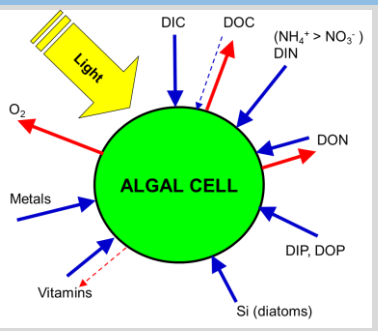
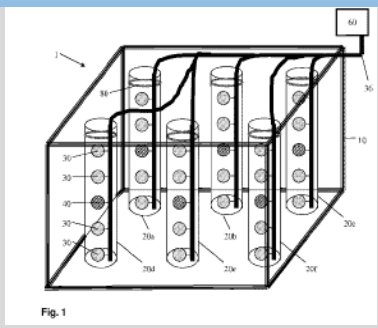
Enhancing Microalgal Production

Constructing Decision Support Tools Using System Dynamics Modelling

Kevin J Flynn





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European Regional Development Fund
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ENHANCE MICROALGAE

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.

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) 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

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

α^{Chl}: the rate of photosynthesis per unit of chlorophyll per photon. α^{Chl} characterises the initial slope of a Chl-specific *PE curve* (e.g., gC $gChl^{-1}$ 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 rate (APR): production rate described in units of area (e.g. gC m^{-2} 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. 3-5 gC m^{-2} d^{-1}. Cf. *volumetric production rate*.

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 HCO_3^- (usually the most abundant form of *DIC*) to 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$_a$, 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: (Cp) 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 CO_2 (the substrate for *RuBisCO*, for photosynthesis), bicarbonate (HCO_3^-) and carbonate (CO_3^{--}).

DIN: dissolved inorganic nitrogen, comprising ammonia (NH_3), ammonium (NH_4^+), nitrate (NO_3^-) and nitrite (NO_2^-). NH_4^+ and NO_3^- are the usual main forms of DIN; 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, PO_4^{---}.

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 α^{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 $cell^{-1}$), or the C content (e.g. gN gC^{-1}). 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 suffic*ient 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 $cell^{-1}$), or the C content (e.g. gP gC^{-1}). The value of P:C relates curvi-linearly 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, CO_2) through photosynthesis. (Cf. *heterotrophy*, mixotrophy, *osmotrophy*, *phagotrophy.*)

PFD: photo flux density (photons m^{-2} 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 (as in "primary production"), but explicitly the *yield* as a one-off output from a process, as distinct from the rate of biomass generation expressed as *volumetric production rate* or *areal production rate*. Cf. *standing crop*, *yield*.

Q_{10}: the proportion by which biological process rates (e.g., growth rate) increases when temperature in increased by 10°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.

ODE: ordinary differential equation. The simulation models described in this book all make use of ODEs.

RuBisCO: ribulose bisphosphate carboxylase; the enzyme responsible for fixing CO_2 (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_2 concentrations (O_2 being a by-product of the *light reaction* of photosynthesis, q.v.), CO_2-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 $cell^{-1}$), or the C content (e.g. gSi gC^{-1}). 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^{-1} 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 $cell^{-1}$ d^{-1}) differs from C-specific (C C^{-1} d^{-1}), and differs from N-specific N N^{-1} d^{-1}), 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^{-1}); 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*, *yield*.

Steady-state: a condition where all processes at progressing in unison, such that the s*pecific 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 though 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*.

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*.**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 Rate (VPR): production rate described in units of volume (e.g. gC m^{-3} d^{-1}). Because of self-shading within the microalgal suspension, optimising high *areal production rate* and a high volumetric production rate 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*.

Yield: production, as a one-off event, akin to harvesting a field. Cf. *production rate*.

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_2 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 kjfplankton@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 (*Spriulina* 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 10µm in diameter (1mm = 1000µm).
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 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 production rate

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 production. Production 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 production rates

Productivity is the effectiveness of the production effort; in crude business terms money in versus money out, or profitability. Of course, you could have a high level of productivity, but a low rate of production. But time is invariably also related to money, so the overwhelming challenge in the commercial exploitation of microalgae centres upon maximising production rates as well as productivity. Specifically, we need to maximise areal and volumetric production rates (APR and VPR respectively). What does that mean?

The Areal Production Rate is the rate of biomass produced per area (i.e., the footprint 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 APR are as (for example) gC m^{-2} d^{-1}. C is the base for organism growth, C-metabolites control organism physiology, 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} × {cell volume}).

The Volumetric Production Rate refers to the rate of biomass production per volume of water per day (e.g. gC m^{-3} 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 APR and VPR, 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 APR and VPR, 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/. 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.

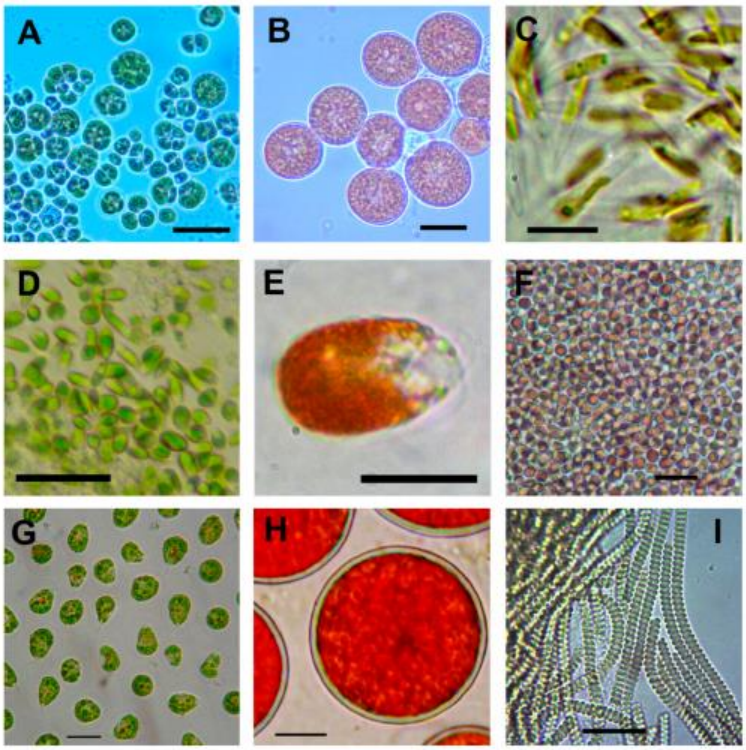


Fig. 2.1 Light micrographs of selected microalgae for commercial cultivation for various biotechnological applications. *Chlorococcum* sp.((A,B); source for mixed carotenoids including -carotene, astaxanthin, canthaxanthin, lutein), *Dunaliella salina* ((D,E); the source for β-carotene) and *Haematococcus 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 µm, except for (C,E), which are 10 µm. 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 compartmentalisations; 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_2-fixation (photosynthesis).

Most cyanobacteria, and all protist microalgae, use fixed (usually inorganic) forms of N-nutrient. Some cyanobacteria, however, can also fix N_2 gas into intracellular ammonium. These "diazotrophs" may either grow in filaments of cells where some cells fix CO_2 and others fix N_2, 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_2 and N_2 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_2-fixation (namely O_2) 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 (Mitra 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 µm in diameter; there are 1000 µm 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 congregate 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 $_{P700}$, 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, mycrosporine-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 β-carotene and phycocyanin (as food colorants) and MAA (for making sun-lotions).

2.6 Photosynthesis

Photosynthesis, the fixation of CO_2 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 bisphosphate 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_2, which is both directly dangerous for the cell and also inhibitory of CO_2-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_2) 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 µM. The solubility of P (as phosphate) is limiting in seawater cultures, as phosphate precipitates out of solution above a concentration of ca. 35 µM (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_2 is usually bubbled in (often as CO_2-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 lead 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.

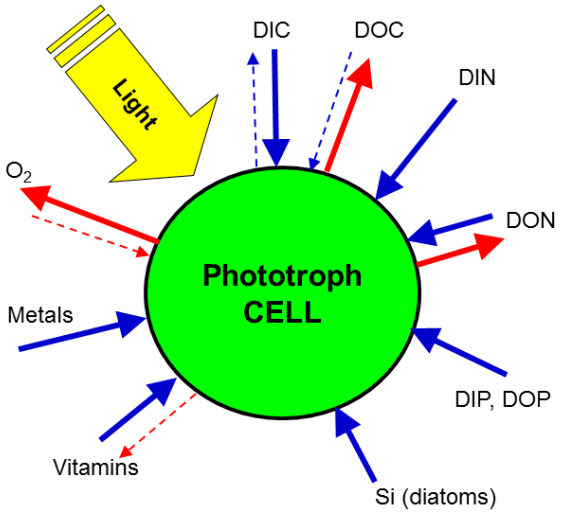


Fig. 3.1. Schematic of resource needs (arrows in), and releases (arrows out), from a phototrophic microalgal cell. Dissolved inorganic C (DIC) is consumed with light-enabled photosynthesis. Flows of O_2 are the converse to those of DIC; a net release of DIC occurs when respiration > C-fixation. A proportion 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_2

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, μ_{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 μ_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 μ 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 ↔ bicarbonate ↔ 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^{-1}), 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 (uC_Alg and uN_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* florescence as this signal (i.e., *in vivo* florescence per unit Chl) varies with nutrient status.

It is important to note that growth rate, technically termed the specific growth rate, has units of $X \cdot X^{-1} \cdot time^{-1}$. "X" could be as cell abundance, gC, Chl fluorescence, or whatever is measured. **Providing the full units for specific growth (and not just $time^{-1}$) 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).

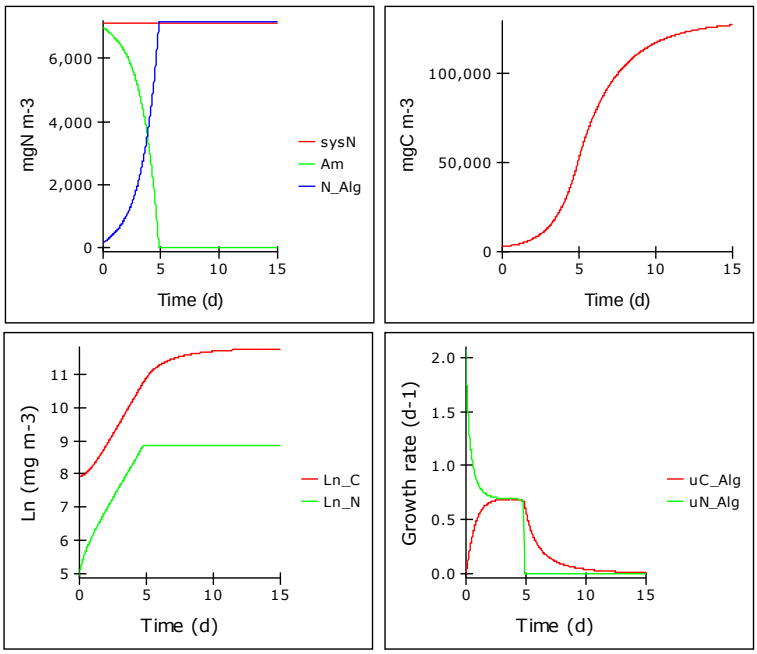


Fig. 3.2. Simulated growth pattern in a system with a shallow (0.05m) optical depth, with ammonium as the limiting nutrient supplied at 500µM (7gN m^{-3}). Illumination is constant. The plots show, over the 15 day period, changes in external and cellular N (Am, N_Alg) with a constant system N (sysN), growth of cellular C (C_Alg), the natural log plot of cellular C and N (Ln_C, Ln_N), and the C- and N-specific growth rates (uC_Alg, uN_Alg). Compare with Fig. 3.3 for a deeper system. The model used for this simulation is described in Chapter 8.

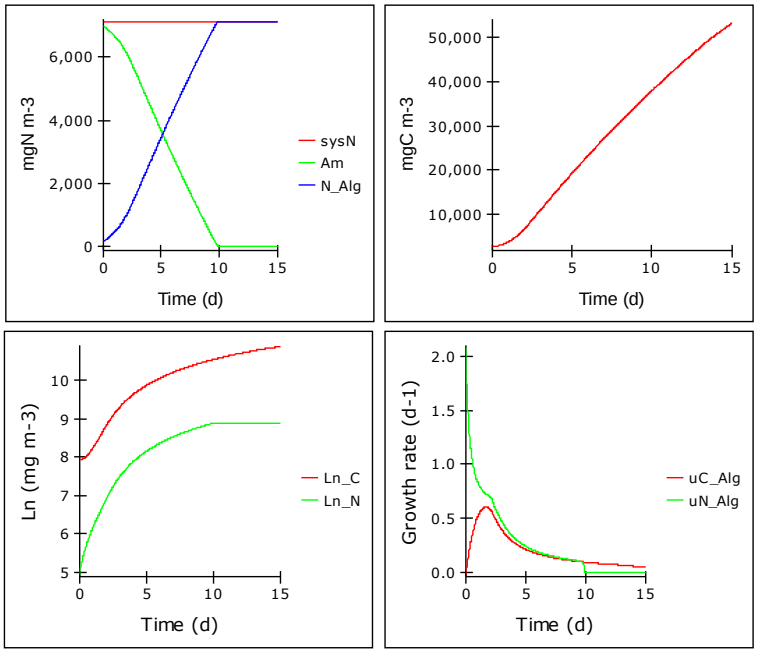


Fig. 3.3. As Fig. 3.2, but for a deeper system, now of optical depth 0.5m. Note that growth is now linear because of the impact of self-shading within the developing culture system.

Only in batch cultures may the growth rate (μ_T) approach the maximum possible rate at that temperature (μ_{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 spectrums (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.

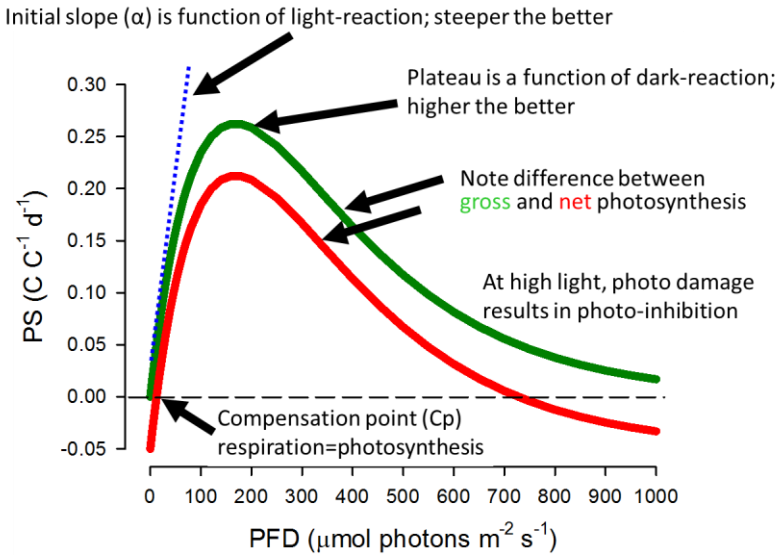


Fig. 3.4 Photosynthesis-irradiance curve showing the relationship between gross vs net photosynthesis, limitations by "light" vs "dark" reactions. Here, respiration is shown as constant; in reality this is not so.

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 (Cp). 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 $C \cdot Chl^{-1} \cdot time^{-1}$. 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 Cp 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 Cp 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 α (α^C) decreases – the initial slope of the PE curve decreases, though the Chl-specific equivalent (α^{Chl}) may alter rather less. Under Fe-limitation, α^{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_2 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_2-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_3^-). However, the "preferred" source for physiology is ammonium (NH_4^+); 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_3), which forms an equilibrium product with ammonium (NH_4^+), 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.

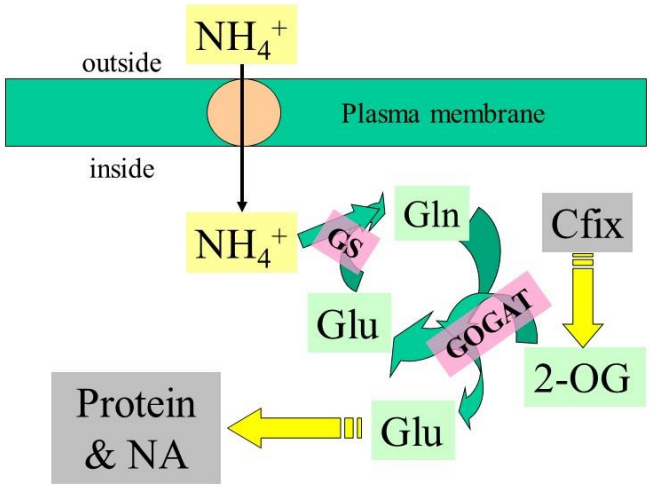


Fig. 3.5. Ammonium assimilation. Ammonium (NH_4^+) is transported into the cell where it combines with the amino acid glutamate (Glu) to give the amino acid glutamine (Gln); this is enabled via the enzyme glutamine synthetase (GS). C is supplied, as shown here via C-fixation from photosynthesis, as 2-oxoglutaric 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.

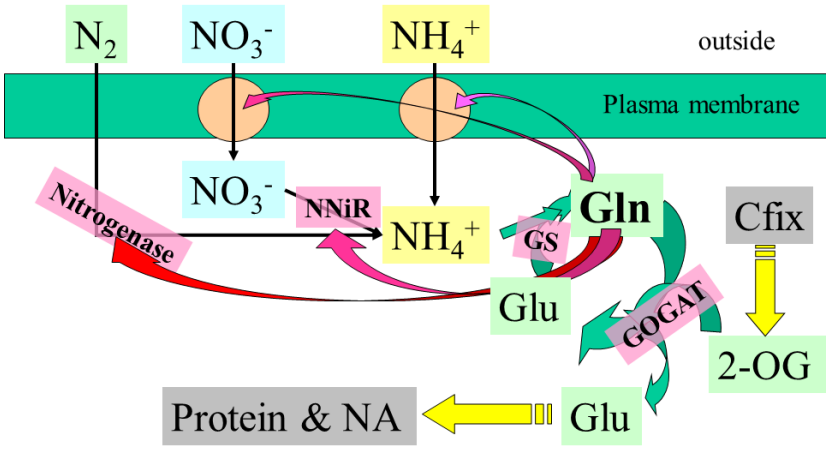


Fig. 3.6. (De)-repressive regulation of N-source acquisition. Through the scheme shown, the internal concentration of the first organic product of inorganic-N assimilation, glutamine (Gln), likely allied to the concentration of a C-substrate such as 2-oxoglutaric acid (2-OG) is implicated in the control 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 (NCmin) and the optimal value (NCopt). N:C can exceed NCopt 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^{---}). 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).

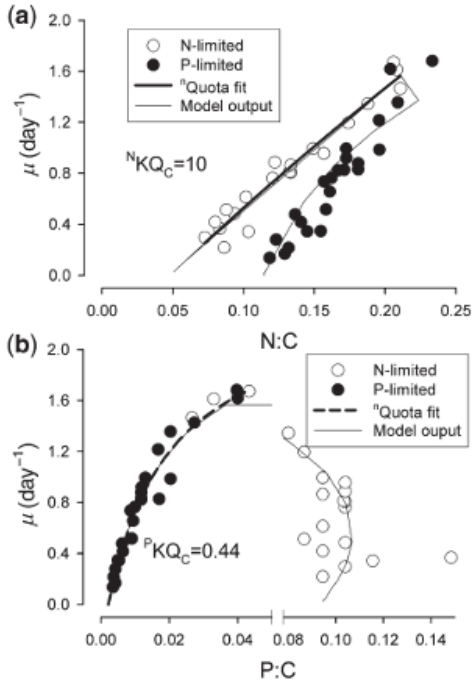


Fig. 3.7. Relationships between N:C and P:C with growth rate. Note how the relationships vary depending on whether N or P is limiting. The dashed line is a regression line fit through the nutrient limited points for N:C (panel (a)) or P:C (panel (b)). The thin lines ("Model output") comes from a simulation, 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. 35μM. This is not to say that the culture cannot be loaded with more P than ca. 1mgP 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 tricornutum*, 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 previous 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_2 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^{-3}), but areal abundance (i.e., cells m^{-2}). 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 rates (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_{12} (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 sloughs 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.

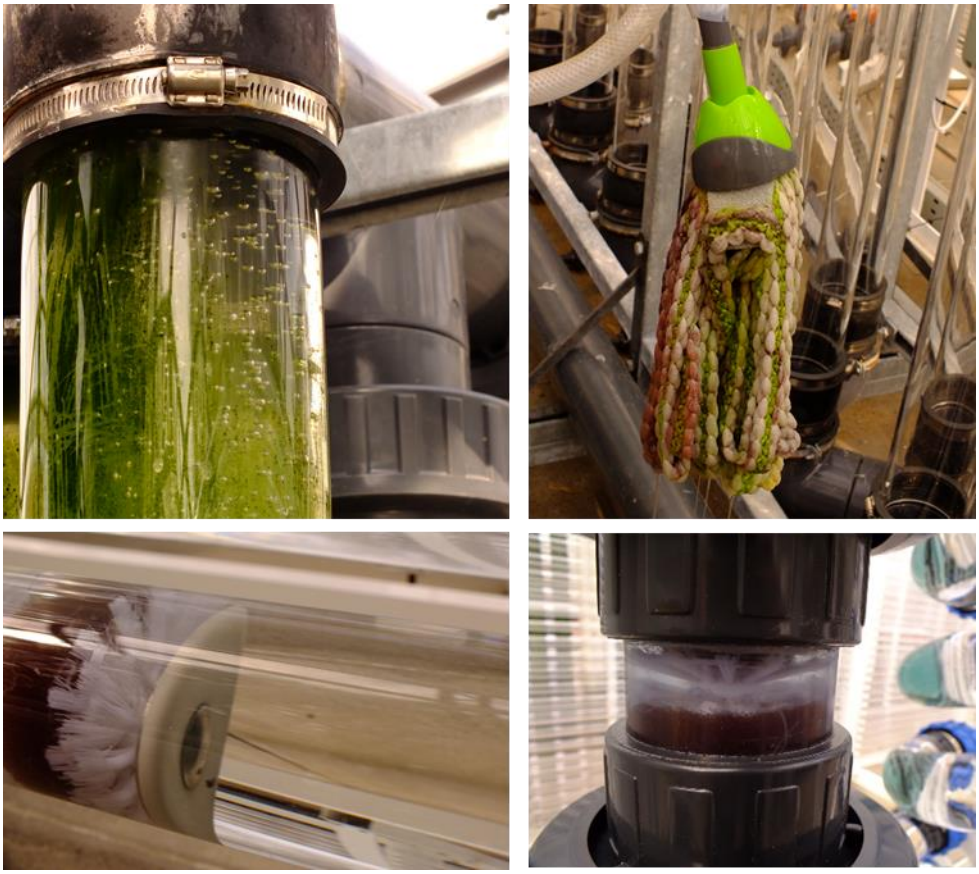


Fig. 4.1. Growth of microalgae on bioreactor walls can rapidly become highly significant (top-left), requiring the manual use of some form of brush or mop (top-right). A more high-tech option, that does not require the dismantling of the reactor, and indeed can be conducted during active culture growth, 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).

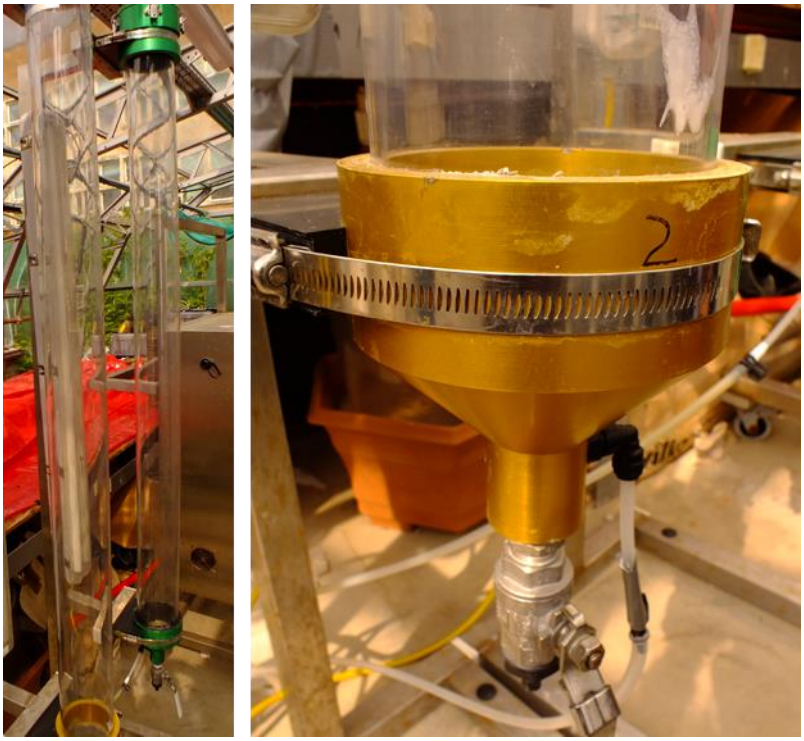


Fig. 4.2. Simple bioreactors for low volume (20-50L) batch cultures. These comprise lengths of acrylic tube with custom made end caps; detail of the lower cap (with aeration input) is shown on the right.

