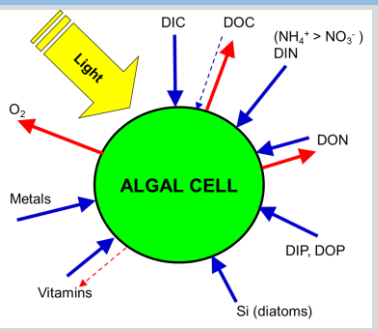
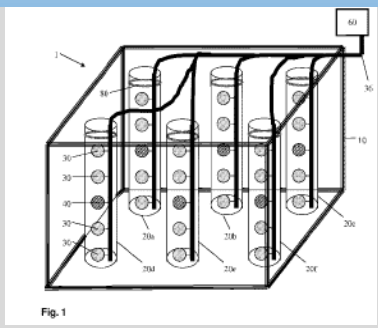
Enhancing Microalgal Production

Constructing Decision Support Tools Using System Dynamics Modelling

Kevin J Flynn





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Foreword

This work comprises part of a deliverable for the EnhanceMicroAlgae (EMA) project; https://www.enhancemicroalgae.eu/. The overarching aim of the EMA project is to promote the commercial exploitation of microalgae. A key sectorial challenge is the conversion of small-scale research-intensive understanding to the large scale production that also needs to return a profit. The technology to bulk grow microalgae ranges from crude plastic-bag cultures or ponds to complex highly engineered small-bore photobioreactors. All approaches, however, share the common logistic challenges of having to handle large volumes of microalgal suspension as the organisms grow (often rather slowly) over many weeks.

The aim of this work is to provide a platform to support *in silico* experimentation with microalgae. Through such a route experiments that would take many years of effort, and cost exorbitant amounts of material and money, can be conducted in minutes, for free. If all that comes from the operation of such simulation models is to rule out most of the options leaving the more plausible approaches, then that is in itself a great advantage. In addition, the operation of such simulation models, the justification for their construction and operation (including all the caveats), also helps to draw attention to matters of importance that may otherwise be overlooked.

Inevitably, this work and its simulation models, will contain errors and gross simplifications. The author welcomes any feedback; please email any comments to kjfplankton@gmail.com.

Thank you

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How to cite this work

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How to access the DST models

The models comprising the DST simulation platform are published by Zenodo as detailed below. To run the models, you need to use the Powersim Studio Cockpit interface. The interface is available for free, to run on a MS Windows platform, from:

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

These are all free-to-end-user models; please read the disclaimer on page viii. The models are also available as open versions that you can edit and develop them further if you have access to Studio 10.

Chapter 7 (Introductory model):
https://doi.org/10.5281/zenodo.4771866

Chapter 8 (A Simple Model of Microalgal Growth in a PBR):
Open version https://doi.org/10.5281/zenodo.4771946
Cockpit version https://doi.org/10.5281/zenodo.4772362

Chapter 9 (An Arrayed Simple Model):
Open version https://doi.org/10.5281/zenodo.4772437
Cockpit version https://doi.org/10.5281/zenodo.4772448

Chapter 10 (An Arrayed Complex Model):
Open version https://doi.org/10.5281/zenodo.4772537
Cockpit version https://doi.org/10.5281/zenodo.4772560

Chapter 11 (Heterotrophic and Coupled Photo-Heterotrophic Nutrition):
Open version https://doi.org/10.5281/zenodo.4772587
Cockpit version https://doi.org/10.5281/zenodo.4772601

Chapter 12 (Production of Dissolved Organics):
Open version https://doi.org/10.5281/zenodo.4772632
Cockpit version https://doi.org/10.5281/zenodo.4772663

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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 exploitation 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 free use under the Powersim Cockpit platform. Neither 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 and degrade over time through bacteria activity or with UV illumination.

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 from a thin suspension through different phases of the culture dynamics, to a dense 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 this refers to an organic compound that binds onto iron, Fe, thus 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 specific 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 added to cultures, but NH_4^+ is the dominant form in most recycled 'grey' waters. NH_4^+ is the "preferred" N-source in algal physiology but it is highly 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, temporally discrete, replacement of a portion of the culture with fresh medium. Discontinuous removal (rather than continuous removal) has the advantage that a more useful volume is removed for processing at a point in time. A period of maximum growth rate is not usually seen. However, if the gap between dilutions is of

sufficient duration, and the proportion of culture replaced also significant, then the discontinuous culture approaches a *stretched-batch culture* system which does allow for a period of maximum growth rate.

DOC: dissolved organic carbon, which typically includes primary metabolites such as glucose, but also potentially exotic chemicals that may be of commercial interest.

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*.

DST: decision support tool.

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* or even as prey for nutrition via *phagotrophy*), 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*.

Mixoplankton: protists (flagellates and some ciliates) that combine *phototrophy* and *phagotrophy*. Cf. *mixotrophy*.

Mixotrophy: combining *phototrophy* and *heterotrophy*. All microalgae are mixotrophic, in that they can photosynthesise and exploit organics such as amino acids. Cf. *heterotrophy*, *mixoplankton*, *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 unless that reality is itself rigorously controlled, as may well be the case in a laboratory situation.

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 in a near-linear fashion 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 net 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. Note that the cessation of growth may occur sometime after the exhaustion of the external nutrient source as growth of a phototrophic organism proceeds using the internal nutrient quota (see *N-quota*, *P-quota*). Cf. *nutrient deplete, nutrient replete, nutrient sufficient*.

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 carbon. 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, at which time net C-specific growth halts.

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). At higher levels of E, P declines due to photoinhibition and then photodamage.

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

Photoacclimation: *acclimation* of microalgae to the supply and demand of the products of photosynthesis balanced against light and nutrient (usually *DIN* or *DIP*) supply. Characterised by changes in *Chl:C* and often also by 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. Full sunlight has a PFD of ca. 2000 µmol photons m^{-2} s^{-1}.

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 assumed. 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 relatively 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), 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 by definition *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 remove particles from the face of the filter that would otherwise rapidly 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 (match) 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 that occurs 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 correctly configured *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 suitably constructed simulation platform, however, allows *in silico* experiments to be conducted quickly and safely.

Part I describes 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 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; see page ii.

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 was spurred on during the early 21st century by interests in 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. It also provides a platform for those engaged in scientific work on microalgae, invariably conducted using very small culture volumes (perhaps just a few 100 mL), to better appreciate the challenges in upscaling their work to commercial levels (to many m^3). This is very important, because growing small volume flasks presents a totally different environment for microalgal growth.

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 serious 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 in the laboratory.

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 www.mixotroph.org/models.

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 5-10µm 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 mixoplanktonic through combining phototrophy and phagotrophy (i.e., they eat their competitors and other organisms, such as bacteria)
- 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 uninitiated. 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. Indeed, evolution of cultured microalgae can occur rapidly over a few months of being forced to grow at a particular rate (Droop 1974).

Microalgae and their physiology are explored in more detail in **Chapters 2 and 3**. The book of Richmond (2004) provides a comprehensive treatise on the subject.

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 recovered at the time of harvest. This analogy to terrestrial farming 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 and, as will be seen when running the simulation models later in this work, the manipulation of the periodicity and proportion of the crop harvested are critical determinants in maximising both the production and also the chemical characterisation of the microalgal crop.

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 when they are starving of N-nutrient in a well-lit environment. However, the growth of microalgal cells requires sufficient light and nutrient. A high biomass of microalgae also 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 - little production per unit of time giving a high profitability margin, but little actual profit. 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. 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}). So, units for APR are most usefully described as (for example) gC m^{-2} d^{-1}.

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 in as small an area as possible. However, very quickly these ideals become self-defeating. A "pea-soup" suspension, and especially one that is optically deep, 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. A route around that may be to supplement the C source by adding sugars; this is explored in **Chapter 11**. 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 time.
ii) by definition, a simulation must represent, or allude to representing, 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 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 also 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 the DST 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; **Fig.2.1** shows some of the types commonly used.

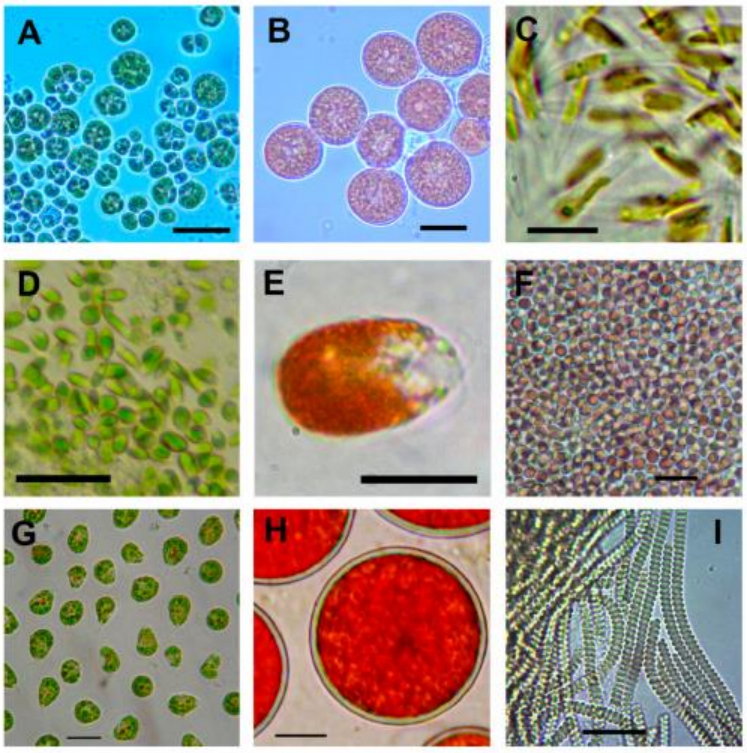


Fig.2.1 Light micrographs of selected microalgae for commercial cultivation for various biotechnological applications. *Chlorococcum* sp.((A,B); source for mixed carotenoids including -carotene, astaxanthin, canthaxanthin, lutein), *Dunaliella salina* ((D,E); the source for β-carotene) and *Haematococcus pluvialis* ((G,H); the source for astaxanthin); these 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). Others are usually cultivated as a single-phase actively growing biomass for the targeted biomolecules: (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). All scale bars are 25 µm, except for (C,E), which are 10 µm. Figure and modified 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 phototrophic growth; it is thus perhaps disingenuous to refer to non-photosynthesising protist cultures as containing "microalgae" unless they are at least occasionally illuminated.

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 upon bacteria, cyanobacteria or other protists); these protists are *mixoplankton* (Flynn et al. 2019).

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 typically termed "phytoplankton", though we now know that many of the non-diatom protist species, and many ciliate protozooplankton are actually mixoplankton.

Some phytoplankton just drift, 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, 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) dissolved 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 the nucleus, 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 heterotrophic through osmotrophy (using dissolved 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; Flynn et al. 2019). 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 just 0.01mm in 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. Many species used in commercial cultivation are non-motile.

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 (as normally 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). ESD is most easily measured using a Coulter counter, or similar, though a good calibrated microscope may suffice.

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 may 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 may be 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 dominant residual visible spectra of light appears green, 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 contain much nitrogen 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-tan 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.

Microalgae acclimate to changes in light by altering both the amount of pigment and the amount of enzymes; in crude terms, they alter the Chl and/or RuBisCO content of the cells. Different microalgae acclimate in different ways (Richardson et al. 1983), but the dominant route is through altering either both Chl and RuBisCO (Vandenhecke et al. 2015).

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 concentrations approaching 1mM, though ammonium (the major N source present in anaerobic digestate liquor) is often toxic at levels above 100 µM, and may become toxic at much lower concentrations. 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 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 seawater media). As we will see below, the ratios of these concentrations does not align well with that of algal biomass.

The above solubility and toxicity levels 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. This dual role for DIC is so important that, throughout this DST, it is assumed that the DIC concentration in the water is held around 2mM.

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, photosynthetic, O_2-releasing 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 small 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 the elements 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 (indirectly) affects both growth rates and the chemical quality of the biomass; a relatively 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 physiological 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 in higher plants) but they can also be highly toxic (such as shell-fish toxins in some dinoflagellates). 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. Some such chemicals exhibit an allelopathic activity, modulating cell-cell interaction with the same species and/or between different species (Śliwińska-Wilczewska et al. 2021).

2.8 Growth rates

Growth rates of microalgae do not even approach those of bacteria such as *Escherichia 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 in microalgal cultures. 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 rate. Many reports do not actually determine this rate correctly, or make measurements over an insufficient period of time (ideally measurement should be averaged over several days) to enable a robust estimation of real growth rate. Only thin (low numeric abundance) cultures of microalgae can actually grow in true exponential phase at a maximum rate. More usually, microalgal culture growth rates are 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 that maximises 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. The various chapters in the work edited by Richmond (2004) provide overviews of various aspects of this subject, as applied to commercial microalgal biomass production.

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 requires the acquisition of N, P, Fe and other factors as well as light and DIC; just the core enzyme, RuBisCO can account for 10-20% or of cell-N. **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.

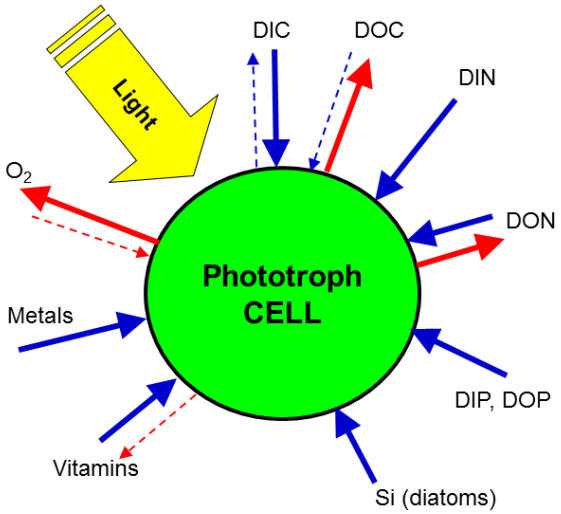


Fig.3.1. Schematic of resource needs (arrows in), and releases (arrows out), from a phototrophic microalgal cell. Dissolved inorganic C (DIC) is consumed with light-enabled photosynthesis. Flows of O_2 are broadly the converse to those of DIC; a net release of DIC occurs when respiration > C-fixation.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

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 metabolites of interest (e.g., Torzillo & Vonshak 2013; Juneja et al. 2013; Lari et al. 2016). Through molecular biology approaches, the normal biochemical regulation can be further manipulated to enhance (over-express) or depress selected facets of growth or of the synthesis of specific metabolites.

The breadth of cell physiologies (Barra et al. 2014), culture system operations and commercial interests presents a myriad of combinations. The selection of which species, or indeed which strain of a species, is a critical issue in mass cultivation (e.g, Griffiths & Harrison 2009; Abdelaziz et al. 2013).

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 DST user.

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 assumed in the DST, this relationship follows the form of:

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

Here, μ_{RT} is the growth rate determined at a reference temperature RT, Q_{10} is a multiplier that defines how much faster is growth when temperature is elevated by 10˚ C, 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˚, 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. A consideration of such matters for simulations is given in Flynn (2018).

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 the growth of competitors or of 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 lower (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^+). A 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 physiology, and the growth of microalgae itself changes the pH of the growth media. As microalgae 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 with growth at high nutrient levels, can occur in 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. Importantly, 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 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 may divide 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 immediate increase in total biomass.

During the course of cellular growth, various resources 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 nucleic acids, 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, as there are typically many millions of cells per mL (10^6 cells mL^{-1}), the system can be considered as operating as a heterogeneous, asynchronous collective. The collective can thus be considered as growing in steady-state, even though individuals cannot. 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, microalgal growth dynamics can usually be considered as following the traditional pattern for microbial growth 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 **Fig.3.2** and **Fig.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 seen 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 **Fig.3.2** and **Fig.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 so as 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 an 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).

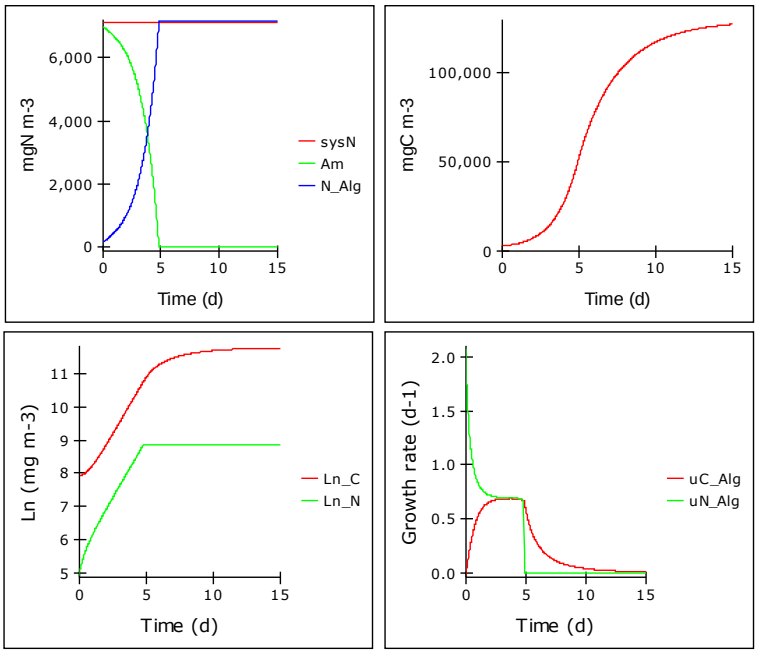


Fig.3.2. Simulated growth pattern in a system with a shallow (0.05m) optical depth, with ammonium as the limiting nutrient supplied at 500µM (7gN m^{-3}). Illumination is constant. The plots show, over the 15 day period: top left - changes in external and cellular N (Am, N_Alg) with a constant system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

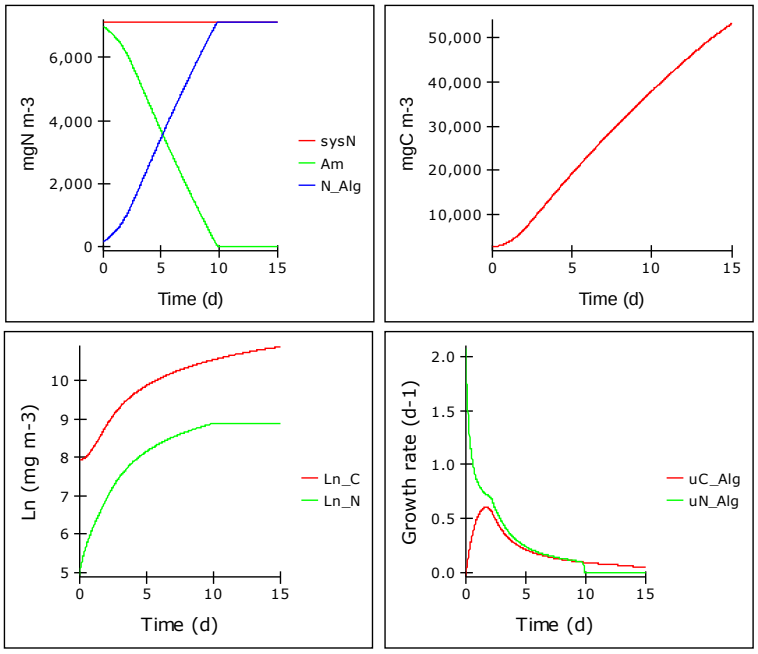


Fig.3.3. As **Fig.3.2**, but for an optically deeper system, now of optical depth 0.5m. Note that growth in terms of C-biomass (top right) is now linear because of the impact of self-shading within the developing culture system.

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 quickly.

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 top-right panels in **Fig.3.2** and **Fig.3.3**. Invariably at the high 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 dynamic.

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 gross 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 the 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.

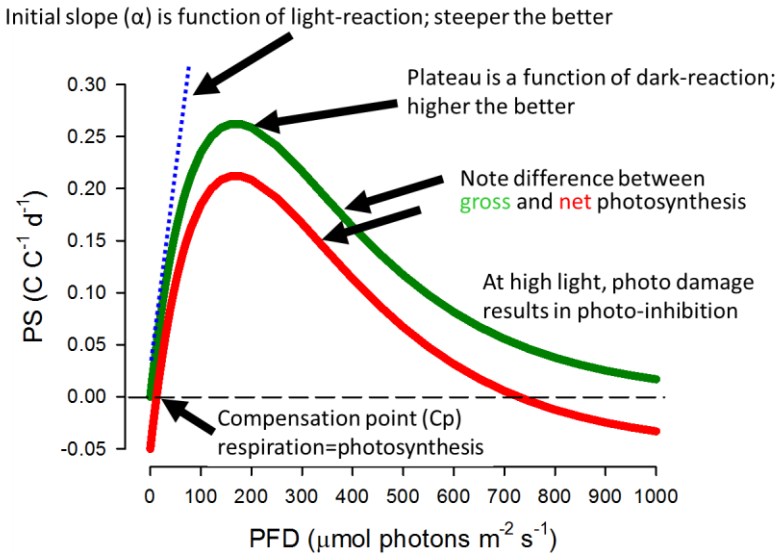


Fig.3.4 Photosynthesis-irradiance curve showing the relationship between gross vs net photosynthesis, limitations by "light" vs "dark" reactions. Here, respiration is shown as constant; in reality this is not so as it will increase with increasing growth rate and/or with increasing damage repair activity.

Note that gross photosynthesis is zero at 0 PFD, while for net photosynthesis the value is negative at 0 PFD (due to respiration); it 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 processes of anabolism increase.

In **Fig.3.4**, photosynthesis is described as a C-specific term. Often in the literature 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. The amount if RuBisCO also changes. Different microalgae show different relative changes in the value of α and the maximum rate of photosynthesis, and thus in the shape of the PE curve (**Fig.3.4**; Richardson et al. 1984). 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 likely 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 within the cell is very expensive, accounting for ca. 20% of total photoreductant.
- Because evolution has led to microalgae being able to transport ammonium at high rates from very low concentrations in nature, 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 microalgae, 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 **Fig.3.5** and **Fig.3.6**.

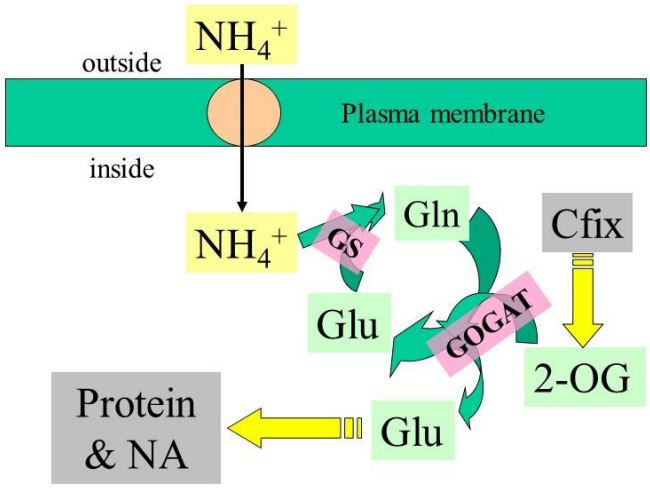


Fig.3.5. Ammonium assimilation. Ammonium (NH_4^+) is transported into the cell where it combines with the amino acid glutamate (Glu) to give the amino acid glutamine (Gln); this is enabled via the enzyme glutamine synthetase (GS). C is supplied, as shown here via C-fixation from photosynthesis, as 2-oxoglutaric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

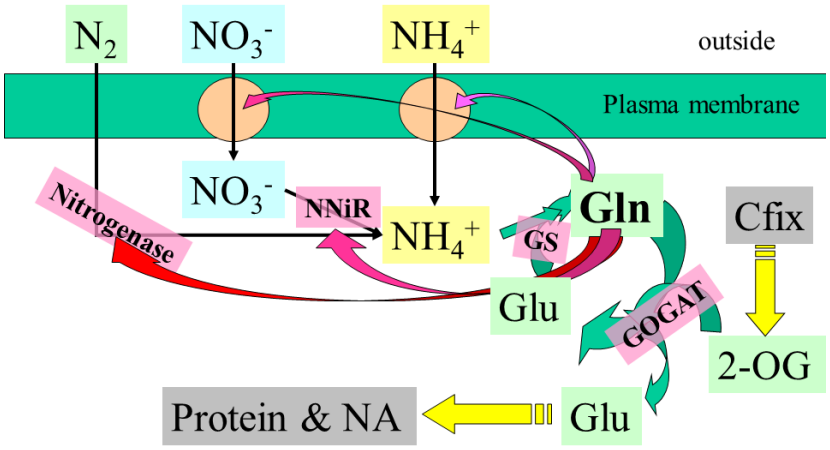


Fig.3.6. (De)-repressive regulation of N-source acquisition. Through the scheme shown, the internal concentration of the first organic product of inorganic-N assimilation, glutamine (Gln), likely allied to the concentration of a C-substrate such as 2-oxoglutaric acid (2-OG) is implicated in the control 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 physically 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).

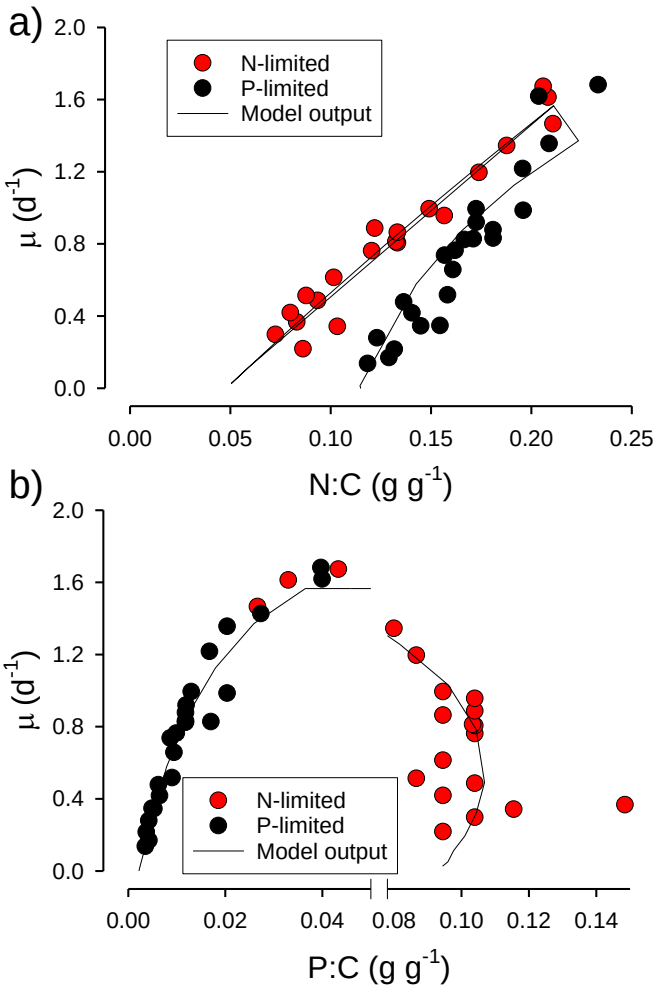


Fig.3.7. Relationships between N:C and P:C with growth rate; the relationship for the nutrient-limiting situations is linear for N and curved for P. Note also how the relationships vary depending on whether N or P is limiting; when P is not limiting (here when N is limiting) P can be accumulated to vast excess, with a high P:C. The thin lines ("Model output") comes from a simulation, showing how well a mechanistic model of algal physiology can align with experimentally derived data. Modified 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 continue to grow in the absence of external nutrient-P, lowering their P:C significantly with little obvious impact on growth rates. This affects the N:P ratio requirements of the growth medium (see **Section 3.7**).

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); 16:1 is the Redfield ratio of N:P, though microalgal C:N:P ratios deviate significantly from Redfield ratios (Geider & LaRoche 2002). Because of the shape of the P-quota curve (**Fig.3.7b**) this likely represents a significant waste of P-nutrient. Cellular ratios of N:P may be increased 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 Si 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 (Flynn & Martin-Jézéquel 2000). This is because 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 readily 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 or 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 through light limitation and increased pH), 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 large-scale microalgal cultures 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 **Fig.3.2** vs **Fig.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 (Śliwińska-Wilczewska et al. 2021). 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. Mixed culture systems thus have the potential to be difficult to control. As an example of the complexity of such interactions, consider the interaction between the microalgal flagellates, *Dunaliella* and *Isochrysis*, growing in the absence or presence of the predatory dinoflagellate *Oxyrrhis*. *Dunaliella* produces compounds that bind 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* becomes unpalatable to *Oxyrrhis* when it is N-starved, so if the timing of the interactions is correct, the outwardly unlikely outcome is that *Isochrysis* can become the dominant organism as the *Oxyrrhis* cannibalises itself (Flynn et al. 1996; Mitra & Flynn 2006). There may be scope for exploiting allelopathic interactions in commercial mixed-species culture systems (Mendes & Vermelho 2013).