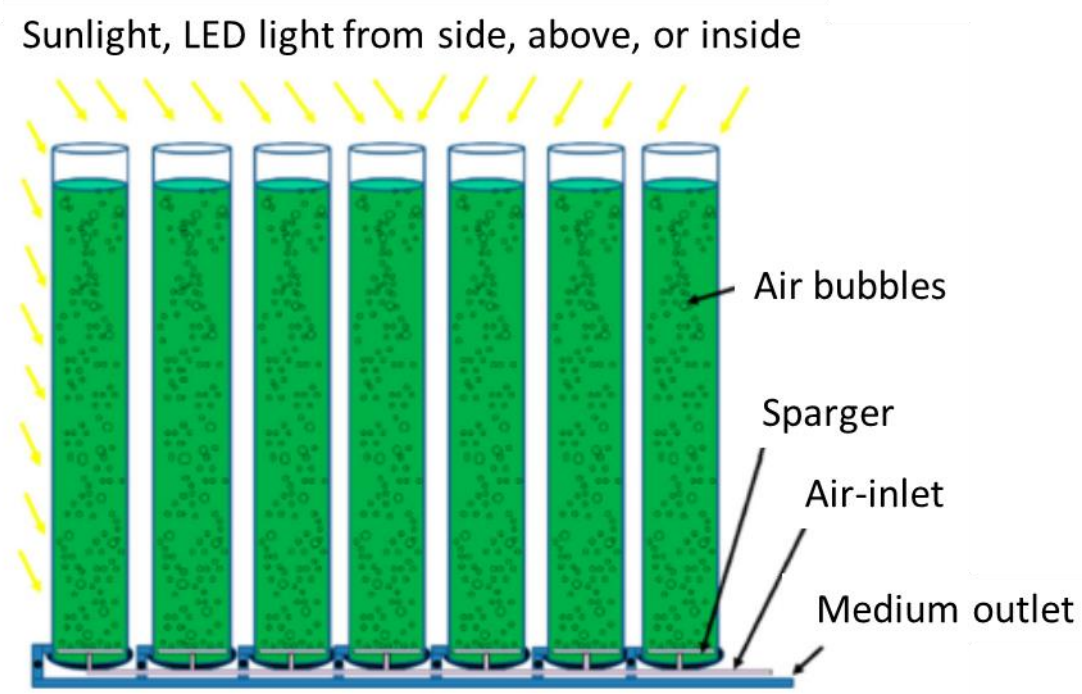


Fig. 4.3. Schematic of a modular PBR. Such a configuration can be scaled indefinitely but is not readily configured as a continuous-culture system but rather for batch growth. From Saha & Murray (2018).

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.

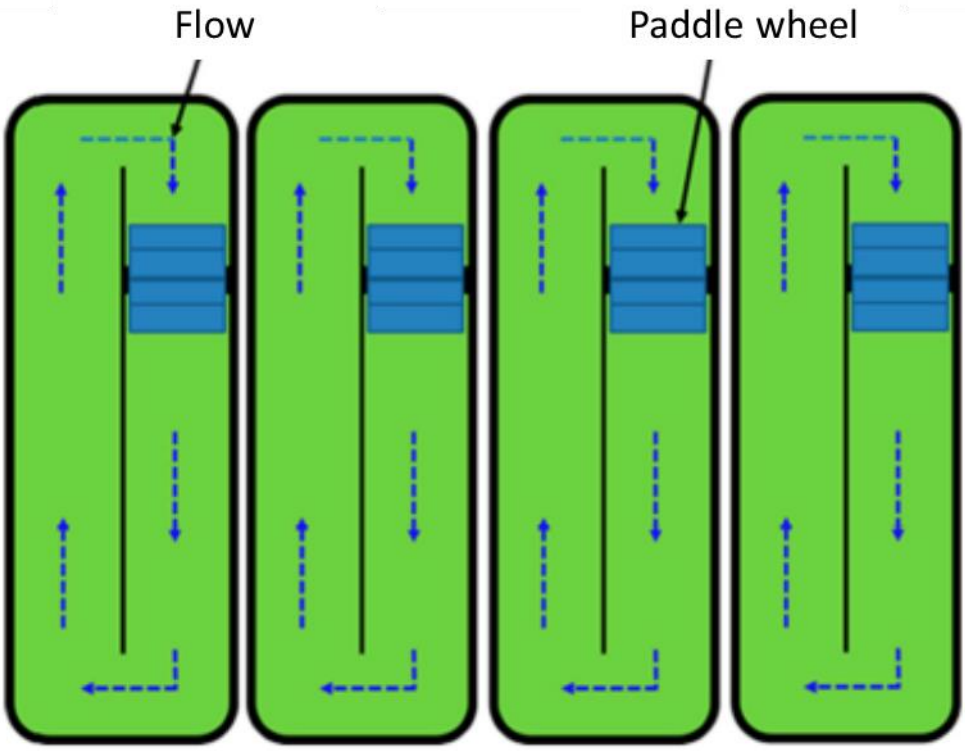


Fig. 4.4. Birds-eye schematic of a simple pond, "race-way", culture system comprising outdoor rectangular ponds and paddle wheels to maintain a flow of mixed suspension. In addition to gas exchange at the surface, additional gas (CO_2-augmented air) may be injected into the system. From Saha & Murray (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 (CO_2 in, 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 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.

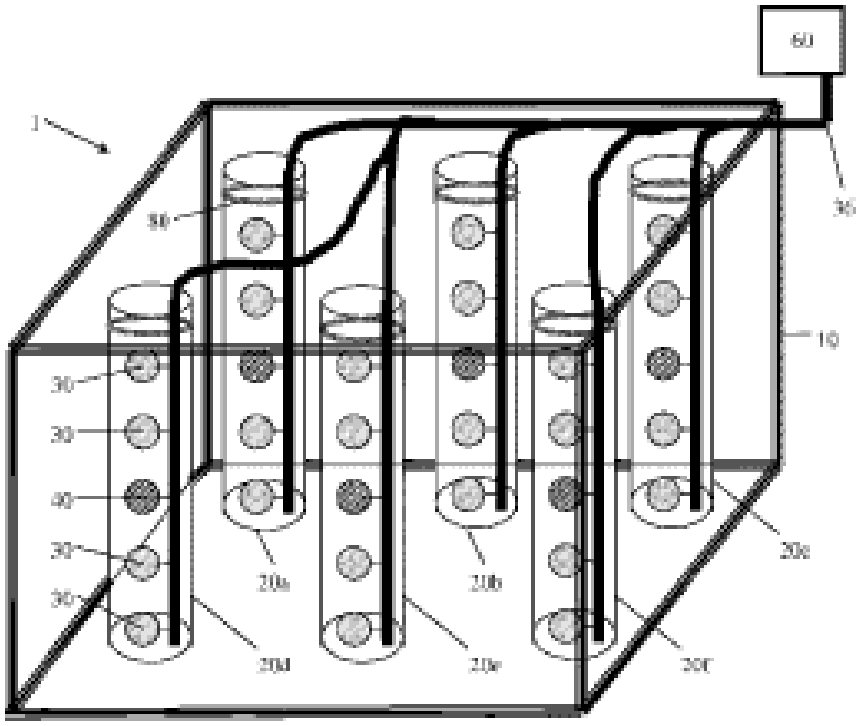


Fig. 1

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 4π 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 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$_a$ content using taxonomic factors).

The unit of light is preferably as photon flux density (PFD; moles of photons m^{-2} s^{-1}), or as energy (W m^{-2}).

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_{10}=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:

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

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_2 removal by photosynthesis and gas exchange of CO_2. CO_2 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_2 aqueous, HCO_3^- (bicarbonate) and CO_3^{--}(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_2 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_2. Microalgae have to depend either directly upon CO_2 in the water, or use carbonic anhydrase to convert HCO_3^- to CO_2 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_2 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_2 gas. This addition not only maintains the DIC concentration but buffers the water as well. Typically, in large systems pH and CO_2 concentrations are simultaneously maintained using a pH-stat which governs the injection of CO_2 gas to balance the removal of CO_2 by algal growth. Usually CO_2 entry is coupled with air in a 5% v/v mixture, or similar.

Another, important, reason to aerate the system is to bring O_2 into the culture medium during the night (to prevent anaerobic conditions developing due to respiration) and conversely to remove excess O_2 during the day. It is important to remove O_2 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_4^+) and ammonia (NH_3). The balance of NH_4^+ vs NH_3 depends on pH; at high pH NH_3 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_2-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 and constant production rate (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 production rates 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 production rate 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., $gC/m^3/d$). 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^{rds} 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), 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 . 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 simulator 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. 1/3rd 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., gN m^{-3}), and the rate of production will thus have units of gN m^{-3} 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 m^3; there are 1000 L in 1 m^{-3}. A volume of pure water of 1 m^{-3} has a weight (mass) of 1 metric ton.
- Ground area of the bioreactor &/or of the facility: This is required as 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 d^{-1}). Organisms growing in a chemostat do so at the same rate as the dilution rate; so, a dilution rate of 0.693 d^{-1} will drive a growth rate of 0.693 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 (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 W m^{-2} or PFD (mole photons m^{-2} $time^{-1}$). 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 mgN and mgP L^{-1} (= g m^{-3}).
- Volume of the microalgae inoculum: This is the volume of culture added as an inoculum to the bioreactor, required as m^{-3}; there are 1000 L in 1 m^{-3}.
- Inoculation concentration: This is the biomass concentration in the inoculum; it is required as gC m^{-3}. This unit is the same numerically as 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 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 2×0.693 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 (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 gN gC^{-1} and 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 gChl gC^{-1}, where Chl is actually 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.
- α^{Chl}: This defines the initial slope of the PE curve expressed per unit of 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^{-3})
- Irradiance at the bioreactor surface (as energy or PFD)
- Cell numbers &/or Chl_a, (numbers or gChl m^{-3})
- 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^{-3}, then transformed to gC m^{-3})
- Biomass elemental content (C,N,P; g m^{-3})
- Pigments (g m^{-3})
- Biomass protein &/or lipid &/or carbohydrate (g m^{-3}, then transformed to gN &/or gC m^{-3})
- DIC if pH is not constant (gC m^{-3})
- Specific metabolites of interest (g m^{-3}). 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 financial 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 products 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 (enhanced by growth without exhausting N-nutrient)
- Lipid production (enhanced by growth typically exhausting the N-nutrient).
- Pigment production (enhanced by growth 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 rate).

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. The 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.

7. Introduction to Using the Models

7.1 Introduction

In the following chapters (Part II) different models will be introduced, concepts explained, and the computational basis of the simulator described. These descriptions are based on what are called (after their originator) Forrester diagrams, which provide pictorial representations of the model structure. These models describe system dynamics, with an explicit description of the flow of materials around a system. The fundamentals of systems dynamics modelling, as applied here, are given in Flynn (2018). What will not be given here, except in Chapter 8, are in-depth descriptions of the models themselves. However, the models are presented for download with descriptions of the components for those who wish to rebuild them and have the skills and enthusiasm to do so.

The models for this DST are provided in two forms:

- a version to run using the free-to-end-user Powersim Studio Cockpit; this allows limited access to changing configurations. You cannot develop the model using this interface, but you can experiment and operate them as a means to aid decision making (i.e., as a DST).
- a version to run using Powersim Studio 10; this allows full access and development options. You can also add in risk analysis and optimisations. You will, however, need to purchase this software, or code the model into an alternative platform.

The first models, described in this and the next chapters, can also be run using Powersim Studio 10 Express (see Section 7.2). While this is a free download, giving access to the full software, Express does, however, place a limitation on the size of the model. The model described below will comfortably run within that, but the model in Chapter 8 is at the maximum size to operate in this software environment. From this you will likely be able to judge whether you wish to delve deeper into using system dynamics models. Please also check Flynn (2018) for further information and a self-taught course on how to develop these types of models.

The EnhanceMicroAlgae project does not endorse Powersim products. Other modelling options are available; some (such as insightmaker; https://insightmaker.com/ and R) are free, though most are not. The prices also typically vary depending on whether the application is for academic or commercial use.

You do not have to make models at all. The models provided with this DST are available as free-to-use for the end user. All you will need is a PC running MS Windows, or an emulator. You then simply need to download Powersim Cockpit for free from:

https://powersim.com/main/download-support/technical_resources/service_releases/studio10cockpit/

You can then open the DST model of interest, make your operational choices (such as the depth of the pond, nutrient concentrations, light levels), and press “run”.

Each of the following chapters provides insight, instructions, and caveats for the systems being simulated.

7.2 Using Powersim Studio – a primer

The following provides a VERY brief introduction to system dynamics prior to guiding you through making a simple model. It is presented here really to satisfy your curiosity. You can undertake this activity using the free Powersim Studio Express download available from:

http://www.powersim.com/main/download-support/technical_resources/free-downloads/

If you simply wish to run the model described in Section 7.3, you just need to download and install the free Powersim Studio Express software, download and open model from the DST website, and use the run buttons to run the model (see Fig. 7.2 to locate these).

Fig. 7.1 shows the start-up screen; depending on how the programme was last exited, there may be other panes visible as well.

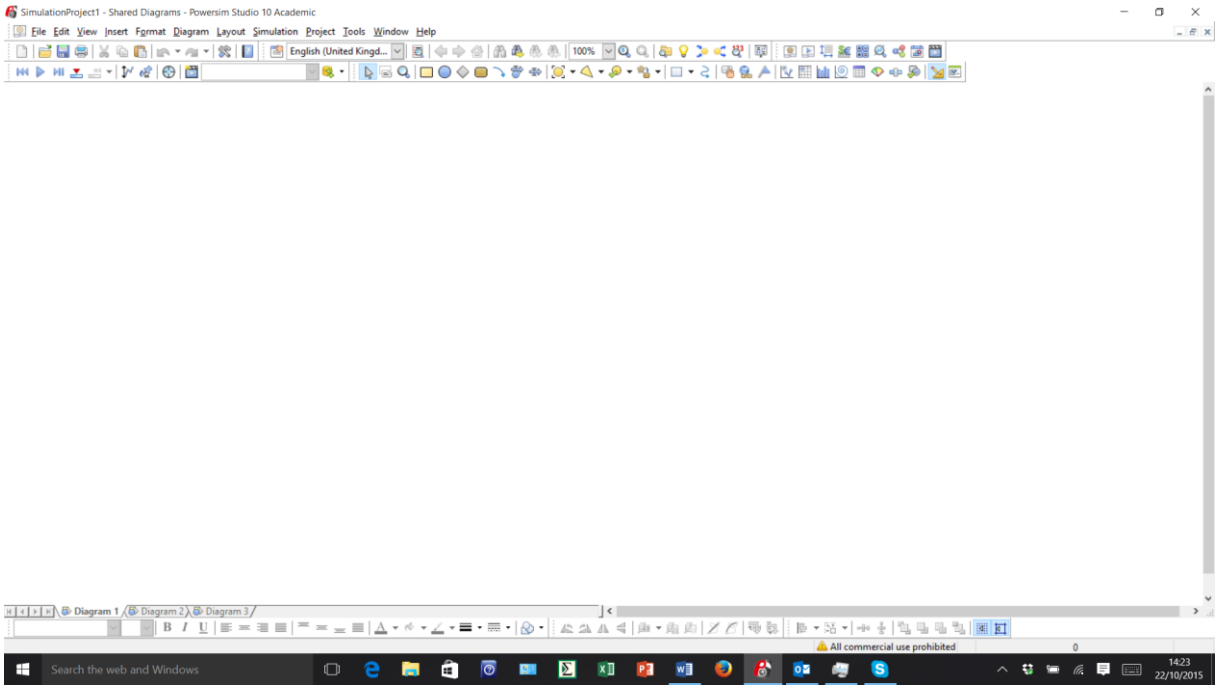


Fig. 7.1. Start-up screen.

Locations of the most important buttons and dialogues are indicated in Fig. 7.2.

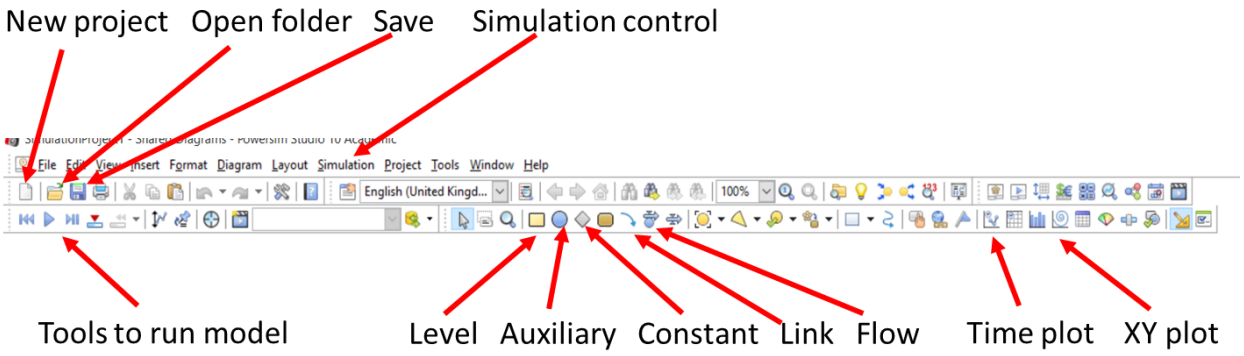


Fig. 7.2. Buttons and dialogues.

Models are made from combining the following components:

"**Levels**" (also often called "state variables") describe variables that can be measured and have a history, such as concentrations and amounts. In Forrester diagrams, levels are shown as rectangles.

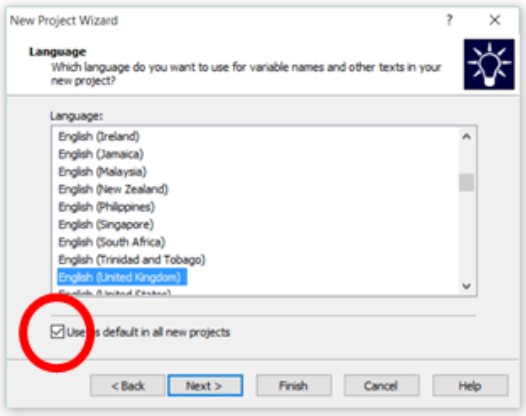
"**Constants**" describe variables that are (at least for the simulation) held fixed, as constant. In Forrester diagrams, constants are shown as diamonds.

"**Auxiliaries**" describe variables that result from functions of other auxiliaries, constants and levels, described by equations. In Forrester diagrams, auxiliaries are shown as circles, with connections to variables described in the equation with links (arrows).

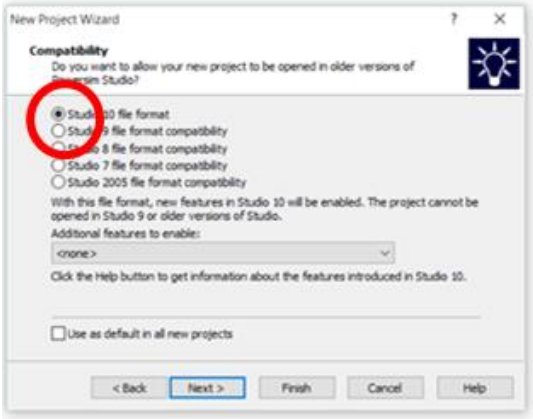
"**Flows**" described additions or subtractions to levels. Flows are described by constants or (more usually) auxiliaries. In Forrester diagrams, the actual flow is shown as pipelines with arrows indicating the flow direction.

To start a new project, click the "New project" button; you will then be asked to go through the "new-project wizard". It is important that you check certain features in this, as follows.

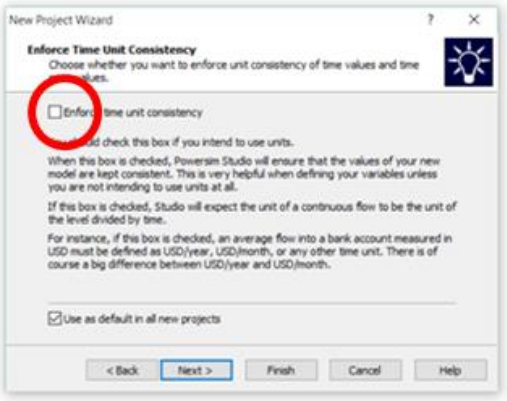
Always enable the wizard – do <u>not</u> check the "Don't show the wizard next time" box.



Check your language of choice. AND check the "Use as default in all new projects" option for this and all the following dialogues.

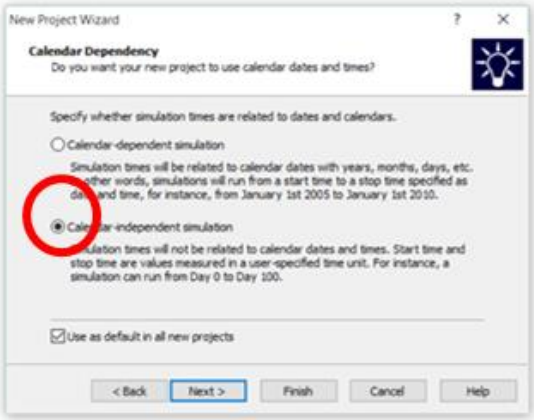


Check the file format. Note that once you pass this step you cannot change the format.

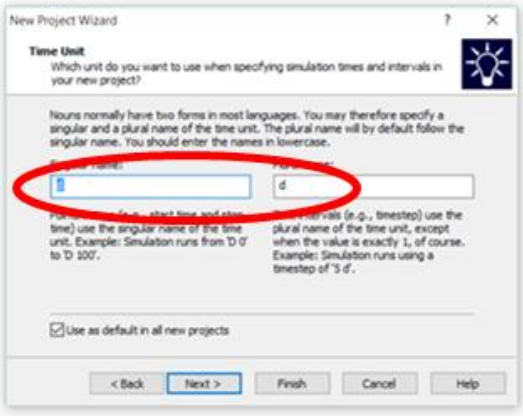


I would suggest that you do not check for unit consistency. In theory this can be really useful, but in practice it can be annoying. However, without this facility be aware that it is up to you to check that units make sense.

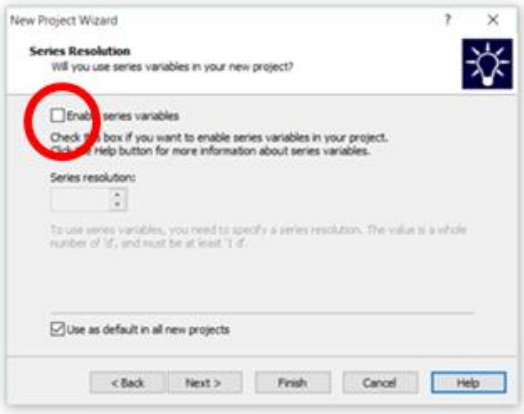
Remember that everything you do with parameters you do with the units. So, if you multiply parameters then the resultant units are dictated by the component units multiplied together. For example, gC * 1/time = gC time^{-1}. You can only add and subtract parameters that share common units. Thus you cannot legitimately do this .. gC + gN . If you wished to do this then you would need to convert one value unit into the other using a transform. So, you may know that in your microalgae, the mass ratio of C:N was 7, so now you could do .. (gC/(7gC/1gN) + gN. Be careful and double check equations; it is very easy to make a mistake, but even without unit checking, Studio will alert you to spelling and various constructional errors.



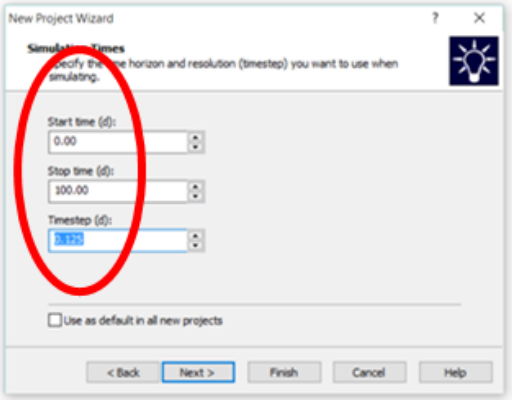
Select calendar **in**dependent simulations. Do not select for calendar dependant simulations, unless you really want time date-stamped with days and months of the year.