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 used for a DST, if only to rule them out as being of little significance for the system being considered. Much research was driven in the early 21st century through interests in manipulating microalgal lipid content for biodiesel production (e.g., Greenwell et al. 2010; Hannon et al. 2010; Verma et al. 2010; Singh & Dhar 2011; Bellou et al. 2014). Although the promise of microalgal biofuels has subsequently been brought into question (Kenny & Flynn 2017), undoubtedly the biofuels research initiatives have brought the attention of these organisms and their cultivation to the public, students, engineers, and even politicians in a way not possible through any other route (except perhaps via Harmful Algal Bloom events). And the only way to really consider whether mass cultivation will deliver, is through simulations.

4.2 Reactor design – overview

The reader is referred to Richmond (2004) and Tredici (2004) for in depth considerations of culture systems for commercial deployment.

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). 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. Some microalgae can be grown on solid substrates such as a biofilm, or on balls of an inert solid floating in the reactor. Indeed, a problem in many systems is to prevent the growth of microalgae on the walls of the bioreactor where they then prevent full light penetration, form a mass that is not readily harvestable, and can slough off causing blockages or otherwise foul the system.

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 even a moving

brush (**Fig.4.1**) may be introduced to pass along tubes of a bioreactor to slough off any biofilm growth.

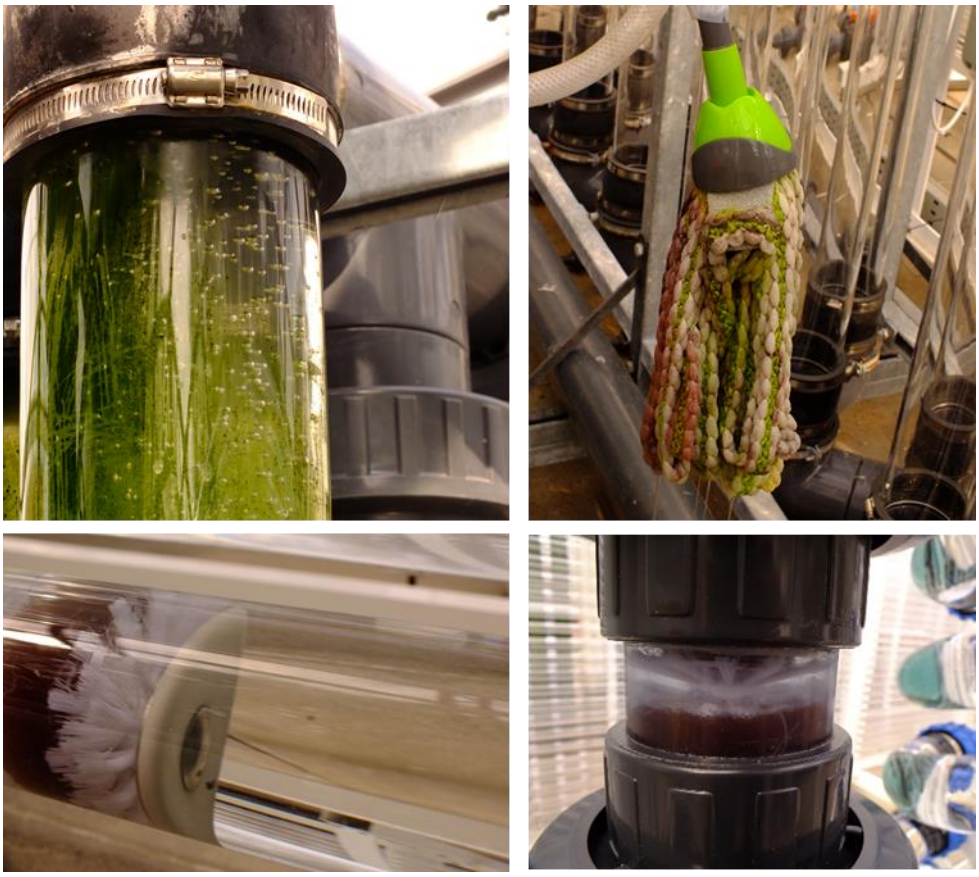


Fig.4.1. Growth of microalgae on bioreactor walls can rapidly become highly significant (top-left), requiring the manual use of some form of brush or mop (top-right). A more high-tech option, that does not require the dismantling of the reactor, and indeed can be conducted during active culture growth,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

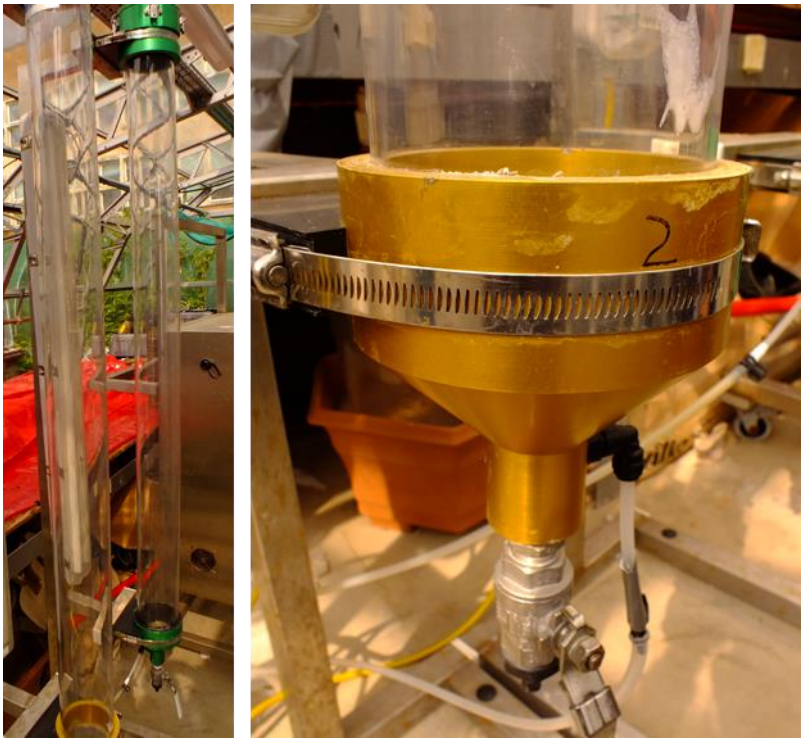


Fig.4.2. Simple bioreactors for low volume (20-50L) batch cultures. These comprise lengths of acrylic tube with custom made end caps; detail of the lower cap (with aeration input) is shown on the right.

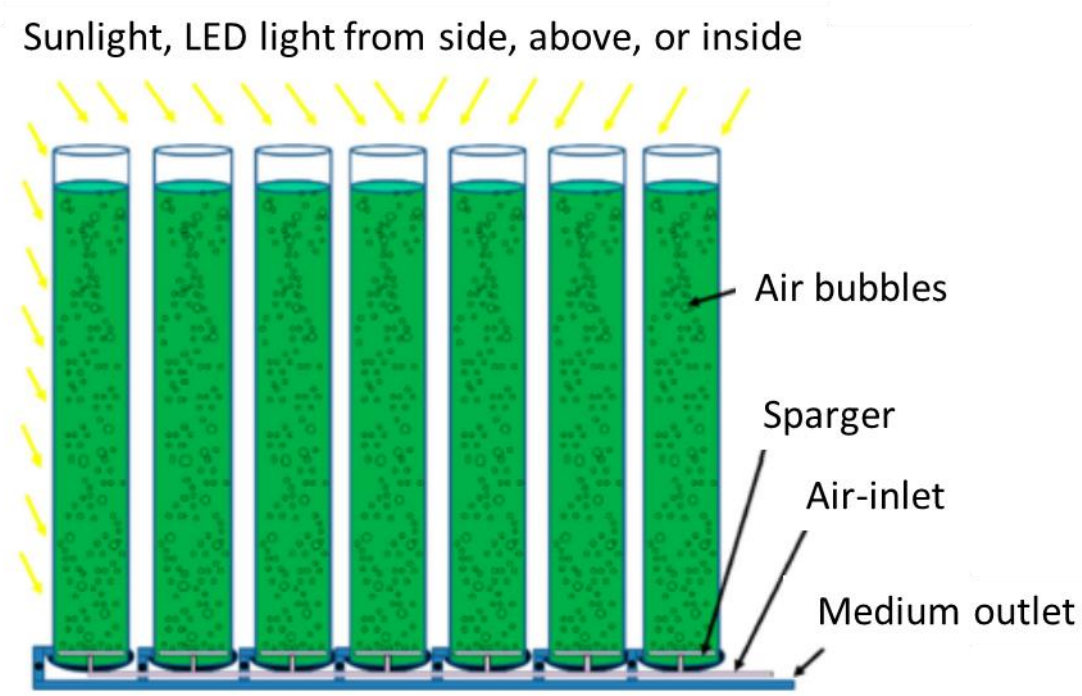


Fig.4.3. Schematic of a modular PBR. Such a configuration can be scaled indefinitely but is not readily configured as a continuous-culture system but rather for batch growth. From Saha & Murray (2018).

At the other extreme, open reactors (as ponds; **Fig.4.4**) can be truly massive and perhaps better suited to vast infrastructures with continuous harvesting. Ponds may at first sight offer a cheap, low technology solution, but they must be manipulated with care to achieve good results (Sutherland et al. 2015; Sreekumar et al. 2016) 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 (**Fig.3.2** vs **Fig.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.

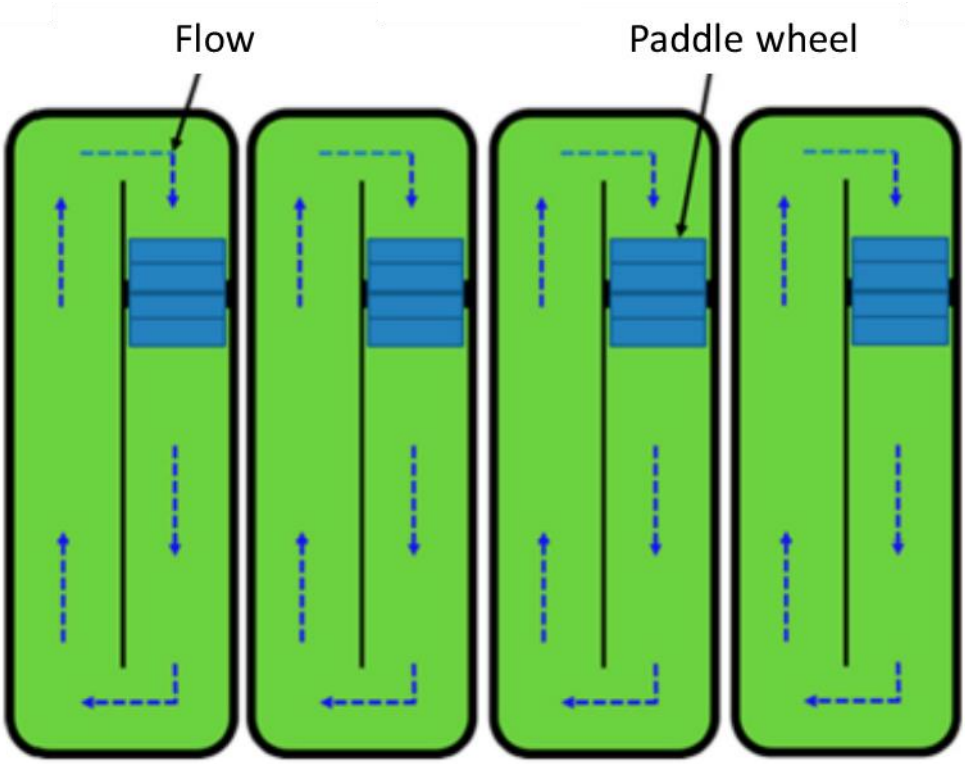


Fig.4.4. Birds-eye schematic of a simple pond, "race-way", culture system comprising outdoor rectangular ponds and paddle wheels to maintain a flow of mixed suspension. In addition to gas exchange at the surface, additional gas (CO_2-augmented air) may be injected into the system. From Saha & Murray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

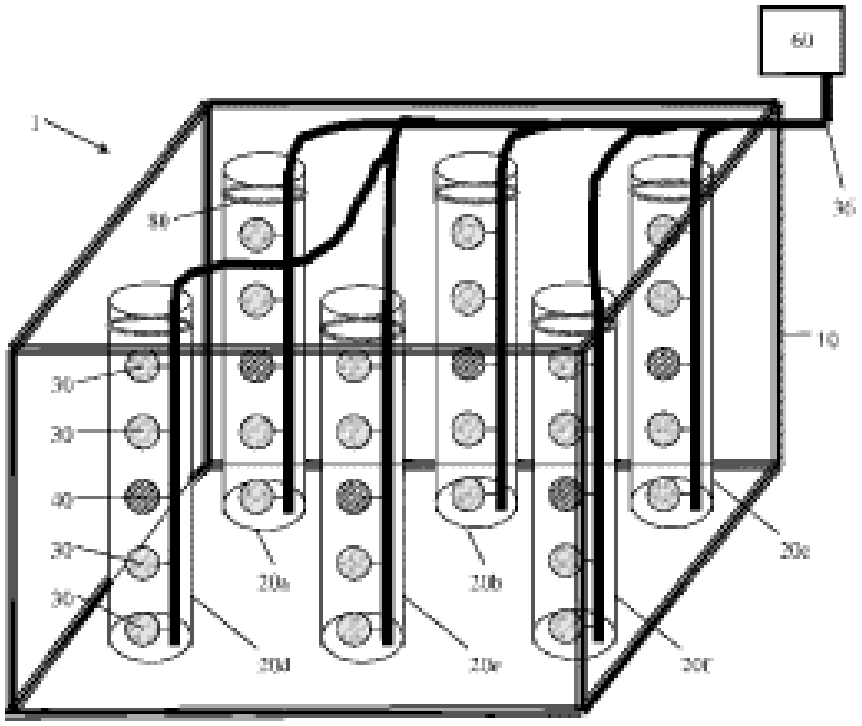


Fig. 1

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 and nutrients

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 greater 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 (for such a model, see Flynn 2018). 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 are 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 may be provided in a tight wavelength band (or several bands) rather than across a wide spectrum (Schulze et al. 2014).

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 this 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}).

Nutrients define the ultimate biomass that can be attained in a culture system. While some nutrients (notable nitrate, bicarbonate and vitamins) can be added to very high concentrations (such that wastage of materials is inevitable), others cannot be added to high concentration either because they are toxic (notably ammonium) or because the precipitate out of solution (phosphate, silicate, iron). Precipitation is a particular problem in marine media because of the presence of other salts. In practice, then, it is the concentration of these toxic or precipitating nutrients that limits the nutrient loading to the system unless they are added carefully during culture growth so that the residual concentrations (or for ammonium, the pH) are controlled.

Chapter 3 discusses individual nutrient types in this context. It is worth noting that the commonly used f/2 growth medium (Guillard 1975) and similar, contains an excess of N over P and Si, and may also lead to exhaustion of DIC (overcome with injection of CO2) and more than likely result in light-limitation through self-shading.

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 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 DIC 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 microalgal 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 produced by photosynthesis 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 the dominant form 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. Entry of NH_3 into cells is also uncontrolled, as ammonia is directly soluble in the plasma membrane.

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 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; this is the assumption made in the DSTs described here.

4.7 Harvesting: when and how much

Harvesting could at the extremes be of the entire reactor contents on one occasion, or of a small volume continuously taken off as part of a chemostat-style operational regime. The former operation is a batch culture system; the later is a continuous culture system.

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, part-spent nutrients and any organics released by the microalgae into the water). 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, is set, 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 ideally requires growth in continuous light. It is also not possible to achieve growth rates exceeding ca. 75% of maximum without an increasing risk of the culture being washed out.

Another culture approach is to use a turbidostat, in which a light sensor is used to monitor the optical state of the culture. If the culture density exceeds a certain set value, then fresh media is pumped in (and excess media plus cells washed out) to dilute the optical density. The turbidostat can be used to grow cultures at rates much closer to the maximum growth rate than may a chemostat. At steady-state, a turbidostat gives a continuous dilution of the culture system, just like a chemostat.

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 physiological 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. Operating a continuous culture system places a different logistic challenge to that of providing large volumes periodically. 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 suspension. 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 to promote flocculation (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 sudden 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 steps 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 and to clean grey water (Sutherland et al. 2015). The wastewater 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). However, the accumulation of allelochemicals may hinder growth of microalgae in recycled water streams. Some argue for an exploitation of such allelopaths in improving production (Mendes & Vermelho 2013).

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 specific to the operation (geographic) site, reactor design and operations, and organism traits. 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 (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 provides 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 simulation 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), which operates within 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.

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

For the reader who does not have the time, or indeed the patience, to develop their own models, much can be explored and learnt by playing with the models provided free to the end user. Just reading the chapters in Part II, will likely also impart useful information.

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 entering alternative values. 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 values 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 or grams 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 an organism 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 of that harvesting (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 value 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). As an approximation, 1L of cell volume equates to ca. 200gC. 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 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. How individual species respond to such conditions depends on how high their RuBisCO activity is compared to the maximum growth rate. The more complex models used here allow for the changing of this relationship.

It is assumed that the culture system is operating at a 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 using 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 may 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) but the more the better.

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 but the data need to represent the dynamics over the duration of the culture growth; ideally there should be a sample taken every day at the start and/or end of the light phase of growth. The units of measurement need to be transformed as required to be consistent with those used by the model; units 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.

Because of the variability in cost options, and their dependence on system configuration, the models provided do not explicitly enable a costing to be made. The models could be readily further developed to provide such insights. However, as a first step it is clear that production output must be maximised while minimising the use of resources (water, nutrients) and minimising the production of wastes that could cause pollution. The models do provide such information, enabling the user to explore at least the initial optimisation of options.

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 quite 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 of microalgae such that its growth does not conform to that of typical microalgae.

For microalgae there are various potential commercially valuable 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 the component of interest; this requires growth, at least for most of the culture period, that is not limited by light nor nutrients).
- Protein production (this is enhanced by growth without exhausting N-nutrient). This will also likely align with production of N-rich metabolites released into the growth medium.
- Lipid, starch or other high-C production (this is enhanced by growth typically exhausting the N-nutrient). This will also likely align with production of C-rich metabolites released into the growth medium.
- Pigment production (usually enhanced by growth 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 decreasing as production increases and as the market reacts to availability); all these and other factors constitute risks. Again, the software may be able to help with this. For each of your input parameters you can assign a range of values (minimum, maximum, average). The simulation is then automatically run many times and the software will output your target interests with the average (typical) result together with confidence limits.

6.4 Conclusions

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

Disclaimer

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

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

Instructions for using the DST are given in Chapter 7

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 most complex model is also described in the **Appendix**.

The models for this DST are that enable you 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).
- 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 (such as GNU Octave – see Akoglu & Flynn 2020).

The first models, described in this and the next chapters, can also be run using Powersim Studio 10 Express (see **Section 7.2**). This is a free download, giving access to the full software; however Studio 10 Express places 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), and also Akoglu & Flynn (2020), 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/ , R and GNU Octave) 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 a suitable 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”. See **Section 7.4**.

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.

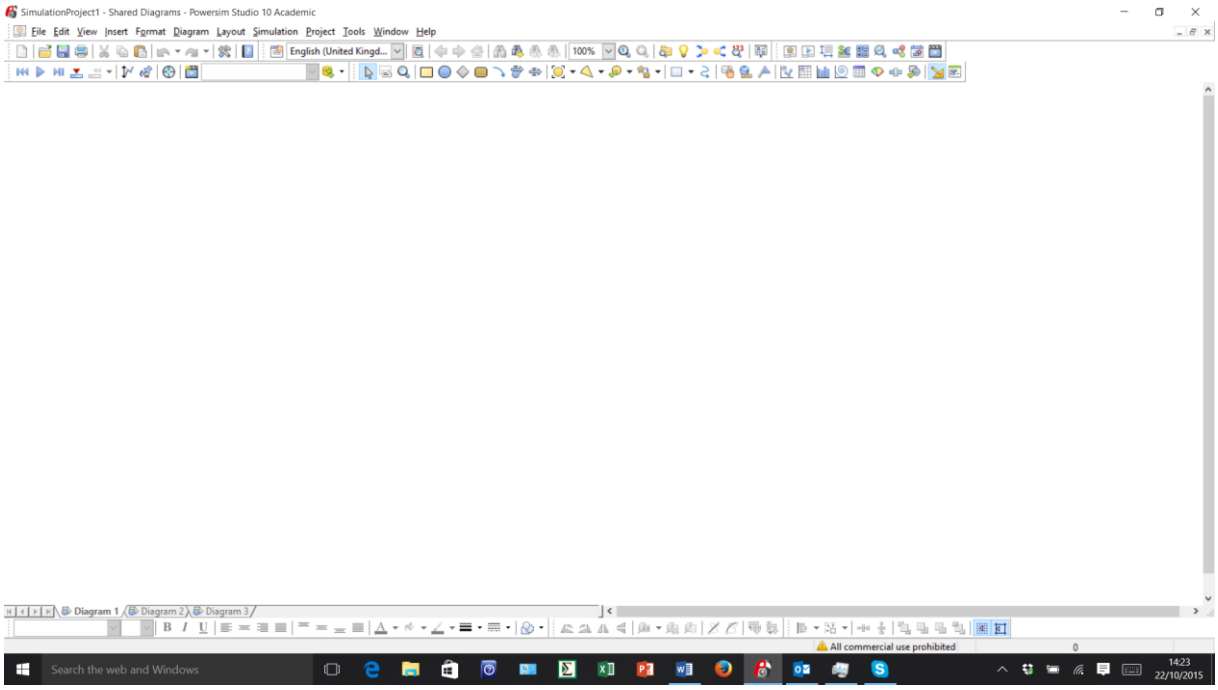


Fig.7.1. Start-up screen.

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

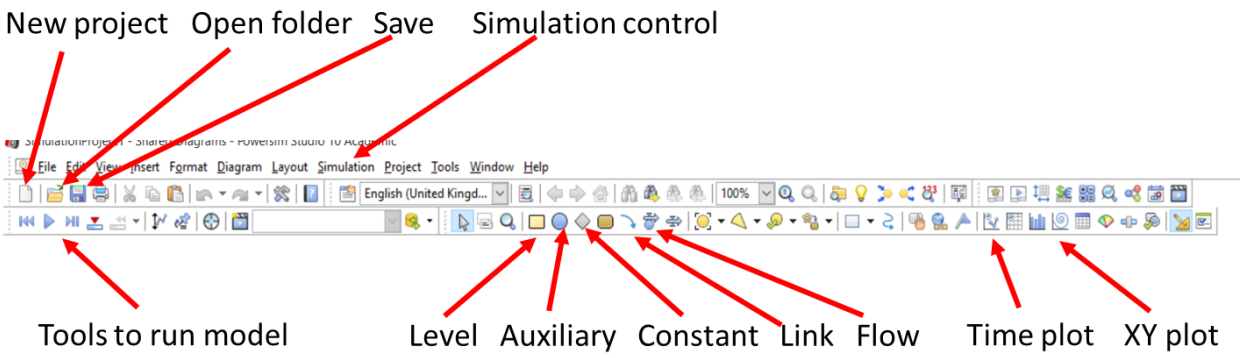


Fig.7.2. Buttons and dialogues.

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.

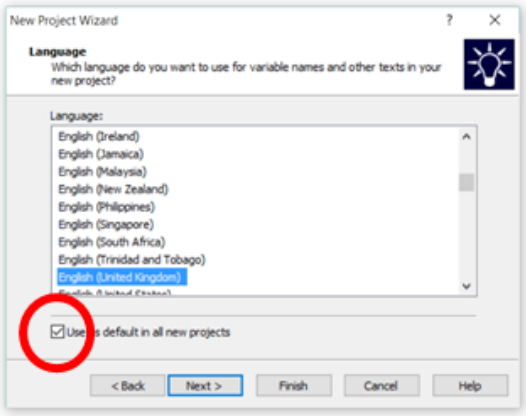
"**Constants**" describe variables that are (at least for the simulation) held fixed, as constant. In Forrester diagrams, constants are shown as diamonds.

"**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).

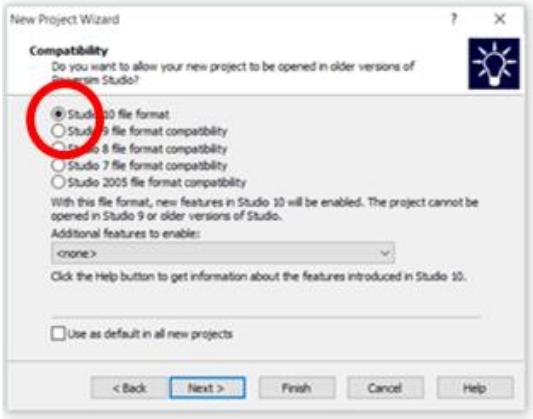
"**Flows**" described additions or subtractions to levels. Flows are described by constants or (more usually) auxiliaries. In Forrester diagrams, the actual flow is shown as pipelines with arrows indicating the flow direction.

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.

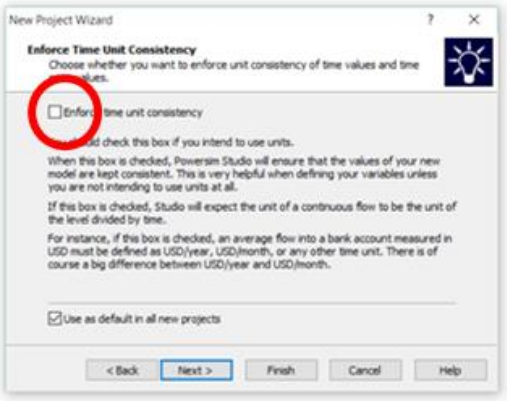
Always enable the wizard – do <u>not</u> check the "Don't show the wizard next time" box.



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



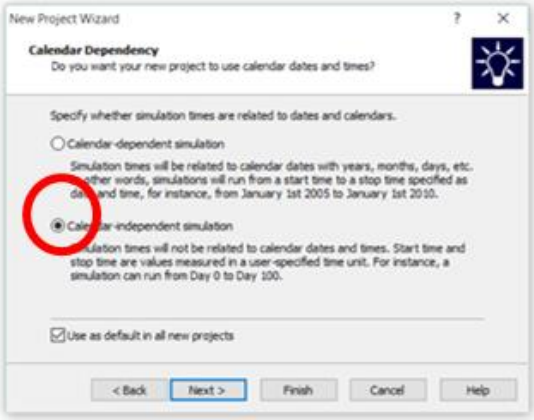
Check the file format. Note that once you pass this step you cannot change the format.



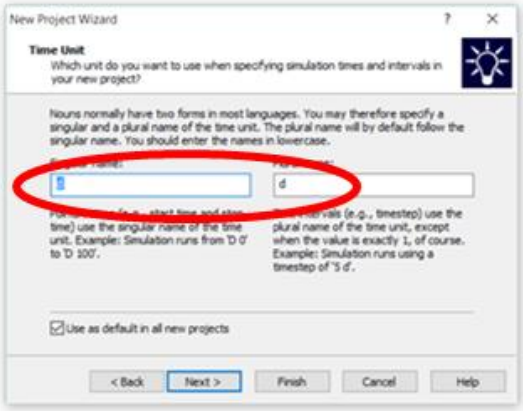
I would suggest that you do not check for unit consistency. In theory this can be really useful, but in practice it can be annoying. However, without this facility be aware that it is up to you to check that units make sense.

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 add information held within parameters with different units then you 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; the answer is in units of gN.

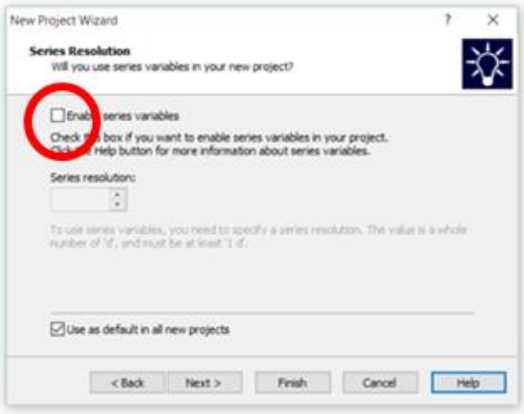
Be careful and double check equations; it is very easy to make a mistake, but even without unit checking, Studio will alert you to spelling and various constructional errors.



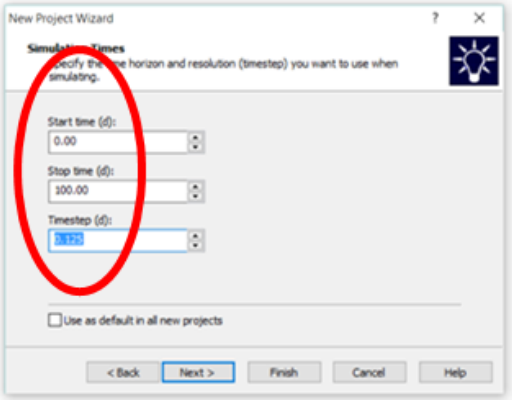
Select calendar **in**dependent simulations. Do <u>not</u> select for calendar dependant simulations, unless you really want time date-stamped with days and months of the year.



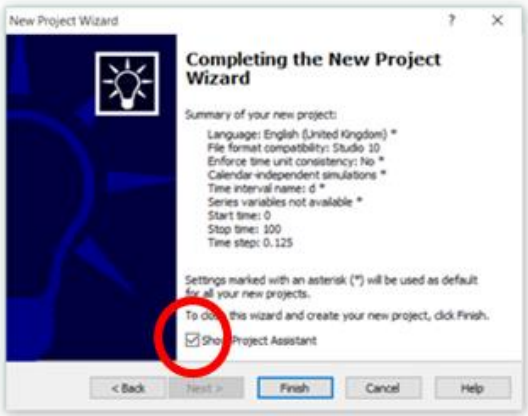
For most biological applications the time unit is most convenient as days. The SI unit for time is seconds, but using this will generate numbers that will not typically be very helpful.



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; this means that integration calculations to make the model work will occur 16 times (i.e., 1/0.0625) each simulated day. **You can alter these values easily later.** See Flynn (2018) for information about selecting integration methods and step sizes.

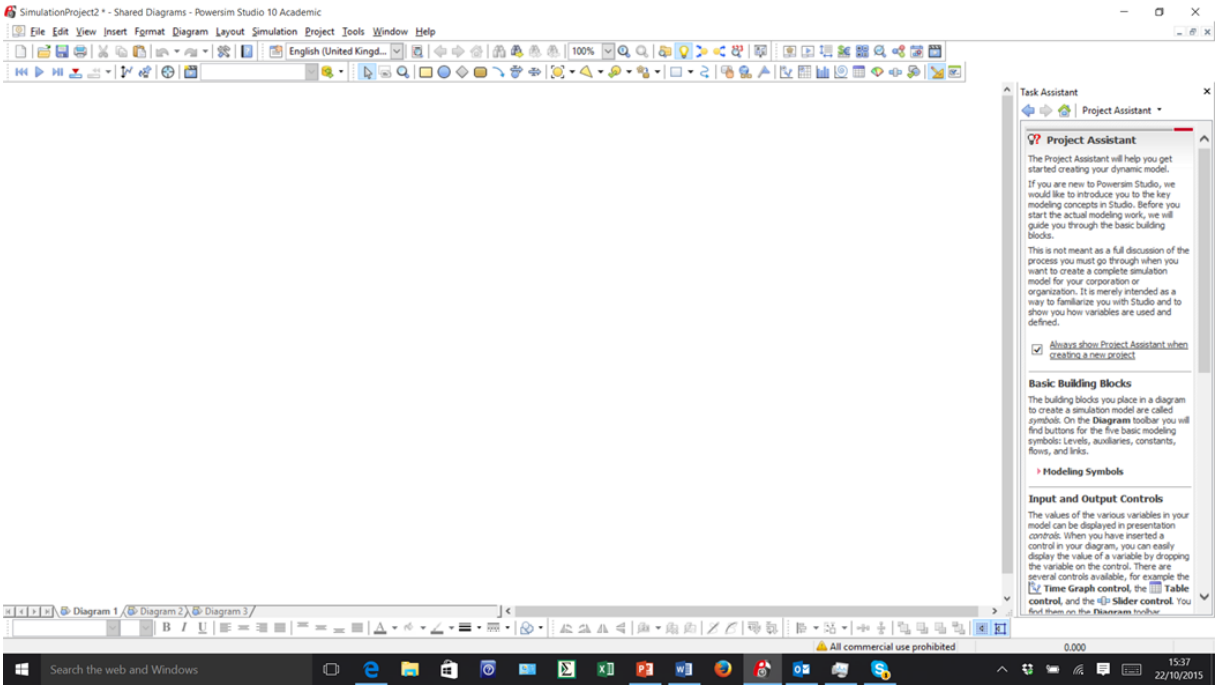


Select whether you want the project assistant to be present. It is easy enough to turn it on or off.

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:

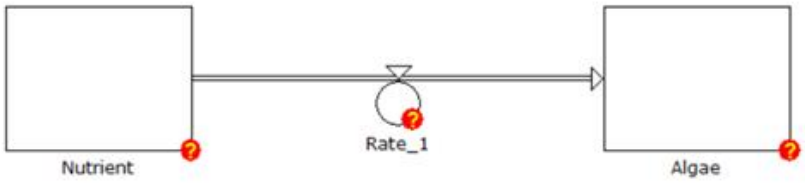


Unless you want the "Project Assistant", shown on the right here, click it out of the way (X in top-right-hand corner of the Project Assistant dialogue).

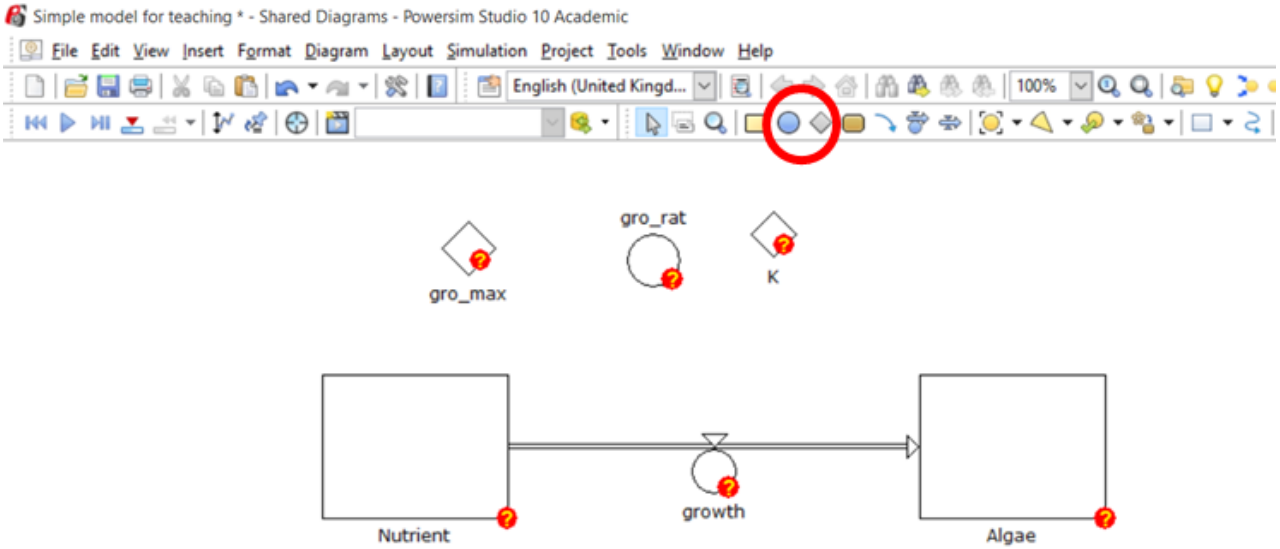
In each of the screen shots below, the menu item to use is identified with a **red circle**.

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*.

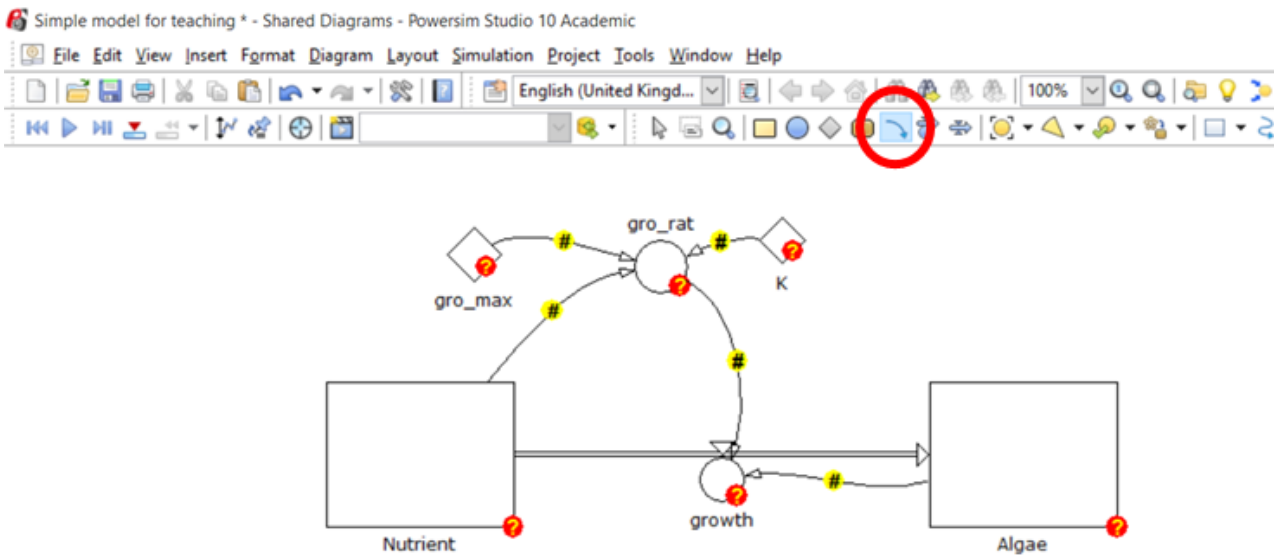


Now select the "auxiliary" tool and place an auxiliary above the flow; label this as *gro_rat*. Move (drag) its title to the top of the circle. Add two "constants" close to *gro_rate* labelled *K* and *gro_max*.



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!



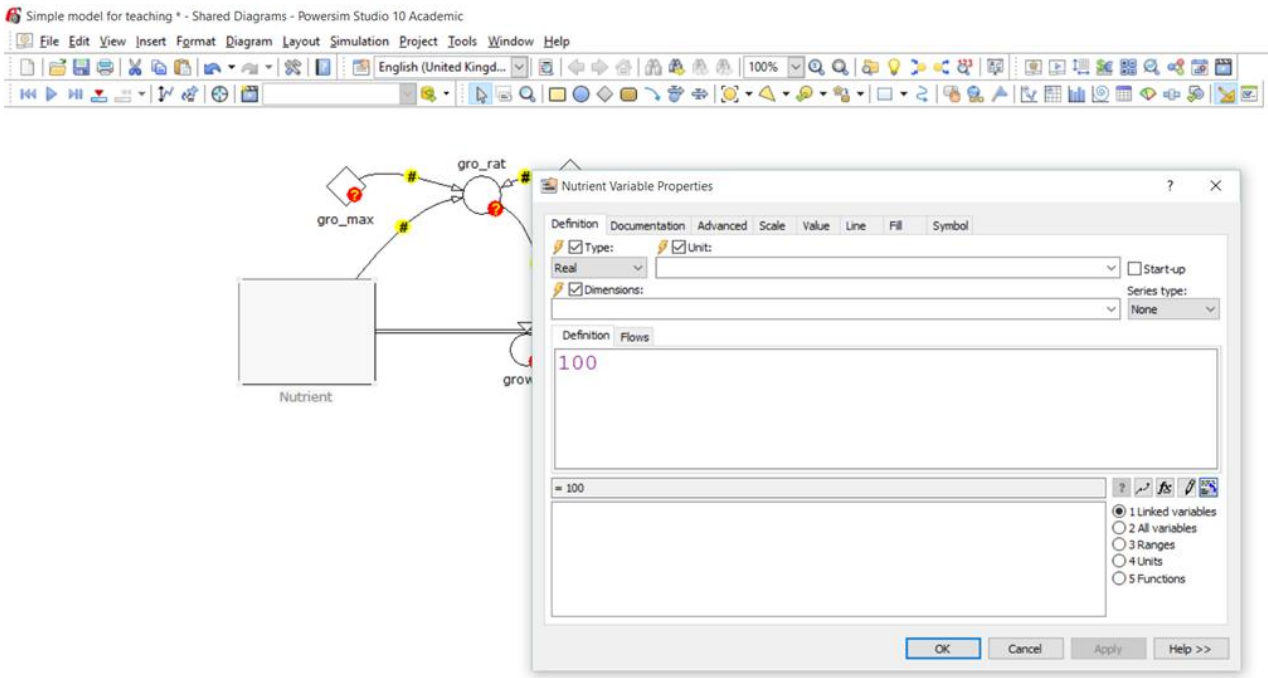
You have now completed the conceptual model. What this is saying is that there is a flow of material from *Nutrient* to *Algae* that is a function of the concentration of nutrient, a maximum possible growth rate (*gro_max*) and a constant *K*, and that the growth of the population (*growth*) is a function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

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!

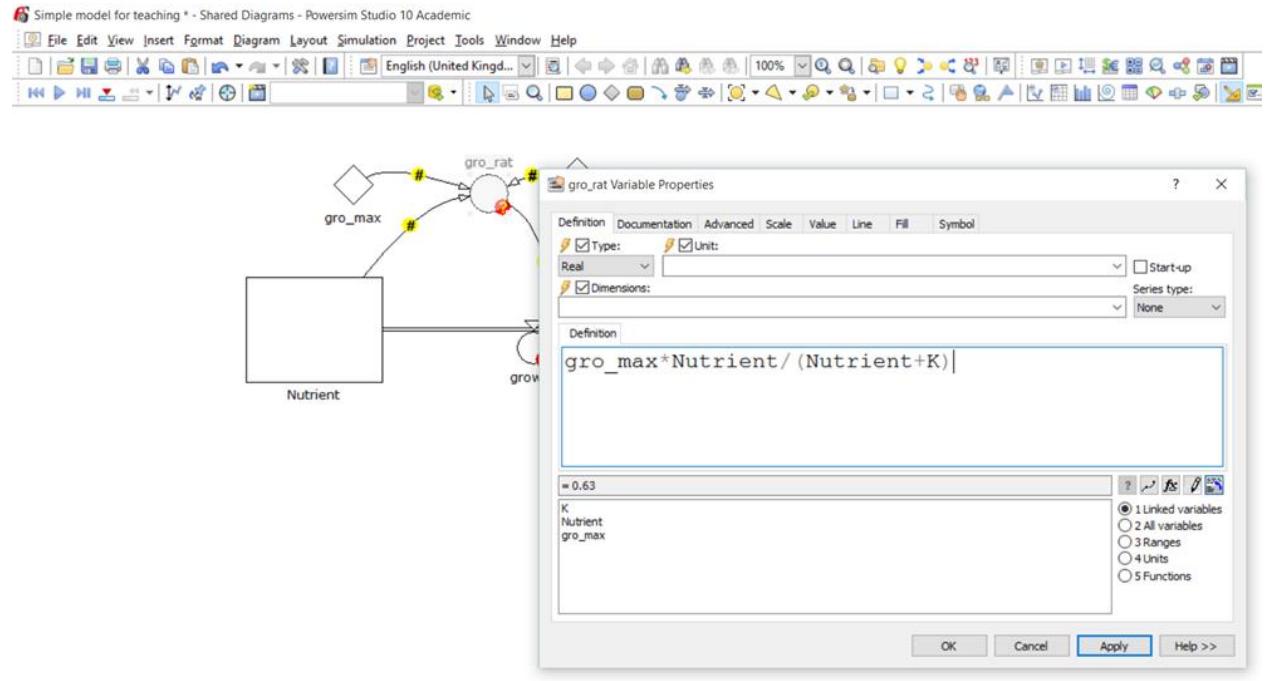
Into the "Documentation" tab enter some text explaining what this parameter is; it is good practice to include the units of g N m-3 (this should actually be g N m^{-3} but the dialogue will not by default accept superscripts). Do NOT use the "unit" part of the dialogue as in the wizard you have said 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 time unit (which we have set as a day), and 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.

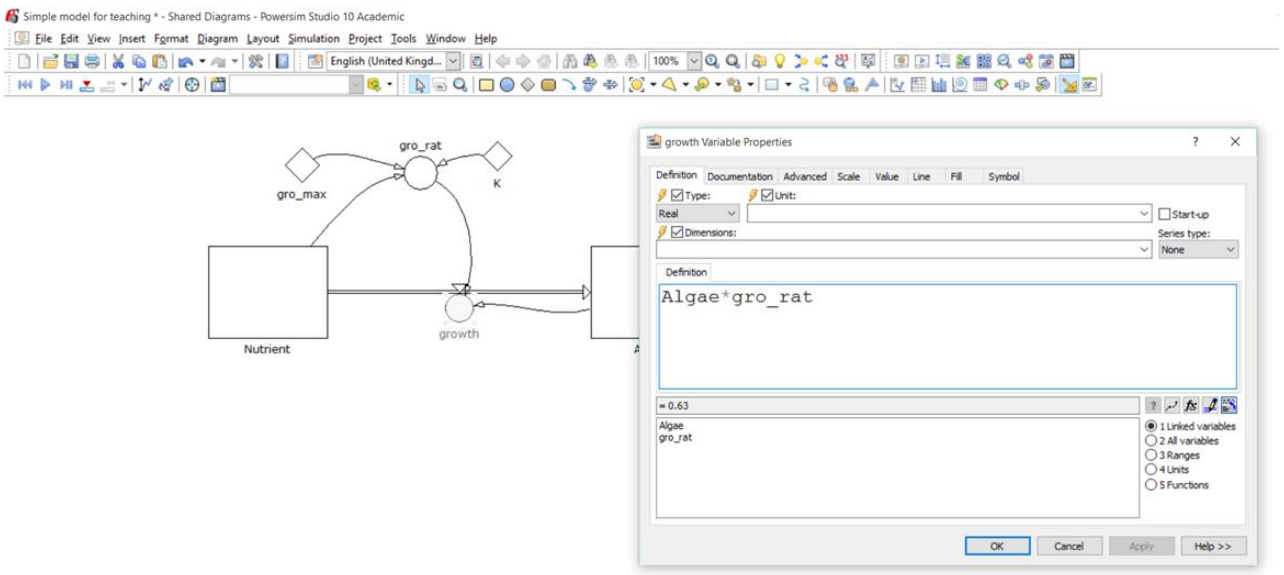
If you later wish to change a parameter name you can easily do so by just editing the name on the symbol in the Forrester diagram; changes to the parameter name in all equations will be made automatically throughout the model.

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 must have units of g N m^{-3} d^{-1}.



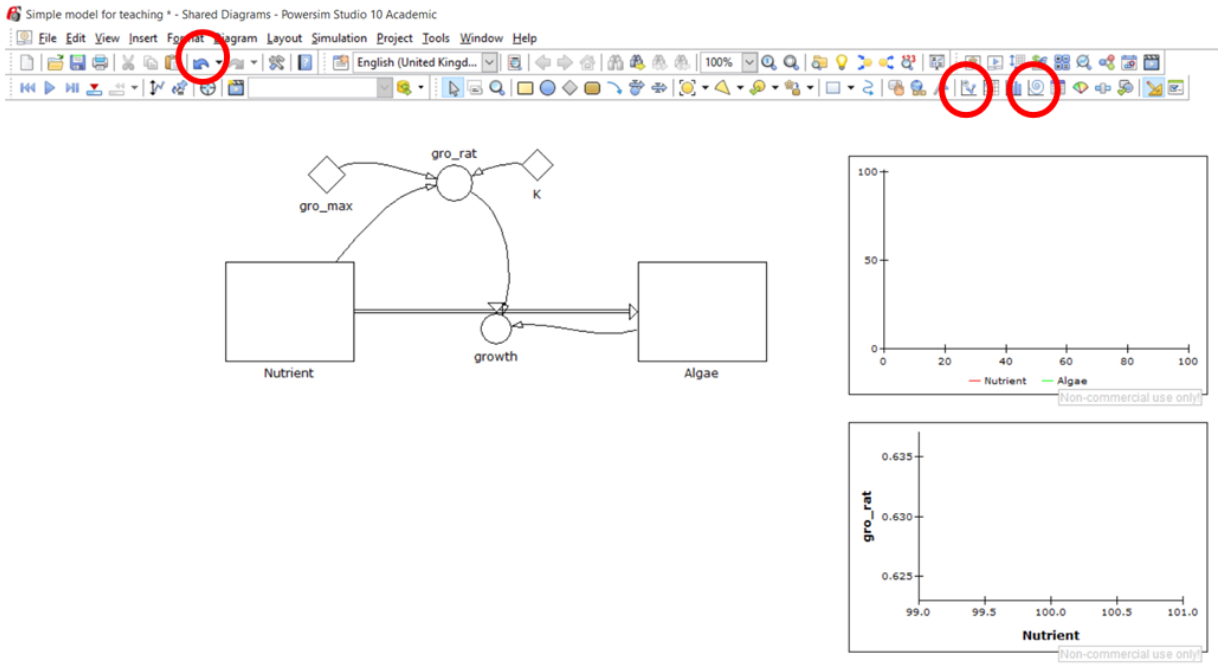
The model is done; remember to save!

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 the graphs 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

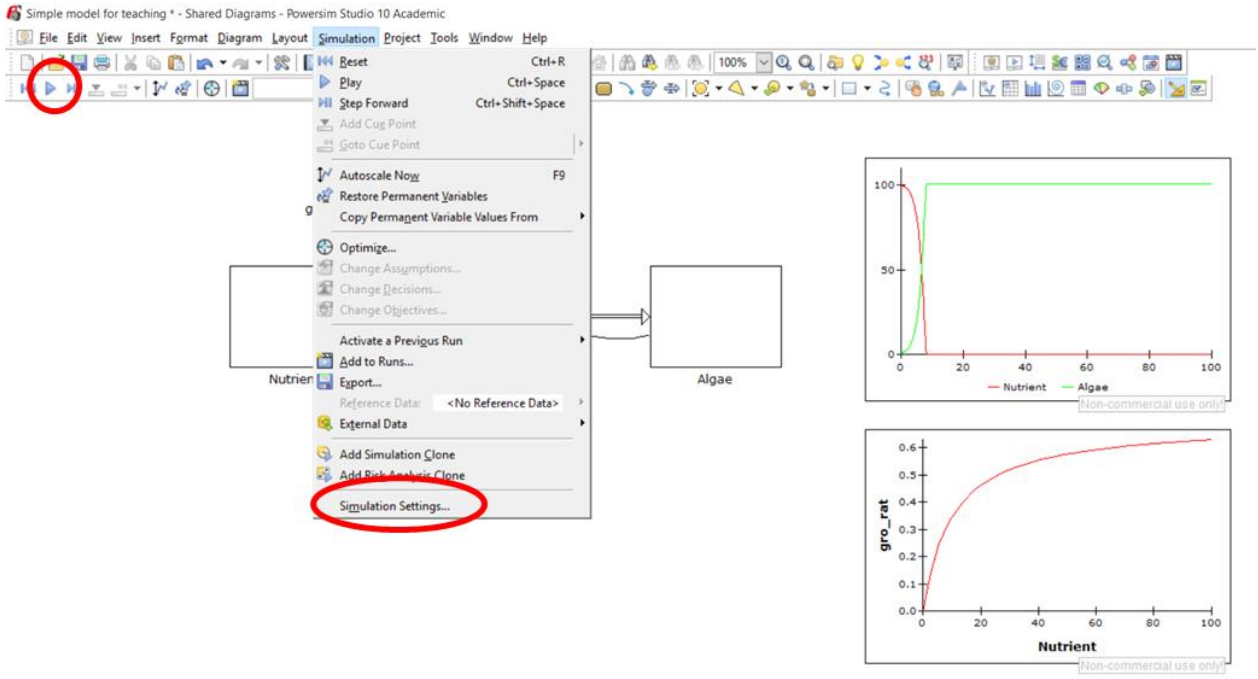
graph box and the cursor symbol has changed. If you do not do it correctly, it is likely that the diagram will be radically changed; if that happens, use “undo” (top line of buttons), and try again.

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”.

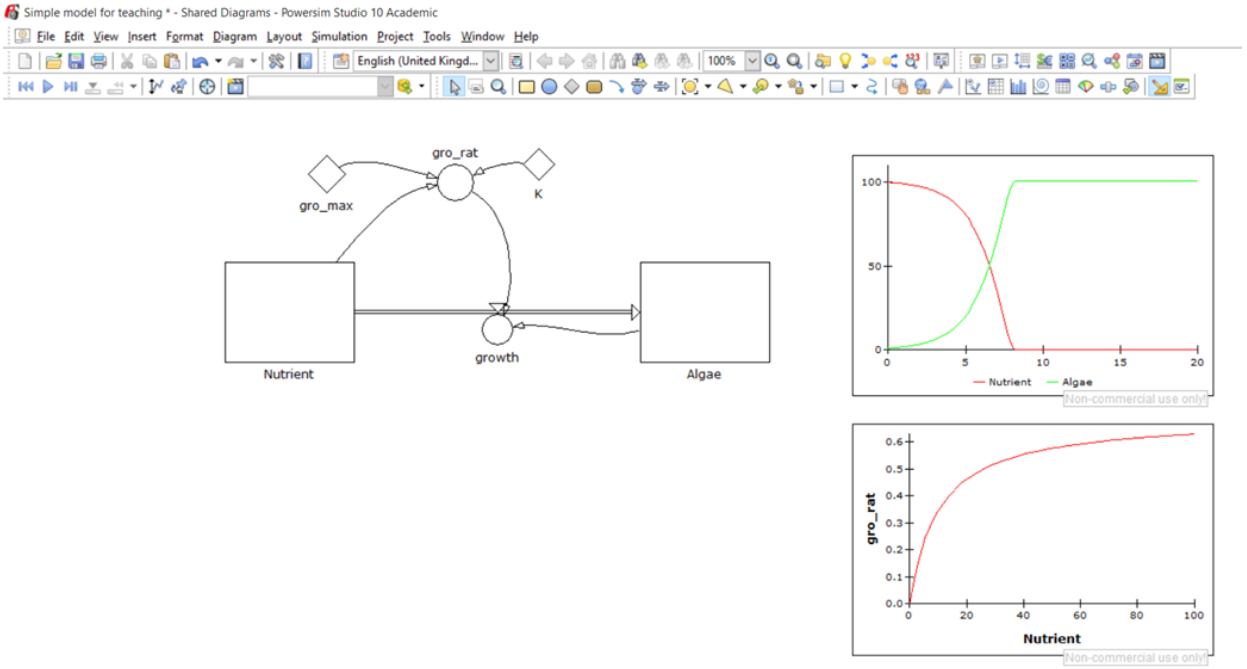


And now you are ready to run the model. Before pressing run for the first time, save the project again.