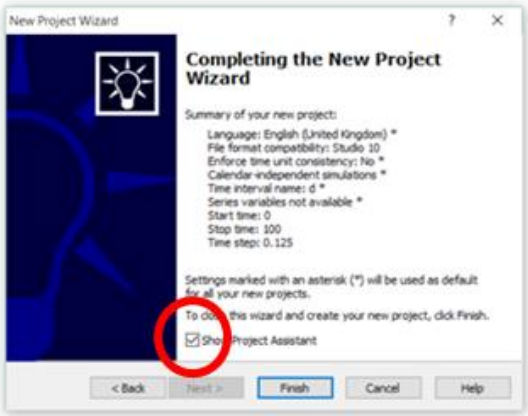
For most biological applications the time unit is most convenient as days. The SI unit for time is seconds, but using this will generate numbers that will not typically be very helpful.



Do not check the series variable box.



As default time and timestep settings use a start of 0, and end of 100, and a timestep of ca. 0.0625 (which means that integration calculations to make the model work will occur 16 times each simulated day). **You can alter these values easily later.** See Flynn (2018) for information about selecting integration methods and step sizes.

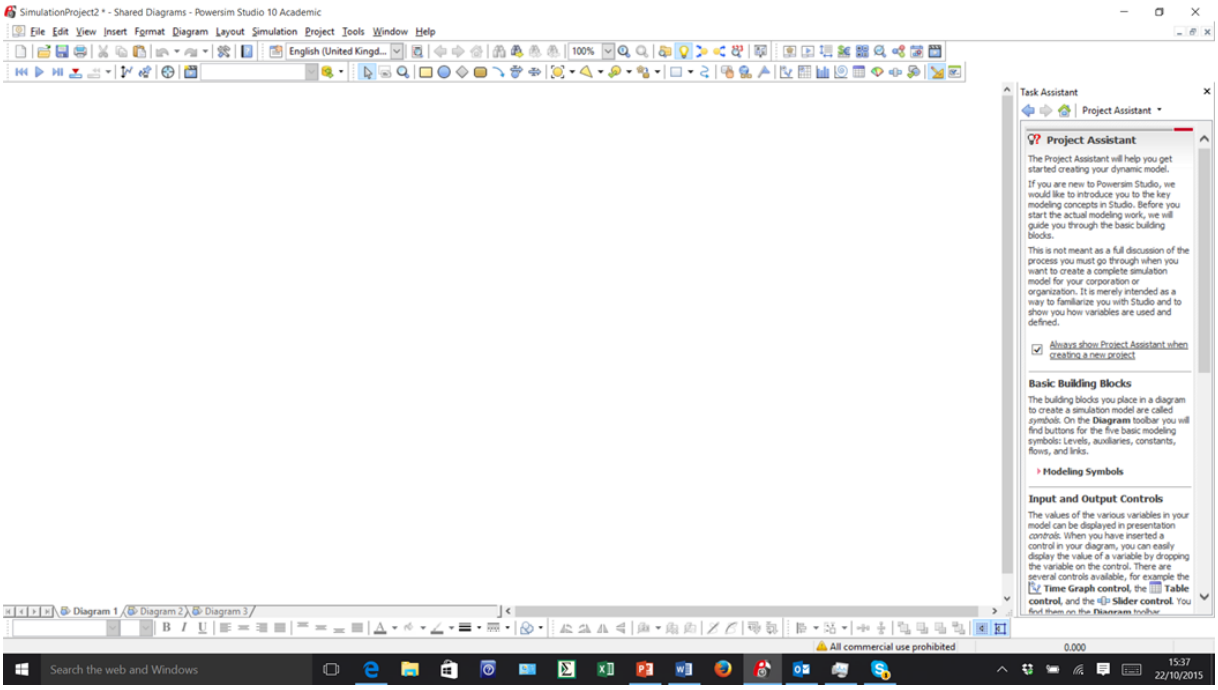


Select whether you want the project assistant to be present. It is easy enough to turn it on or off.

7.3 Making a simple model

This section will describe a model simulating nutrient-limited growth of a microalgal culture using Powersim Studio 10.

The start screen should look something like this:

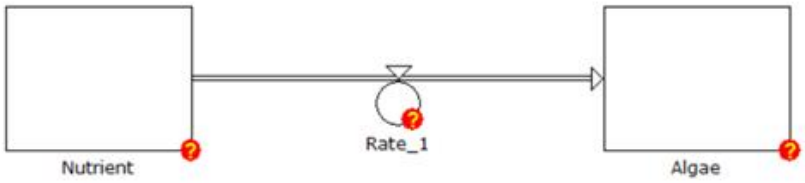


Unless you want the “Project Assistant” click it out of the way (X in top-right-hand corner of the Project Assistant dialogue).

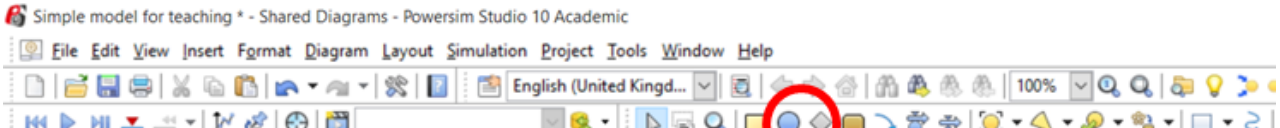
In each of the screen shots below, the menu item to use is identified with a **red circle**.

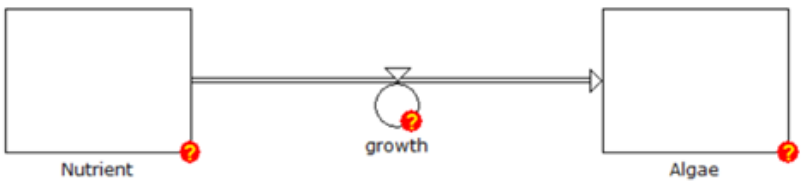
Start by using the “level” tool to make two levels, approximately horizontal with each other and equal size, and label them as “Nutrient” and “Algae”. From here on, variables names will be given in this document in italics; thus *Nutrient* and *Algae*. The “?” indicates that you have not defined the parameter yet; ignore this just now.

Next add a flow between the levels. To do that, select the "flow-with-rate" tool by left-clicking on the button, move the cursor to the centre of the *Nutrient* symbol, click-and-hold-and-drag over until the cursor is in the centre of the *Algae* symbol (ignore the cloud!), and un-click. Title the "Rate_1" as *growth*.



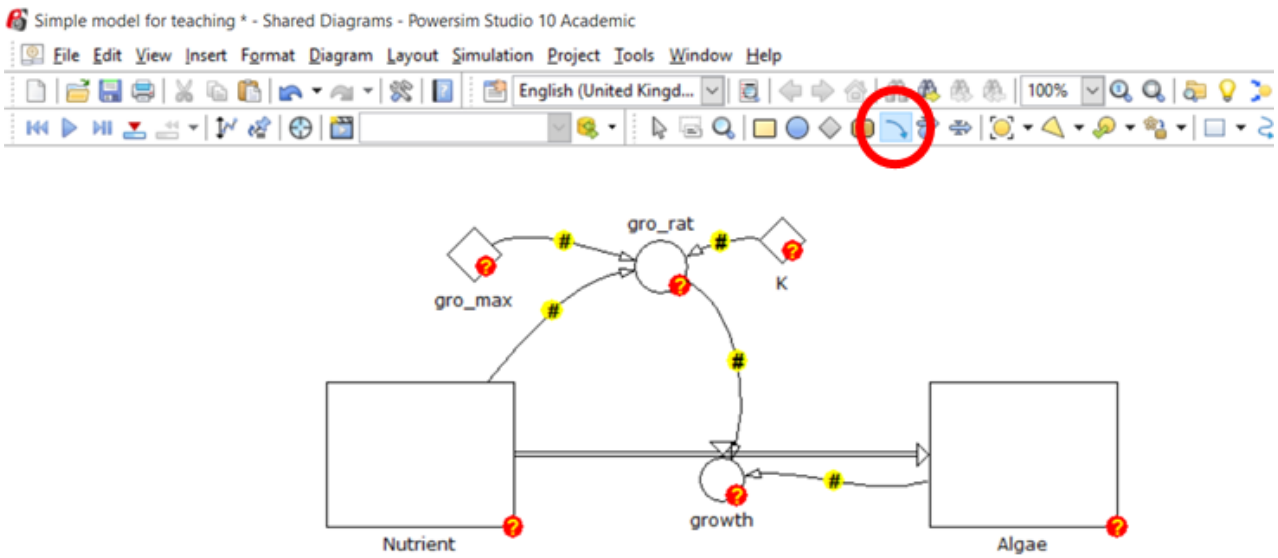
Now select the "auxiliary" tool and place an auxiliary approximately above the flow; label this as *gro_rat*. Move (drag) its title to the top of the circle. Add two "constants" close to *gro_rate* labelled *K* and *gro_max*.





Now would be a good time to "save" the project.

Next use the "link" tool to connect the levels, auxiliaries and constants as shown; note the direction of the arrows!



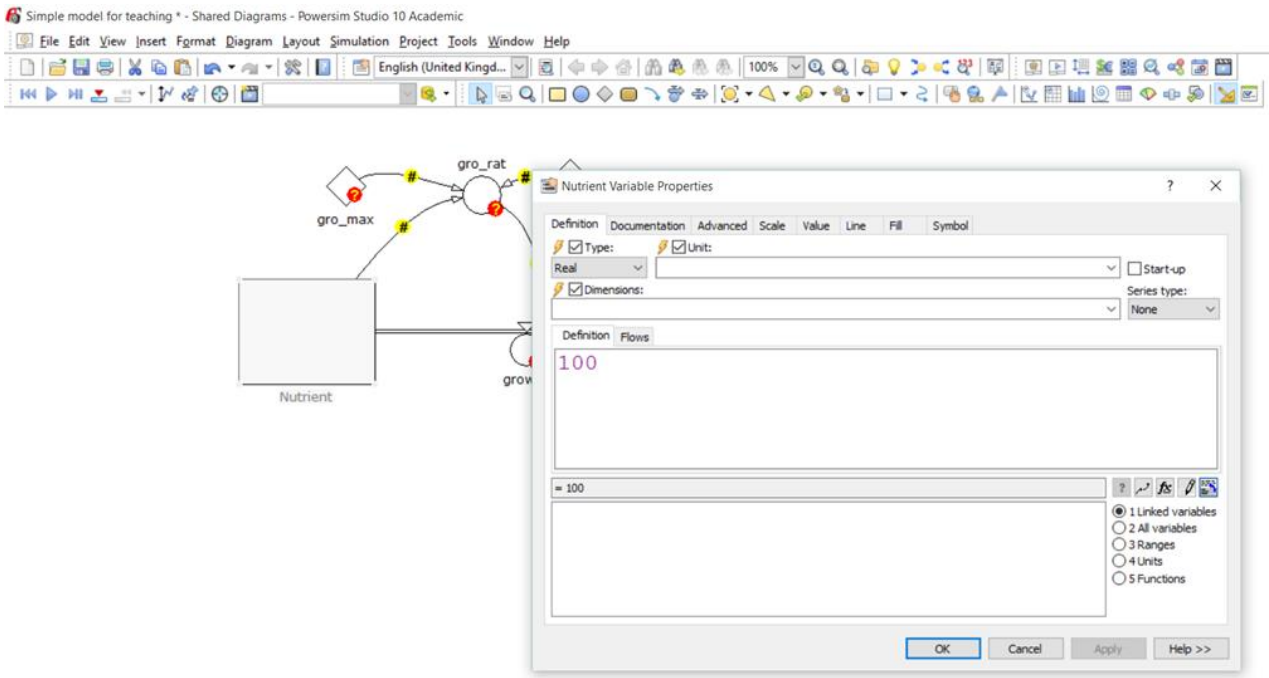
You have now completed the conceptual model. What this is saying is that there is a flow of material from *Nutrient* to *Algae* that is a function of the concentration of nutrient, a maximum possible growth rate (*gro_max*) and a constant *K*, and that the growth of the population (*growth*) is a function of the growth rate of the individual (*gro_rat*) and of the size of the algal population.

To turn this conceptual model into a mathematical model, you now need to enter information into each of the components. Do this by double-clicking on the component, and a dialogue box opens.

Start with the levels, *Nutrient* and *Algae*, and enter 100 and 1 (respectively) into the "Definition" box. This is shown below for *Nutrient*. Once you have entered the number and clicked "Accept", if you then right-click with your cursor in the centre of the dialogue box, and select "options" you can increase the default font size of text that you enter into all future dialogues. This is really helpful when it comes to checking that you have entered equations correctly!

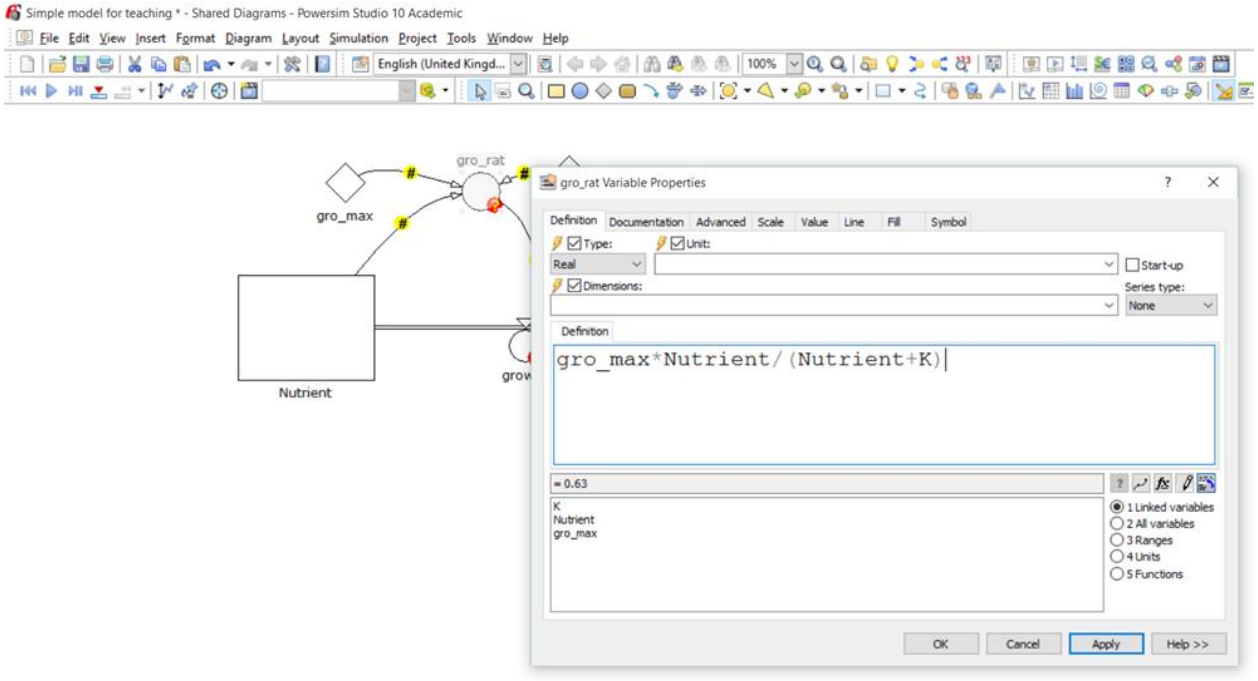
Into the "Documentation" tab enter some text explaining what this parameter is; it is good practice to include the units of g N m-3 (this should actually be g N m^{-3} but the dialogue will not quickly accept superscripts). Do NOT use the "unit" part of the dialogue as in the wizard you have said you are not using this functionality.

Note that once you have entered a definition the "?" disappears. The # symbols indicate that the link is not used correctly.



Into *gro_max*, enter 0.693; this will give a maximum growth rate of a doubling per day, it is the value of Ln(2). Into *K*, enter 10. In their "Documentation" tabs enter respectively for these constants, "maximum growth rate d-1" and "half saturation constant g N m-3".

Now select *gro_rat*.



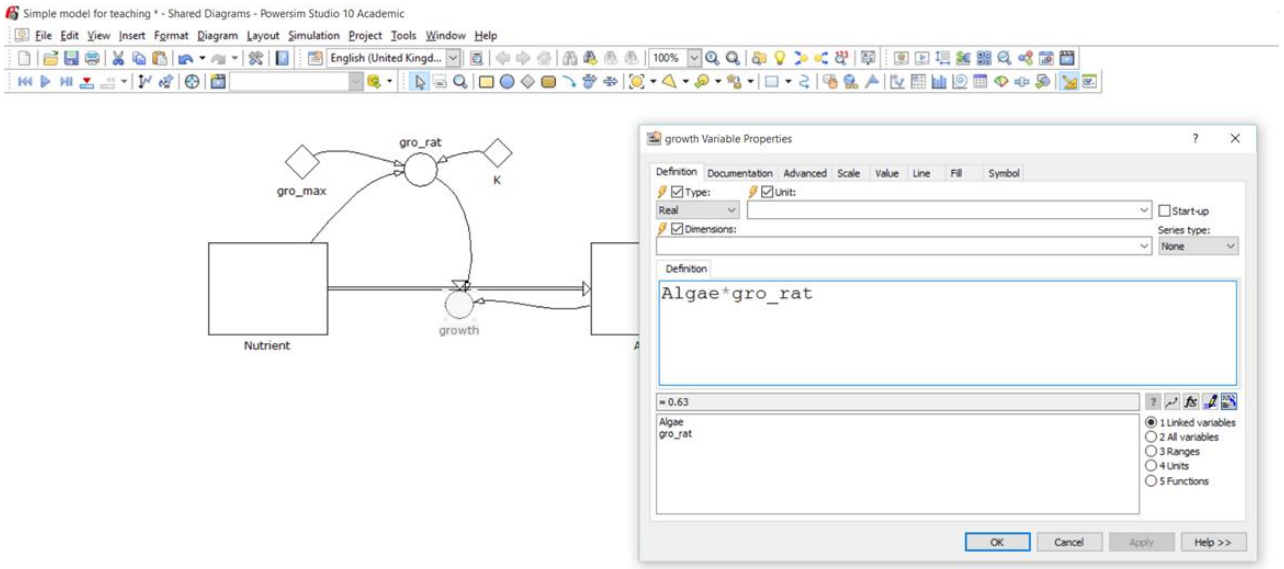
In this dialogue the lowermost box lists the parameters that are connected to *gro_rat*. You must use each of these at least once in the definition else the programme auto-checks will not pass the entry. So, in the "Definition" box itself, enter the equation as shown above; be careful to do so exactly as shown. As in Excel, and for computers in general, "*" means multiply. You can enter the parameter names either by double clicking on them in the list, or by typing their names; if you do the former you can be sure that the name will be entered correctly.

Under the "Documentation" tab, enter "growth rate d-1". If you inspect the equation, you will see that *K* and *Nutrient* have the same unit (otherwise you could not properly add them together!), and that the units of *Nutrient*/(*Nutrient*+*K*) thus cancel out. So, the unit of *gro_rat* must be the same as that for *gro_max* (which is d^{-1}).

When you "Accept" and exit the dialogue for *gro_rat* the # and ? will disappear.

Now select the *growth* auxiliary attached to the flow. Again the parameters that must be involved in the "Definition" are provided. Enter the equation as shown below. In "Documentation" enter "population growth rate (g N m-3 d-1)".

How can these units be correct? Well, *Nutrient* has units of g N m^{-3}, and *gro_rat* has units of d^{-1}, and these parameters are multiplied together. However, another way of thinking about this is that this parameter *growth* defines a flow of material. A flow is a rate, and rates always have as units "per time", or "1/time". Here time is in days, and 1/day = d^{-1}. The material we are moving has a concentration of g N m^{-3}. And so the flow rate has units of g N m^{-3} d^{-1}.

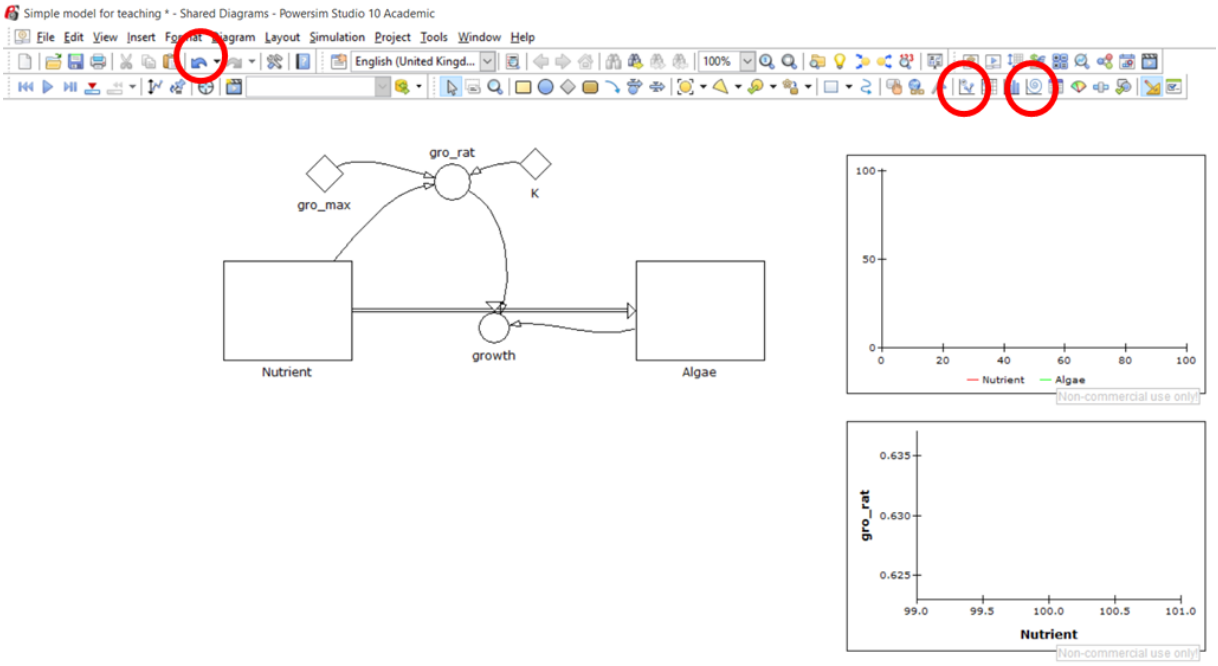


The model is done; remember to save!

Next let us make some graphs. Select a time graph and also an x-y scatter graph. For each, click on the button (see below), put the cursor where you wish the top-left-hand corner, click-and-drag to expand, and release the click when done. As with everything, you can later move and resize as you wish.

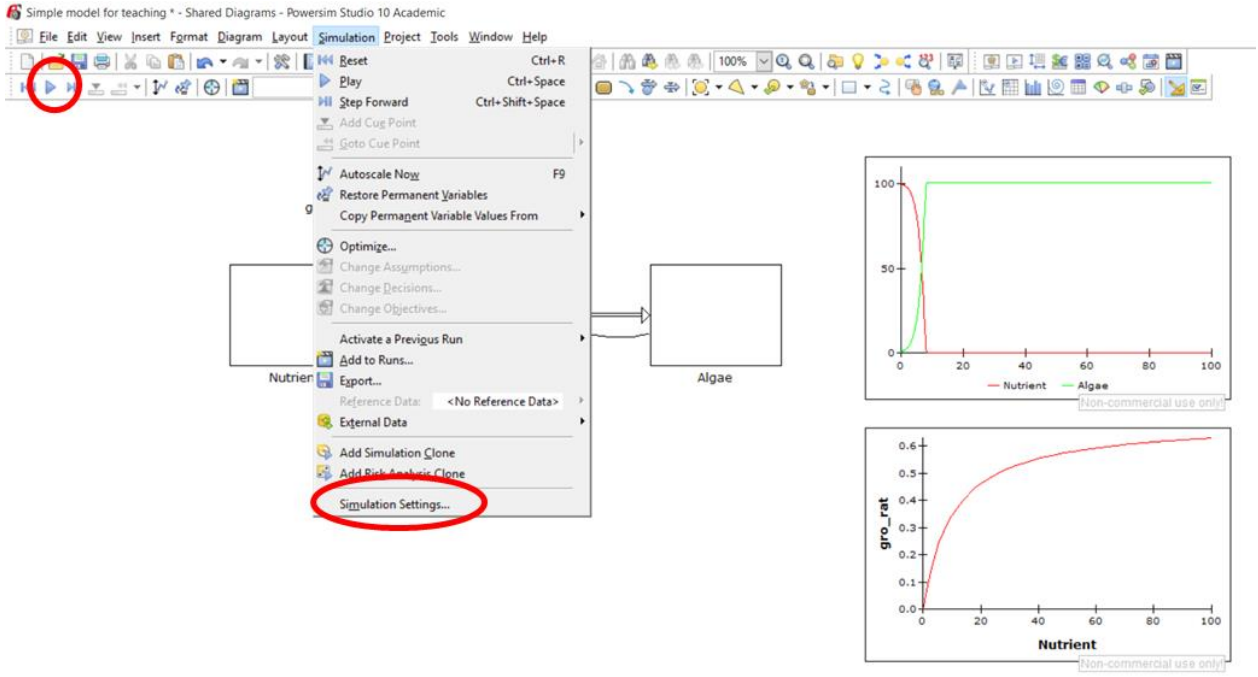
To select the data to be plotted, simply place the cursor over the parameter, left-click-and-hold-and-drag over into the centre of the plot. Do NOT let go of the button until the cursor is within the graph box and the cursor symbol has changed. If you do not do it correctly, it is likely that the diagram will be radically changed; if that happens, use "undo" (top line of buttons), and try again.

For the time graph drag in *Nutrient*, and then *Algae*. For the x-y scatter, drag in *Nutrient* (which then goes on the x-axis) and then *gro_rate* (which then goes on the y-axis). The screen should now look like this, with the red circles indicating the graph buttons and also “undo”.

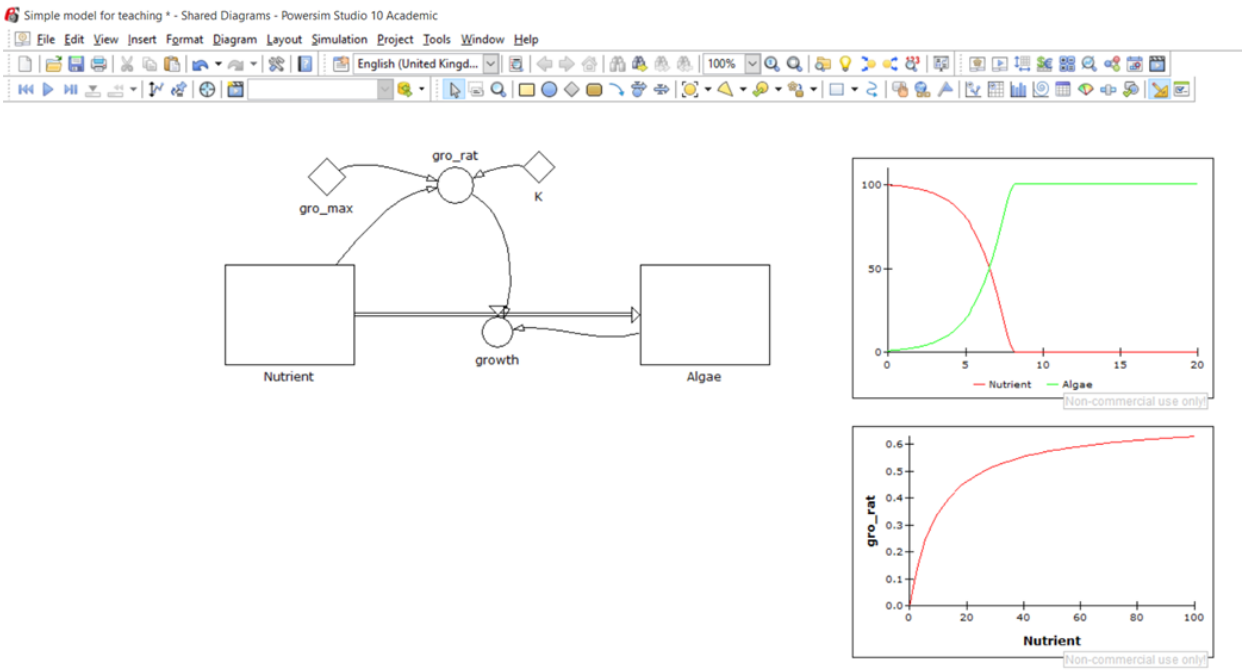


And now you are ready to run the model. Before pressing run for the first time, save the project again.

Now, press the run button and watch what happens. The events have concluded within 20 simulated days, so it is pointless running the simulation for 100 days. So click on the “Simulation” tab, and then on the “Simulation settings” option.



Alter the “stop time” in the dialogue that appears to the new value of 20, accept and re-run the model. It should look something like this:



You can see that the model is simulating the decrease in nutrient concentration as it is transferred to the algae. The x-y plot shows that as the nutrient level declines, so does the growth rate of the algae; note that the plateau value (which is off scale here) will be 0.693 d^{-1}, equal to the value of *gro_max*, while if you read off a value of *Nutrient* equal to the value of *K* (10 g N m^{-3}), you get a value of *gro_rat* that is equal to half that of *gro_max* (around 0.35 d^{-1}). From this you can see why parameter *K* is termed a "half saturation constant".

Note that the levels must have the same units, else you cannot simulate the flow of material between them. So, if you want to simulate changes in cell abundance you will need to know the relationship between gN and cells. For a typical microalgal cell of 10µm diameter, the C content is approximately 0.12 ngC $cell^{-1}$, and you could assume a mass ratio for C:N of 6, so the N content would then be 0.02 ngN $cell^{-1}$. There are 10^9 ng in 1g. At the peak of the simulated culture growth there are 101 gN m^{-3} of algal biomass. So that would be 0.02e9 *101 cells in 1 m^3 of culture. There are 10^6 mL in 1m^3, so in 1mL of this suspension we may expect something like (0.02e9*101 / 1e6) = 2.02e3 = 2020 cells. Of course, to do this continuously in the model output all you need is a constant describing this transform between gN m^{-3} and cells mL^{-1}; you then just need to link this and the level describing algal biomass to a new auxiliary called, for example, cells_mL .

7.4 Conclusion

This concludes a simple demonstration of how to make a system dynamics model. Here it just describes the growth of a microalgae which is only limited by the availability of nutrient. In reality, as the microalgal population grows so it self-shades itself from the light. So, the more nutrient you put in (which according to this model you have just made will just increase production) the greater the level of self-limitation due to light limitation. We will explore this interaction further in the next chapter.

8. A Simple Model of Microalgal Growth in a PBR

8.1 Introduction

Here we will build a simple model describing growth of a microalgae in a bioreactor limited by light &/or N-nutrient. Why light &/or N-nutrient? Because as you will find out when you are operating the model, that it is very difficult to grow a dense culture of microalgae without it self-shading and hence of that culture becoming light limited. It is also difficult to grow a dense suspension of a marine microalgae without it exhausting P-nutrient because of phosphate solubility issues, but we will explore than later.

Why is biomass abundance density important? If the emphasis on microalgal growth was to grow a given biomass (gC), then biomass abundance (gC m^{-3}) would be of lesser concern. You would just grow the crop in a reactor of sufficiently large volume to attain the biomass required. Thus, in the oceans, the biomass abundance is very low, but of course the planetary scale of production means that the total biomass is massive. For commercial reasons, of course, abundance is important; with a low abundance you need more land, more growth medium, and it will cost much more to harvest the crop.

You may think that if you just keep adding nutrient (i.e. increase the concentration as gN m^{-3}), then the culture will attain a higher cell abundance. But as you will see, and as shown by the plots in Chapter 3 (Fig. 3.2, which was actually generated using the model describing in this chapter), the growth rate ceases to be exponential (indeed, the production rate falls). Somewhere between these extremes lays the target of optimal culture conditions for commercial success. This model allows you to explore the core interactions.

The model as presented should operate in the free Powersim Studio Express; however, you will not be able to expand the model as it is at the limit of permitted size.

Studio Express is available from here:

http://www.powersim.com/main/download-support/technical_resources/free-downloads/

Of course, you could build the model in another platform, or develop and hence operate the Powersim model in the full Studio platform.

If you just want to use the model, download either the full (open) version, or that running under Studio Cockpit (see Chapter 7).

8.2 The model – constructional basis

The Forrester diagram for the whole model is given in Fig. 8.1. Variables are defined in the accompanying tables. Before considering the details, we will consider the general form of the model and concepts upon which it is built. The models in later chapters are more complex, and will not be described in details beyond the level described in this chapter section for this model. While the model (Fig. 8.1) is described in separate blocks or modules, there are significant levels of cross-talk between these.

The "**Constants**" module simply contains the variables that are all held constant in the model. As befits Forrester diagrams (Chapter 7), these are all shown as diamonds. They are used elsewhere in the model, where they appear as diamonds surround by corners (any variable shape that appears in multiple places as a copy, or short-cut, has these 4 corners around the symbol).

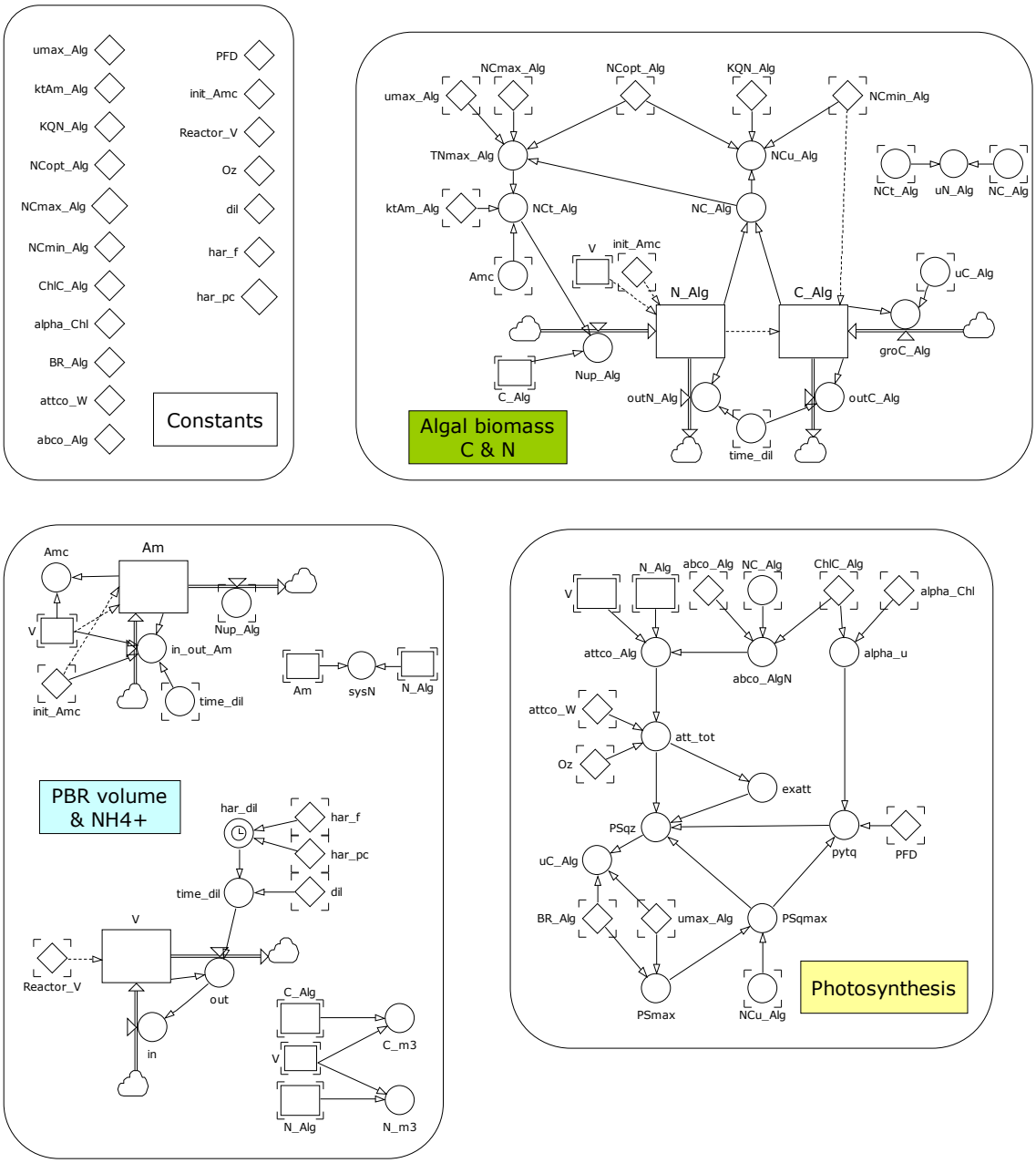


Fig. 8.1 Forrester diagram schematic for the model. This shows the modules describing the algal biomass, control of photosynthesis, and the bioreactor and nutrients. See also Tables 8.1 – 8.3.

The "**PBR volume and NH4+**" module describes changes in the volume of the photobioreactor, including changes with harvesting the crop, and in the amount of nutrient. The PBR is described here with respect to just two features – the volume (m^3) and the optical depth (m). The optical depth is used in the "Photosynthesis" section and affects the light penetration. The meaning of optical depth is discussed in various sections in Chapter 4; it is a critical parameter in microalgal cultivation. In natural systems, the depth may be 10's, or even 100's of metres. In commercial ponds

it may be 0.5m. The optical depth in specialist bioreactors is a few cm (ca. 0.05m). In simple terms we can consider the area occupied by the reactor as {volume}/{optical depth}. Obviously, that is a gross simplification for anything other than a pond. The nutrient considered here is just ammonium (NH_4^+; or *Am* in the model). This is the most important nutrient from waste-water streams. All other nutrients are considered to be present in excess in this model. It should be noted though that if this were considered to be a seawater system, then phosphate (and silicate for diatoms) cannot be added to high concentrations because these nutrients precipitate out of solution.

Variable	Value	Unit	Definition
abco_Alg	20	m^2 $(gChl)^{-1}$	Light absorbance coefficient for chlorophyll
alpha_Chl	7.00E-06	$(m^2$ g^{-1} chl.a)*(gC μmol^{-1} photon)	Slope of Chl-specific PE curve
attco_W	0.05	m^{-1}	Absorbance coefficient for growth medium (water)
BR_Alg	0.05	dl	Scaler for basal respiration rate
ChlC_Alg	0.06	gChl $(gC)^{-1}$	Mass ratio content of chlorophyll:C in the phytoplankton
dil	0	d^{-1}	Background dilution rate
har_f	5	d	Frequency of harvesting
har_pc	0.5	dl	Proportion harvested at frequency of har_f
init_Amc	7	gN m^{-3}	Input ammonium-N concentration
KQN_Alg	10	dl	KQ for N-quota
ktAm_Alg	0.014	gN m^{-3}	Half saturation constant for ammonium transport
NCmax_Alg	0.2	gN $(gC)^{-1}$	Maximum NC_Phy
NCmin_Alg	0.05	gN $(gC)^{-1}$	Minimum NC_Phy
NCopt_Alg	0.15	gN $(gC)^{-1}$	Optimal NC_Phy
Oz	0.1	m	Water (optical) depth
PFD	500	µmol photon m^{-2} s^{-1}	Surface irradiance
Reactor_V	1	m^3	Reactor volume
umax_Alg	0.693	gC $(gC)^{-1}$ d^{-1}	Maximum C-specific growth rate

Table 8.1 Variables held constant

Variable	Initial Value & Flows	Unit	Definition
Am	init_Amc*V*0.98 + in_out_Am - Nup_Alg	gN	Ammonium-N
C_Alg	N_Alg/NCmin_Alg - 1e-6 + groC_Alg - outC_Alg	gC	Phytoplankton-C
N_Alg	init_Amc*V*0.02 + Nup_Alg - outN_Alg	gN	Phytoplankton -N
V	Reactor_V + in - out	m^3	Reactor volume

Table 8.2 Variables described as levels (state variables). Shown are the initial values and (in red) the changes at each integration step.

Variable	Initial Value & Flows	Unit	Definition
abco_AlgN	abco_Alg * ChlC_Alg/NC_Alg	m^2 $(gN)^{-1}$	Phytoplankton-N specific coefficient for light absorbance
alpha_u	alpha_Chl*ChlC_Alg	(m^2)*$(\mu mol^{-1}$ photon)	Specific slope of PE curve
Amc	Am/V	gN m^{-3}	Ammonium concentration
att_tot	Oz*(attco_W + attco_Alg)	dl	Total attenuation
attco_Alg	abco_AlgN*N_Alg/V	m^{-1}	Attenuation coefficient to phytoplankton N-biomass
C_m3	C_Alg/V	gC m^{-3}	C-biomass abundance
exatt	EXP(-att_tot)	dl	Negative exponent of total attenuation
groC_Alg	C_Alg*uC_Alg	gC d^{-1}	Growth rate in phytoplankton-C
har_dil	IF((TIME>0), 1, 0)*IF((FRAC(TIME/har_f)=0), 1, 0)*har_pc/TIMESTEP	d^{-1}	Harvesting dilution rate
in	out	m^3 d^{-1}	Wash-in of medium to balance *out*
in_out_Am	time_dil*(init_Amc*V-Am)	gN m^{-3} d^{-1}	nutrient exchange
N_m3	N_Alg/V	gN m^{-3}	N-biomass abundance
NC_Alg	N_Alg/C_Alg	gN $(gC)^{-1}$	Phytoplankton N:C quota
NCt_Alg	IF(Amc>0,TNmax_Alg*Amc/(Amc +ktAm_Alg),0)	gN $(gC)^{-1}$ d^{-1}	Phytoplankton C-specific N transport rate
NCu_Alg	MIN(1,((1+KQN_Alg)*(NC_Alg-NCmin_Alg))/((NC_Alg-NCmin_Alg)+KQN_Alg*(NCopt_Alg-NCmin_Alg)))	dl	Quotient for N-status
Nup_Alg	C_Alg*NCt_Alg	gN m^{-3} d^{-1}	Phytoplankton population uptake of ammonium-N
out	time_dil*V	m^3 d^{-1}	washout of medium
outC_Alg	time_dil*C_Alg	gC m^{-3} d^{-1}	Washout of C_Phy
outN_Alg	time_dil*N_Alg	gN m^{-3} d^{-1}	Washout of N_Phy
PSmax	umax_Alg*(1+BR_Alg)	d^{-1}	Maximum gross photosynthetic rate required to enable u_Phy=umax_Phy
PSqmax	PSmax*NCu_Alg	d^{-1}	Maximum photosynthetic rate down-regulated by nutrient stress
PSqz	PSqmax*(LN(pytq+SQRT(1+pytq^2))-LN(pytq*exatt+SQRT(1+(pytq*exatt)^2)))/att_tot	d^{-1}	Phytoplankton N-specific growth rate
pytq	(alpha_u*PFD*24*60*60)/PSqmax	dl	Intermediate in depth-integrated photosynthesis rate
sysN	Am+N_Alg	gN m^{-3}	System N
time_dil	dil+har_dil	d^{-1}	Total dilution rate
TNmax_Alg	IF(NC_Alg<NCopt_Alg,umax_Alg*NCopt_Alg,umax_Alg*NCopt_Alg*(NCmax_Alg-NC_Alg)/(NCmax_Alg-NCopt_Alg))	gN $(gC)^{-1}$ d^{-1}	Maximum C-specific N-transport rate
uC_Alg	PSqz-(umax_Alg*BR_Alg)	d^{-1}	Net growth rate
uN_Alg	NCt_Alg/NC_Alg	gN $(gN)^{-1}$ d^{-1}	N-specific growth rate

Table 8.3 Variables described as auxiliaries.

The PBR module also describes the harvesting of the crop. In contrast to traditional (terrestrial) agriculture, the dynamics of harvesting a microalgal crop is an important determinant of commercial success. In this model, you can alter the two most fundamental features of the harvesting process: the proportion of the crop harvested on any occasion, and the frequency of undertaking that task. Concurrent with the removal of crop during harvesting (unless the entire reactor is drained), the

remainder is diluted by the addition of fresh growth medium. The residual algal population thus acts an inoculum for the next cycle.

The "**Algal biomass C & N**" module describes the algal biomass through reference to its C and N content in the whole bioreactor (as gC and gN). There are thus state variables for C and N in the algal biomass component. The model thus gives what is termed a "variable stoichiometric" description of the biomass; that is, the N:C ratio varies depending on the matching of photosynthesis and respiration (for C) and nutrient uptake (for N). The ratio of N:C in the algae usefully describes its nutritional status;

- A low N:C indicates that growth is limited by N-supply. Being limited by N may be exactly what you want; a high lipid or carbohydrate content (the details depend on the physiology of the organism you are growing) is attained during N-limited growth.
- A high N:C just means that growth is not N-limited. It does not mean that the growth rate is maximal. To attain a high growth rate requires cells to have a high N:C under conditions that are conducive to high rates of photosynthesis.

As explained in Chapter 4 (Section 4.6), pH and the supply of dissolved inorganic C (DIC) are interlinked; we assume here that DIC is supplied to the bioreactor using an injection system linked to pH, and hence neither the concentration of DIC nor the value of pH limit growth. We also assume temperature is fixed and that light is constant (though you can change it in the model.

The "**Photosynthesis**" module describes the depth-integrated photosynthetic rate of the algae; thus it considers the activity of the algal cells as they are being moved around within the bioreactor of a stated depth and supplied with a stated amount of light at the surface. Because the C enters the algal biomass via photosynthesis, we need reference not only to light at the surface but also to factors that absorb light before it reaches the individual cells. Those factors absorbing light includes the colour of the growth medium; pure water absorbs little light but some growth media (especially those containing anaerobic digestate) can be quite coloured. The main factor absorbing light, however, is usually the coloured algal biomass itself. The ability of the cell to perform photosynthesis at a given rate depends on the amount of pigment and the cellular nutrient status (described here as its N:C). In this model we will not explicitly describe pigment content (in models this is often described as gChl gC^{-1}, or Chl:C).

The value of Chl:C varies both with the amount of light (increasing to a maximum as the amount of light received by the cell declines), and also with the nutrient status (decreasing as N:C declines). We are describing N:C, so here we simply relate pigment content to N:C. What this allows us to do is consider that the culture, expressed per cell or per unit of biomass, becomes paler as nutrient is exhausted. The rate of photosynthesis is controlled by the light received by the cells (note, by the cells, not by the culture!) and the ability of the cells to process the light energy (which relates to the cellular N:C). See also Chapter 3, Section 3.3.

8.3 The model – the detail

In this section we consider the model in greater detail, including the underlying mathematics. **You do not need to read or understand this section to use the model, though flicking through it may give you some insight into various aspects of the endeavour.**

The model is a merging of models described previously in Flynn (2018) {Chapters 7, 8 and 15 in that book}. This model comprising 4 state variables describing the bioreactor volume, the N-nutrient (stated as ammonium, though it could equally be nitrate), the biomass in terms of algal- C and algal-N.

Reactor volume and harvesting

This part is shown in Fig. 8.2. This describes the volume (state variable *V*, set by constant *Reactor_V*), and the controls of input of fresh medium (*in*) and simultaneous (happening within the time frame of model processing) removal of reactor volume containing spent medium and algal biomass (out). The removal is in total described by *time_dil* and can be described as a continuous, chemostat-style, dilution (set by *dil*). Alternatively, it can be set as a proportion of the total reactor volume (set by *har_pc*) removed with a frequency set by *har_f*. Thus, to sample the system continuously for monitoring, *dil* would be set at a low value (perhaps 0.05 d^{-1}) and every 5 days (i.e., *har_f* = 5), 95% (i.e., *har_pc* = 0.95) of the reactor is harvested. The remaining 5% of the culture would then act as a seed inoculum for the next crop.

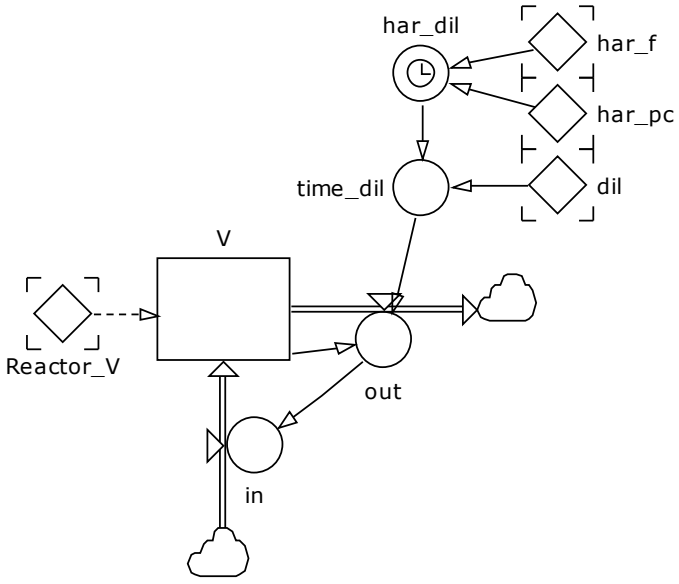


Fig.8.2. PBR volume and harvesting.

Reactor ammonium (N-source) content

This part is shown in Fig. 8.3. The state variable *Am* describes the ammonium-N content in the reactor volume *V*. Here the concentration of the ammonium is entered as gN m^{-3} (*init_Amc*) and the model calculates, with reference to reactor volume *V*, how much nutrient-N enters (as part of *in_out_Am*). The amount of ammonium (as gN) in the reactor accounts for the input, output of ammonium in the growth medium, and also the removal of ammonium by the microalgae,

Nup_Alg). The resultant concentration of ammonium is given by *Amc*, by reference to the amount of ammonium (*Am*) and the volume of the reactor (*V*). *Amc* is then used to inform the microalgal module on the availability of the N-nutrient to support algal growth. The value of *in-out_Am* is related to *time_dil* to account for dilution and harvesting.