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:



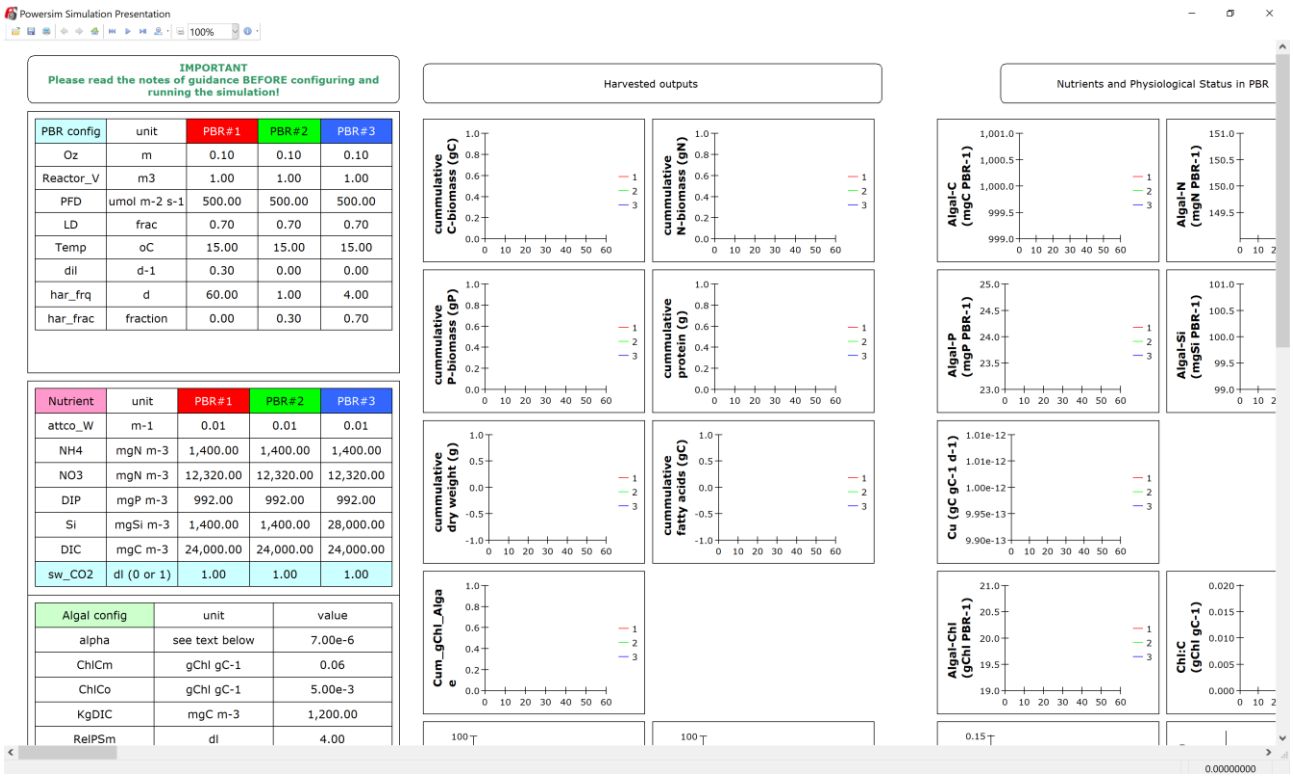
You can see that the model is simulating the decrease in nutrient concentration as it is transferred to the algae. The x-y plot shows that as the nutrient level declines, so does the growth rate of the algae; note that the plateau value (which is off scale here) will be 0.693 d^{-1}, equal to the value 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 will understand why parameter *K* is termed a “half saturation constant”.

Note that the levels must have the same units, else you cannot correctly 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 $cell^{-1}$. Recall that there are 10^9 ng in 1g. At the peak of the simulated culture growth in this simulation 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. To obtain a plot of changing cell numbers over time 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 . Why not try it?

7.4 Studio 10 Cockpit

Unless you have the full version of the Powersim Studio 10 software, you will need to operate the models using the free software, Cockpit. See **Section 7.1** for download information.

The Cockpit interface is very simple. Below is a screen shot of the Cockpit model for **Chapter 10**. The model is controlled by entering values into the parameter tables; these are described in detail for each model. You will need to use the window sliders and alter the zoom magnification as you desire to see all the tables and graphs.

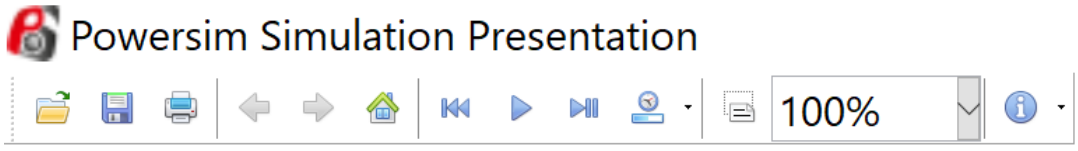


PBR config	unit	PBR#1	PBR#2	PBR#3
Oz	m	0.10	0.10	0.10
Reactor_V	m3	1.00	1.00	1.00
PFD	umol m-2 s-1	500.00	500.00	500.00
LD	frac	0.70	0.70	0.70
Temp	oC	15.00	15.00	15.00
dil	d-1	0.30	0.00	0.00
har_frq	d	60.00	1.00	4.00
har_frac	fraction	0.00	0.30	0.70

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

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

The simulation is controlled using the interface in the top left of the screen, shown enlarged below. The only selections you need are the reset, run and advance-one-step buttons in the middle, the zoom-in %, and just to left of that is a facility for copying parts of the screen to the clipboard (so you can copy graphs of your results out to another programme). The copy function is akin to the snapshot function in Adobe Acrobat Reader.



Cockpit will allow other functionalities, but they are not enabled here.

Pressing Ctrl+space at any time will pause and run the simulation and will also autoscale the graphs.

7.5 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. 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.

If you want to explore other facets of building these types of models, see Flynn (2018).

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 and/or N-nutrient. Why light and/or N-nutrient? Because, as you will find out when you are operating the model, 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 and not worry about the space requirements. Thus, in the oceans, the biomass abundance is very low, but of course the planetary scale of production means that the total biomass across Earth is massive. For commercial reasons, of course, abundance is important; with a low crop 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 in that free download.

Studio Express is available from here:

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

For information as to how to access the model, please refer to page ii of this work.

Of course, you could build the model in another platform, or develop and hence operate the Powersim model in the full Studio platform. For example, see Akoglu & Flynn (2020) for models in GNU Octave which can also run in Linux.

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); note that if you want to change a parameter name, you can only do so with the original version and not a copy.

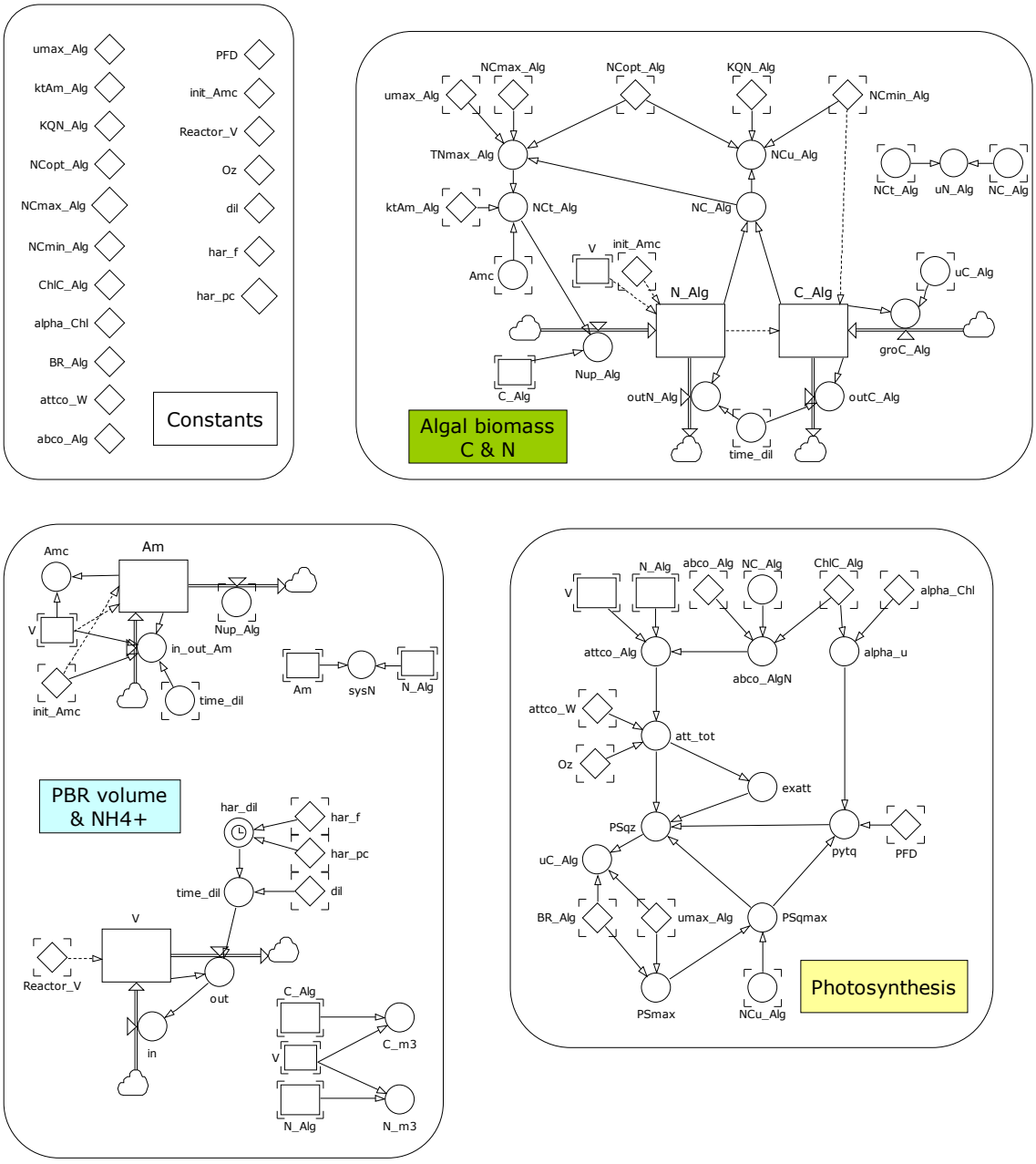


Fig.8.1 Forrester diagram schematic for the model. This shows the modules describing the algal biomass, control of photosynthesis, and the bioreactor and nutrients. See also **Tables 8.1 – 8.3**.

The "**PBR volume and NH4+**" module (**Fig.8.1**) describes changes in the volume of the culture in 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 culture 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 while 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 (see **Chapter 3** and **Section 4.4**).

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 that are defined as constant.

The PBR module (**Fig.8.1**) also describes the harvesting of the crop. In contrast to traditional (terrestrial) agriculture, which is usually harvested on one occasion for a given batch of plants, 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.

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 culture volume

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

The "**Algal biomass C & N**" module (**Fig.8.1**) 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 (**Table 8.2**). 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 (**Fig.8.1**) 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-absorbing materials in 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).

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 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). As we are describing N:C, 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 the nitrogenous 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**, **Sections 3.3 – 3.5**.

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}. The model described here 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 (**Fig.8.1**; **Table 8.2**).

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 culture 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 culture 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 culture volume is harvested. The reactor is considered to be immediately refilled, so the 5% of the culture remaining after harvesting would then act as a seed inoculum for the next crop.

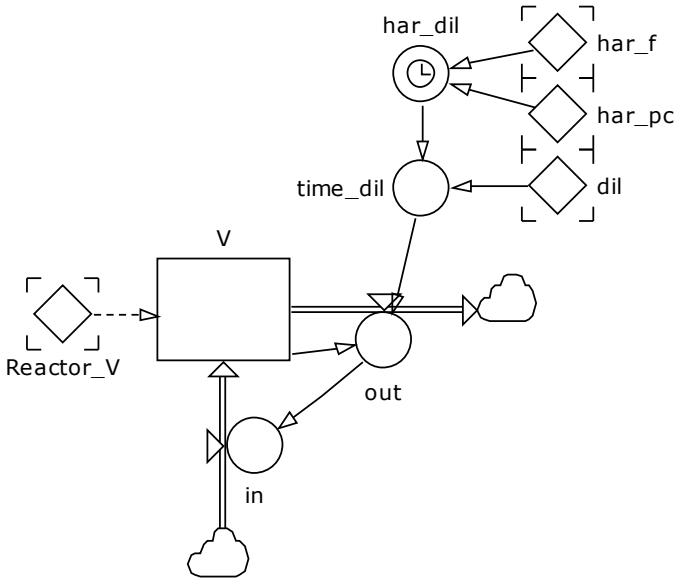


Fig.8.2. PBR volume and harvesting.

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 culture volume *V*. Here the concentration of the ammonium is entered as gN m^{-3} (*init_Amc*) and the model calculates, with reference to reactor culture 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 and 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.

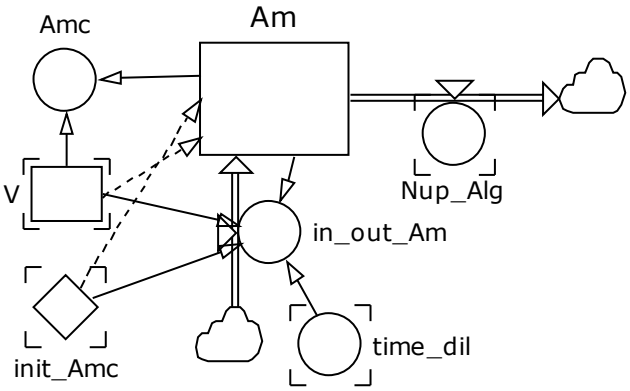


Fig.8.3. Reactor ammonium (N-source) content.

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 3.5**). 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. This operates via a NC-quota function; see **Fig.3.7a** and adjoining text for how growth rate is related to growth rate (also Flynn 2008a, 2008b). 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 (see **Fig.3.7a**).

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 the 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 3.5**). Actual ammonium transport then relates to the current value of the potential transport, *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.

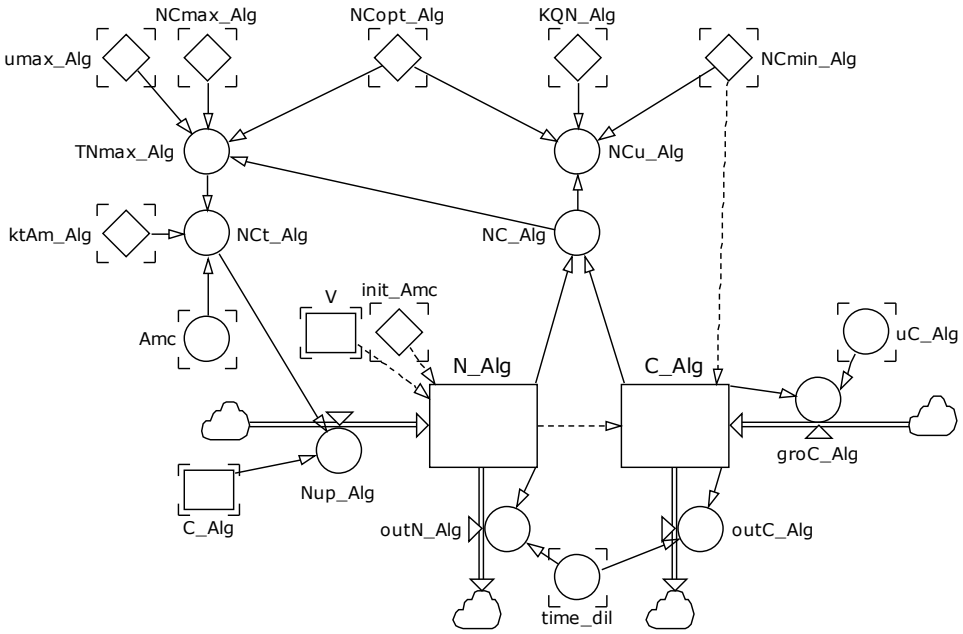


Fig.8.4. Microalgal biomass.

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 current value of N:C for the algae (*NC_Alg*) and the absorbance of the algae with reference to Chl:N. Together with the reactor culture volume (*V*) and the N-biomass (*N_Alg*) we obtain the light attenuation due to the algae (*attco_Alg*). Together with the absorbance due to coloured material in 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**.

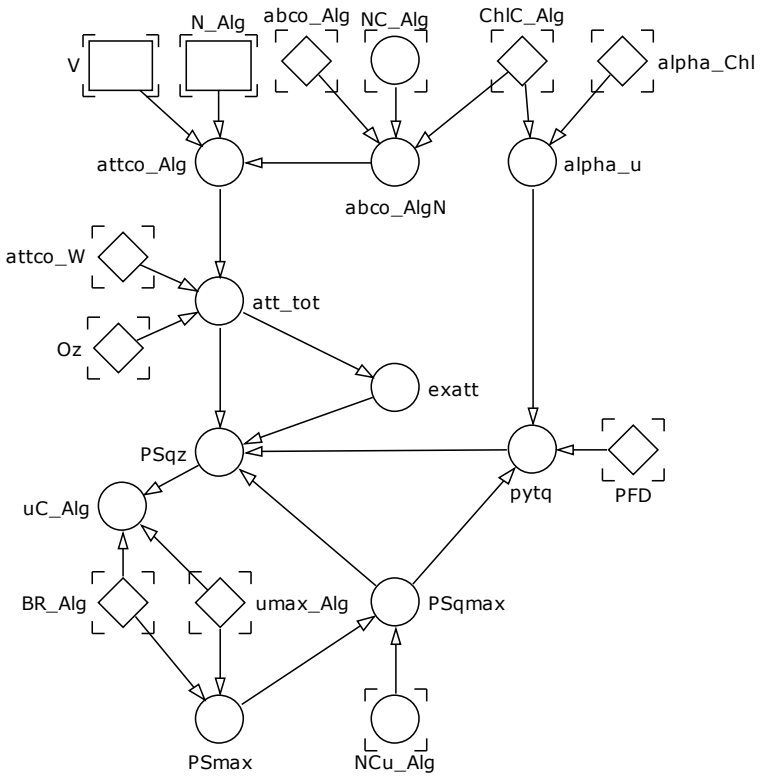


Fig.8.5. Light and photosynthesis.

Other outputs

Shown in **Fig.8.1**, within the "PBR volume & NH4+" module, are also calculations of the total system N content (sysN), which should stay constant; if it does not when operating the system as a closed batch culture then likely you have done something wrong! 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 are also calculated (C_m3, N_m3; as gC m^{-3} or gN m^{-3}).

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 N-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 (i.e., 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}

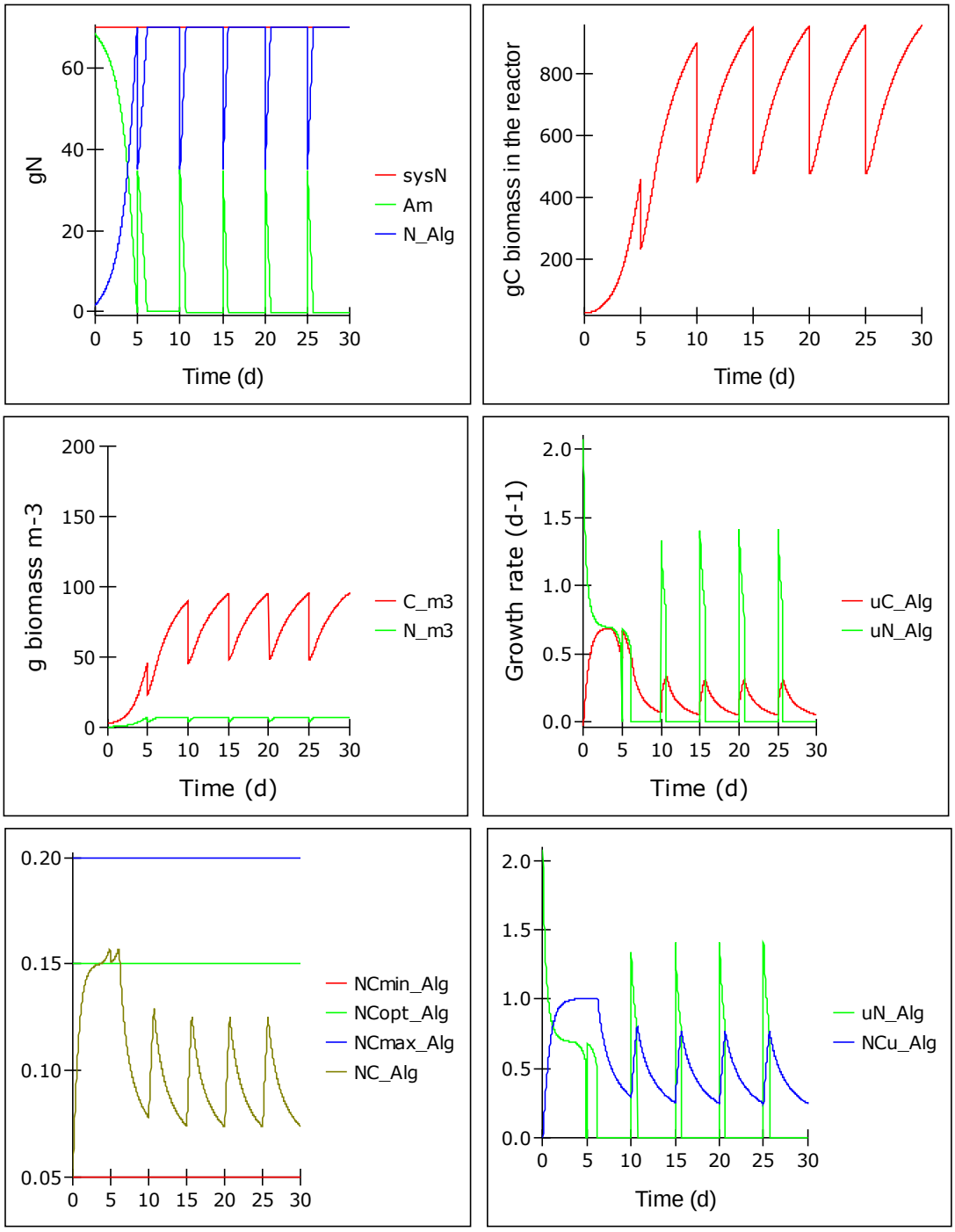


Fig.8.6 Example output. Working left to right and down the page, the plots show: system-N (sysN) remaining constant while ammonium-N (Am) is converted into algal-N (N_Alg) – oscillations occur every 5 days when 50% of the biomass is harvested and the volume made good with fresh medium; changes in C-biomass 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). This is because they become light limited.
- 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? How much nutrient is wasted?

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 with temperature) 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), the 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 growing 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).

To run the model you need the Powersim Studio Cockpit interface, available from:

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

For information as to how to access the model, please refer to page ii of this work. See also Section 7.4.

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 {so you can independently configure 3 PBRs}
- species : this has a size of 4 {so you can independently configure 4 microalgae}

In an arrayed model, members of each variable now have a number identifier as well as the name. Thus, the culture volume of each of the three PBRs, which each use the same 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 will 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] growing 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 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 so the software understands the instruction.

Note that:

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

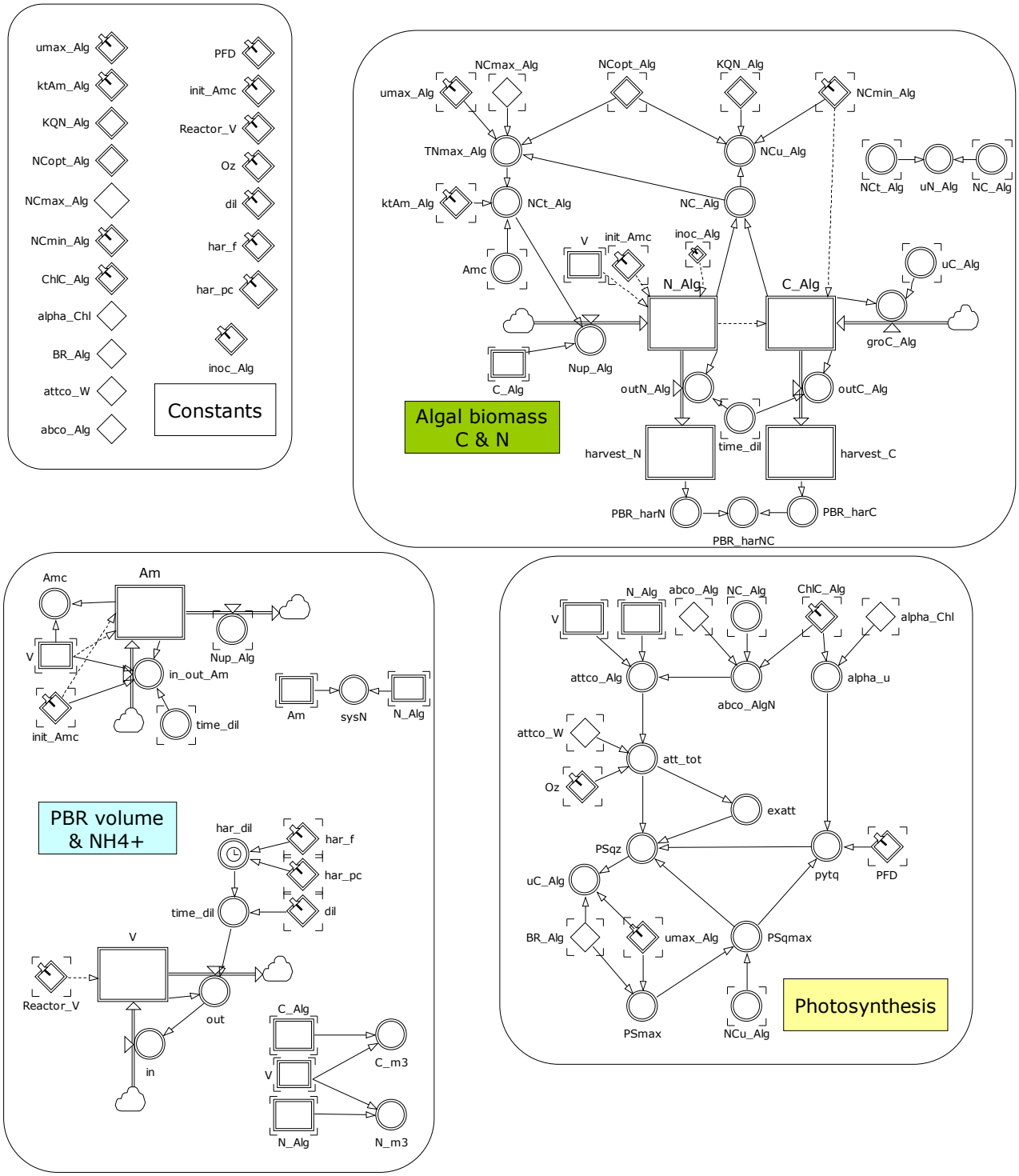


Fig.9.1 Forrester (Studio) diagram of the arrayed model. Arrayed variables are indicated by double walls. Those constant (diamond) inputs that preserve their values between simulation runs have a map-pin symbol on the top left-hand side.

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 microalgae 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 assume 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 some 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 need to 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**.

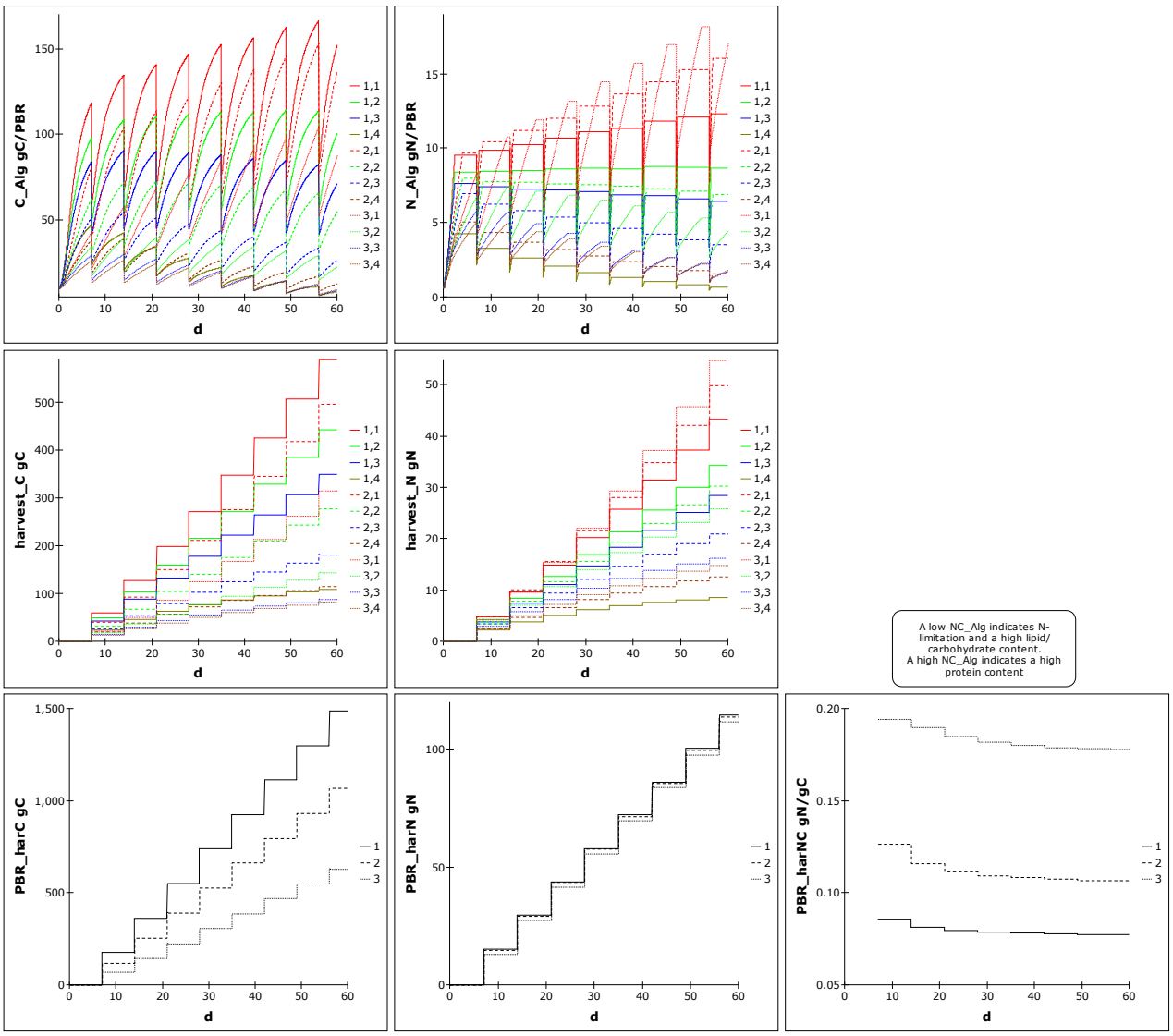


Fig.9.3 Example graphic output. The input values are as shown in **Fig.9.2**. See text for explanations.