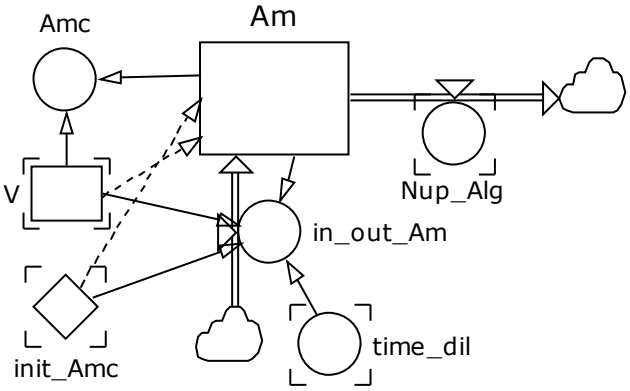


Fig.8.3. Reactor ammonium (N-source) content.

Microalgal biomass

On first impressions it looks like the N-growth and C-growth are poorly connected (the biomass description being separate). Actually, they are intimately connected. As we have seen above, N:C affects photosynthesis. N:C also affects N-growth; it does so in the model in a mode analogous to the processes of (de)repression described in Chapter 3 (Section 3.4). If N:C is low, the cell needs N-nutrient and the maximum rate at which it could take up nutrient (if available) is high. If N:C becomes elevated above a certain level, then the ability to take up nutrient is decreased, and eventually stopped altogether. This is because without C entering the organisms no more N can be assimilated.

The module is shown in Fig. 8.4. The two state variables for C and N biomass (*C_Alg* and *N_Alg*) define the total biomass (gC or gN) in the reactor volume V. The initial values of these state variables are configured here as a 2% inoculum (relative to the potential maximum N-content, set by *Init_Amc*), and assuming this initial inoculum is not N-replete (i.e., that starting algal N:C is close to or the same as the minimum set by *NCmin_Alg*).

The critical part of the model linking C and N growth relates to the emergent value of N:C. There are 4 constants involved in this linkage, 3 of which relate directly to the N:C value:

- *NCopt_Alg* Optimum N:C (maximises together photosynthesis and N uptake)
- *NCmin_Alg* Minimum N:C (maximises the ability to take up N, and minimises photosynthesis)
- *NCmax_Alg* Maximum N:C (minimises the ability to take up N)

The 4th constant (*KQN_Alg*) is involved in describing the relationship between N:C and photosynthesis. This relationship is usually close to linear.

The N-status of the microalgae relates to the N:C (*NC_Alg*), via the value of *NCu_Alg*. This is calculated through reference to the current N:C, the minimum cellular N:C (*NCmin_Alg*), the optimum value (*NCopt_Alg*) and a response curve constant (*KQN_Alg*). A value of *NCu_Alg* = 0

indicates extreme N-stress, while a value of 1 indicates optimal status. *NCu_Alg* is then used to define the current maximum photosynthetic rate (see below).

The maximum potential ammonium transport (*TNmax_Alg*) is controlled by the current N:C value (*NC_Alg*) so that as N:C exceeds the optimum (NCopt_Alg) and starts to approaches the maximum value (*NCmax_Alg*) then *TNmax_Alg* decreases to zero. This conforms with expectations (see Chapter 3, Section 3.4). Actual ammonium transport then relates to the current value of *TNmax_Alg*, the residual ammonium concentration in the reactor (*Amc*), and a half saturation constant for transport into the cell (*ktAm_Alg*). This gives a C-specific transport rate (*NCt_Alg*; gN $(gC)^{-1}$ d^{-1}), which is converted into a biomass uptake rate (*Nup_Alg*; gN) through reference to the C-biomass (*C_Alg*; gC).

Changes in the algal C-biomass (*C_Alg*) occur with reference to the C-specific growth rate, *uC_Alg* (gC $(gC)^{-1}$ d^{-1}), which is defined via the photosynthesis module.

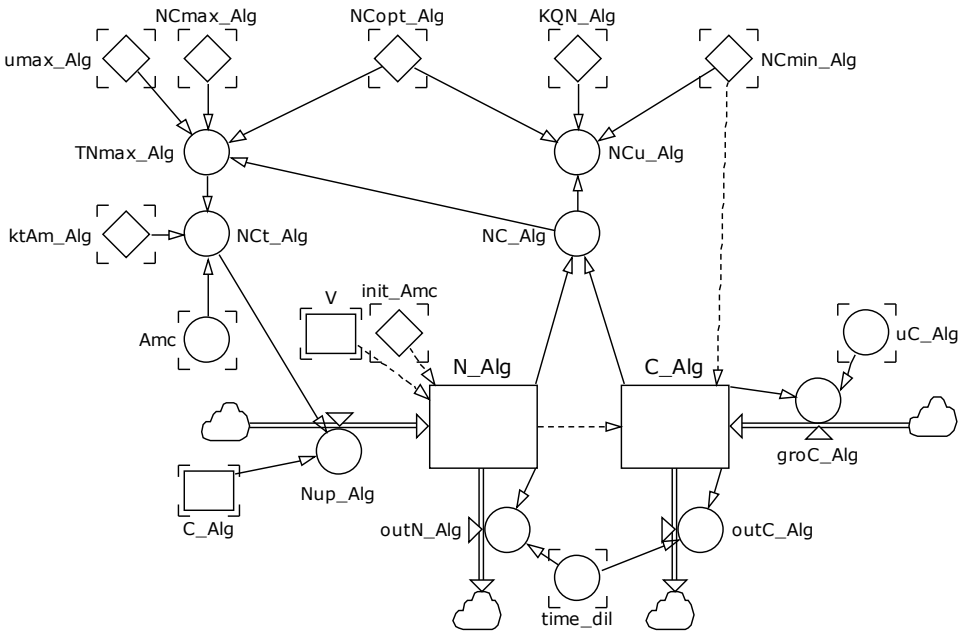


Fig.8.4. Microalgal biomass.

Light and photosynthesis

This module is shown in Fig. 8.5. The upper left hand section of this figure considers the attenuation of light as a function of the maximum Chl:C of the cells (*ChlC_Alg*; set as a constant because photoacclimation is not considered here), the N:C of the algae (*NC_Alg*) and the absorbance of the algae with reference to Chl:N. Together with the reactor volume (*V*) and the N-biomass (*N_Alg*) we obtain the light attenuation due to the algae (*attco_Alg*). Together with the colour of the growth medium (*attco_W*), and the optical depth (Oz) we then obtain the total attenuation (*att_tot*).

The maximum gross rate of photosynthesis, sufficient to account for respiration (*BR_Alg*) and supportive of the maximum growth rate (*umax_Alg*), is given by *PSmax*. The operational maximum (*PSqmax*) takes into account the N-status of the algae (*NCu_Alg*).

Light at the surface of the liquid in the bioreactor is input as a constant (PFD). Photosynthesis (i.e., the gross growth rate) is then computed using an integration of the Smith function (this is described in Flynn 2018, Chapter 8), with reference to the initial slope of the PE curve (*alpha_u*), *PSqmax*, *PFD*, and light attenuation *att_tot*. The net rate of photosynthesis is the C-specific growth rate, *uC_Alg*; this value controls the growth of algal C-biomass (*groC_Alg*) in the "Microalgal biomass" module in Fig. 8.4.

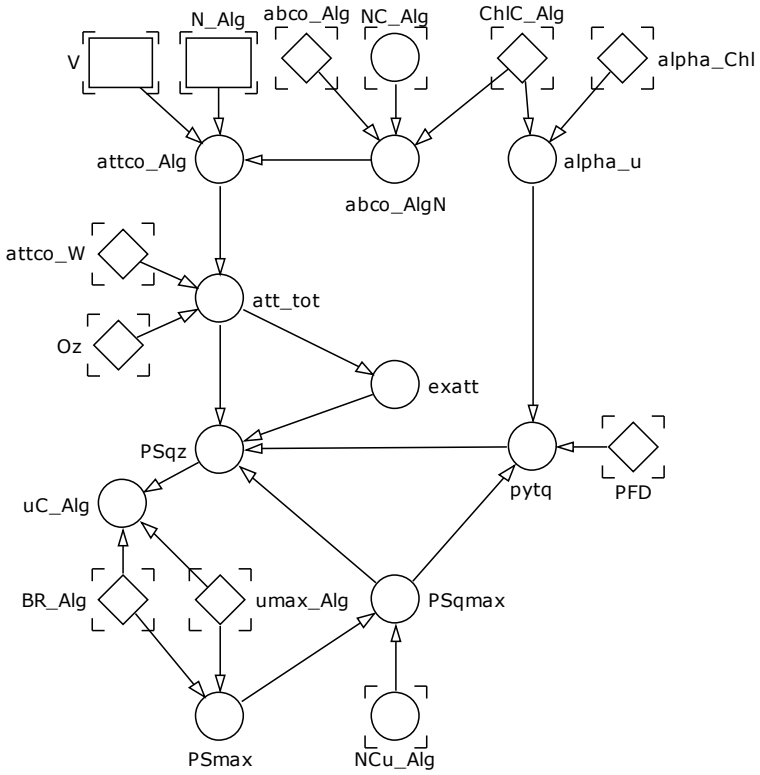


Fig.8.5. Light and photosynthesis.

Other outputs

Shown in Fig. 8.1, within the "PBR volume &d NH4+" module, are also calculations of the total system N content (sysN), which should stay constant. The N-specific growth rate (uN_Alg) is calculated for comparison with the C-specific growth rate, uC_Alg. To describe the algal abundance, C and N biomasses (as gC or gN m^{-3}) are also calculated (C_m3, N_m3)

8.4 The model – controls

As presented, the model is equipped with some simple push-button controls for changing the following:

- Reactor volume (0.5, 1 or 10 m^3)
- Optical depth (small and medium bore tubular or flat-plate configurations, and a 0.5m deep pond)
- N-nutrient loading (100, 500 or 1000 µM ammonium; 880 µM equates to the content in f/2 medium)
- Dilution rate (0, 0.05 or 0.1 d^{-1})
- Harvest frequency (2, 5, or 10 d)
- Harvest proportion (0.25, 0.50 or 0.95)
- PFD (200, 500 or 2000 µmol photons m^{-2} s^{-1}; artificial light is often ca. 200, while a cloud-free day may supply 2000)
- Maximum growth rate of the algae (0.35, 0.693 or 1.39 d^{-1}; 0.693 d^{-1} equates to a doubling each day)

The simulations run for 30 d. Buttons can be pressed during the simulations, but you should select your initial options before pressing the run button (see Chapter 7).

An example of model output, and explanations for what is happening, is given in Fig. 8.6. In this instance the following conditions were used:

- Reactor volume 10 m^3
- Optical depth 0.1m (10cm)
- N-nutrient loading 500 µM ammonium (=7gN m^{-3})
- Dilution rate 0 d^{-1}
- Harvest frequency 5 d
- Harvest proportion 0.50
- PFD 500 µmol photons m^{-2} s^{-1}
- Maximum growth rate of the algae 0.693 d^{-1}

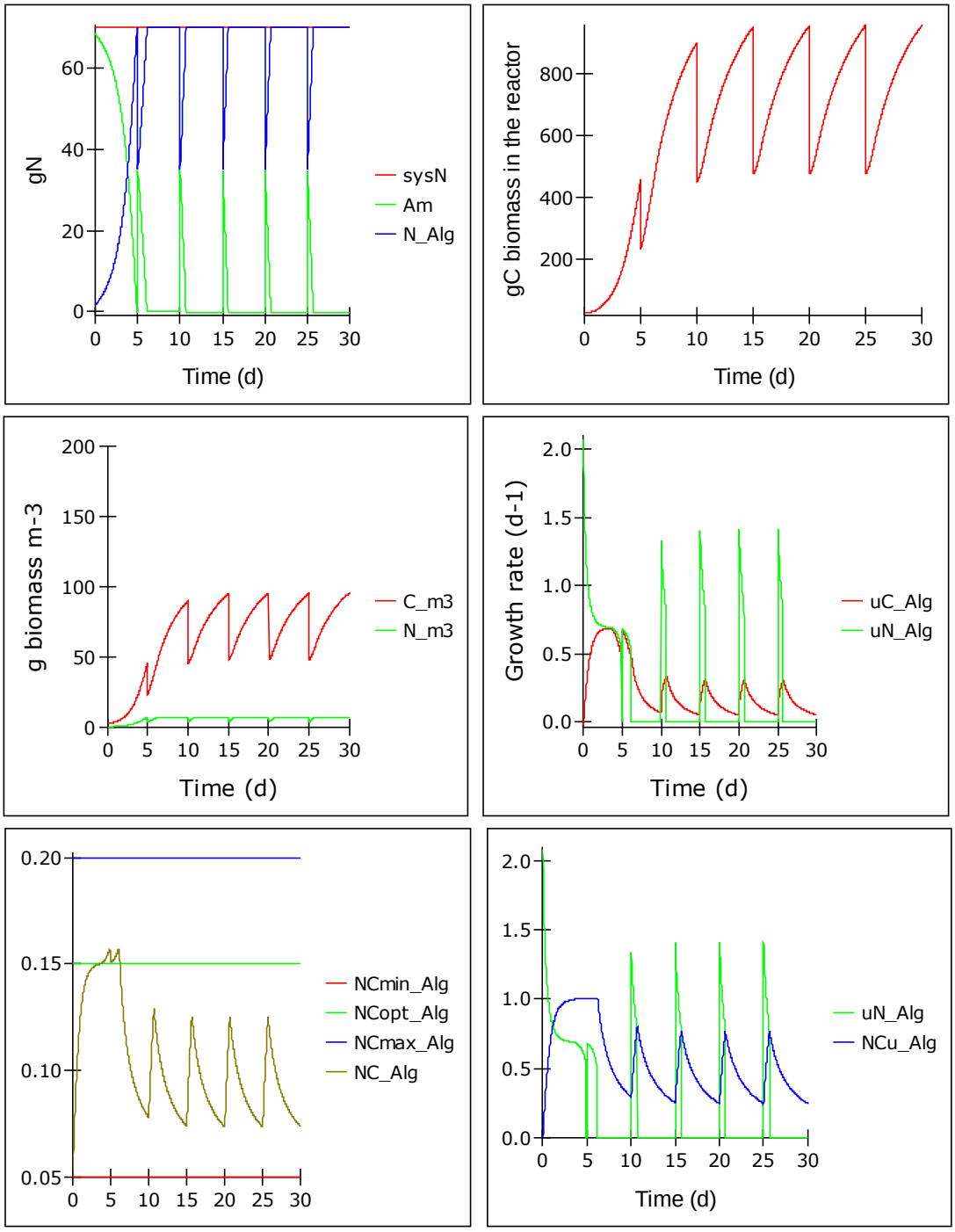


Fig. 8.6 Example output. Working left to right and down the page, the plots show: system-N (sysN) remaining constant while ammonium-N (Am) is converted into algal-N (N_Alg) – oscillations occur every 5 days when 50% of the biomass is harvested and the volume made good with fresh medium; changes in C-biomass within the 10 m^3 PBR – it takes 10 days from inoculum for the system to approach a maximum biomass content; changes in biomass concentration as gC m^{-3} (C_m3) and gN m^{-3} (N_m3); changes in C-specific growth rate (uC_Alg) and N-specific growth rate (uN_Alg) – only for a brief period around day 3 is cell physiology approaching steady state such that C- and N- specific growth rates are similar; changes in the N-status of the cells as indicated by the N:C value (NC_Alg) – this oscillates between minimum (NCmin_Alg) and maximum (NCmax_Alg) being only around the optimum value (NCopt_Alg) around day 3; the N-status (NCu_Alg) is only maximum, at 1, around day 3 and otherwise oscillates with the harvest and refeeding events, the latter causing sharp temporary spikes in the N-specific growth (uN_Alg).

8.5 Some things to explore

Even with this simple model, you can demonstrate many key features of importance for the commercial growth of microalgae. Here are some examples:

- How does changing the optimal depth affect the nutrient status and production? Deeper systems are far less likely to give nutrient-limited biomass, and they also give a slower growth rate (lower rate of production).
- How does increasing or decreasing the growth rate affect nutrient exhaustion and production? Growth rate is a function not only of the strain of microalgae used, but also of temperature. Within bounds, an increase in T by 10°C doubles growth rate.
- How does altering the frequency and proportion of biomass harvesting affect production? And how does it affect whether the product is nutrient limited or not?
- To what extent does altering the nutrient concentration affect the above?

You will realise that many of these topics interact in quite complex ways. And this is why playing with a simulation model gives you a good idea of what might happen in your PBR. You could, of course, make summary tables of the results and not bother running a dynamic simulation at all. But the advantage of a simulation model is that you can test what happens if the weather suddenly changed (alter PFD &/or growth rate) or if your harvesting equipment was out of action for a few days.

8.6 Caveats and what next

All models come with caveats. Some obvious examples for this model include the following:

i. Only one nutrient (ammonium) is considered. The concentration of this can be increased to levels above that at which a balancing amount of phosphate (or for diatoms, silicate) may precipitate and thus become limiting.
ii. The description of the algal photo-physiology is limited, with a fixed Chl:C.
iii. Inputs and outputs from the model are limited by the size of the model that can run on the free Studio Express

In the next chapter, while still restricted by caveats (i) and (ii), this model is developed to enable it to be more readily used in a DST scenario. To do this the model is arrayed; this is explained in Chapter 9.

9. An Arrayed Simple Model

9.1 Introduction

In Chapter 8 you will have used, and perhaps have built, a variable stoichiometric (C,N) description of a single species of microalgae growing in a single PBR. While you can readily alter the description of both the algae and the PBR, short of recording the results and amassing a series of comparable plots, this does not provide a ready approach to comparing options as required for a DST.

Here the same model as detailed in Chapter 8 is modified so that it now describes as many as 4 different species of microalgae within 3 PBRs. Any of the 4 species can be inoculated, grown and harvested into any of the 3 PBRs. Or you could consider different growth rates (due to assumed different temperatures) of the same species. The model outputs are also developed to now portray the changing C and N biomass for each species, the harvested biomass of each species (even though in reality it would be de facto impossible to separate them), the total harvest and its N:C (quality).

9.2 Arrayed models

Arraying a model provides a means by which the complexity of the description can be readily and massively enhanced but without also massively increasing the computer coding. The catch is that the syntax of the code itself becomes slightly more complex (not that you need to worry about that), and the naming of variables is different (which you do need to understand).

Within reason (depending on computer memory and software), you could have any number of arrays each of any size. Here, the model contains two arrays of different sizes:

- PBR : this has a size of 3
- species : this has a size of 4

In an arrayed model, members of each variable now have a number identifier as well as the name. Thus, the volume of each of the three PBRs, which use the name *V*, are identified as *V*[1], *V*[2] and *V*[3]. Likewise, the ammonium concentration, *Am*, will have 3 identities because ammonium is within each of the three PBRs.

There are four species of algae, so a variable that is owned solely by the algae will have 4 identities. So, there are 4 maximum growth rates, one for each species, *umax_Alg*[1] .. *umax_Alg*[4].

However, many of the algal-related variables not only have an identifying number for the species, but they may also have a number for the PBR into which they are inoculated. Thus, the C-biomass of a given species of algae within a given PBR carries the name *C_Alg*[{PBR},{species}]. For example, the C-biomass of species 4 in PBR 2 is given by the value of *C_Alg*[2,4].

Unless you want to know how this operates in the context of the syntax of the model coding, you can stop here, and go directly to Section 9.4.

9.3 Equation syntax of arrayed models

Where the programmer has to be particularly careful is in the syntax of equations to ensure they reference the correct component of each array.

As an example, consider the definition of *abco_AlgN*.

In the non-arrayed version of the model in Chapter 8, this was described as this –

abco_AlgN = abco_Alg * ChlC_Alg/NC_Alg

Recall that *abco_Alg* is a constant, *ChlC_Alg* is unique to a given species, and *NC_Alg* is an emergent property of growth for the single species described.

In an arrayed version, first we declare the dimensions are as follows …

FIRST(PBR) .. LAST(PBR), FIRST(species)..LAST(species)

This tells the software to work through the arrays sequentially, and also that there will in total be PBR x species (=3 x 4 = 12) elements. That is, there will be 12 values of *abco_Alg*, identified as [{PBR},{species}].

And *abco_AlgN* is now defined as ..

Abco_AlgN = FOR(A=FIRST(PBR)..LAST(PBR), B=FIRST(species)..LAST(species) | abco_Alg * ChlC_Alg[B]/NC_Alg[A,B])

The array identifiers are in red to help you. The character "|" indicates the split between the instruction and the equation.

Note that:

- *abco_Alg* has no array identifier (there is only one version of this constant)
- *Chl_Alg* belongs to a species, and so carries the identifier B
- *NC_Alg* belongs to a species growing in a PBR, so it carries identifiers A (for the PBR) and B (for the species).

The mathematics of the actual equation is the same between the non-arrayed and arrayed versions.

9.4 The model

The model is as before, except with a few additional input options and outputs. The Forrester diagram is similar (Fig. 9.1) except that the variables that are arrayed have a double walled symbol.

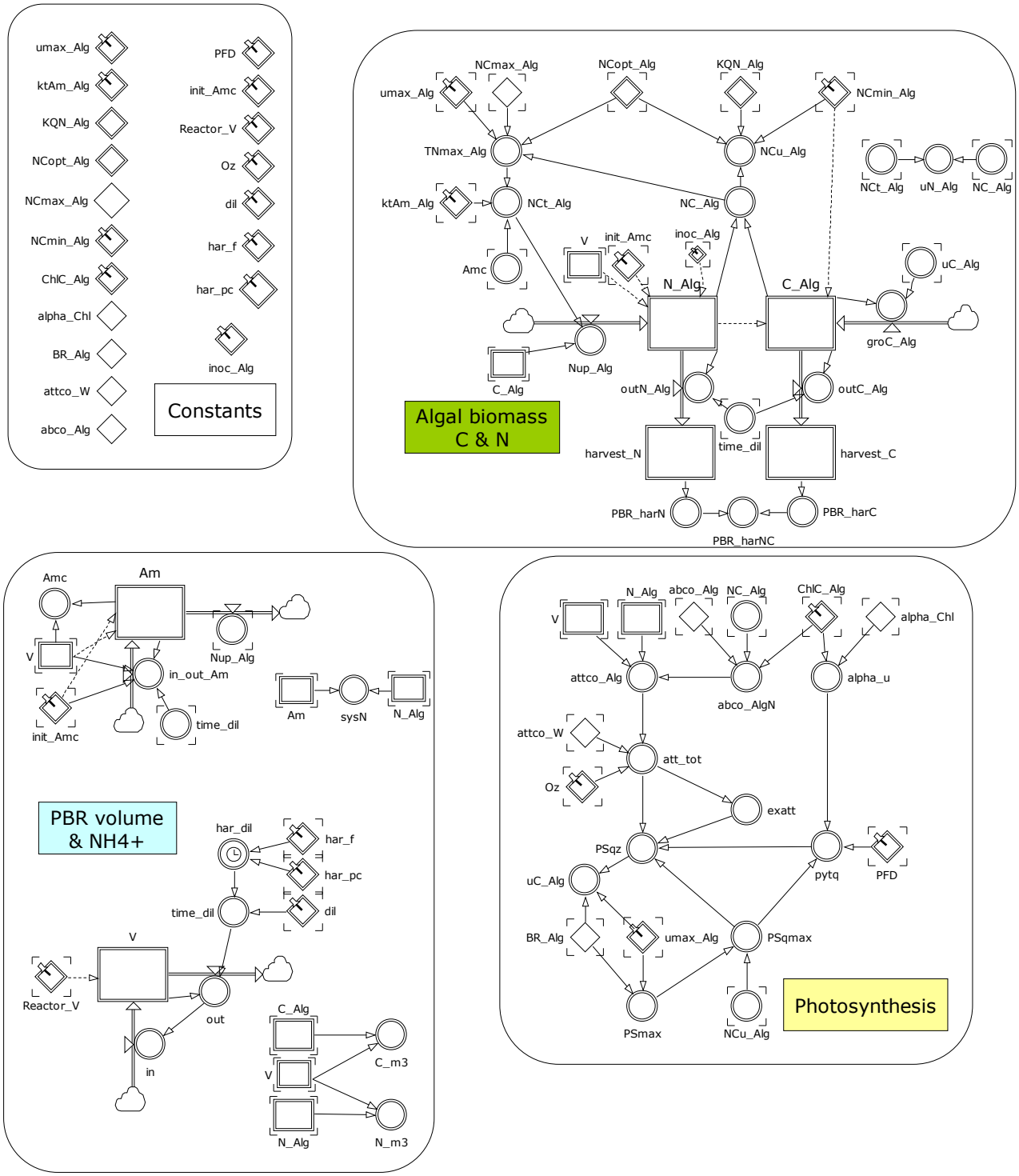


Fig. 9.1 Forrester (Studio) diagram of the arrayed model. Arrayed variables are indicated by double walls. Those constant (diamond) inputs that preserve their values between simulation runs have a map-pin symbol on the top left-hand side.