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., the plots for 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 shown 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 also 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 that has been harvested. 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 lower levels of self-shading in the narrow-bore system.
- 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 to purchase and operate. 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** because 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 physically with the PBR (e.g., pump or pH/CO_2 control failure) or biologically (e.g., contamination). 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 remains 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 now 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**), 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/

For information as to how to access the model, please refer to page ii of this work. See also Section 7.4.

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).

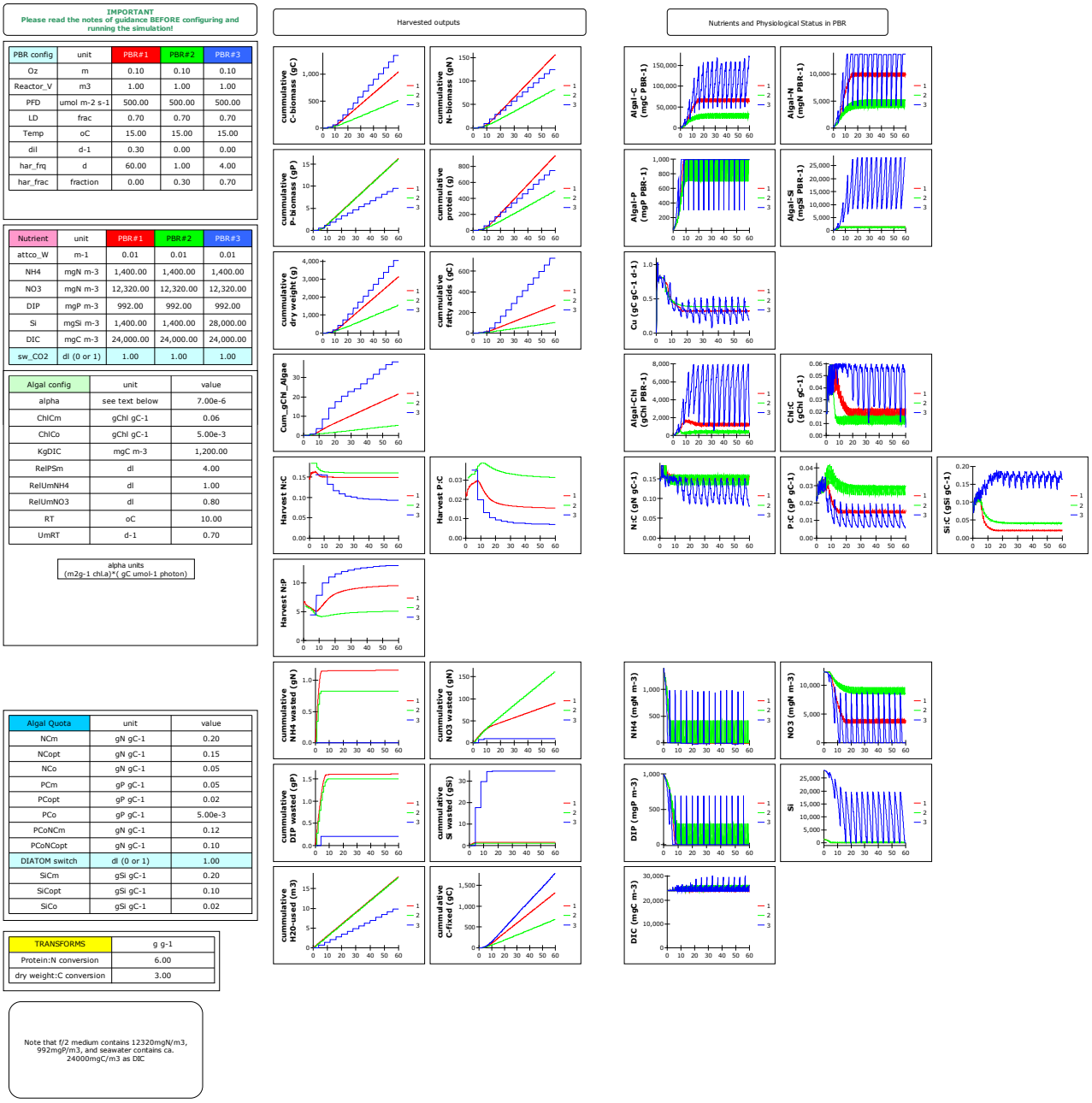


Fig.10.1 Snapshot of the entire model screen, as an aid to navigation. On the left-hand side are the data entry tables; information and instructions on how to use these are given in **Section 10.3**. The next (middle) block show graphs for the outputs of the model from harvesting the crop; some of these 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 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 also by 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 (using Si) 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 (note that mg m^{-3} is numerically the same as µg L^{-1}):

- 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 also :Si; all with respect to mass)
- Variable (photo-acclimative) Chl:C (mass ratio) and the content of RuBisCO (as activity relative to the maximum growth rate)
- 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* whose growth could be described here as an osmotroph – 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. Si is then also required as a nutrient.

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 and/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* (hereafter, **AP**) 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 1: Normalised Acquisition Potential Control Mechanism (nAPCM)

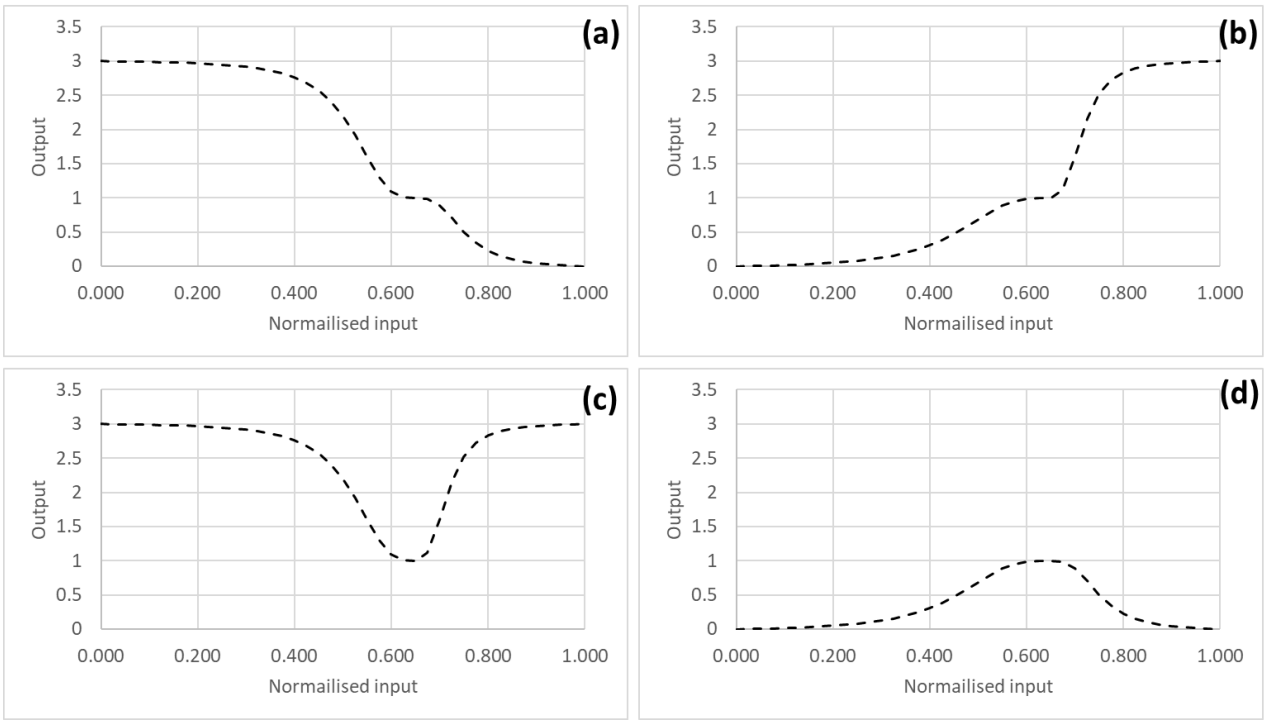


Fig.10.2 Generalised form of the acquisition potential control (APC) for nutrient transport. On the x-axis is the normalised input value of the nutrient quota, for example for N-acquisition (plot (a); x-axis as N:C), P-acquisition (plot (a); x-axis as P:C), DOC-acquisition (plot (b); x-axis as N:C), 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-form 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 used 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 (see Flynn 2018 for further information).

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 and/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 exceeding plausible values.

C and N increase 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 (note mg m^{-3} is numerically the same as µg L^{-1}):

- 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 (CO_2-fixation) 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. Here 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 culture 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 culture 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, and the culture volume remaining from the previous harvest provides an inoculum.

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 (tannins) 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 most 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 output syntax is simpler to understand that that 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 here 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.

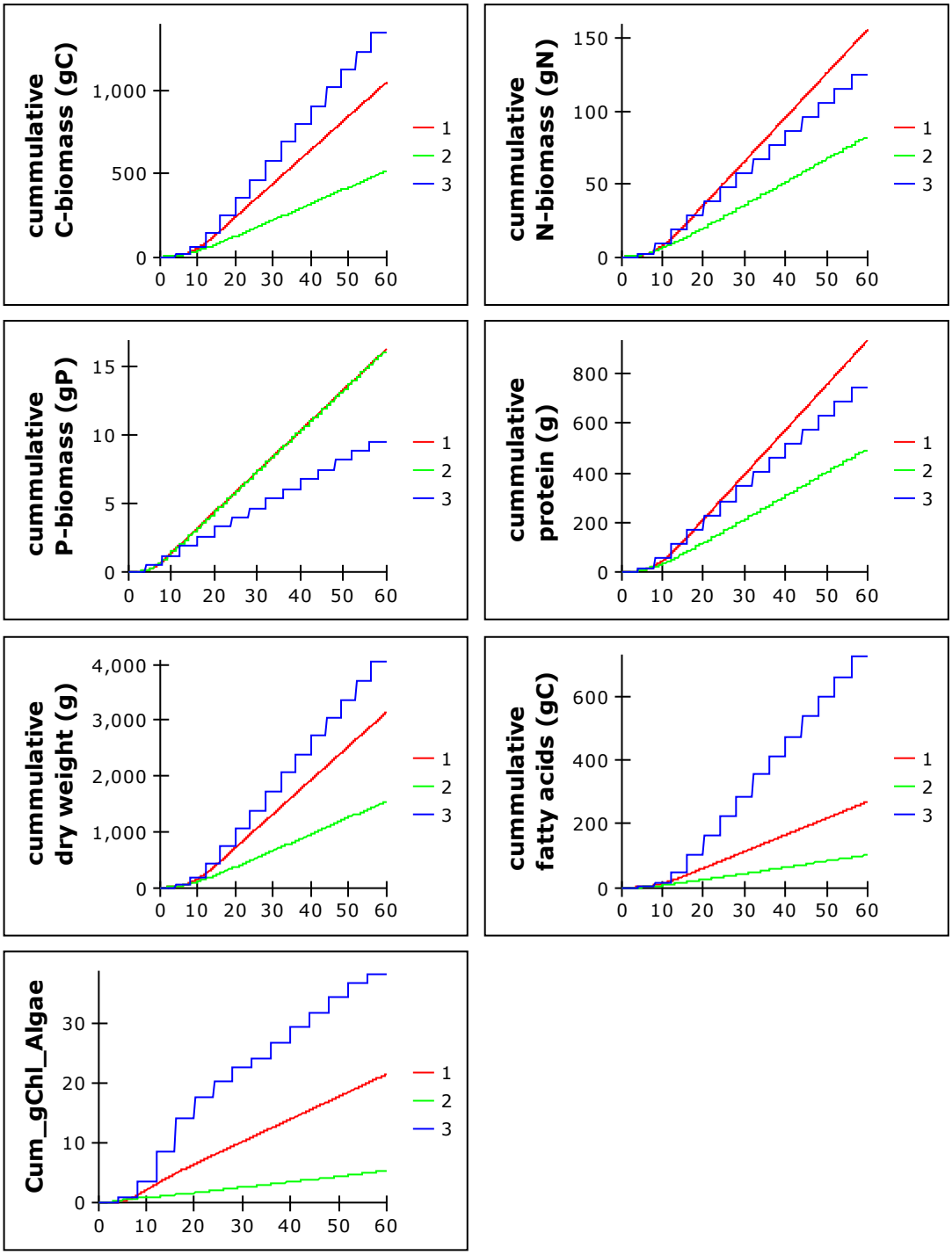


Fig.10.8 Snapshot of the cumulative harvest biomass.

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.

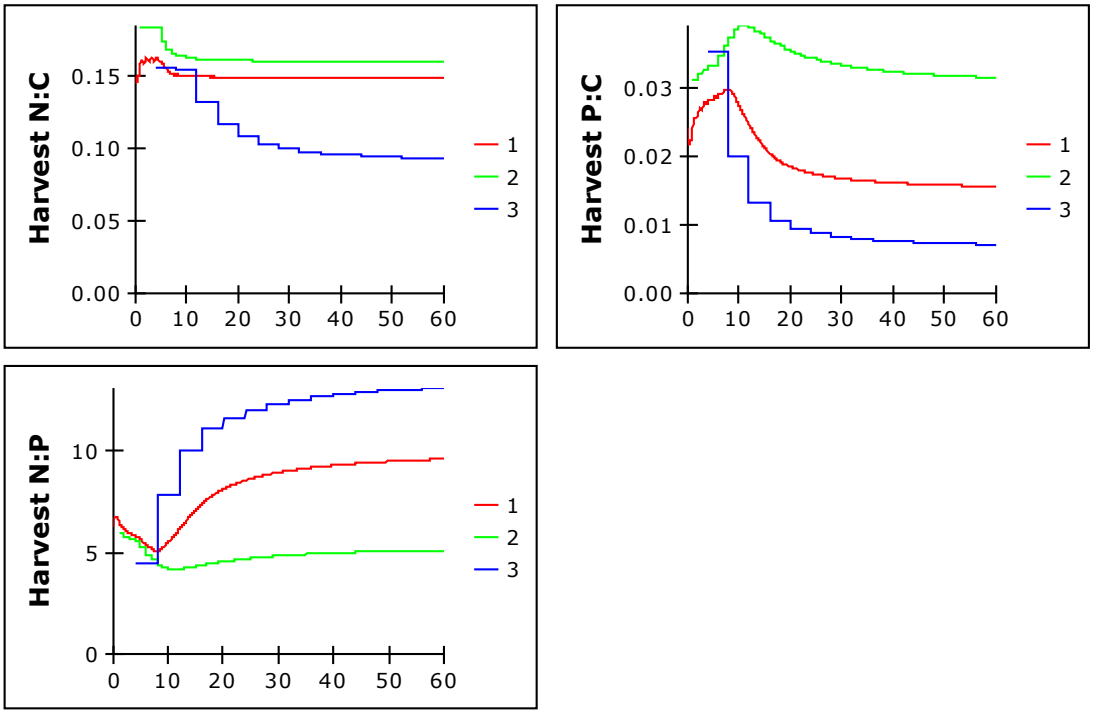


Fig.10.9 Snapshot of the C:N:P quality of the cumulative harvest.

The flip side of production is the wastage of resources (**Fig.10.10**). Because ammonium is used by priority as a N-source, 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**).

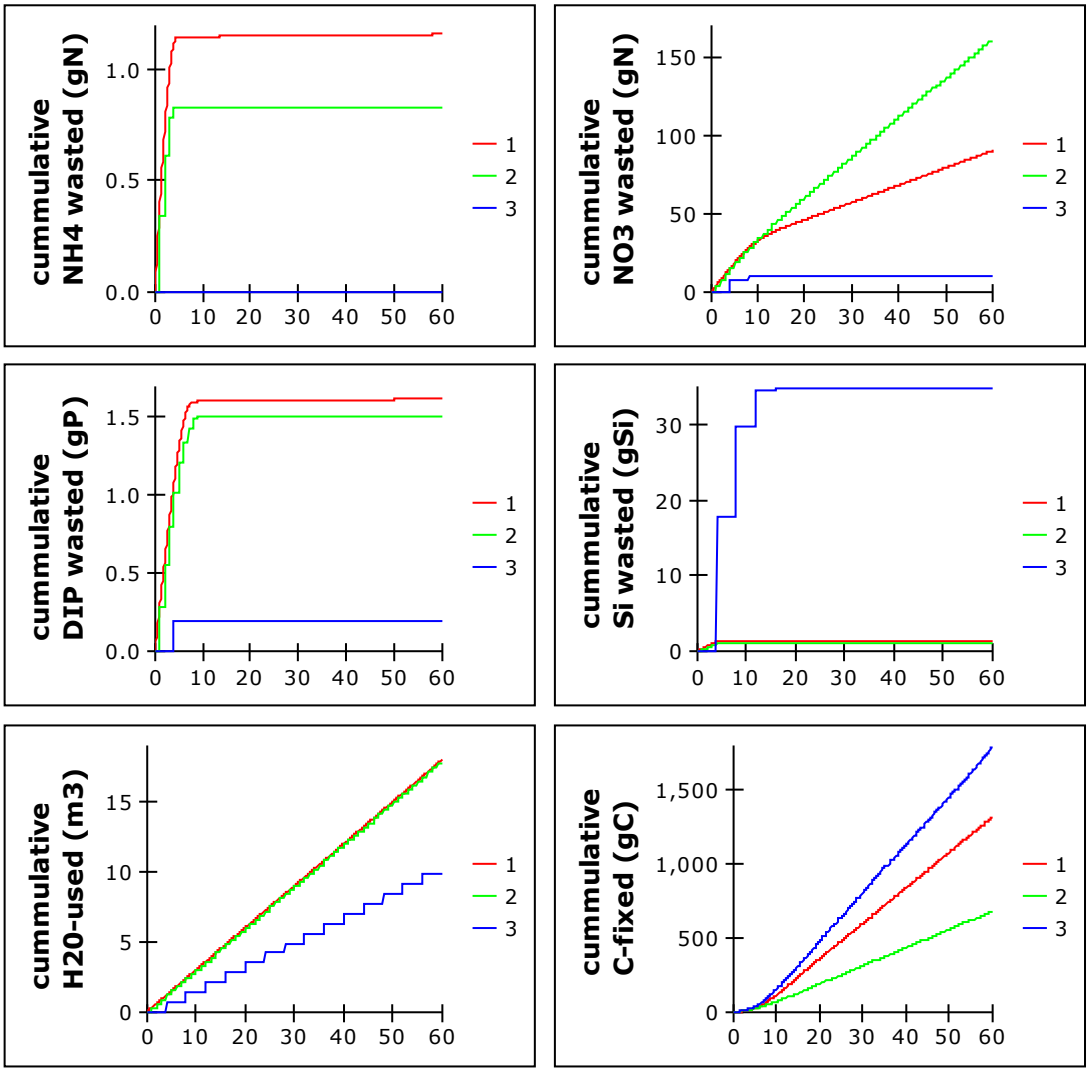


Fig.10.10 Snapshot of the resource waste and C-fixation.

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 biomass 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.

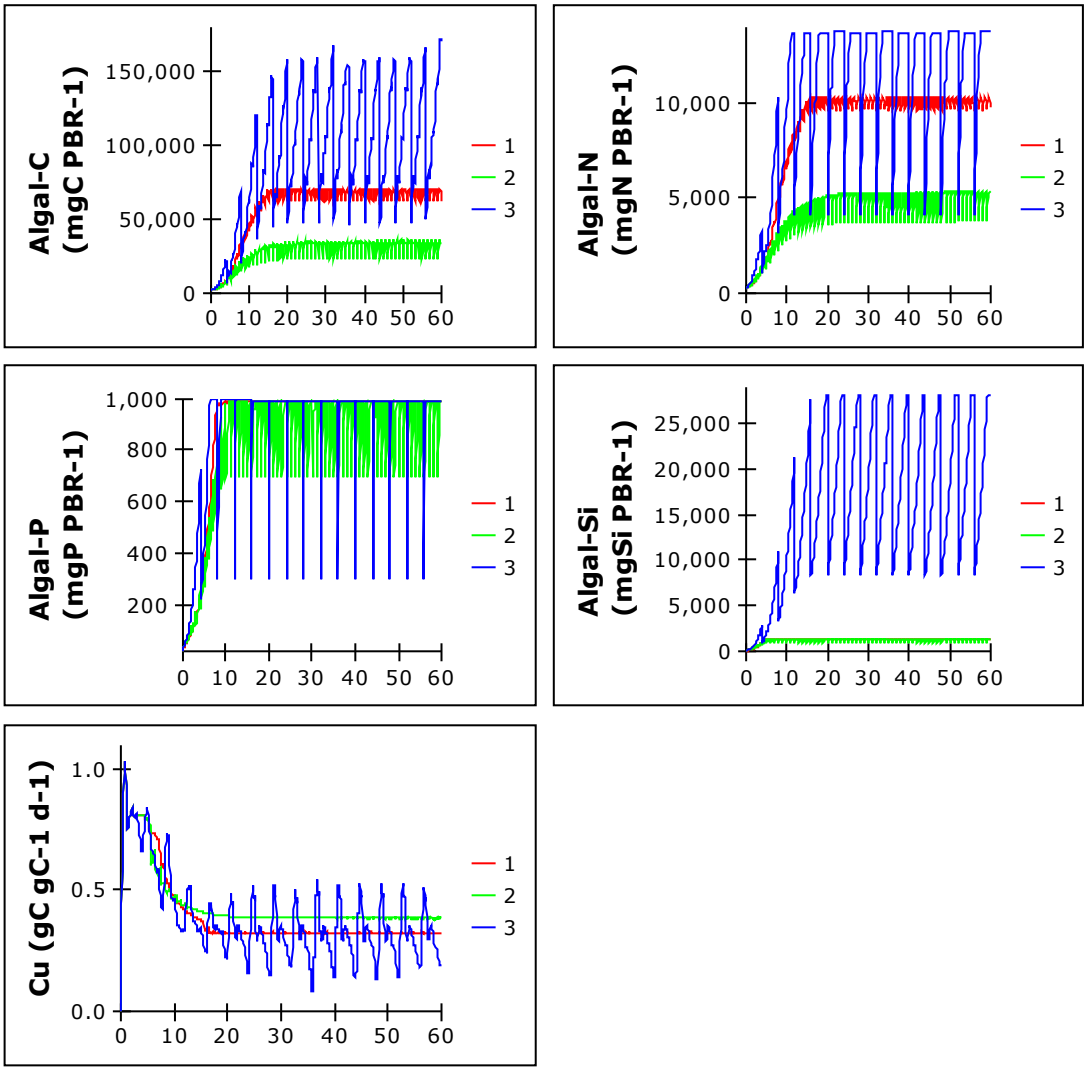


Fig.10.11 Snapshot of the plots for algal biomass in each PBR in terms of C, N, P and (because this simulation is for a diatom) Si. Changes in the C-specific growth rate (Cu) are also shown.

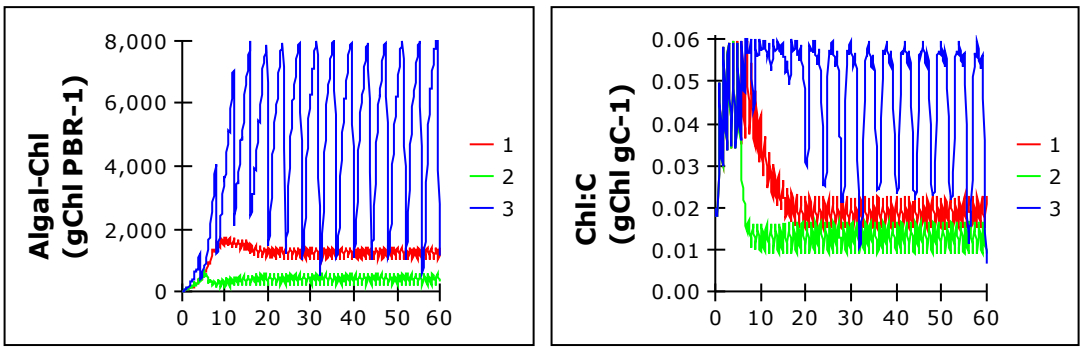


Fig.10.12 Snapshot showing changes in algal chlorophyll and of the Chl:C ratio.

The plots in **Fig.10.13** 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).

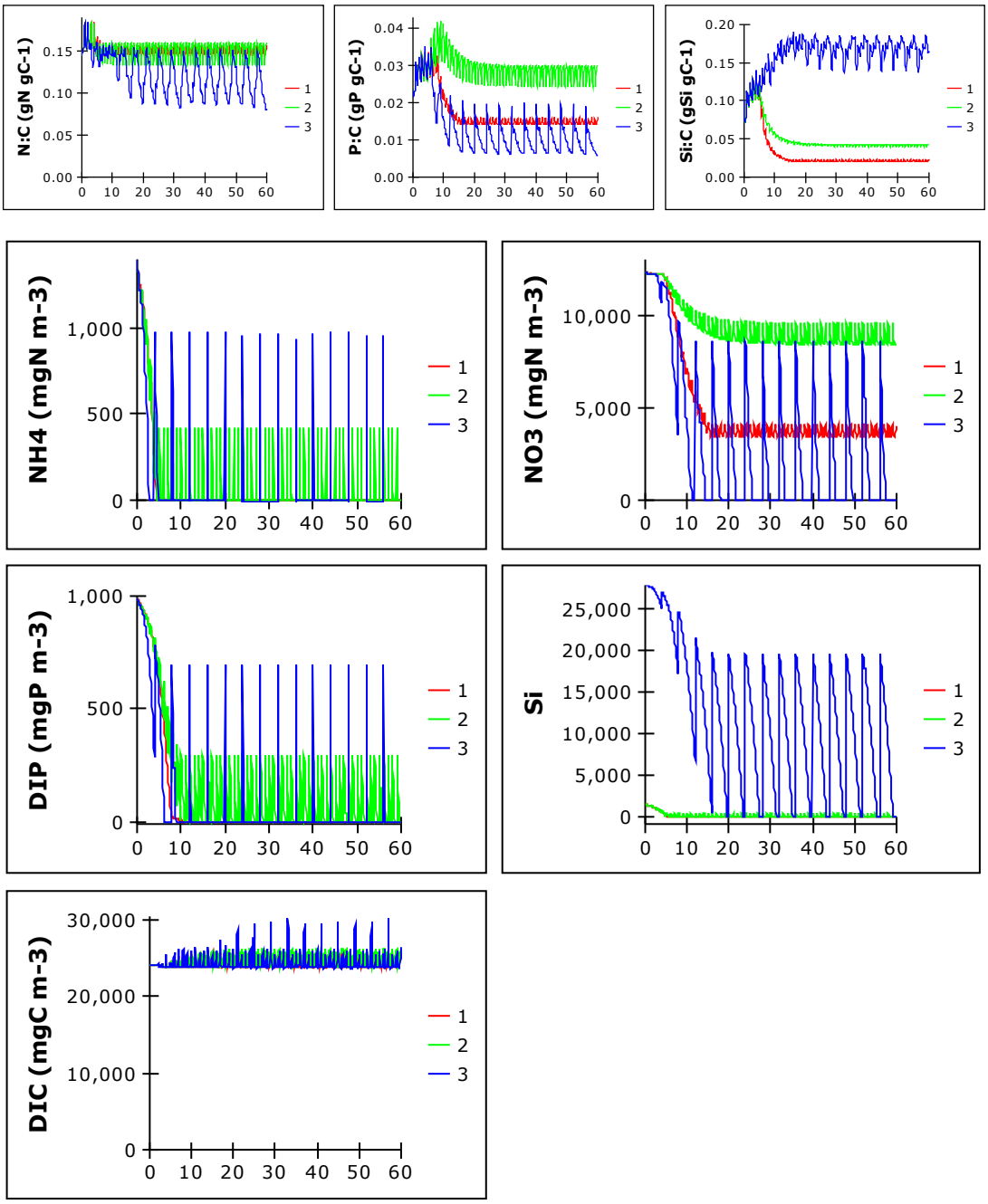


Fig.10.13 Snapshot showing residual nutrient concentrations and (upper line) the algal biomass nutrient quotas (N:C, P:C, Si:C). DIC is high because these simulations assume a DIC-stat which injects CO_2 to compensate for C-fixation.