While the inputs to the model in Chapter 8 were controlled by simply pressing buttons to select between options, here inputs are made into tables. These are shown in Fig. 9.2.

Sp config	umax_Alg	ktAm_Alg	NCmin_Alg	ChlC_Alg
unit	d-1	gN m-3	gN (gC)-1	gChl (gC)-1
sp#1	1.20	0.01	0.05	0.06
sp#2	1.20	0.01	0.05	0.04
sp#3	1.20	0.01	0.05	0.03
sp#4	0.69	0.01	0.05	0.06

PBR config	unit	PBR#1	PBR#2	PBR#3
Oz	m	0.05	0.10	0.20
Reactor_V	m3	1.00	1.00	1.00
PFD	umol m-2 s-1	500.00	500.00	500.00
dil	d-1	0.00	0.00	0.00
har_f	d	7.00	7.00	7.00
har_pc	fraction	0.50	0.50	0.50
init_Amc	gN m-3	28.00	28.00	28.00

Enter "0" not to inolculate with this species, or "1" to inoculate.

Do NOT enter numbers other than "0" or "1" !

inoculate			
0 or 1	PBR#1	PBR#2	PBR#3
sp#1	1.00	1.00	1.00
sp#2	1.00	1.00	1.00
sp#3	1.00	1.00	1.00
sp#4	1.00	1.00	1.00

Fig. 9.2 Input tables to change the values of variables controlling the model. The PBRs are numbered as #1 .. #3, while the four microalgal species configurations are numbered sp#1 .. sp#4.

You have the option to change various parameters the are key to the microalgal description. These are:

- maximum growth rate (noting that if you consider growing at different temperatures in this model you can simply alter the value of *umax_Alg* as required and keep other algal-specific values the same; at the simplest you could use Q_{10}=2)
- nutrient affinity (*ktAm_Alg*; 0.014 gN m^{-3} equates to 1µM ammonium)
- minimum N:C quota (*NCmin_Alg*; the lower this value the greater the potential for accumulating carbohydrate or fatty acids; values are typically between 0.1 and 0.05 gN $(gC)^{-1}$)
- Chl:C ratio (*ChlC_Alg*; this controls how "green" is a microalga – this is the subject of genetic modification studies as a lower value enhances population growth by decreasing self-shading; consider values between ca. 0.08 and 0.01)

There are then the controls for the PBR:

- optical depth (*Oz*; for tubular or flat pate reactors with lighting from both sides you could consider this as the radius or half the plate thickness; for a pond, it is the depth)
- reactor volume (*Reactor_V*; the total volume of the reactor)
- illumination (PFD; here you can only control the value of what is considered to be continuous illumination; full sunlight can exceed 2000 µmol photons m^{-2} s^{-1}, while artificial illumination may be only a few 100's at the surface of the reactor)
- continuous, chemostat-style, dilution (*dil*; this removes a proportion of the culture at a continuous rate, topping up the reactor with an equivalent volume of fresh medium)
- harvesting frequency and proportion (*har_f* and *har_pc*; this control dis-continuous harvesting, with the reactor being topped up with an equivalent volume of fresh medium)
- nutrient content of the fresh medium (*init_Amc*; this is the concentration of ammonium added to the medium, coming in with the fresh medium; the residual concentration is of course much lower as the microalgae remove it to support their growth; 1mM ammonium equates to 14gN m^{-3})

And finally, you decide which PBR configuration is to be inoculated with which microalgal configuration. This is achieved by just entering "0" or "1" for no or yes, respectively. The inoculation equates to 2% of the maximum yield, so there will be a period of well illuminated nutrient-replete growth for the first week.

It is important, as for all models, to only input values that are plausible. For example, *umax_Alg* should be less than ca. $3d^{-1}$, and *init_Amc* should not exceed 28 gN m^{-3} (and for marine media probably < 14, as a balancing amount of phosphate would precipitate out above such a level).

9.5 Interpreting the model outputs

Firstly, it is important that you appreciate the syntax used in the outputs. Any output of the form {name} x,y is referring to the contents of PBR "x" and species "y". If there is only one number, it will refer to the PBR identity.

An example of the most complex type of output (where every species is present (competing for light and nutrients) in every PBR is shown in Fig. 9.3.

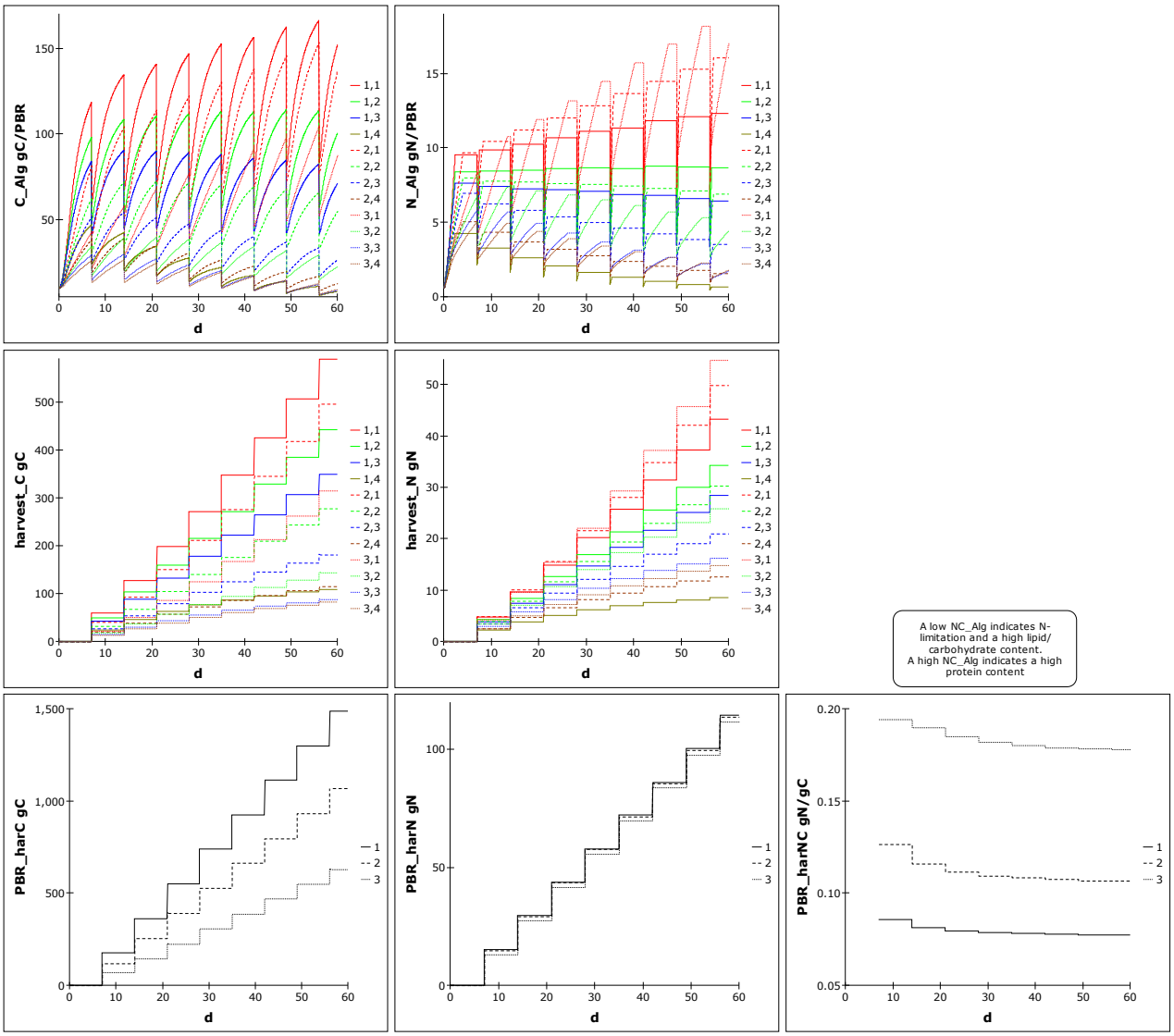


Fig. 9.3 Example graphic output. The input values are as shown in Fig. 9.2.

The output graphs show (from left to right, working down the panels):

- C-biomass for each species in each reactor. Over time species 1, in each reactor (i.e., plots 1,1, 2,1 and 3,1) is gradually taking over the reactor. The sudden drops are due to harvesting events, followed by growth of the remaining biomass on the fresh nutrient that is introduced to return the volume to $1m^3$ after each harvest.

- N-biomass for each species in each reactor. Note that unlike the C-biomass plots these plots flat-line; this is a consequence of the exhaustion of ammonium in the reactor (*Am* is not plotted here).
- Harvest C-biomass for each species in each reactor. This shows the cumulative amount of biomass harvested over the 60d simulation period. The step-like appearance reflects the harvest events. In reality it would not be possible to separate the species within a given reactor during harvesting, so what these plots are showing are changes in biochemical composition over time.
- Harvest N-biomass for each species in each reactor. This is the N-counterpart for the C-biomass.
- Harvested PBR C-biomass. This gives the sum of the species biomass in each reactor. What is immediately obvious is that the narrow-bore PBR (PBR #1) is very much more effective. This is because of the decreased level of light limitation impacting the cultures due to self-shading.
- Harvest PBR N-biomass. This is the N-counterpart for the C-biomass. The reason that these plots are similar between PBRs is because the ammonium-nutrient was in all instances exhausted (hence the flat-topping microalgal N-biomass plots mentioned above).
- N:C of the harvested biomass. This shows that the product from the small bore PBR (PBR #1) has a higher C-content (lower N:C) than the others.

From these simulations we deduce that:

i) If you want to maximise C-biomass production you need to use a small bore PBR

ii) If your interest is in microalgal protein, then a larger bore (greater depth) PBR may be quite acceptable, and these are easier to maintain and may be cheaper. However, you need to run additional simulations to optimise the amount of ammonium-nutrient added.

iii) Certain configurations of microalgae outcompete others. Species #1, #2, #3 differ only with respect to their Chl:C content. Grown alone, species #3 will be better, as the lower Chl:C will limit self-shading. However, in competition, a species with a high Chl:C will win. Genetically modifying microalgae to give a lower Chl:C is thus not a stable mutation. Species #4 grows slower and is unsurprisingly less competitive.

As mentioned earlier in this book, system dynamics models work by describing the flow of materials around a system. This is why the model works on gC and gN. If you are interested in dry weights and protein contents, you will need to transform the model outputs accordingly (see Sections 5.4 and 5.5). These transforms will never be exact, but as rough-and-ready approximations:

- Dry weight (g) = 3x gC
- Protein (g) = 6x gN

And the C mass (as g) of storage carbohydrate + fatty acids + lipids can be estimated as:

{g C-biomass} – 5x {g N-biomass}

The number 5 is the reciprocal of the value of N:C_{max} (usually around 0.2), which is the maximum N quota in N-replete microalgae.

9.6 Caveats

Caveats are similar to those in Chapter 8 as the base model is the same. There are, however, some additional caveats associated with simulated these arrayed systems.

- It is assumed that there is no interaction between species growing in the same PBR other than competition for ammonium (the only limiting nutrient described) and for light. In reality, there may be allelopathic interactions that can totally change the production. See Section 3.10, and search the book for “allelopath”.
- Except in laboratory conditions, it would be very difficult to set up PBRs in the same conditions. Likewise, algal cultures rarely behave exactly as expected. It is thus important to trial configurations with plausible deviations between simulations to see how robust are the simulations.
- These are 60d simulations. The longer a culture system is operating the more likely it is that something will go wrong. That may be mechanically with the PBR or biologically. Again, you need to balance theory with the possibility for deviations in reality.

10. An Arrayed Complex Model

10.1 Introduction

Chapter 9 considered an arrayed simple model. The model was simple in that it only described the microalgae in terms of C and N, with light and nutrient-N limitation. It did not consider P, nor Si for diatoms, and neither did it describe photo-acclimation or differentiate between nitrate and ammonium nutrition. However, the model was complex in the way that it was arrayed, allowing the user to include various species together in different PBR systems (making the likely gross simplification that there were no allelopathic interactions between these species).

In this chapter, we revert to the typical "single species growing in a reactor" configuration. However, the model is now arrayed so that three different reactors, or perhaps different operational configurations of a common reactor design, can be considered. Thus, the reactors can be configured with respect to temperature, lighting, nutrient levels (including CO_2 injection), and also the harvesting protocols as described for the culture system in Chapter 9.

The enhanced complexity of the model comes with the description of the algal physiology. This describes the organism with respect to variable acclimative stoichiometry with respect to C,N,P,Chl (and for diatoms Si). It also describes nitrate versus ammonium use.

Unless you have a specific desire to re-code the model onto another platform (for which purpose the equations are provided in the Appendix {to be done}), the most important topics covered in this chapter are the justifications (with caveats) for the model structure, and considerations for operating the model using the Powersim Studio Cockpit interface. The interface is available from:

https://www.powersim.no/main/products-services/powersim_products/end-user-tools/cockpit/

In this model, the array only operates to describe the PBR (with an array size of 3, though if you build the model yourself, it could be reconfigured to describe any number of reactors). Nonetheless, the model is large, and so too is the interface panel for its control and reporting. To help you navigate the control screen, a snapshot of the implementation for this chapter is shown in Fig.10.1.

The same model base is used also in Chapter 11 (for considering osmotrophy, feeding with sugar &/or amino acids), and in Chapter 12 (for considering production of metabolites that are released into the growth medium).

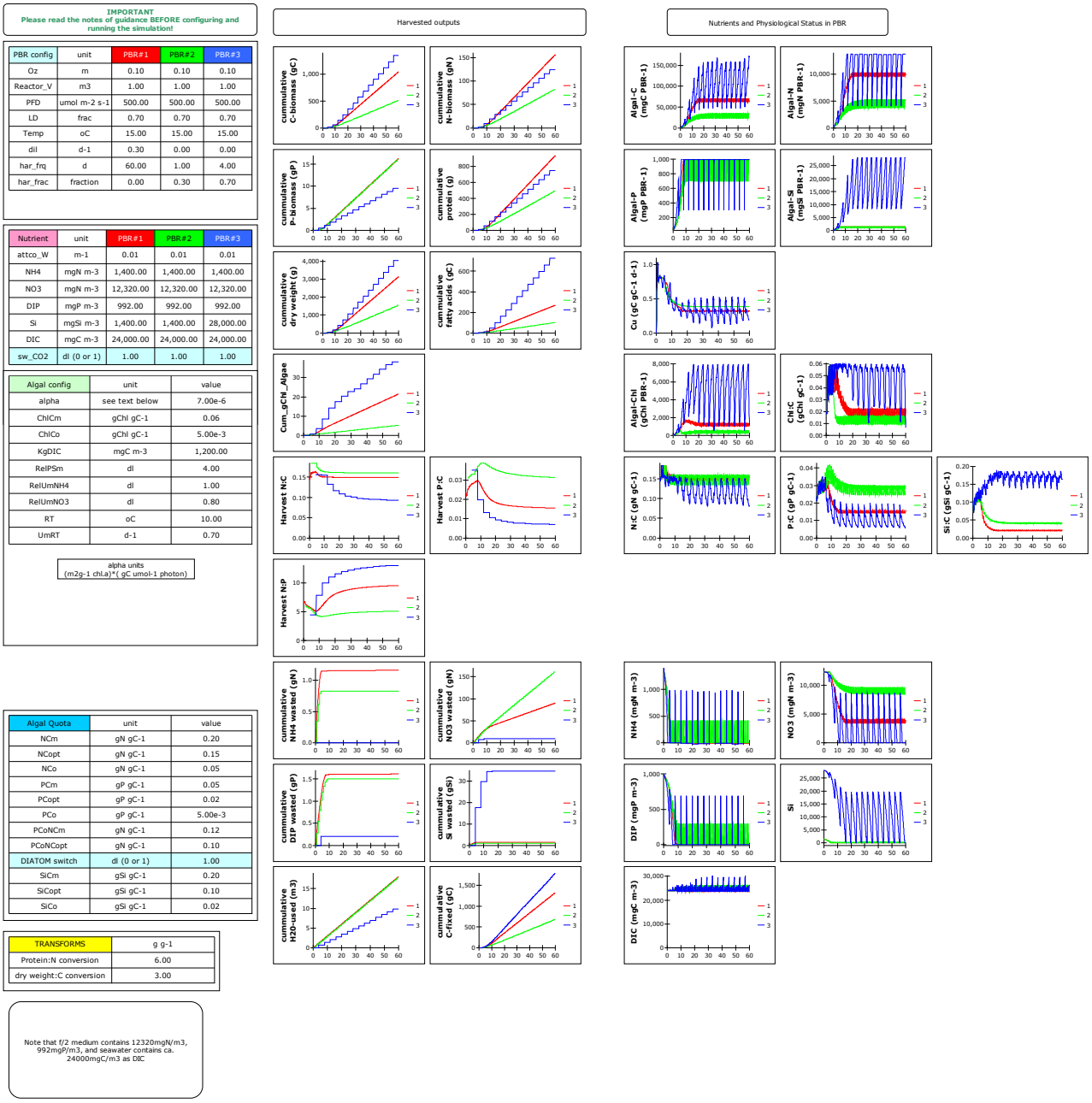


Fig.10.1 Snapshot of the entire model screen, as an aid to navigation. On the left-hand side are the data entry tables; information and instructions on how to use these are given in Section 10.3. The next (middle) block show graphs for the outputs of the model from harvesting the crop; some of these show cumulative changes over the 60d simulation period. The right-hand graphs give more details on growth rates, concentrations etc. The three different colours in the plots are for data from each of the three different colours represent the three PBR configurations, enabling comparisons to be made between the advantages of operating the biomass production system in different ways.

10.2 The algal model

If you do not wish to know anything of the model structure, you can skip this section and go directly to *Section 10.3 Configuring the simulations*.

The basis of the model is an ODE-based system dynamics model capable of describing the growth and activities of contrasting protist plankton functional types of different allometries (cells size) and C:N:P:Si:Chl stoichiometries, and displaying acclimation to changes in the environment. The full model can thus describe purely heterotrophic growth supported by osmotrophy and phagotrophy (as befits a protozooplankton), various mixoplankton variants (see Flynn et al. 2019), and non-phagotrophic osmo-photo-trophic protists. It is this last group that is configured for this application; thus, the model describes diatom and non-diatom microalgae.

Although designed originally for protists, the same model structure as given here is suitable for describing non-diazotrophic (non N_2-fixing) cyanobacterial growth.

State variables describe microalgal:

- C-biomass (mg m^{-3})
- N-biomass (mg m^{-3})
- P-biomass (mg m^{-3})
- Si-biomass; diatom only (mg m^{-3})
- Chl-biomass (mg m^{-3})
- Average growth rate (gC gC^{-1} d^{-1})
- Average gross photosynthetic rate (gC gC^{-1} d^{-1})

The microalgae can additionally be described with respect to:

- Range of stoichiometry (C:N:P and for diatoms, :Si; all with respect to mass)
- Variable (photo-acclimative) Chl:C (mass ratio)
- Exploitation potential for NH_4^+, NO_3^-, DIP and for diatoms Si, all linked to nutritional status and scope for growth

The characteristics that demand particular attention are as follows, ordered alphabetically by variable name.

ChlCm : the maximum cellular Chl:C ratio; this must be zero for the purely phagotrophic protoZ as these are not pigmented (such as the heterotrophic dinoflagellate *Oxyrrhis marina* grown here as an osmotrophy – see Chapter 11).

NCo and **PCo** : the minimum cellular N:C and P:C values, which affect the capacity to accumulate storage C (as fatty acids &/or starch).

RelPSm : the relative value of the maximum photosynthetic rate, PSmax (which de facto is set in reality by the cellular enzyme activity of RuBisCO), compared to the maximum growth rate. This may be <1 for mixotrophs but is more likely to be ca. 2-4 so that phototrophic growth in L:D cycles can approach the maximum growth rate at a given temperature (set by **UmT**).

RelUmNH4 : the relative growth rate compared to UmT that can be supported by growth using ammonium-N as the sole N-source. Typically this would be 1. This value must be set as 0 in the (unlikely) event that the organism cannot use NH_4^+.

RelUmNO3 : the relative growth rate compared to **UmT** that can be supported by nitrate-N. Often this may be less than 1, and it would not be greater than the value of RelUmNH4. This value must be set as 0 if the organism is unable to use NO_3^-.

sw_diat : the switch selecting for "diatom" which thus enables Si uptake.

UmRT : the maximum growth rate at the reference temperature. The actual maximum growth rate (**UmT**) depends on temperature. Diatoms can typically exceed a division per day (0.693 d^{-1}), but most non-diatom species do not exceed a division per day (=< 0.693 d^{-1}). Care must be taken if the RT is very different to the optimal T, else UmT may not be plausible &/or the organism may be killed by that temperature.

10.2.1 Nutrient transport

The nutrients described for potential use by microalgae in the model are:

- Ammonium
- Nitrate
- Phosphate
- Silicate (required for diatoms other than *Phaeodactylum tricornutum*)
- DIC (dissolved inorganic C, CO_2)

Of these nutrients, usage of all but silicate are described using a similar general construct that relates the *acquisition potential control* (hereafter, **APC**) for that nutrient to the C:N:P stoichiometry of the organism. See Chapter 3 for a physiological (mechanistic) basis for this approach. The generalised form of the APC curves for different nutrient types are shown in Fig. 10.2.

For further information, see:

Appendix: Normalised Acquisition Potential Control Mechanism (nAPCM)

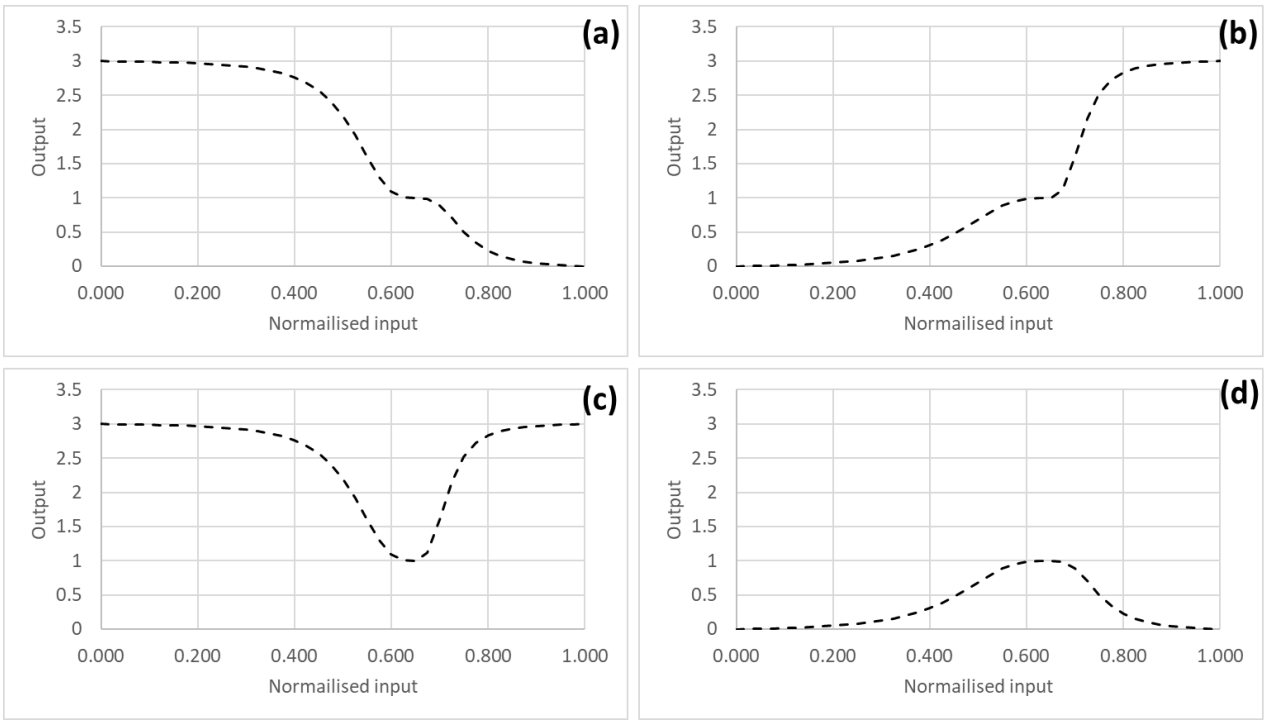


Fig.10.2 Generalised form of the acquisition potential control (APC) for nutrient transport. On the x-axis is the normalised input value of the nutrient quota, for example for N-acquisition (plot (a); x-axis as N:C), P-acquisition (plot (a); x-axis as P:C), DOC-acquisition (plot (b); x-axis as N:C), Dissolved Free Amino Acid-acquisition (plot (c); x-axis as N:C). x-axis value of 0 equates to the minimum quota; a value of 1 is the maximum quota. The value at around 0.66 aligns here with the quota value at the optimum growth conditions. At the optimum conditions the output value (y-axis) is 1. If the nutrient is in short supply, or in excess, then the value of the normalised quota is used to enhance or depress (respectively) the APC for that nutrient. Depression will typically turn the APC off (goes to zero), but there is great variability in the upper value. Thus, for nitrate, the value may be only around 1 (no enhancement) but for ammonium it may be 10 or so. Plot (c), for amino acid transport potential the APC shows it increasing at both extremes of N:C because amino acids are both a source of C and for N.