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**, **Fig.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 year's performance if you have been growing the same species in the same enforced culture regime. 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 physically 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 (Droop 1974; Flynn & Skibinski 2020).

11. Heterotrophic and Coupled Photo-Heterotrophic Nutrition

11.1 Introduction

In **Chapter 10** a complex arrayed model was presented, which described the growth of a single species growing in three different bioreactors. This allows comparisons between the growth of the same organism type (species, strain) within reactors of different configuration, and/or the same PBR configuration operating under different conditions of nutrient loading, harvesting, lighting etc.

The algal physiology in the model provides a description with respect to variable acclimative stoichiometry for C,N,P,Chl (and for diatoms Si). It also describes nitrate versus ammonium use. What was missing, however, was any potential to consider the addition of a dissolved organic nutrient source. An organic nutrient source, usually a sugar or amino acid, may be included to enhance growth, especially in the often inevitable light-limiting conditions in dense microalgal suspensions. For further considerations of such nutrition for commercial production, see Harel & Place (2004).

The model used to describe this usage differs from that used in **Chapter 10** by the inclusion of the opportunity to provide organics as nutrients. There is no detailed consideration of the type of organics added other than that the concentration is in terms of C concentration; sugar is described as dissolved organic C (DOC), and amino acids are described as dissolved free amino acids (DFAA) with a set N:C mass ratio. These sources are intended merely as surrogates for C-rich or N-rich organic substrates.

Unless you have a specific desire to re-code the model on to another platform (for which purpose the equations are provided in the **Appendix**), 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/

For information as to how to access the model, please refer to page ii of this work. See also Section 7.4.

As with the model in **Chapter 10**, the array in this model 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). Please see **Chapter 10** for a description of the array syntax.

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 is shown in **Fig.11.1**.

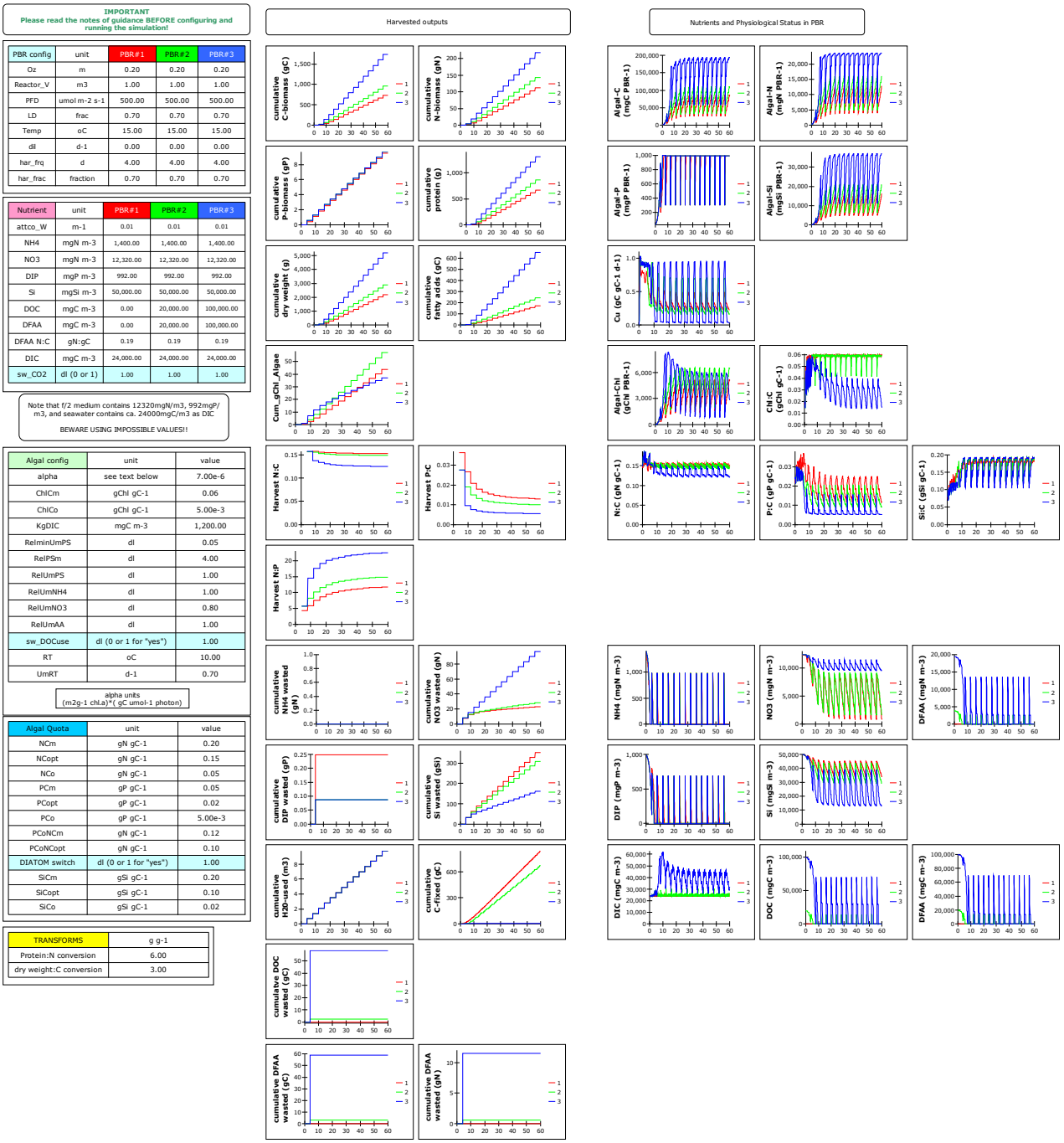


Fig.11.1 Snapshot of the entire model screen, as an aid to navigation. On the left-hand side are the data entry tables; information and instructions on how to use these are given in **Section 11.3**. The next block, on the right, show graphs for the outputs of the model from harvesting the crop; some of these show cumulative changes over the 60d simulation period. The far right-hand graphs give more details on growth rates, concentrations etc. The three different colours in each plot are for data from each of the three PBR configurations, enabling comparisons to be made between the advantages of operating the production system in different ways.

11.2 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 11.3 Configuring the simulations*.

The basis of the model is the same ODE-based system dynamics model described in **Chapter 10**. This describes 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 model is further developed here to enable osmo-heterotrophy. Parts of what follows are repeated from **Chapter 10**.

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. Cyanobacteria cannot, however, consume particles; if phagotrophy is to be considered then the model describes protists only.

State variables describe microalgal (note that mg m^{-3} is numerically the same as µg L^{-1}):

- 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 (acclimative) Chl:C (mass ratio)
- Exploitation potential for, NH_4^+, NO_3^-, DIP, DOC, DFAA (of a stated N:C), all linked to nutritional status and scope for growth
- Obligatory need for photosynthate (affecting capacity for heterotrophic growth in darkness)

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 osmotroph).

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).

RelminUmPS : the minimum proportion of microalgal growth to be supported by photosynthesis; this is to account for the fact that many microalgae capable of phototrophy seem to have an absolute requirement for light and thence for some level of phototrophy else they cannot grow. They may be able to survive in darkness using organic substrates for energy, but they cannot grow.

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*).

RelUmPS : the maximum relative rate of growth on phototrophy. This may be less than 1 if *UmT* can only be attained through the osmotrophic use of DOC, or DFAA.

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.

11.2.1 Nutrient transport and osmotrophy

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

- Ammonium
- Nitrate
- Phosphate
- Silicate (required for diatoms other than *Phaeodactylum* which usually can obtain sufficient Si just dissolved from the glassware of the culture vessel)
- DIC (dissolved inorganic C, CO_2)
- DOC (e.g., sugar)
- DFAA (e.g., dissolved free amino acids)

Of these nutrients, usage of all but silicate are described using a similar general construct that relates the *acquisition potential* (hereafter, **AP**) 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 AP curves for different nutrient types are shown in **Fig. 10.2**.

Inorganic nutrient transports

These are all described in **Section 10.2.1**.

DOC transport

This is like the control for DIN, in that the AP for DOC also references N:C, but it operates in the reverse direction, maximised at the opposite end of the N:C spectrum (**Fig. 10.2**). Thus, while AP for ammonium and nitrate increase at low N:C and transport is curtailed at high N:C, the DOC AP does the opposite and increases as N:C increases (and hence when the cell is C-limited).

The ability to bring in and use DOC to support significant growth is not atypical in microalgae. In the model controls there is a switch to enable this physiological feature (*sw_DOCuse*).

DFAA transport

This differs from the other AP controls because amino acids comprise both a C and N source. DFAA AP is thus increased at both low and high N:C (Flynn & Syrett 1985, 1986a,b).

Depending on the settings for an absolute requirement for some proportion of C coming via phototrophy (set by *RelminUmPS*), growth can proceed at rates even as high as *UmT* on just osmotrophy (using DOC+DIN, or DFAA, plus the use of DIP of course).

11.2.2 Phototrophy

This is as described in **Section 10.2.2**. However, there are the following differences:

- Osmotrophy using DOC can now depress the need for C-fixation and thus decrease the emergent Chl:C
- The maximum growth may not be attainable by phototrophy alone (set by *RelPSm*)
- A critical minimum amount of C coming through phototrophy is set by *minPhotUm*.

11.2.3 Growth

C-specific growth is the balance of all C-inputs and outputs. Inputs are from photosynthesis and osmotrophy (use of DOC and/or DFAA). Outputs include respiration associated with anabolic and catabolic activities, and nitrate reduction. The control of DFAA uptake, and of DOC uptake, versus leakage, are also described.

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).

11.2.4 Biomass

As in **Chapter 10**, 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 exceeding plausible values.

Biomass C and N increase by osmotrophy &/or phototrophy (C) or nutrient uptake (N).

Biomass 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 (not described).

Chl synthesis and degradation is described related to C-demand and nutrient status. Thus, Chl content increases during 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 and/or low nutrient supply and/or with elevated osmotrophy. 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.

11.2.5 External nutrients

The following external nutrients may be included (note mg m^{-3} is numerically the same as µg L^{-1}):

- Ammonium (mgN m^{-3})
- Nitrate (mgN m^{-3})
- Phosphate (mgP m^{-3})
- Silicate (mgSi m^{-3})
- DOC (mgC m^{-3})
- DFAA (mgC m^{-3} and mgN m^{-3}); the N:C of the feed DFAA is fixed
- 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 (CO_2-fixation) by photosynthesis.

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.

11.3 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.

11.3.1 PBR configuration

The configuration table from the simulator is shown in **Fig.11.2**; 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	0.20	0.20	0.20
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	0.00	0.00	0.00
har_frq	d	4.00	4.00	4.00
har_frac	fraction	0.70	0.70	0.70

Fig.11.2 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.

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 culture 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 to even-out gas exchange rates; the volume set by this constant is thus the total PBR culture 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 volume ratio of {light tank}:{total PBR}.

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 will 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 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, and the culture volume remaining from the previous harvest provides an inoculum.

11.3.2 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.11.3**. 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	50,000.00	50,000.00	50,000.00
DOC	mgC m-3	0.00	20,000.00	100,000.00
DFAA	mgC m-3	0.00	20,000.00	100,000.00
DFAA N:C	gN:gC	0.19	0.19	0.19
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.11.3 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 (containing 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 (killing the microalgae) 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. 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).

DOC Dissolve organic C (mgC m^{-3}) in the feed, considered to be added as a sugar (typically glucose).

DFAA Dissolved free amino acid (mgC m^{-3}) in the feed, considered to be added with an average C:N as set by **DFAA C:N** (gC gN^{-1}). The identity of the amino acid is set by the user, as appropriate for the organism. Good forms of DFAA to support growth include L isomers of glutamate, glutamine, and arginine. Some forms, notably histidine, support only poor growth rates. 'DFAA' could be substituted by other N-rich organics, such as nucleic acids.

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 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).

11.3.3 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.11.4**, while that for the quotas is shown in **Fig.11.5**.

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
RelminUmPS	dl	0.05
RelPSm	dl	4.00
RelUmPS	dl	1.00
RelUmNH4	dl	1.00
RelUmNO3	dl	0.80
RelUmAA	dl	1.00
sw_DOCuse	dl (0 or 1 for "yes")	1.00
RT	oC	10.00
UmRT	d-1	0.70

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

Fig.11.4 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.11.4** is as follows:

Alpha Initial slope of the PE curve (m^2g^{-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}).

DOC use Switch to enable DOC usage (dl, 0 for no, 1 for yes). The model by default releases a proportion of newly-fixed C as DOC. That DOC may be a secondary metabolite and would accumulate in the growth medium. However, the model does not discriminate between that DOC and any other, so to explore such a production the user must not introduce DOC as a nutrient (Section 10.3.2), and needs to set this switch to 0.

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*RelUmPS*UmT* gives the maximum plateau value for the net PE curve. (dl; typical values may be between 1 and 4). NOTE, this value could be <1 if *UmT* can only be attained by mixotrophy.

RelUmPS Maximum growth rate supported by phototrophy relative to *UmT* (if <1, then *UmT* can only be attained by mixotrophy).

RelUmNH4 Maximum growth rate supported by ammonium-N relative to the maximum possible growth rate (dl; typically this will be 1 unless the maximum growth rate can only be attained by consumption of DFAA).

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).

RelUmAA Maximum growth rate supported by amino acid -N relative to the maximum possible growth rate (dl; this may be 1 for an amino acid such as arginine, but may be very low for some, such as histidine).

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 most phototrophic dinoflagellates.

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

An explanation of the options shown in **Fig.11.5** 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 for "yes")	1.00
SiCm	gSi gC-1	0.20
SiCopt	gSi gC-1	0.10
SiCo	gSi gC-1	0.02

Fig.11.5 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.

11.3.4 Transforms

The model operates, as a system dynamics model must, on common units. However, often in the commercial microalgal sector, operates 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.11.6**).

TRANSFORMS	
Protein:N conversion	6.00
dry weight:C conversion	3.00

Fig.11.6 Snapshot from the screen of the model showing the transform table. Units are g g^{-1}.

11.4 Interpreting the model outputs

Before you run the model, first set the options as described in **Section 11.3**.

The model is not particularly fast. It will also be slower depending on the graphics-chip of your PC as there are a lot of plots. This rate of progress does however have the advantage that you can watch what is happening.

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

To make the model run (much!) faster, just minimise the window after pressing "run", give it a few seconds and maximise the window again.

Abiotic conditions for the simulations shown are given in **Fig.11.7.**

PBR config	unit	PBR#1	PBR#2	PBR#3
Oz	m	0.20	0.20	0.20
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	0.00	0.00	0.00
har_frq	d	4.00	4.00	4.00
har_frac	fraction	0.70	0.70	0.70

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	50,000.00	50,000.00	50,000.00
DOC	mgC m-3	0.00	20,000.00	100,000.00
DFAA	mgC m-3	0.00	20,000.00	100,000.00
DFAA N:C	gN:gC	0.19	0.19	0.19
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.11.7 Snapshot from the screen of the abiotic parameter values. PBRs differ with respect to the DOC and DFAA content; they al have the same Oz.

11.4.1 Syntax of the output

The syntax used in the outputs often includes 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.

11.4.2 Harvested biomass

The graphs detailing the harvested biomass (**Fig.11.8**) show the cumulative harvest over the 60d simulation period. The step-style of the outputs reflect the form of the harvesting schedule, which involves a 70% harvest every 4th day. PBR#1 has no DOC or DFAA, and hence is totally phototrophic. PBR#2 is partly heterotrophic, while PBR#3 is sufficient DOC and DFAA added that C-fixation is suppressed and light limitation in the 0.2m optical depth PBR is of no consequence.

The quality of the harvest, as indicated by the elemental C:N:P, is shown in **Fig.11.9**. These plots different from the light-limited (non-heterotrophic-supported) plots shown in **Fig.10.9**. The light (and hence C) -limited PBR#1 has a higher quality product in that the N:C and P:C are higher. Addition of DOC provides excess C for especially PBR#3.

The waste of resources and the cumulative C-fixation is shown in **Fig.11.10**. Clearly there is scope for modifying the inflow concentrations to minimise waste.

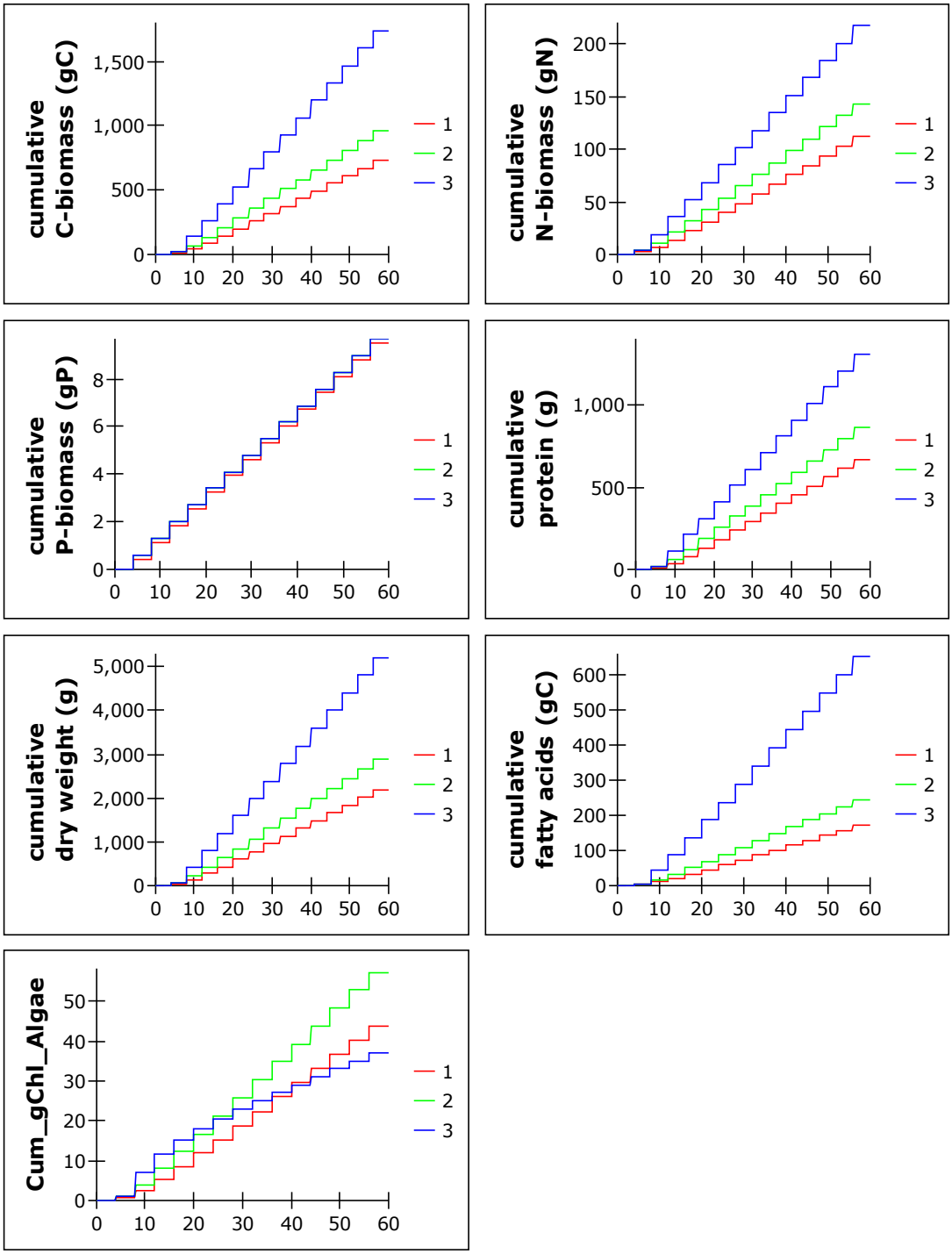


Fig.11.8 Snapshot of the cumulative harvest biomass.

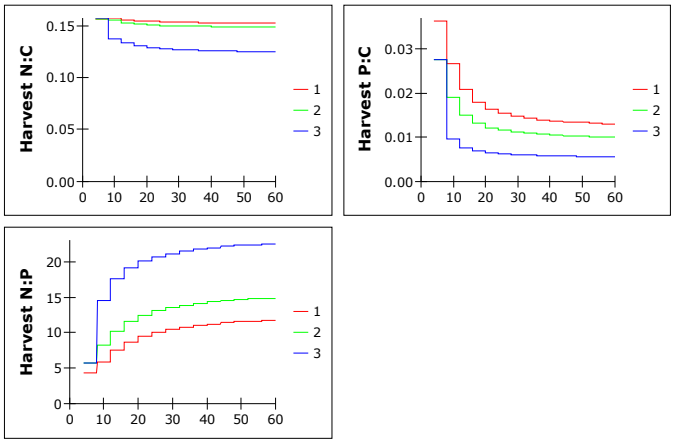


Fig.11.9 Snapshot of the C:N:P quality of the cumulative harvest.

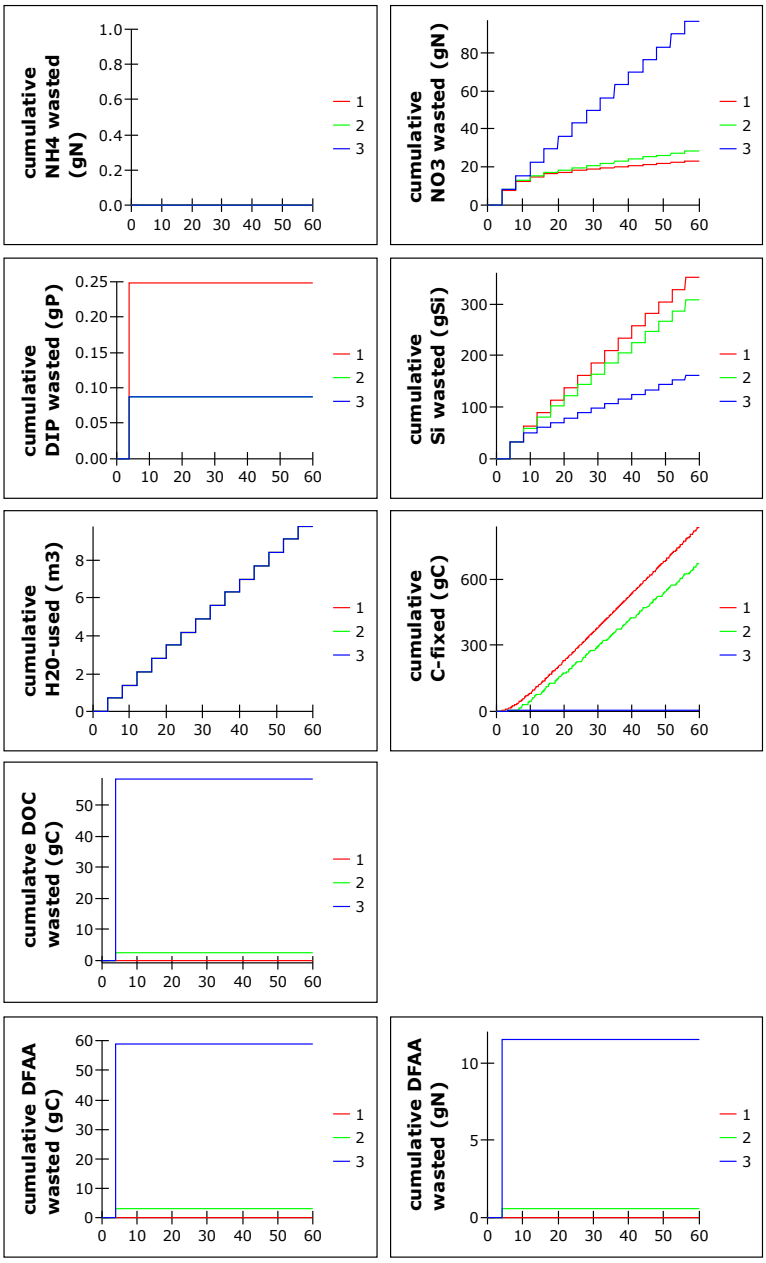


Fig.11.10 Snapshot of the resource waste and C-fixation.

10.4.3 Physiological status

The graphs in the right-hand part of the project window (**Fig.11.1**) show the physiological status of the simulated microalgae.

Fig.11.11 shows biomass content in each of the PBRs; note this is not concentration but the total PBR content. The inclusion of so much organic nutrient in PBR#2 and even more so for PBR#3, supports a massive increase in biomass. Note however, and also in **Fig.11.9**, that the DIP level cannot support a high P-content. The growth rate (Cu), while higher for PBR#3, also drops to zero which in reality could cause cell death.

Fig.11.12 shows the pigment content. Whether the microalga in PBR#3 would have much pigment depends in reality on the species.

Fig.11.13 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 diatom cell walls).

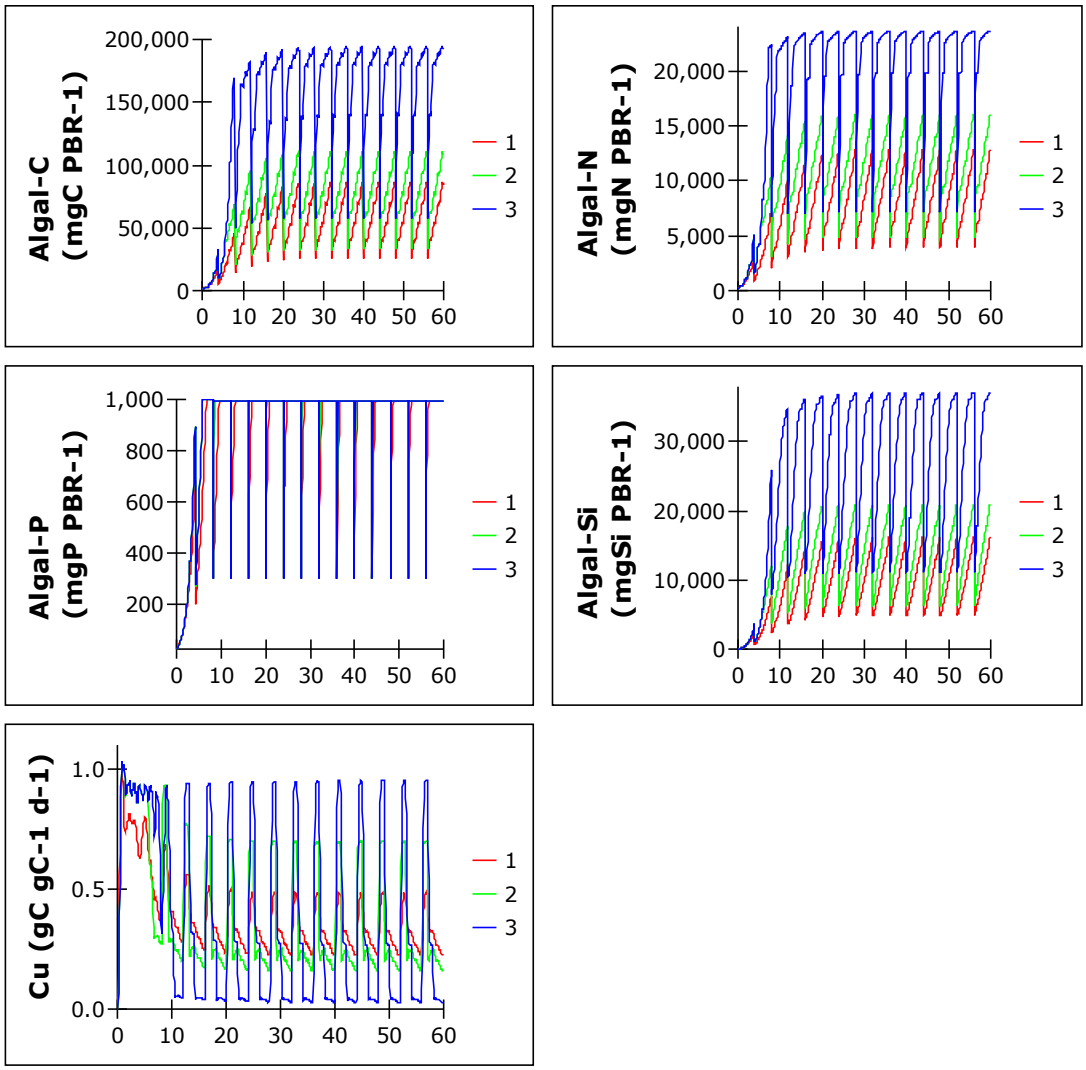


Fig.11.11 Snapshot of the plots for algal biomass in each PBR in terms of C, N, P and (because this simulation is for a diatom) Si. Changes in the C-specific growth rate (Cu) are also shown.