Ammonium and nitrate (DIN) transports

The APC for these nutrients operate by reference to the N:C quota. When this ratio declines, so the AP increases. AP for ammonium starts to develop from a higher N:C and also develops more rapidly than for nitrate. This difference between AP for ammonium vs nitrate enables:

i) the description of the ammonium-nitrate interaction, with ammonium usage being preferred,
ii) the potential for a higher growth rate using ammonium (and indeed for nitrate usage to be zero).

The optimal N:C controlling ammonium and nitrate AP is itself a function of P:C; this gives the expected decrease in N:C with P-stress (Flynn 2008a).

Phosphate (DIP) transport

By analogy with control of DIN transport, that for DIP is controlled by reference to the P:C quota, with the AP increasing as P:C declines. Like the control of ammonium and nitrate, reference to the quota uses the normalised quota construct of Flynn (2008b).

Silicate transport (for diatoms)

This is different to the controls for DIN and DIP because the control and fate of this nutrient relates to the cell-division cycle of the diatom. The description of silicate uptake follows that of the short-cut version of Flynn & Martin-Jézéquel (2000), as per Flynn (2001).

10.2.2 Phototrophy

The phototrophy description is developed from the approach described by Flynn (2001). This relates the level of photoacclimation to the demand for C. This contrasts with the approach of Geider et al. (1996), who relate photoacclimation to the provision of photoreductant, as that approach does not lend itself to modulation in consequence of osmotrophy (Chapter 11).

Further modifications to Flynn (2001) include the following:

- A stated minimum Chl:C to prevent the value going too close to zero on nutrient starvation.
- A capacity for the maximum photosynthetic rate to exceed that required for maximum growth. This is set by RelUmPS, and *de facto* describes the value of RuBisCO activity. This modification permits growth rates in L:D cycles to approach those in continuous light by increasing the rate of C-fixation during the L phase of the diel cycle. To enable this functionality, a state variable is required to record the average growth rate over the last day.

Photosynthesis is computed as previously implemented (using an integration of the Smith equation), to give a depth-integrated value.

10.2.3 Growth

C-specific growth is the balance of all C-inputs and outputs to the algal biomass. Input in this model is only from photosynthesis; the model described in Chapter 11 includes osmotrophy (including the use of DOC &/or DFAA). Outputs include respiration associated with anabolic and catabolic activities, and in support of nitrate reduction to ammonium.

As part of growth regulation, and the control of phototrophy, the model refers to the moving averages of net growth and net photosynthetic rate.

Temperature is involved here simply at the level of calculating the operational maximum growth rate (UmT) with reference to the reference maximum (UmRT) at a stated reference temperature (RT), current temperature (T) and a value for Q_{10}. Note that temperature does not affect alpha (the slope of the Chl-specific photosynthesis-light curve). Changes in temperature thus change the form of the relationship between the net photosynthesis rate and light (affected also by photo-acclimation).

10.2.4 Biomass

Biomass is described by state variables (with units of mg element m^{-3}), for C, N, P, and also for diatoms, Si. Chl also has a state variable.

There are outputs for C (respiration and DOC), N (regeneration) and P (regeneration). These latter releases include an overflow release from cells to prevent the stoichiometric ratios of N:C and P:C becoming too large.

C and N increases by phototrophy (C) or nutrient uptake (N).

P increases by nutrient uptake. There is no explicit description of DOP usage; that is usually supported by expression of an external phosphatase and the actual uptake is then of DIP.

Si usage accumulates into the biomass (of diatoms). Si would only be released on death of the diatom followed by dissolution of the organically-bound Si (not described).

Chl synthesis and degradation is described related to C-demand and nutrient status. Thus, Chl content increases during nutrient-replete growth at low light (in response to increased C-demand), and decreases (or at least increases at a lower rate than does C-biomass), at high light &/or low nutrient supply. Stoichiometric allocations to photosystems are not explicitly defined, so C,N,P associated with Chl and phototrophy are all included within the bulk C,N,P state variables.

10.2.5 External nutrients

The following external nutrients may be included:

- Ammonium (mgN m^{-3})
- Nitrate (mgN m^{-3})
- Phosphate (mgP m^{-3})
- Silicate (mgSi m^{-3})
- DIC (mgC m^{-3})

It is assumed that the pH is controlled either explicitly (via addition of acid or alkali) with no input of CO_2, or by injection of CO_2. In the former case, DIC-limitation can develop, and photosynthesis is then limited with respect to a half saturation for DIC-limited growth (K_GDIC; see Clark & Flynn 2000). In the latter case, the supply of DIC keeps pace with the removal by photosynthesis thus maintaining the pH.

Light is described with respect to the at-surface-of-PBR value of PFD, and also by the L:D cycle. The available light for microalgal cells is then also affected by light attenuation as functions of PBR optical depth, attenuation by the water itself, and attenuation by the Chl-containing biomass.

10.3 Configuring the simulations

In the simulation platform provided, values for different features of the PBR and algal physiology can be input. It is important that these are made with reference to the information provided below and to any empirical information held by the programme user.

WARNING: there is no error checking in the model for the entry of implausible parameter values. It is the responsibility of the user to verify the appropriateness of such values.

10.3.1 PBR configuration

The configuration table from the simulator is shown in Fig.10.3; this gives access to the following features that can be configured independently for each of the three arrayed PBRs (PBR#1, PBR#2, PBR#3).

PBR config	unit	PBR#1	PBR#2	PBR#3
Oz	m	0.10	0.10	0.10
Reactor_V	m3	1.00	1.00	1.00
PFD	umol m-2 s-1	500.00	500.00	500.00
LD	frac	0.70	0.70	0.70
Temp	oC	15.00	15.00	15.00
dil	d-1	0.30	0.00	0.00
har_frq	d	60.00	1.00	4.00
har_frac	fraction	0.00	0.30	0.70

Fig.10.3 Snapshot from the screen of the model showing the simulator PBR configuration table, with example entries. The user completes this table using entries appropriate to the system being explored. The simulation runs for 60d, so PBR#1 is only subjected to continuous harvesting through a chemostat-style dilution (hence the value for dil for PBR#1 is 0.3 d^{-1}). The other PBRs are subjected to periodic harvesting, with no continuous dilution.

An explanation of these options follows:

Oz This is the optical depth of the PBR in m. For a tubular reactor this approximates to the radius of the tube. For a pond it would be the depth. The actual effective depth, or more importantly the light field over that depth, will depend on many factors such as the evenness of illumination, wall growth, reflectance and refraction etc.

Reactor_V This is the volume of the PBR in m^3. There are 1000L in $1m^3$. This particular model does not discriminate between light and dark tanks as used by some PBR configurations to help even-out gas exchange rates; the volume set by constant Reactor_V is thus the total PBR volume. To account for the light:dark tank volumes with this model the easiest route is to decrease the value for PFD (see below) pro rata with the volumes of the {light tank}:{total PBR} ratio.

PFD The photon flux density at the surface of the PBR. Please note the comment about light:dark tank volumes in the Reactor_V description above.

LD The light:dark periodicity of illumination. For full (continuous) illumination this value will be 1; for full darkness for pure heterotrophic growth this would be 0.

Temp The temperature of the water in the PBR in °C.

dil The continuous dilution rate as d^{-1}. If this is used to operate the facility as a chemostat-style system, then the value of dil sets the net growth rate of the organisms. Set dil to zero if there is no continuous dilution.

har_frq and **har frac** These, respectively, set the frequency (in days) of harvesting, and the fraction of the PBR harvested on each occasion. The harvest volume is assumed to be replaced immediately by the addition of fresh growth medium.

10.3.2 Nutrient configuration

Nutrients are assumed to be supplied at a fixed concentration in the feed water to the PBR. Note that all concentrations are of the elements, (i.e., C, N, P, Si) and not of nutrient molecules. In configuring these concentrations, it may be useful to consider that the classic f/2 medium of Guillard (1975), contains 12320 mgN m^{-3} (usually as nitrate-N), 992 mgP m^{-3}, and that seawater contains ca. 24000 mgC m^{-3} as DIC.

The configuration table from the simulator for nutrients is shown in Fig.10.4. This gives access to a range of features that can be configured independently for each of the three arrayed PBRs (PBR#1, PBR#2, PBR#3).

Nutrient	unit	PBR#1	PBR#2	PBR#3
attco_W	m-1	0.01	0.01	0.01
NH4	mgN m-3	1,400.00	1,400.00	1,400.00
NO3	mgN m-3	12,320.00	12,320.00	12,320.00
DIP	mgP m-3	992.00	992.00	992.00
Si	mgSi m-3	1,400.00	1,400.00	28,000.00
DIC	mgC m-3	24,000.00	24,000.00	24,000.00
sw_CO2	dl (0 or 1)	1.00	1.00	1.00

Fig.10.4 Snapshot from the screen of the model showing the simulator nutrient configuration table, with example entries. The user completes this table using entries appropriate to the system being explored.

An explanation of these options follows:

attco_W Absorbance of the growth medium (m^{-1}). This is the absorbance coefficient for the blank growth medium. Although this is often very low, if digestate or soil-extract (tanins) are present then the value may be elevated enough to be of significance.

NH4 Ammonium-N (mgN m^{-3}) in the feed. While ammonium is the primary form of DIN in anaerobic digestate, it should be noted that high concentrations of ammonium are usually

toxic and that feed values may in reality need to be ramped up carefully. High concentrations in the feed can thus be used provided that the residual concentrations in the PBR are not allowed to rise too high (ca. maximum of 100 µM = 1400 mgN m^{-3}).

NO3 Nitrate-N (mgN m^{-3}) in the feed.

DIP Phosphate (mgP m^{-3}) in the feed. Care must be taken not to specify amounts that would, in reality, precipitate out of suspension. This becomes likely at levels in excess of ca. 1000 mgP m^{-3} in seawater-based media.

Si Silicate (mgSi m^{-3}) in the feed; this is required only when simulating the growth of diatoms other than *Phaeodactylum tricornutum* (which can obtain what little Si it requires from Si dissolving off glassware in the PBR). In reality, care needs to be taken to prevent silicate from precipitating out of solution at high concentrations (increasingly likely above 10000 mgSi m^{-3} depending on salinity, temperature and medium preparation methods).

DIC Dissolved inorganic C (mgC m^{-3}) in the feed, usually added as bicarbonate and/or as CO_2 bubbled into the system, and then allowed to equilibrate between carbonate, bicarbonate and CO_2(aq) in proportions set by the pH of the medium.

sw_CO2 Switch to control whether the automatic injection of CO_2 is enabled. Set a value of 0 for no injection; 1 for injection. Injection of CO_2 is quantified only with respect to that which is required to dissolve into the water in the PBR; excessive addition (which would just bubble out of the system) is not accounted for. If no CO_2 injection is allowed, then the model assumes that pH is held constant by addition of acid/alkali. Under that condition, phototrophic growth can rapidly become limited by DIC availability (Clark & Flynn 2000).

10.3.3 Algal physiology and quota configurations

The model describes one microalgae, growing in the 3 PBRs. The configuration table from the simulator for the physiology is shown in Fig.10.5, while that for the quotas is shown in Fig.10.6.

An explanation of the options shown in Fig.10.5 is as follows:

Alpha Initial slope of the PE curve ($m^2 g^{-1}$ chl.a)*(gC µmol^{-1} photon).

ChlCm Maximum ratio of chlorophyll to cellular C (gChl gC^{-1}). This controls how "green" is a microalga – this is the subject of genetic modification studies as a lower value enhances population growth by decreasing self-shading; values are usually between ca. 0.08 and 0.01.

ChlCo Maximum ratio of chlorophyll to cellular C (gChl gC^{-1}).

KgDIC Half saturation for DIC usage (mgC m^{-3}). This is only of consequence if there is no CO_2 injection (sw_CO2 = 0; Section 10.3.1). See Cark & Flynn (2000)

RelPSm Maximum value of photosynthesis relative to maximum (day-averaged) growth rate on phototrophy. Thus, RelPSm* UmT gives the maximum plateau value for the net PE curve. (dl; typical values may be between 1 and 4).

RelUmNH4 Maximum growth rate supported by ammonium-N relative to the maximum possible growth rate (dl; typically this will be 1).

RelUmNO3 Maximum growth rate supported by nitrate -N relative to the maximum possible growth rate (dl; typically this will be 1, or a little less, but it could be zero if the microalgae cannot transport or reduce nitrate through to ammonium inside the cell).

RT Reference temperature at which UmRT is achieved (°C).

UmRT Maximum growth rate, typically that using NH_4-N, at reference T (gC gC^{-1} d^{-1}). The actual maximum growth rate at temperature **Temp** (Section 10.3.1) is **UmT**. It is very important that the value of UmRT is a C-specific value. The maximum value is one that likely will not give a value of UmT at the operational temperature exceeding ca. 3 d^{-1} (see Flynn & Raven 2017). More likely the value will be around 1 d^{-1}, and less than 0.5 d^{-1} for phototrophic dinoflagellates.

The half saturation constants for the use of nutrients other than DIC are all set to be equal to 1µM, except P at 0.1µM. In a PBR nutrients are supplied at such excess that the values of these parameters in unialgal culture is usually of little consequence.

Algal config	unit	value
alpha	see text below	7.00e-6
ChlCm	gChl gC-1	0.06
ChlCo	gChl gC-1	5.00e-3
KgDIC	mgC m-3	1,200.00
RelPSm	dl	4.00
RelUmNH4	dl	1.00
RelUmNO3	dl	0.80
RT	oC	10.00
UmRT	d-1	0.70

alpha units
(m2g-1 chl.a)*(gC umol-1 photon)

Fig.10.5 Snapshot from the screen of the model showing the microalgal physiology configuration table, with example entries. The user completes this table using entries appropriate to the microalga being explored.

An explanation of the options shown in Fig.10.6 is as follows; the most important are those underlined.

NCm Maximum possible microalgal N:C (gN gC^{-1}).

NCopt Optimal microalgal N:C for P-replete growth (gN gC^{-1}).

NCo Minimum possible microalgal N:C (gN gC^{-1}). The lower this value the greater the potential for accumulating carbohydrate or fatty acids; values are typically between 0.1 and 0.05.

PCm Maximum possible microalgal P:C (gP gC^{-1}).

PCopt Optimal microalgal N:C for P-replete growth (gP gC^{-1}).

PCo Minimum possible microalgal P:C (gP gC^{-1}).

PCoNCm NCm when P:C=PCo (gN gC^{-1}).

PCoNCopt NCopt when P:C=PCo (gN gC^{-1}).

DIATOM switch Switch to define the microalga as a Si-requiring diatom (dl; 0 for non-diatom, 1 for diatom). Set as 0 for *Phaeodactylum* as this microalga has no significant demand for Si as long as part of the culture vessel is made of glass.

SiCm Maximum possible diatom Si:C (gSi gC^{-1}).

SiCopt Optimal diatom Si:C (gSi gC^{-1}).

SiCo Minimum possible diatom Si:C (gSi gC^{-1}).

See Fig.3.7 and allied text (Chapter 3) for an explanation for the meaning and importance of PCoNCm and PCoNCopt.

Algal Quota	unit	value
NCm	gN gC-1	0.20
NCopt	gN gC-1	0.15
NCo	gN gC-1	0.05
PCm	gP gC-1	0.05
PCopt	gP gC-1	0.02
PCo	gP gC-1	5.00e-3
PCoNCm	gN gC-1	0.12
PCoNCopt	gN gC-1	0.10
DIATOM switch	dl (0 or 1)	1.00
SiCm	gSi gC-1	0.20
SiCopt	gSi gC-1	0.10
SiCo	gSi gC-1	0.02

Fig.10.6 Snapshot from the screen of the model showing the microalgal C:N:P:Chl:Si quota configuration table, with example entries. The user completes this table using entries appropriate to the microalga being explored.

10.3.4 Transforms

The model operates, as a system dynamics model must, on common units. However, often in the commercial microalgal sector, operators refer to production in terms of protein or dry weight. To facilitate an understanding of the results, harvested production is also reported in these units. To achieve that the model uses transform values. There are no fixed transforms (the values depend on the microalgal species, and indeed often on the nutritional status as well), so the user can enter their own values. This is done using the transform table (Fig.10.7).

TRANSFORMS	g g-1
Protein:N conversion	6.00
dry weight:C conversion	3.00

Fig.10.7 Snapshot from the screen of the model showing the transform table. Units are g g^{-1}.

10.4 Interpreting the model outputs

Before you run the model, first set the options as described in Section 10.3.

The model is not particularly fast. It will also be slower depending on the power of the graphics-chip of your PC as there are a lot of plots. This rate of simulation progress does however have the advantage that you can watch what is happening, which can be insightful, especially as you can change the values of the constants in the configurations tables (Section 10.3) while the model runs. Even at the slow simulation speed, it is still 1000's of times faster than doing real experiments, and it is free!

Pressing Ctrl+space while the model is running will pause the simulation (allowing you to change the input parameters if you so wish), and it will also rescale the graphs. Press Ctrl+space again to continue the simulation.

WARNING: the plots are self-scaling so be sure to observe the range of values on the y-axes.

To make the simulations run (much!) faster, just minimise the window after pressing "run", give it a few seconds and maximise the window again. Or switch to another application for a few seconds.

The simulation outputs shown is for a diatom, configured as per Fig.10.6.

10.4.1 Syntax of the output

The syntax used in the outputs has a number given within [ ]. That number refers to the identity of the PBR as you configured it for its physical and chemical features, and its mode of operation. There is only 1 species described here, so the outputs are simpler to understand that those in Chapter 9, though there are far more parameters (more detail) than in the simple model used in that earlier chapter.

10.4.2 Harvested biomass

The graphs detailing the harvested biomass (Fig.10.8) are shown in the middle section of the project window (Fig.10.1).

These plots show the cumulative harvest over the 60d simulation period. The step-style of the PBR#3 series reflect the form of the harvesting schedule, which involves a 70% harvest every 4th day. PBR#1 is harvested continuously; PBR#2 is harvested frequently (30% every day). There are differences in the biomass with respect to C, N and P biomass.

The protein harvest aligns with that for N-biomass because there is a simple (fixed) transform between N and protein (Fig.10.7). By the same token, dry weight aligns with C-biomass.

Fatty acid production aligns with the N:C of the microalgae at the time of harvest. The model does not describe the composition of that fatty acid, and indeed does not discriminate between fatty acid and polysaccharide. More properly, this plot reports excess (storage) C.

The cumulative Chl harvest may be expected to align with that of various other photo-pigments, though some others may align (depending on the species) with C-biomass rather than N-biomass.

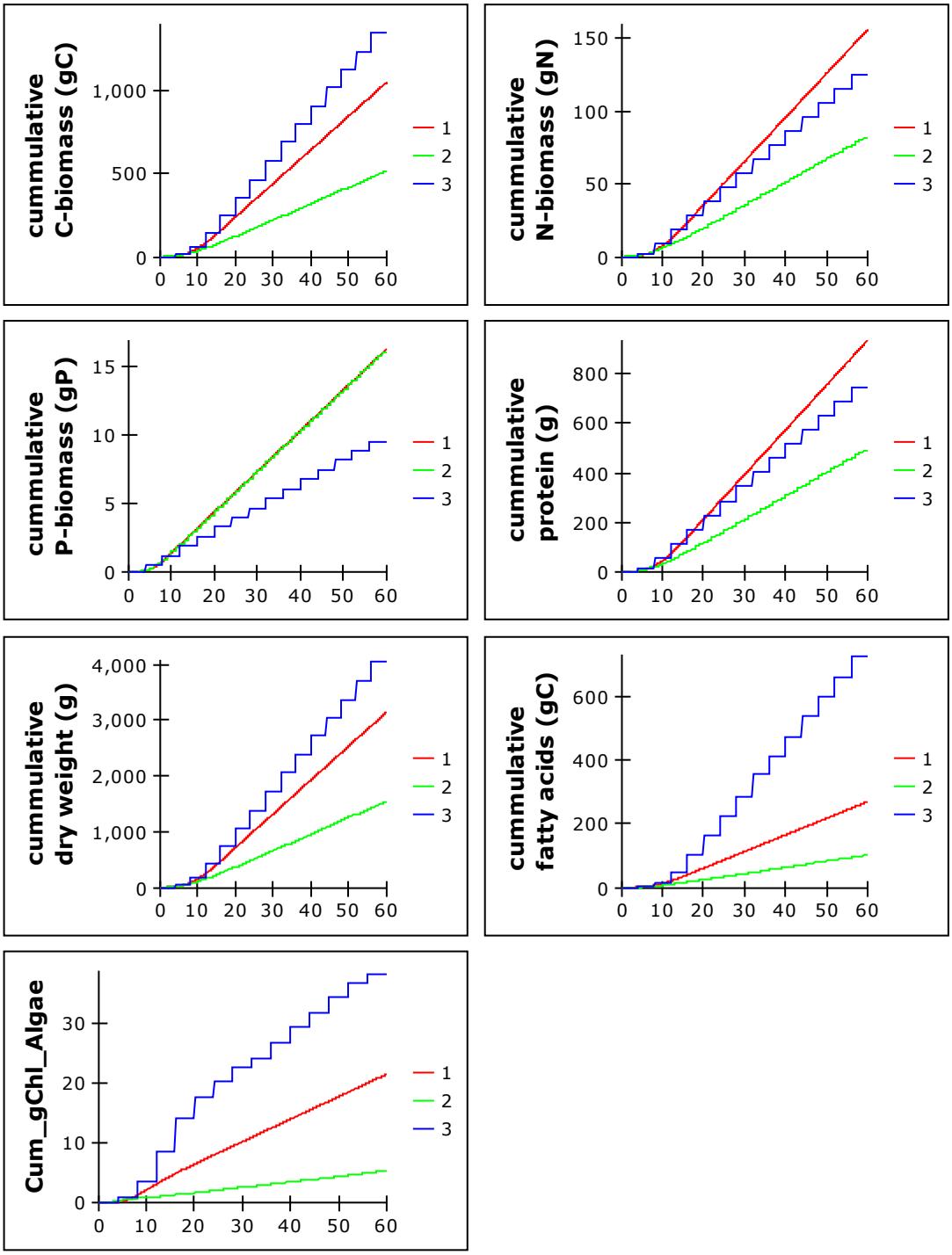


Fig.10.8 Snapshot of the cumulative harvest biomass.

The quality of the harvest, as indicated by the elemental C:N:P, is shown in Fig.10.9. This shows that the conditions of operation in PBR#3 is supportive of combined N and P deprivation (low N:C and P:C), PBR#1 and PBR#2 are more representative of light-limitation for N (high N:C), but there are P-stressed (low P:C) for PBR#3.

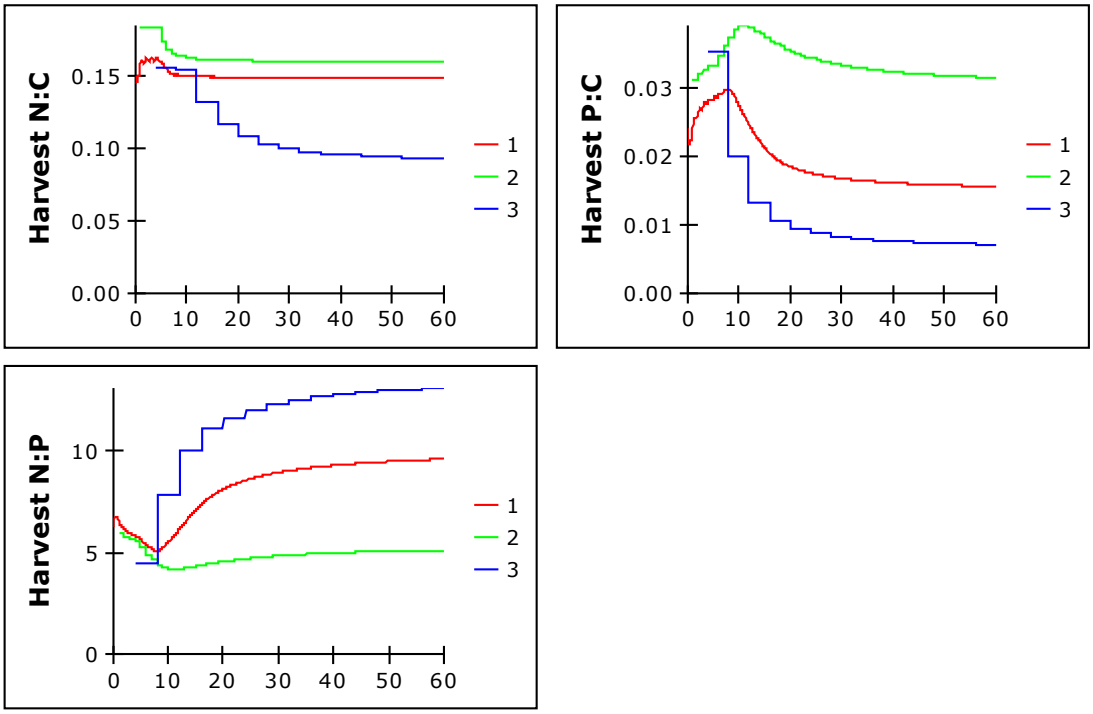


Fig.10.9 Snapshot of the C:N:P quality of the cumulative harvest.

The flip side of production is the wastage of resources (Fig.10.10). Because ammonium is used by priority, and most nutrient-N here is supplied as nitrate, there is no ammonium in the waste stream. Consistent with the production of N-sufficient or P-sufficient biomass (Fig.10.9), PBR#2 is most wasteful of nitrate, while all the supplied P is accumulated into biomass. There is only a minor waste of Si.

PBR#3 wastes least water, and fixes most C.

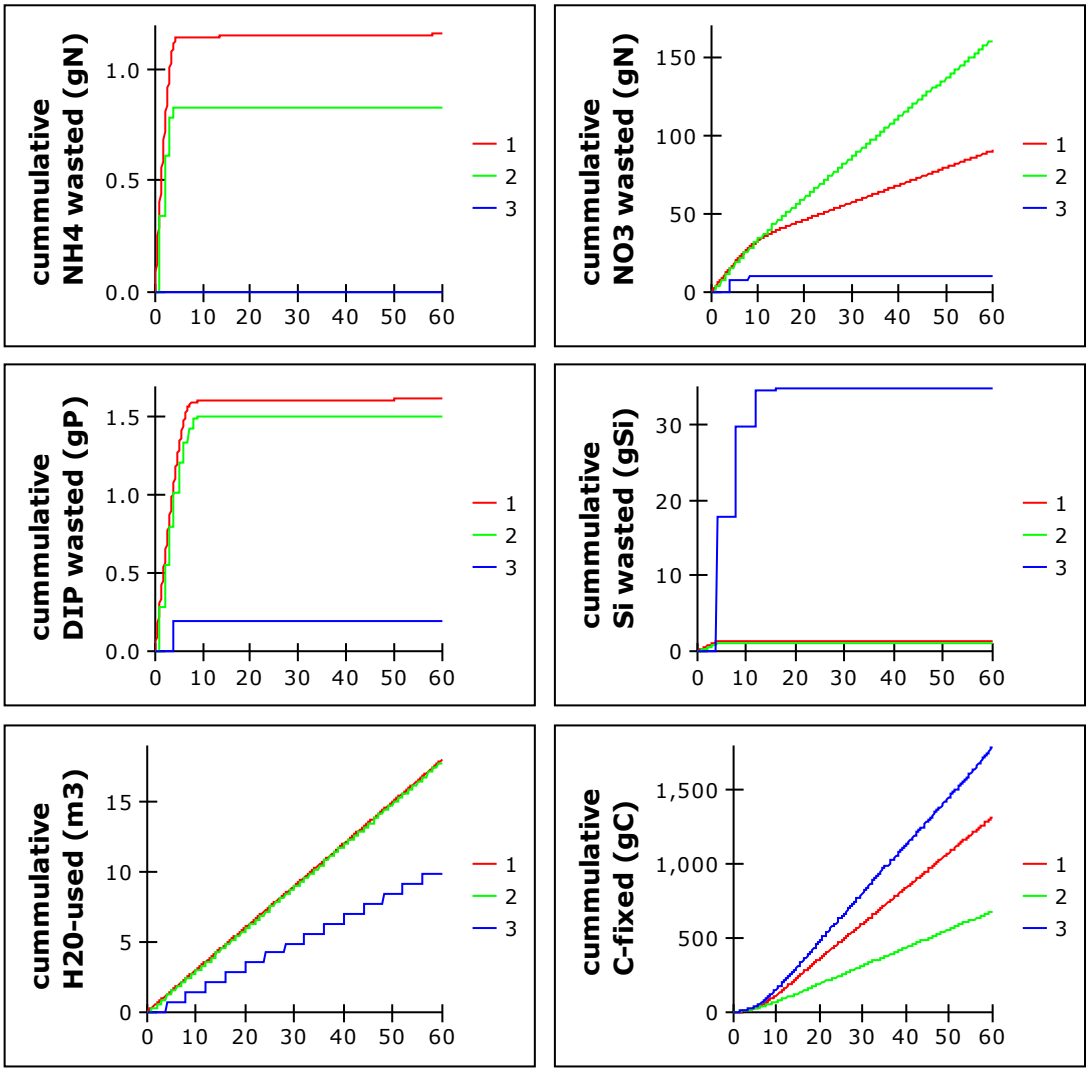


Fig.10.10 Snapshot of the resource waste and C-fixation.

10.4.3 Physiological status

The graphs in the right-hand part of the project window (Fig.10.1) show the physiological status of the simulated microalgae.

Fig.10.11 shows biomass content in each of the PBRs; note this is not concentration but the total PBR content. Note also here that the content of PBR#3 bounces depending on where in the growth-harvest cycle the time is. The growth rate (Cu) also bounces; harvesting of PBR#3 is of cells that have passed through a transient of fast growth and then slower growth when nutrients (specifically N) becomes limiting. These transients also explain the change in Chl:C (Fig.10.12) and N:C (Fig.10.13) in the biomass growing in PBR#3.

The plots in Fig.10.14 show the N:C, P:C and Si:C quotas as well as the residual nutrient concentrations. Note that PBR#2 contains biomass with the highest P:C and PBR#3 contains the highest Si:C (the latter because any non-Si limitation of diatom growth results in deposition of thicker cell walls).

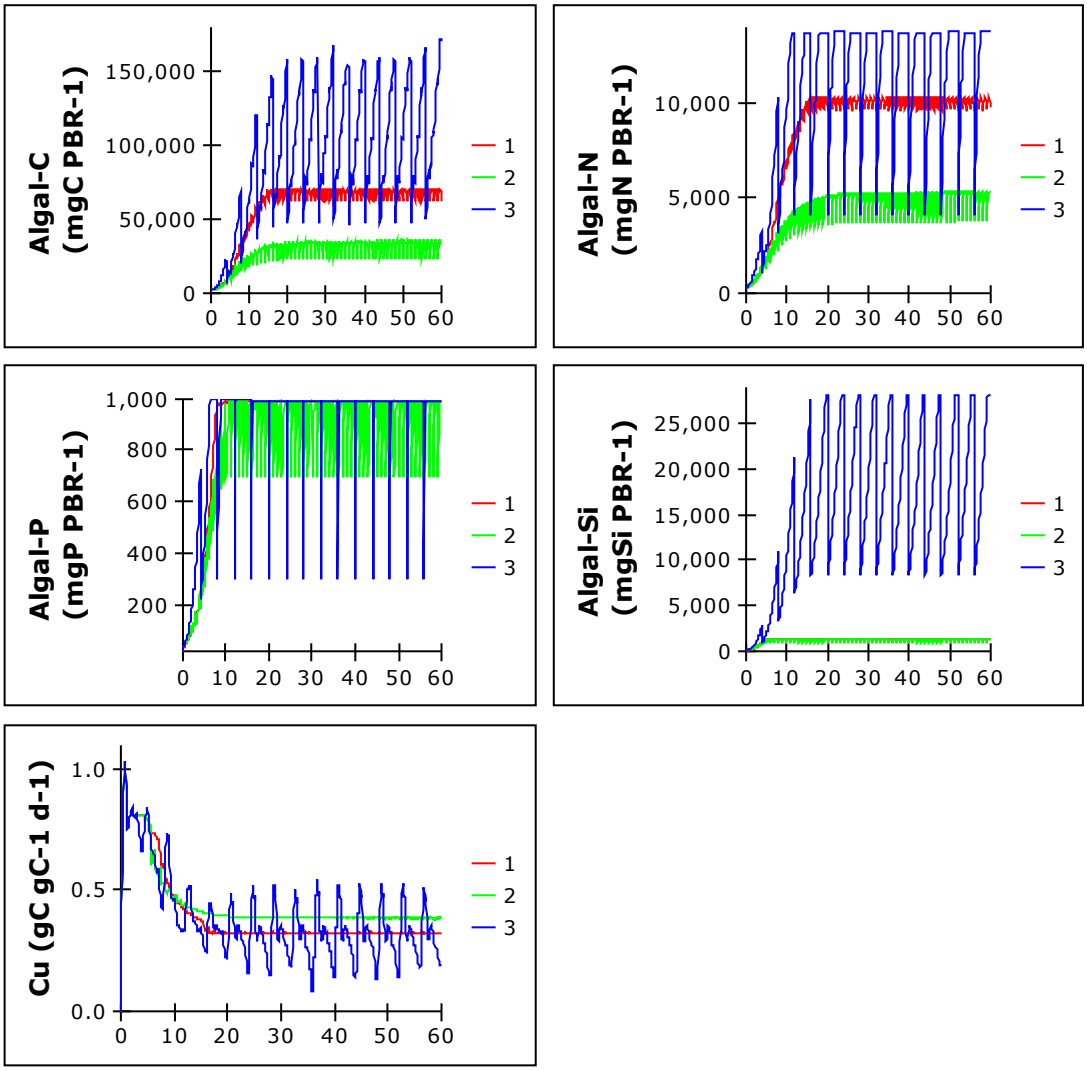


Fig.10.11 Snapshot of the plots for algal biomass in each PBR in terms of C, N, P and (because this simulation is for a diatom) Si. Changes in the C-specific growth rate (Cu) are also shown.

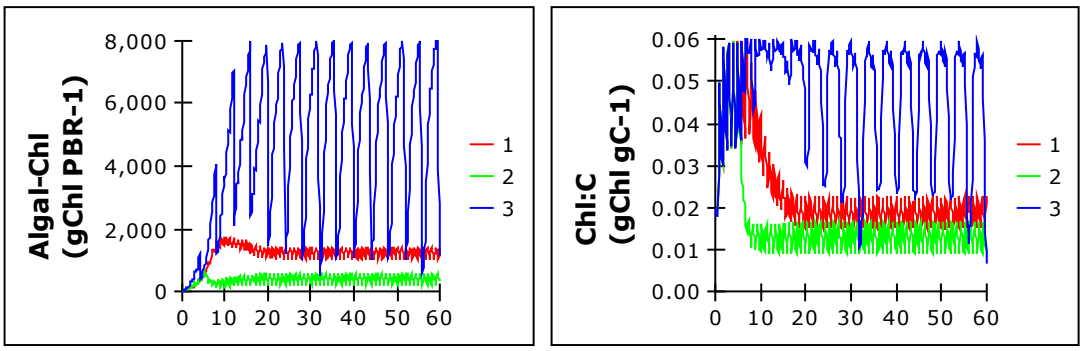


Fig.10.12 Snapshot showing changes in algal chlorophyll and of the Chl:C ratio.

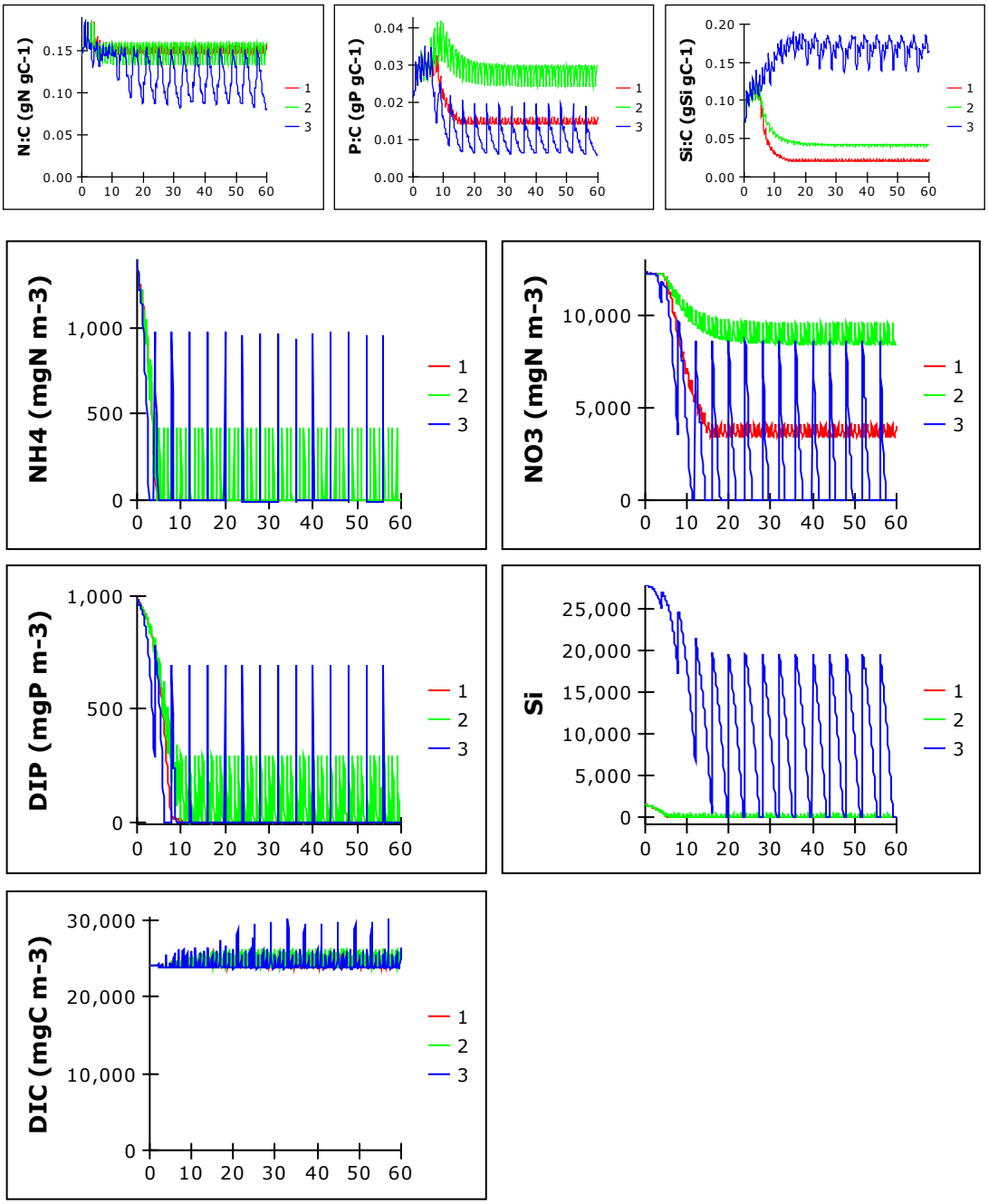


Fig.10.13 Snapshot showing residual nutrient concentrations and (upper line) the algal biomass nutrient quotas (N:C, P:C, Si:C). DIC is high because these simulations assume a DIC-stat which injects CO_2 to compensate for C-fixation.

10.4.4 Some summary observations

Collectively the graphs reveal how complex is the whole process of growing, and then optimising, microalgal crops. How good the simulation model is in describing real events depends on both how closely the model conforms to reality with respect to its underpinnings, and also in its configuration.

By altering the physiological parameters (Fig.10.5, 10.6) you can judge how sensitive is the output to microalgal physiology. It is important to recall that microalgae evolve and so what your real

system does this year may not align well with last years performance. That is so unless you have started your culture with source material kept under cryopreservation and the PBR configuration (including lighting and heating) are also the same.

10.5 Caveats

Many of the caveats given in Chapters 8 & 9 apply here also, but there are also the following caveats to consider.

- Except in laboratory conditions, it would be very difficult to set up PBRs in the same conditions. Likewise, algal cultures rarely behave exactly as expected. It is thus important to trial configurations with plausible deviations between simulations to see how robust are the simulations.
- These are 60d simulations. The longer a culture system is operating the more likely it is that something will go wrong. That may be mechanically with the PBR, or biologically. Again, you need to balance theory with the possibility for deviations in reality.
- As is apparent from the configuration tables for controlling this model (which give scope for less than half those present in the whole model, though the others are of less consequence for the purpose at hand), there are a great many physiological constants involved in a variable stoichiometric model. For many of these there will likely be scant data to support a rigorous parameterisation. Furthermore, how these may change in consequence of growth at different temperatures and different growth limitations is poorly understood for even the best studied organisms. It is thus a good idea to test model runs with different parameter values for physiological constants.
- The longer a culture system is run, especially under steady-state dilution conditions, the more likely it is that the maximum growth rate of the microalgae will evolve downwards to more closely align with the enforced realised growth rate.

Normalised Acquisition Potential Control Mechanism (nAPCM)

Introduction

- The control of resource acquisition makes reference to normalised quotas rather than to absolute quotas. This has the advantage that quota constant values can be more readily changed without changing the form of the control mechanism.
- The Acquisition Potential (AP) for different resources is controlled using a Goldilocks construct (*not too much; not too little; just right*) to simulate (de)repression of physiological processes. This permits complex multi-nutrient and multi-stressor interactions to be considered.
- Collectively, and given the use of normalised quota controls, this approach is the normalised (resource) acquisition potential control mechanism (nAPCM).
- The nAPCM can be readily used to control alternative Goldilocks interactions, and is thus readily used to also control phagotrophy, osmotrophy.
- The same concept could also be used to regulate allometric interactions through reference to a normalised allometric scale (where 0 and 1 indicate the extreme sizes of the scale in question).

General Construction

The control makes use of combinations selected from 4 sigmoidal curves which on being paired, between them describe increases or decreases towards, or away from, an index for optimum physiological behaviour. Sigmoidal curves are used because they well represent (in general terms) allosteric biochemical reactions, and mathematically they also produce robust (non-sensitive) feedback response curves.

In the following, mention is made of data or information availability “from the literature”. It is most unlikely that all such information is available for any individual organism. The user must thus use their own judgement in configuring the model in line with their needs and understanding.

For the application at hand, the construction of these curves requires the following:

- The range of the input (e.g., N:C quota from minimum to maximum); this information comes from the literature.
- The value of the input control in optimally configured (low-stressed) organisms expressed as a normalised value within the range (i.e., between 0 and 1); this information comes from the literature.
- The additional enhancement on de-repression (controlling the extent to which the output reaches beyond that seen in optimally configured, non-stressed, organisms); this information comes from the literature.
- Values of K and H for controlling the form of the sigmoidal curves; these values can be estimated from curve fits to experimental data, noting that operationally these controlling sigmoidal functions in models are very robust to values of these constants.
- Ki and Hi values control increasing AP
- Kd and Hd values control decreasing AP

In total then, equations make reference to:

- nI normalised quota input (referenced to a variable, such as N:C)
- nOI normalised optimum quota input (value between 0 and 1) at which output =1
- Ki (typically in the range of 0.1 – 0.5)
- Hi (typically 2 or 4)
- APadd (0 gives no uplift, 1 doubles the output relative to the output at nOI)
- Kd (typically in the range of 0.1 – 0.5)
- Hd (typically 2 or 4)

An example of all combinations of the resultant curve combinations is shown in Fig.1.

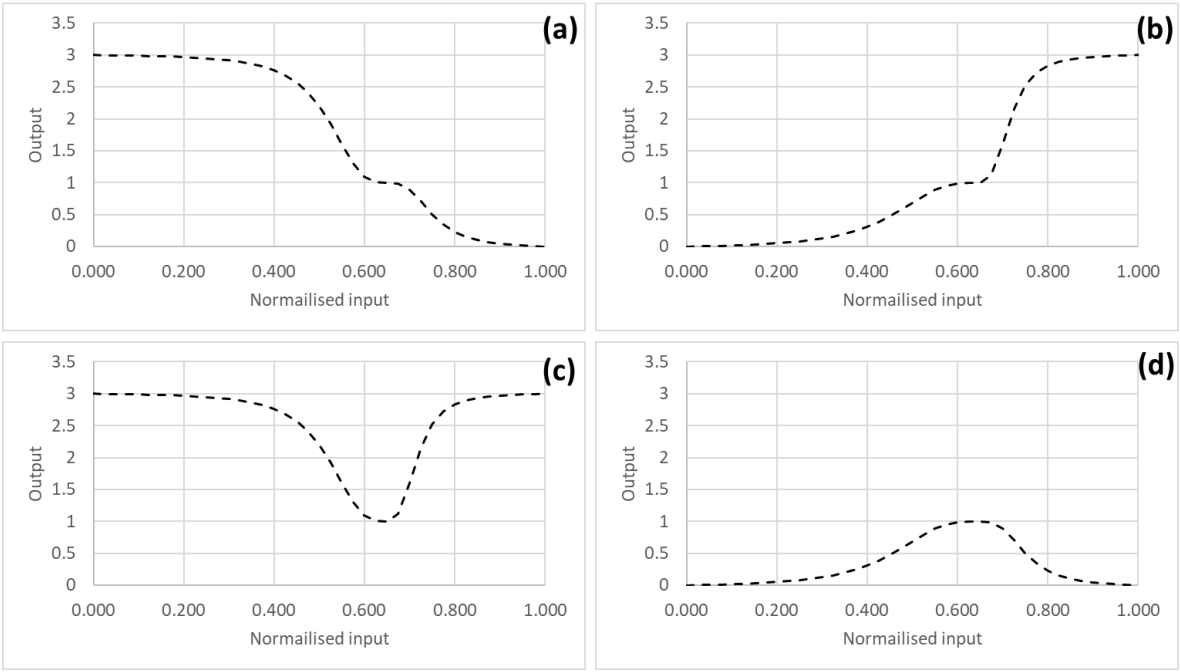


Fig. 1. Four different response curve configurations. In each instance the optimum is at 0.65, at which point the output is 1. Curve (a) could represent a de-repression of DIN transport as N:C decreases below the optimum and a repression above the optimum. Curve (b) could represent a de-repression of DOC transport as N:C increases. Curve (c) could control increases in phagotrophy in a protist in response to either a decrease in N-status (N:C decreases from the optimum) or a decrease in C-status (N:C rises from the optimum). Curve (d) could control the need for another nutrient as N:C varies either side of the optimum.

For a given curve shown in Fig.1, there are two sigmoidal functions controlling the acquisition potential (AP), one for either side of the optimum nutritional point. One curve type (APd) decreases the AP as there is too much nutrient already in the organism, while the other type (APi) increases AP because there is insufficient nutrient in the organism.

There are 4 sigmoidal equations in total, each with an output of 1 when nI = nOI. These curves are alternates for use before or after nI = nOI:

- LL : low input gives low output {APd type, for nI<nOI}
- HH : low input gives high output { APi type, for nI<nOI}
- HL : high input gives low output { APd type, for nI>nOI}
- HH : high input gives high output {APi type, for nI>nOI}

These curves are defined thus:

LL = 1-(1+Kd^Hd)*(((nOI-nI)/nOI)^Hd)/((((nOI-nI)/nOI)^Hd)+Kd^Hd)

LH = 1+APadd*(1+Ki^Hi)*(((nOI-nI)/nOI)^Hi)/((((nOI-nI)/nOI)^Hi)+Ki^Hi)

HL = 1-(1+Kd^Hd)*(((nI-nOI)/(1-nOI))^Hd)/((((nI-nOI)/(1-nOI))^Hd)+Kd^Hd)

HH = 1+APadd*(1+Ki^Hi)*(((nI-nOI)/(1-nOI))^Hi)/((((nI-nOI)/(1-nOI))^Hi)+Ki^Hi)

With reference to the curve types shown in Fig.1, the equations are used thus:

AP(a) = IF(nI<nOI,LH,HL)

AP(b) = IF(nl<nOI,LL,HH)

AP(c) = IF(nl<nOI,LH,HH)

AP(d) = IF(nl<nOI,LL,HL)

The value of nOI and APadd may be altered, for example for changing of the optimal and minimum AP controls for N-sources during P-limitation. This results in N:C decreasing during P-limitation even though the organism is not N-limited by external N-source availability (see Flynn 2008).

The AP value sets (with reference to the maximum growth rate Umax) the maximum acquisition rate, with further reference to a half saturation for nutrient transport (Kt) and the external substrate concentration.

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