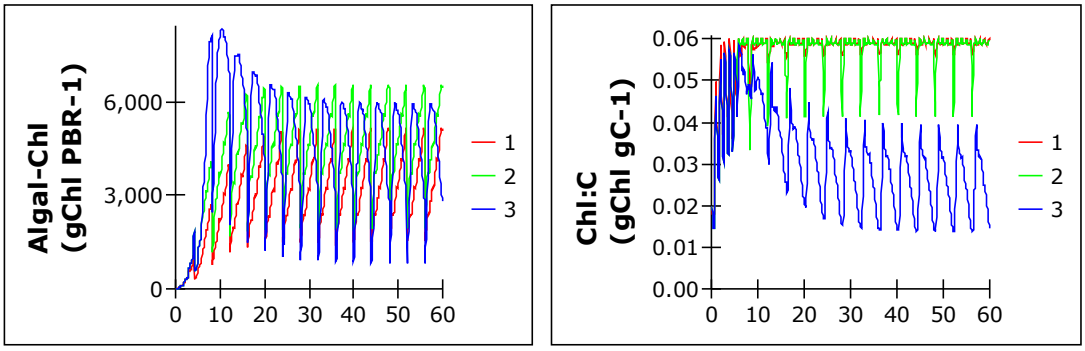


Fig.11.12 Snapshot showing changes in algal chlorophyll and of the Chl:C ratio.

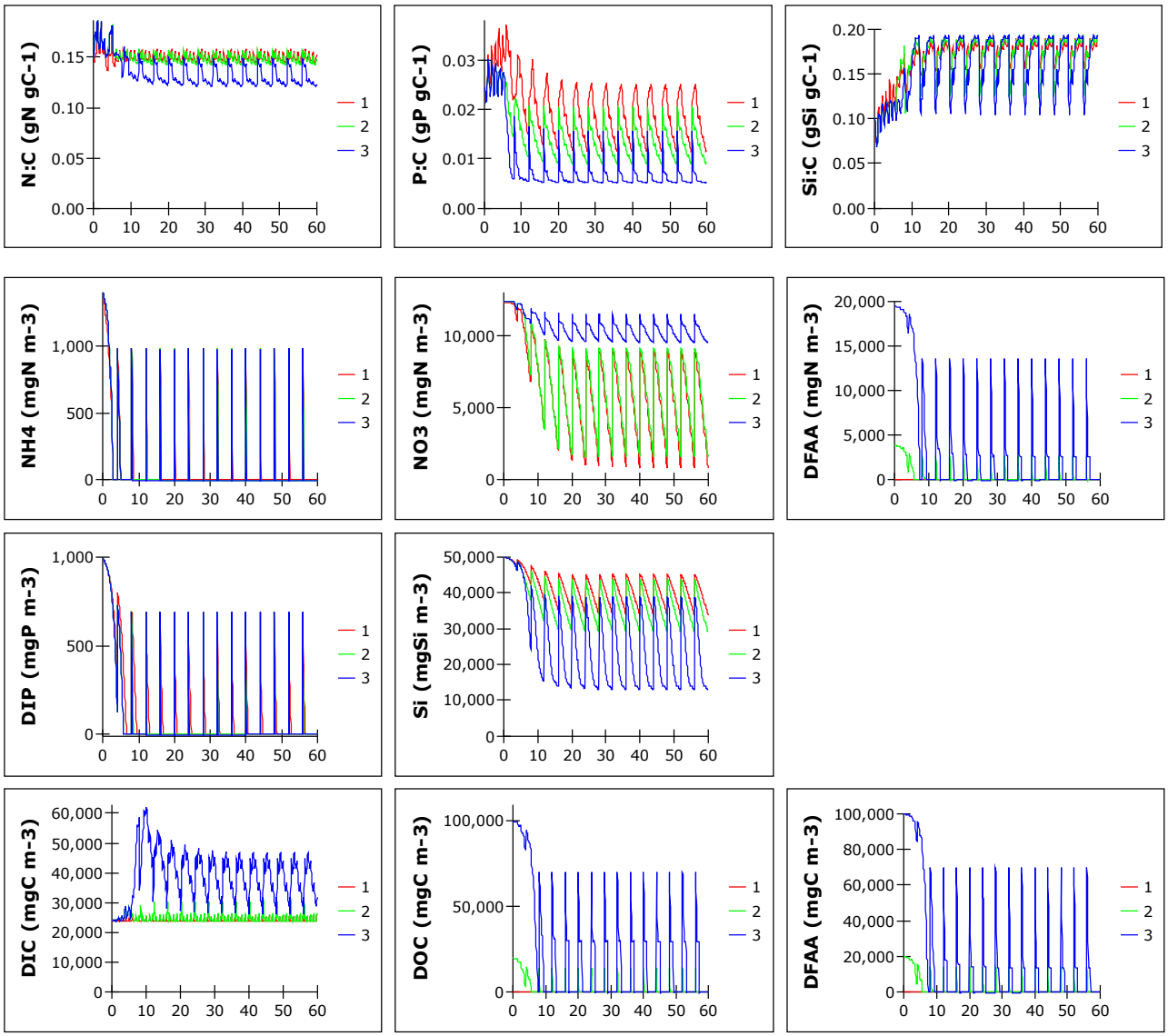


Fig.11.13 Snapshot showing residual nutrient concentrations and (upper row) the algal biomass nutrient quotas (N:C, P:C, Si:C). DIC is high because these simulations assume a DIC-stat which injects CO_2 to compensate for C-fixation.

11.4.4 Some summary observations

Collectively the outputs show the potential for the addition of organic nutrients to overcome light limitation. To what extent that addition, and the consequential suppression of phototrophy, also affects the fatty acid content, and other aspects of the biochemical composition that would damage the financial value of the crop would depend on the species. It should, however, be noted that here the microalga was configured to have an absolute requirement for a minimum level of photosynthesis (*RelminUmPS* = 0.05); growth will not proceed in continuous darkness.

As always, 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 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 year's 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.

11.5 Caveats

Many of the caveats given in **Chapters 8, 9 & 10** apply here also, but there are also the following caveats to consider.

- The inclusion of organic sources of nutrition presents a very real risk of bacterial or fungal contamination. Strict aseptic control measures must be observed at all stages to minimise such a risk. Even so, it is likely that the system will on occasion become contaminated.
- Contamination risks will be lessened by minimising residual concentrations of DOC and DFAA (see **Fig.11.13**). Even though this will in theory result in a lower microalgal production, in practice the added risks of downtime with a fully heterotrophic system (here PBR#3) may well compensate.
- Any contamination events will likely require the closure and cleaning of the system that will add significantly to loss of revenue. This needs to be considered in making any use of the DST for planning.

12. Production of Dissolved Organics

12.1 Introduction

Hitherto in this book we have considered production of microalgal biomass. Here, we consider the released organics as being the product of interest. Microalgae, like all organisms, release excess products of metabolism, together with waste products. Very often, phototrophic organisms produce an excess of metabolites, especially when their physiology is disturbed. For example, production of mucus, a polysaccharide, is often promoted in high light conditions with the transient exhaustion of nutrients. Amino acids are leaked from actively growing cells, and taken back in on nutrient exhaustion.

Such sugars and amino acids (primary metabolites) may themselves not be of commercial interest, unless they are of exotic form, as secondary metabolites. However, it is likely that the release of other, more interesting compounds, occurs concurrently with losses of primary metabolites. This, then, has an analogy with the production of intracellular compounds by microalgae at different states of growth (for example, paralytic shellfish toxins are produced by P-stressed N-replete dinoflagellates; John & Flynn 2002).

The subject of the release and uptake (or recovery) of dissolved organics is complex and very poorly understood in the detail required to support the construction and testing of system dynamics modelling. Flynn et al. (2008) explored modelling approaches; little has changed from that time with a paucity of publicly available data for quantitative production coupled with bulk microalgal growth dynamics.

The model described here considers the release of dissolved organic C (DOC) and of dissolved free amino acids (DFAA). These are described as surrogates for chemicals that may be of real interest, the identity of which requires knowledge by the user of this DST. If the user has explicit knowledge of the dynamics of the synthesis and release of a compound, then the model could be further developed to explicitly describe such events.

The model is similar to that used in **Chapters 10 & 11**. It is thus a complex arrayed model describing growth of a single species in three different bioreactors. This allows comparisons between the growth of the same organism type (species, strain) within reactors of different configuration, and/or the same PBR configuration operating under different conditions of nutrient loading, harvesting, lighting etc.

The algal physiology in the model provided a description with respect to variable acclimative stoichiometry for C,N,P,Chl (and for diatoms Si). It also describes nitrate versus ammonium use. The model is like that in **Chapter 11**, which considered the use of externally provided DOC and DFAA to boost biomass growth. Here, of course, such organics would likely not be provided as nutrients by the user, and the ability to consume these compounds may be suppressed; different microalgae have very different capabilities to use or produce organics under different conditions and the user needs to configure the model accordingly to reflect such physiological capabilities.

The model differs from that used in **Chapter 11** by the inclusion of an ability to configure the leakage of DOC and DFAA. In addition, to aid the user in conducting *in silico* experiments, the model can be configured to pause with a frequency that can be adjusted by the user.

Unless you have a specific desire to re-code the model onto another platform (for which purpose the equations are provided in the **Appendix**), 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/

For information as to how to access the model, please refer to page ii of this work. See also Section 7.4.

As with the model in **Chapter 9** (and **10**, **11**), the array in this model 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). Please see **Chapter 9** (**Sections 9.2 & 9.3**) for an explanation of the array syntax.

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 is shown in **Fig.12.1**.

12.2 The algal model

The core model is exactly the same as that described in Chapter 11, and developed from Chapter 10. The only additional features are as documented below.

12.2.1 C-rich metabolite leakage

Here we use DOC leakage as a surrogate for the release of C-rich compounds of interest.

DOC leakage has been a feature of the models described in **Chapter 10** onwards. However, the significance of this has been rather minor, running in a default setting of a maximum of 10% of newly fixed C being leaked. Typically, PBRs are light-limited so in reality the leakage is indeed small. Here we explore the increase of this proportion to boost excretion.

To increase DOC release, cells need to be grown at high light, which also requires that the optical depth is shallow so that self-shading is minimised. In essence, we are trying to make the cells over-produce C-rich metabolites. It is also likely that you would specifically select a microalgal strain with an exaggerated loss of DOC, or that you will have modified its physiology in some way. Here that eventuality is described by altering a parameter *pcDOC*. The location of this option in the control panel is shown in **Fig.12.2**.

The typical value of *pcDOC* is 0.05 or 0.1. Here you can experiment with higher values. Note that in doing so, you are directing newly fixed C away from biomass production, although that can be mitigated by allowing the organism to take it back in.

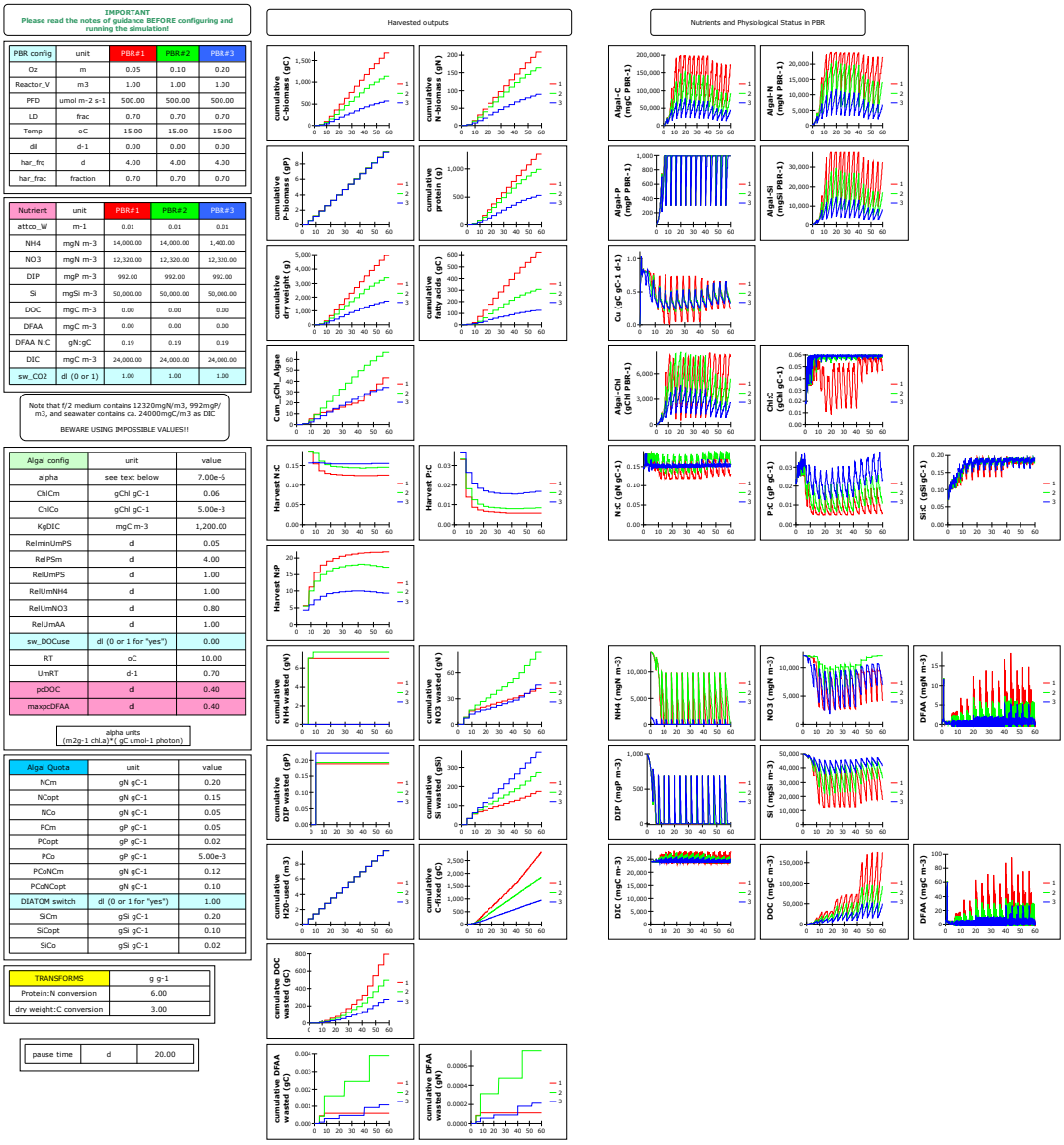


Fig.12.1 Snapshot of the entire model screen, as an aid to navigating the DST. On the left-hand side are the data entry tables; information and instructions on how to use these are given in **Section 12.3**. The next block, on the right, show graphs for the outputs of the model from harvesting the crop; some of these show cumulative changes over the 60d simulation period. The far right-hand graphs give more details on growth rates, concentrations etc. The three different colours in each plot represent the three PBR configurations, enabling comparisons to be made between the advantages of operating the production system in different ways.

12.2.2 N-rich metabolite leakage

Here we use DFAA leakage as a surrogate for the release of N-rich dissolved organic N (DON) compounds of interest.

Microalgae leak DFAA, and they also take it up. Indeed, DFAA uptake may primarily be a mechanism to enable the recovery of leaked DFAA (Flynn & Berry 1999). Whether a non-DFAA compound of interest is recovered or not will affect the dynamics of net production, but in large measure the dynamics are a consequence of the continued availability of DIN during growth, and of the system dilution rate which will washout the solute as well as the biomass. A system with long periods between harvesting, more likely to exhaust its nutrients, is less likely to accumulate DFAA and other

forms of DON. The default leakage of DFAA is 0.1 (i.e., 10% of incoming DIN is leaked as DFAA); this can be altered as required by the user, noting that high values will decrease biomass growth.

The location of this option in the control panel is shown in **Fig.12.2**.

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
RelminUmPS	dl	0.05
RelPSm	dl	4.00
RelUmPS	dl	1.00
RelUmNH4	dl	1.00
RelUmNO3	dl	0.80
RelUmAA	dl	1.00
sw_DOCuse	dl (0 or 1 for "yes")	0.00
RT	oC	10.00
UmRT	d-1	0.70
pcDOC	dl	0.40
maxpcDFAA	dl	0.40

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

Fig.12.2 Snapshot of the physiology control panel, showing *pcDOC* (maximum proportion of C fixed that may be released as DOC) and *maxpcDFAA* (maximum proportion of incoming nutrient-N released as amino acids and other N-rich compounds), both set here at 0.4 (40% of incoming fixed C and of N-assimilation being leaked). See also **Section 12.3.3**.

12.3 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 DST 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.

12.3.1 PBR configuration

The configuration table from the simulator is shown in **Fig.12.3**; 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	0.05	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
LD	frac	0.70	0.70	0.70
Temp	oC	15.00	15.00	15.00
dil	d-1	0.00	0.00	0.00
har_frq	d	4.00	4.00	4.00
har_frac	fraction	0.70	0.70	0.70

Fig.12.3 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.

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 culture 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 this constant is thus the total PBR culture 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 volume ratio of {light tank}:{total PBR}.

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 will 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 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, and the culture volume remaining from the previous harvest provides an inoculum.

12.3.2 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.12.4**. 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	14,000.00	14,000.00	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	50,000.00	50,000.00	50,000.00
DOC	mgC m-3	0.00	0.00	0.00
DFAA	mgC m-3	0.00	0.00	0.00
DFAA N:C	gN:gC	0.19	0.19	0.19
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.12.4 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 (containing tannins) 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 (killing the microalgae) 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. 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).

DOC Dissolve organic C (mgC m^{-3}) in the feed, considered to be added as a sugar (typically glucose). For this deployment, as you are trying to produce DOC, logic is that this value is zero.

DFAA Dissolved free amino acid (mgC m^{-3}) in the feed, considered to be added with an average C:N as set by **DFAA C:N** (gC gN^{-1}). The identity of the amino acid is set by the user, as appropriate for the organism. Good forms of DFAA to support growth include L isomers of glutamate, glutamine, and arginine. Some forms, notably histidine, support only poor growth rates. For this deployment, as you are trying to produce N-rich metabolites, logic is that this value is zero.

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 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).

12.3.3 Algal physiology and quota configurations

The model describes one microalgae strain, growing in the 3 PBRs. The configuration table from the simulator for the physiology is shown in **Fig.12.5**, while that for the quotas is shown in **Fig.12.6**.

An explanation of the options shown in **Fig.12.5** is as follows:

Alpha Initial slope of the PE curve (m^2g^{-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 12.3.1). See Cark & Flynn (2000).

RelminUmPS Relative contribution to the maximum growth rate that must be supported by C-fixation. Only if this value is 0 can growth proceed in complete darkness.

RelPSm Maximum value of photosynthesis relative to maximum (day-averaged) growth rate on phototrophy. Thus, *RelPSm*RelUmPS*UmT* gives the maximum plateau value for the net PE curve. (dl; typical values may be between 1 and 4). NOTE, this value could be <1 if *UmT* can only be attained by mixotrophy.

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
RelminUmPS	dl	0.05
RelPSm	dl	4.00
RelUmPS	dl	1.00
RelUmNH4	dl	1.00
RelUmNO3	dl	0.80
RelUmAA	dl	1.00
sw_DOCuse	dl (0 or 1 for "yes")	0.00
RT	oC	10.00
UmRT	d-1	0.70
pcDOC	dl	0.40
maxpcDFAA	dl	0.40

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

Fig.12.5 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.

RelUmPS Maximum growth rate supported by phototrophy relative to *UmT* (if <1, the *UmT* can only be attained by mixotrophy).

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).

RelUmAA Maximum growth rate supported by amino acid -N relative to the maximum possible growth rate (dl; this may be 1 for an amino acid such as arginine, but may be very low for some, such as histidine).

Sw_DOCuse Switch to enable DOC usage (dl, 0 for no, 1 for yes). The model by default releases a proportion of newly-fixed C as DOC. That DOC may be a secondary metabolite and would accumulate in the growth medium. However, the model does not discriminate between that DOC and any other, so to explore such a production the user must not introduce DOC as a nutrient (**Section 12.3.2**), and needs to set this switch to 0.

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 12.3.1**) 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 most phototrophic dinoflagellates.

pcDOC proportion of newly fixed C released as DOC. Usually this value is only a few % (i.e., ca. 0.05 = 5%), but if you are exploiting a strain that has a particularly high rate of release, you can alter this value as appropriate.

maxpcDFAA maximum proportion of incoming N released as organic-N metabolites (nominally here as dissolved free amino acids).

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

An explanation of the options shown in **Fig.12.6** 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 diatom 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)	0.00
SiCm	gSi gC-1	0.20
SiCopt	gSi gC-1	0.10
SiCo	gSi gC-1	0.02

Fig.12.6 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.

12.3.4 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.12.7**).

TRANSFORMS	
Protein:N conversion	6.00
dry weight:C conversion	3.00

Fig.12.7 Snapshot from the screen of the model showing the transform table. Units are g g^{-1}.

12.4 Interpreting the model outputs

Before you run the model, first set the options as described in **Section 12.3**.

The model is not particularly fast. It will also be slower depending on the graphics-chip of your PC as there are a lot of plots. This rate of progress does however have the advantage that you can watch what is happening. And 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 also rescale the graphs.

To make the model run (much!) faster, just minimise the window after pressing "run", give it a few seconds and maximise the window again.

Abiotic conditions for the simulations shown are given in **Fig.12.8.**

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
LD	frac	0.70	0.70	0.70
Temp	oC	15.00	15.00	15.00
dil	d-1	0.00	0.00	0.00
har_frq	d	4.00	4.00	4.00
har_frac	fraction	0.70	0.70	0.70

Nutrient	unit	PBR#1	PBR#2	PBR#3
attco_W	m-1	0.01	0.01	0.01
NH4	mgN m-3	14,000.00	14,000.00	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	50,000.00	50,000.00	50,000.00
DOC	mgC m-3	0.00	0.00	0.00
DFAA	mgC m-3	0.00	0.00	0.00
DFAA N:C	gN:gC	0.19	0.19	0.19
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.12.8 Snapshot from the screen of the abiotic parameter values. PBRs differ with respect to the nutrient loading and Oz.

12.4.1 Syntax of the output

The syntax used in the outputs often includes 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.

12.4.2 Harvested biomass

An important difference between interpretation of the model run for production of solutes rather than biomass is that the output plots labelled as cumulative *waste* of DOC and DFAA, actually describes production of materials of commercial interest. For reference, the biomass outputs are shown in **Fig.12.9**, while the DOC and DFAA outputs are shown in **Fig. 12.10**.

It is also important to note that the units of production of that organic *waste* is in gC or gN from each PBR. In contrast, plots of DOC and DFAA concentration within the PBR are in units of mg m^{-3} (**Fig.12.11**). Note that while the concentration plot for DFAA PBR#1 (**Fig.12.11**) is higher than for the other PBR configurations, the actual production of harvested DFAA from PBR#1 is the poorest (**Fig.12.10**). The reason for this is that in PBR#1, which has the shallowest optical depth (**Fig.12.8**), the inorganic nutrient resources are exhausted and so the DFAA released into the growth medium is taken back up. Whether that would actually occur depends on the system. If the PBR is contaminated by bacteria, for example, DFAA may never accumulate in the first instance. If your product of interest is not recovered as are amino acids, then you may need to consider other options. That may include setting RelUmAA (**Fig.12.5**) at a low value.

How such models outputs relate quantitatively to materials of actual interest will depend on the application. It may be, for example, that the compound of interest shows a physiological synthesis that aligns with that of DFAA but equates to just a few % of the DFAA mass. And, of course, there is then the non-trivial complication of actually extracting and purifying the product to consider; a high proportion of valuable materials may be lost in the harvesting and purification steps.

The step-style of the outputs reflect the form of the harvesting schedule, which involves a 70% harvest every 4^{th} day (see **Fig.12.8**).

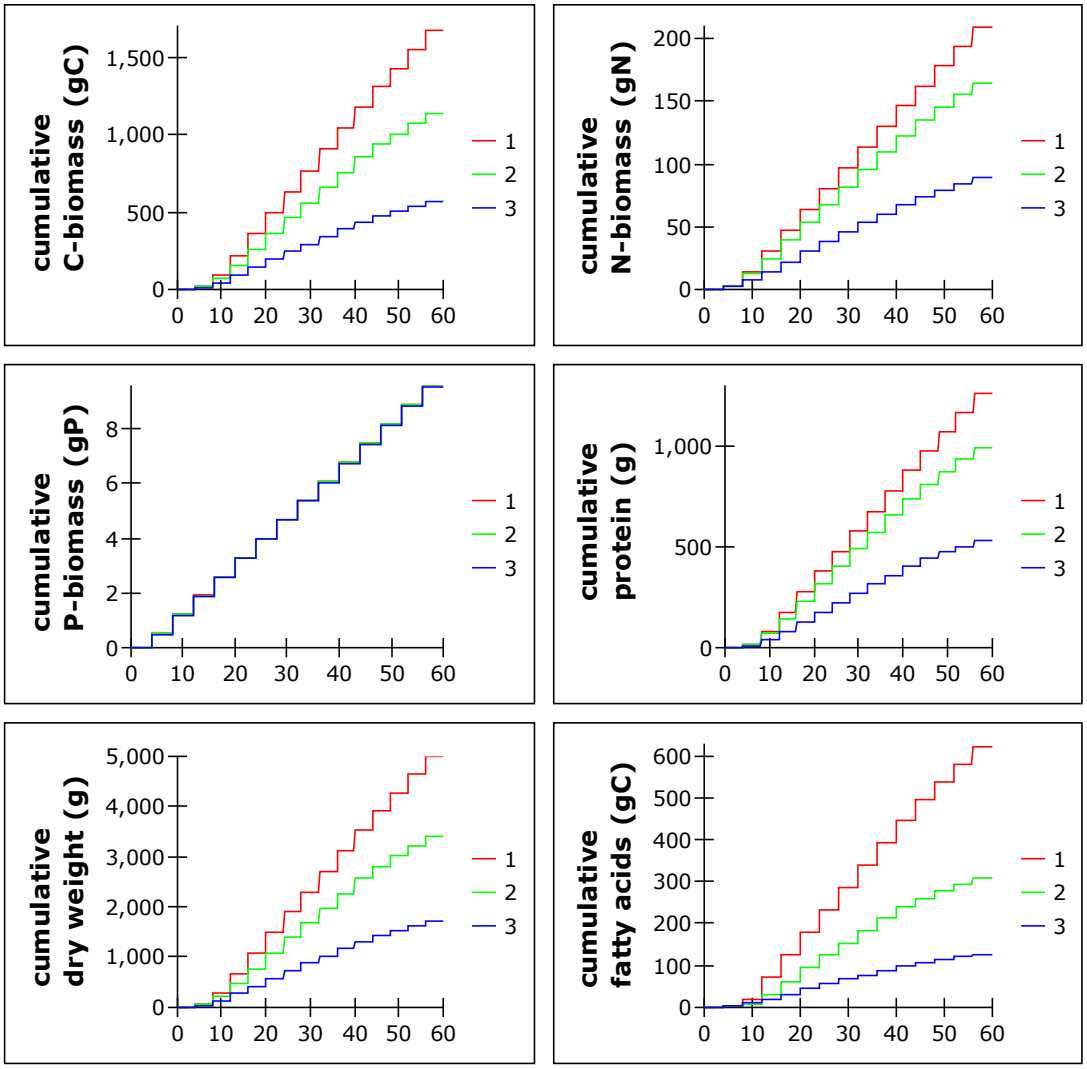


Fig.12.9 Snapshot of the cumulative harvest biomass. For this application, this harvest is not the primary commercial target (see **Fig.12.10**), though of course it may have a useful secondary value. The value of *har_frq* (**Fig.12.8**) was changed during the run – see legend to **Fig.12.10**.

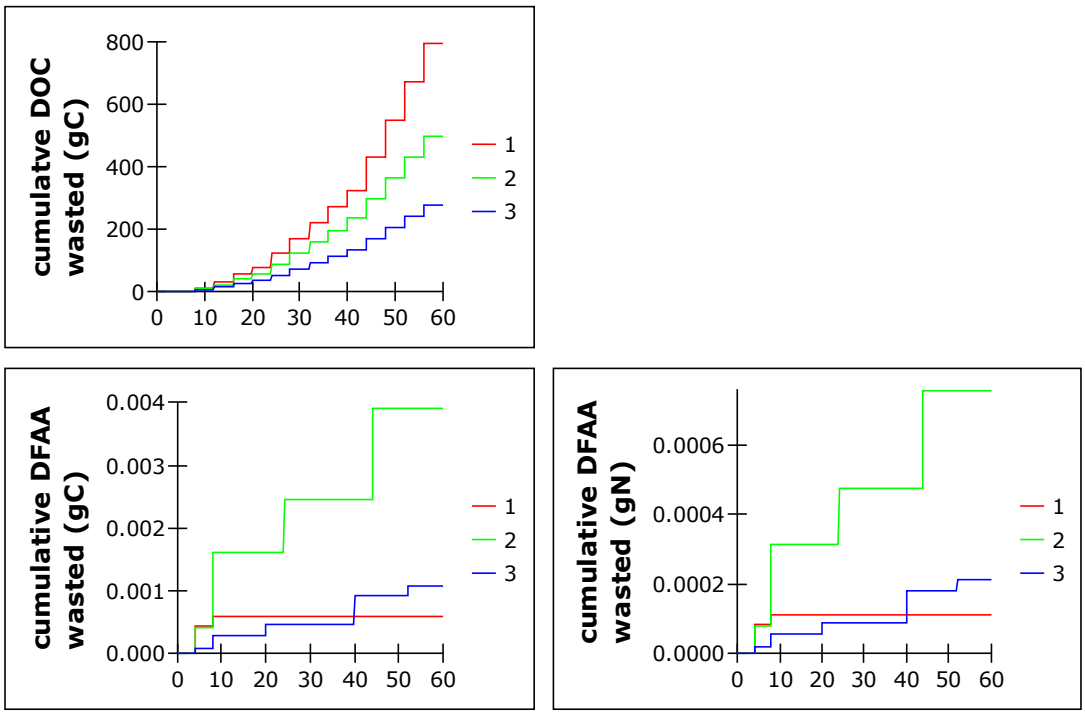


Fig.12.10 Snapshot of the cumulative harvest of dissolved organics. This is a system promoting production of DOC. Nonetheless, note the difference in the values between DOC production shown here and C-biomass production in **Fig.12.9**. The difference in periods between the step size shows the value of *har_frq* (**Fig.12.8**). See also the legends to **Fig.12.11** and **Fig.12.12**.

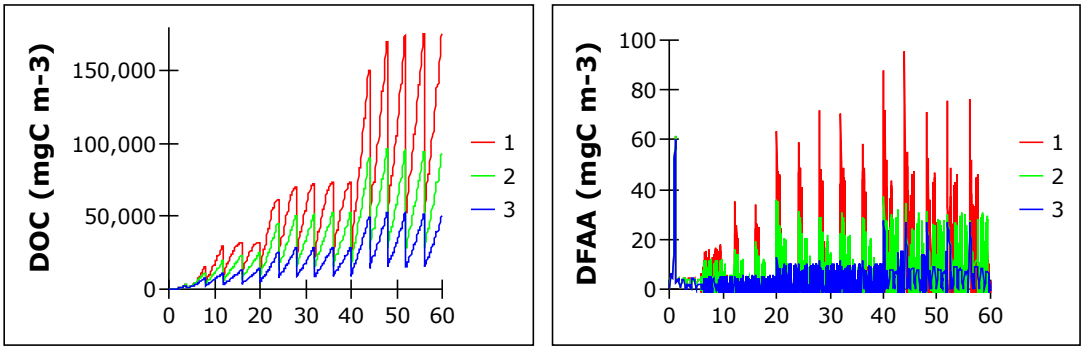


Fig.12.11 Snapshot of the concentration of DOC and DFAA (here shown in C units) in the PBRs. The value of *har_frq* (**Fig.12.8**) was changed during the run – see legend to **Fig.12.10**. Although it looks like PBR#1 produces more DFAA, actually the cells take it up again as the DIN is exhausted, hence the 'waste' of DFAA shown in **Fig.12.10** from PBR#1 is low.

12.4.3 Physiological status

The graphs in the right-hand part of the project window (**Fig.12.1**) show the physiological status of the simulated microalgae. Some of these are shown here in **Fig.12.12**.

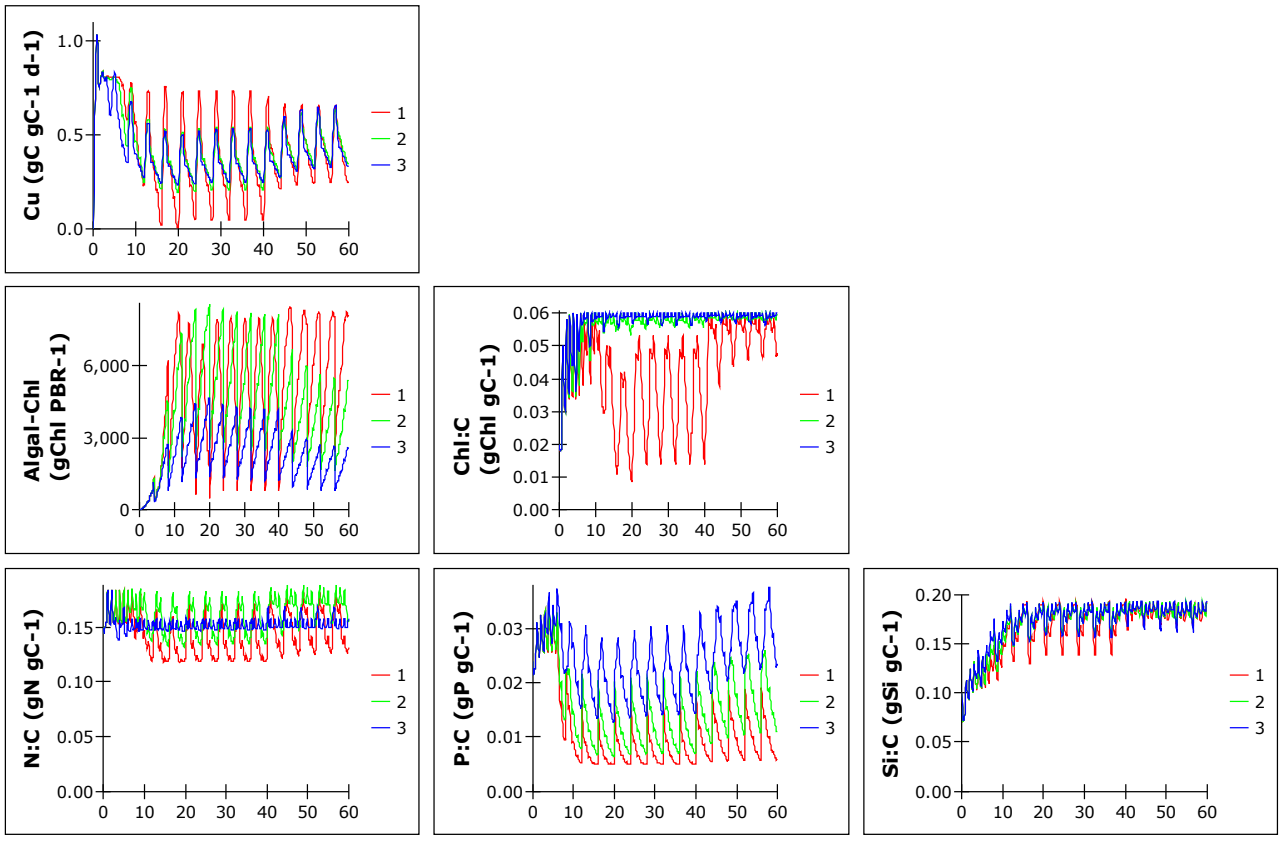


Fig.12.12 Changes in physiological parameters during growth in the different PBRs. Note that PBR#1 drove a level of N-stress (indicated by lower N:C, Ch:C and thence a greater fluctuation in growth rate Cu) that resulted in the recovery of previously leaked DFAA, hence there was no effective production of nitrogenous organics in this system (**Fig.12.10**). The value of *har_frq* (**Fig.12.8**) was changed during the run – see legend to **Fig.12.10**.

12.4.4 Some summary observations

Collectively the outputs show the potential for the manipulation of PBR's to improve the production of metabolites released by microalgae into the growth medium. Unless additional information is available, to better align released metabolites of interest to that of DOC and/or DFAA as modelled here, then the simulations can only provide a rough idea of that potential. What is clear, however, is how important are the PBR design and operational conditions in modulating production.

If the product of interest aligns with DOC production then light limitation must be minimised, and nutrient limitation may provide a route to further enhancing production. However, continual nutrient limitation is not only bad for the microalgae (risking death by physiological damage or over-running by contaminating bacteria), but production is itself low. A better approach may be to grow cells in good conditions (rapid healthy growth to a high biomass) followed by down-shock due to nutrient exhaustion. The availability of light at the point of nutrient exhaustion will control the

rapidity of the down-shock and the production and release of metabolites. The interconnection with P-stress (and for diatoms, of Si) will also play a role here.

If the product of interest aligns with DFAA production, then it is likely important that nitrogenous nutrients are not exhausted. Whether that is achieved by using PBRs with a greater optical depth (Oz; **Fig.12.8**), or supplying additional (excess) N-nutrient depends on the operator. **Fig.12.13** shows output when N-status is controlled by an increased frequency in harvesting. An alternative, is to explore the consequences of N-refeeding; a batch culture would be grown into N-limitation, and then under conditions of good illumination they would be refed. This results in up-shock and an over production of N-rich metabolites which then leak from the cell. Again, interconnection with P-stress (and for diatoms, of Si) will also play a role here.

By altering the physiological parameters 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 year's 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. As always, 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.

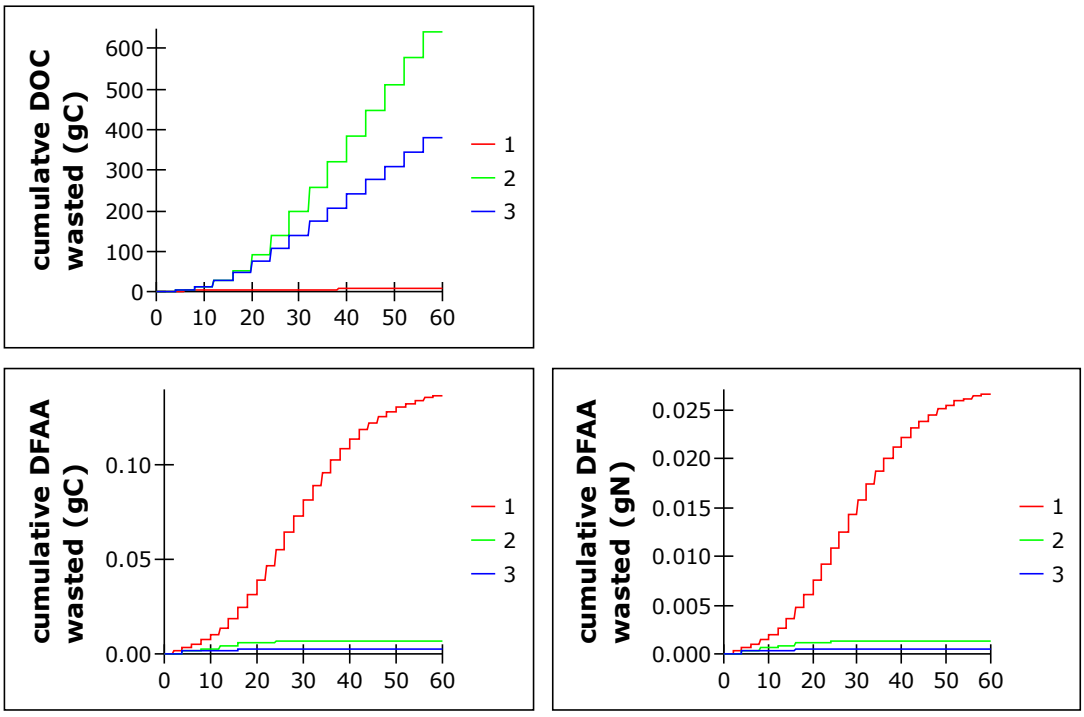


Fig.12.13 Model output when the value of *har_frq* was held as 4d throughout the simulation. This prevented PBR#1 from exhausting nutrients; the production of DFAA is now much higher while production DOC is trivial – compare with **Fig.12.10**.

12.5 Caveats

Many of the caveats given in **Chapters 8, 9, 10 & 11** apply here also, but there are also the following caveats to consider.

- The production of released metabolites is particularly poorly understood from a rate-process angle, such that building a DST for this function carries additional risks and challenges.
- The production of organic sources of nutrition, especially by what are often physiologically stressed microalgae, presents a very real risk of bacterial or fungal contamination. Strict aseptic control measures must be observed at all stages to minimise such a risk. Even so, it is likely that the system will on occasion become contaminated.
- The model, as presented, is not suitable for exploring the production of organic solutes supported by the addition of similar types of solutes as nutrients. Thus, production of a N-rich solute promoted by growth on a N-rich solute, cannot be explored as both compound groups are here labelled as “DFAA”. Likewise for C-rich compounds labelled here as “DOC”.
- Any contamination events will likely require the closure and cleaning of the system that will add significantly to loss of revenue. This needs to be considered in making any use of the DST for planning.

13. Other platforms

13.1 Introduction

This work has concentrated on the physiology and growth of microalgae, and then on their growth in PBRs. There are various other ways that microalgal growth can be exploited to commercial, or at least near-market, advantage. Here we briefly consider two such applications.

To develop models for such deployments, you will need to build or modify the (non-Cockpit) versions of the models provided (see **page ii**). You will also need access to a full licence for Powersim Studio 10 (see **Chapter 7**), or rebuild the model from the equations given in the **Appendix** in the platform of your choice.

13.2 Using microalgae to remove nutrients from grey water

In this work the emphasis has been on producing microalgal biomass, or products of microalgal metabolism. During growth, microalgae are capable of stripping nutrients from the water to levels that approach or are below those of chemical detection. In a world of diminishing resources, recovery of nutrients that are otherwise seen as problematic wastes is increasingly questioned.

Nitrogenous and phosphorous containing waters cause serious environmental damage through promoting eutrophication. Much of these wastes come from agricultural runoff or from sewage treatment, and from farm effluent. Phosphate fertilizer is itself forecast to become increasingly expensive as stocks of readily minable salts are exhausted (Blackwell et al. 2019). Recovery of especially P is thus important. If it were possible to derive some additional value from the microalgal biomass then all the better.

To explore the use of microalgae in stripping water of DIN and DIP, the same model as used in **Chapter 10** can be used. All that is necessary is to configure the incoming nutrient streams to be consistent with those of the grey water, and to then configure the PBR systems, including harvesting regimes, to best effect.

There are a few things to note in exploring this system:

- The P content relative to N in grey waters, and especially of those from digestates, is often very low, and conversely the N content as ammonium very high. It is likely that additional P will need to be added to enable the extraction of the N. If this is not done, then there will be much N remaining. If the incoming water is diluted, such that the N content is low (and hence does not drive high levels of self-shading), it is quite likely that the residual P content will be very low. Either way, care will be needed to get the balance of DIN and DIP, and the absolute concentrations, right.
- The mode of harvesting will need careful consideration. It is likely that a simple continuous-flow chemostat system will be most appealing. At least for this application the usual chemostat-centric problems of media preparation are not an issue. However, microalgae will likely evolve to a lower maximum growth rate (Droop 1974; Flynn & Skibinski 2020) when grown in such systems, having an adverse impact on the efficiency of the system over time.
- It is most likely that such systems will rely at least partly on natural lighting. Variations in sunlight with the weather will likely have important implications for system efficiency.
- The usefulness of the microalgal biomass will be affected by laws controlling the recycling of nutrients from waste waters. While no such constraint applies to the recycling of animal

wastes via sileage back into the human food chain (practices that have continued for centuries), the situation with microalgal systems is unclear.

13.3 Coupled systems

A major challenge in working with microalgae is that of harvesting organisms that are so small and also that are present at very low levels of abundance even in the most dense suspensions. One obvious solution is not to harvest the biomass at all, but to use it to directly support the growth of plankton-feeding animals, such as bivalves.

To explore such scenarios, it is necessary to construct and then couple a PBR model (as described in this work) with an animal tank model. Components of such a combined model are found in this work and in that of Flynn (2018).

It is important to consider the following aspects:

- Food quality is very important for the growth of animals. This may require the simultaneous provision of several microalgal species, likely grown in separate PBRs. It is also important that the balance of protein and (especially essential) fatty acids is optimised; that requires modulation of the nutrient and light regimes, as described in **Chapters 9 & 10**.
- The microalgal biomass abundance (mgC m^{-3}) does not need to be high for effective feeding of the animals. This permits a significant disparity in tank (PBR) sizes between microalgae and animal.
- Flow rates need to be controlled to account for changing consumption rates as the animals grow. This may be controlled using turbidostat systems.
- The outflow water from the animal tank will contain both dissolved inorganic nutrients (regenerated by the animals during respiration) and organics (dissolved and particulate). The DIN and DIP could be useful recycled back to the PBR, while digested organics (in another tank-system, e.g., anaerobic digestion) may also provide a source of nutrients for recycling.
- Robust systems, with some level of redundancy, are important else if PBRs fail then the animals may starve.

14. Conclusions

14.1 Overview

The aim of this work was to provide a Decision Support Tool for the planning of bulk microalgal growth and production. The target was very deliberately generic, not linked to specific species. To enable this, a mechanistic model structure was exploited in which the user can control fundamental characteristics (traits) of the organism(s) of interest. If you want a species-specific model, then if you have sufficient data you can make simple regression function and not use a mechanistic model. However, regression and other empirical description cannot provide a platform for testing scenarios as can be explored using simulation models.

Inevitably, this DST as it stands cannot describe all the details of a real system. It is in any instance important to recall that microalgae may evolve rapidly in culture systems where their growth is forced along a fixed pattern. Thus, Droop (1974) observed that when he grew the microalga *Monochrysis* in a slow dilution rate chemostat it lost the ability to grow rapidly; in terms of the models described in this book, the value of the maximum growth rate decreased. Not only that, but the minimum P:C quota also changed.

It is thus important to explore the risks of growing large cultures with a careful eye on what even the most comprehensively tuned DST model can describe. This, recall, is before including a multitude of other operational problems surrounding the handling of metric tons of growth media, the vagaries of natural sunlight and variable temperature for an outdoor PBR, the risks of contamination, etc. etc.

At the end of the day, however, a DST can still provide you with a much better understanding of the system than trying to conduct an endless stream of real experiments, with their attendant cost in time and money.

14.2 Challenges for future resolution

The construction and testing of simulation models is a supreme test of our knowledge. And it will have become clear that we understand surprisingly little about microalgal cultivation in specific terms. Detailed, high level science on microalgae, exploiting molecular biological tools and detailed chemical analyses, are very rarely coupled with the levels of data measurements that typified the science in the 1960-1980's. In that period, cell numbers, nutrient concentrations, pigment levels, C,N,P biomass, protein and lipid analyses formed the bedrock of microalgal science. Critically for system dynamics models of the type that are needed to support DSTs, such data are vital. More importantly, high resolution time series data of as many types of these data are needed.

The value of the data from the 'omics revolution lays in the detail of how to manipulate organisms to emphasise particular pathway in microalgal production. Such details then need to be translated into system dynamics models. A whole new branch of biology, 'systems biology', has developed over the last decade or so. There are clear overlaps between 'systems biology' and 'system dynamics' but the former tends to be more specific in its detail, and the latter (as used in this DST) more holistic.

The challenge for the science of microalgae extends beyond the complexity and expense of measurements of core parameters and detailed biochemical processes, to the need to undertake studies in bulk culture systems. At the least, cultures need to be of a 10L volume, grown in a culture vessel with an optical depth (usually a diameter) similar to that in true bulk culture systems, such as tubular PBRs or similar.

So, to progress the science, funding directed at commercial end products needs to acknowledge that culture systems need to be of at least a certain size, and that good "old-fashioned" measurements need to be taken frequently. This problem is far from unique to commercial-facing microalgae studies; they apply equally to plankton science in general. These core measurements of elemental stoichiometry and nutrients are expensive, often problematic in marine systems because of interferences from salt, and perhaps viewed as boring; however without them the value of the exciting science is greatly diminished. This problem is demonstrated perhaps most clearly in the context of the microalgal biofuels agenda, where too many extrapolations have been made using questionable starting figures generated from very small-scale culture studies (Kenny & Flynn 2017).

14.3 Improving DSTs

Improving DSTs in support of microalgal growth requires comprehensive data series for both the abiotic system and the biotic system. It also requires integration with a finance model so that the costs associated with different strategies can be considered.

First and foremost, the conceptual basis upon which the biotic component is built needs to be accepted as being plausible. The model used here contains a mixture of components that have been developed over several decades, and shown to be robust and capable of tuning to fit experimental data well. I certainly do not claim the model or the underling approaches to be perfect. No model can be perfect as they are all inevitably a simplification of reality. Hopefully through the descriptions given, coupled with those in Flynn (2018), others will be spurred on to do a better job with their own models.

It is easy to find fault, but much harder to correct the errors without messing up some other part of the model. Indeed, various parts of the model used here in **Chapters 9** onwards, have already been replaced in my new models in the search for a better way to describe plankton functional types (not just microalgae). I hasten to add that the behaviour of the latest model aligns closely with that of the model used in this DST, it is just better suited to other scenarios.

Any financial submodel needs to be married to a risk analysis. Profitability depends on the costs and profits. Costs are constantly changing, and there is the perennial problem that the value of a product declines as production ramps up and the market becomes less niche. Establishing the bounds of profitability depends on a level of foresight in future demand and selling costs at least as much as it relates to manufacturing costs. One of the factors that cripples the argument for microalgal biofuels is the fact that fossil fuels are simply so cheap and plentiful (though Kenny & Flynn 2017 argue that environmentally such a production is most unlikely ever to be even environmentally acceptable given the low areal production rates of microalgae).

14.4 Final words

Hopefully the foregoing will stimulate further developments in microalgal research and applications. The promise of commercialised microalgal exploitation has been around from at least the 1970's, but other than a very few niche markets (for what are usually rather crude products), little has come to pass despite a great deal of expense. Perhaps, with the rise of "green economy" thinking, the microalgal dream will finally become a reality. For it to do so requires a thorough understanding of the complexity of growing the organism in the first instance. Satisfying that need, I would argue, is focussed most clearly through simulation modelling, and thence through the development and application of DSTs.

Appendix 1. Model Description

The model in this Appendix describing microalgal physiology (SAPPM) was developed within the EU-funded MixITiN project.

*Project **MixITiN** received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 766327. This document reflects only the author's view; the REA and the European Commission are not responsible for any use that may be made of the information it contains.*

Descriptions of the culture systems, and allied linkages between the SAPPM model and those systems to form the DST was developed within the ERDF funded EMA project.

*Project **EnhanceMicroAlgae** was funded by the European Regional Development Fund (ERDF) Interreg Atlantic Area programme (EAPA_338/2016; "High added-value industrial opportunities for microalgae in the Atlantic Area").*

Glossary for commonly occurring terms and abbreviations in this Appendix

See also Appendix 2 for model parameters

AP Acquisition Potential; potential for resource acquisition. To achieve the maximum potential requires a saturating amount of substrate.

APCM Acquisition Potential Control Mechanism. The means by which AP is modulated; this is linked to quota values, such as N:C or P:C.

Diazotrophy N_2-fixation (a process performed by some species of cyanobacteria if they are N-stressed and hence the synthesis of nitrogenase is de-repressed).

DIP Dissolve Inorganic Phosphate.

DOC Dissolved Organic Carbon (e.g., sugars)

DFAA Dissolved Free Amino Acids

DST Decision Support Tool

ESD Equivalent Spherical Diameter

Microalgae microscopic phototrophic organisms, as chlorophyll.a containing cyanobacteria and/or protist plankton.

Mixoplankton protist plankton that are capable of both phototrophy and phagotrophy.

Mixotrophy a means of nutrition involving both autotrophy (typically phototrophy) and heterotrophy.

Osmotrophy a means of heterotrophy involving the acquisition and exploitation of dissolved sources of energy and C, such as the use of *DOC* and *DFAA*. A process ubiquitous in microbes.

Phagotrophy a means of heterotrophy involving the acquisition and exploitation of particulate sources of energy and C (typically involving predation and engulfment).

Phototrophy a means of autotrophy exploiting energy from light.

Protist single celled eukaryote organisms.

RuBisCO Ribulose-1,5-bisphosphate carboxylase-oxygenase – the primary enzyme in CO_2 fixation.

SAPPM Switchable Acclimative Protist Plankton -Model. A single model construct that through switches can be used to describe various protist functional types.

SCEB Satiation Controlled Encounter Based predation model. An approach to describing the interactions between prey encounter, capture, assimilation and modulation thereof by consumer satiation. See Flynn & Mitra (2006).

UmT Maximum growth rate at a stated temperature.

UmRT Maximum growth rate at a stated reference temperature.

A1.1 Introduction

This appendix describes the core variable stoichiometric physiological model for the microalgae in **Chapters 10, 11 & 12** in the DST. The model was originally developed as a single structure for describing protist plankton, although it could also be used to describe other plankton microbes.

Explicitly, the model describes the following organism groups:

- Protozooplankton (osmotrophy & phagotrophy)
- Phytoplankton as non-diatoms (osmotrophy & phototrophy)
- Phytoplankton as diatoms (osmotrophy & phototrophy)
- Constitutive mixoplankton (osmotrophy, phagotrophy & phototrophy)

The model, in the form of non-diatom phytoplankton, is also applicable to describing non-diazotrophic cyanobacteria.

While for the DST phagotrophy is not considered, a full description of the whole model is provided here. This is in reflection of the newly found appreciation of the importance of mixoplankton (protists that engage in osmotrophy, phagotrophy & phototrophy; Flynn et al. 2019), and that production of metabolites (notably toxins) by these organisms may prove to be of commercial interest. It should be noted that heterotrophy in microalgae which are now (Flynn et al. 2019) identified as "phytoplankton" is via osmotrophy alone.

The model as described here has the operational title of the –

Switchable Acclimative Protist Plankton -Model "**SAPPM**"

From here on, and in the context of this DST, "protist" and "microalgae" are considered as interchangeable terms, including the instance of non-diazotrophic cyanobacteria as "phytoplankton".

The model as described, and as detailed in Appendix 2, was built and operated within the ODE-modelling platform provided by Powersim Studio 10. The equations, and exact same model output can, however, be produced in other ODE platforms, such as GNU-Octave (Akoglu & Flynn 2020).

A1.2 Providence

The construction of SAPPM follows a series of models dating from Flynn et al. (1997), including Flynn (2001, 2003, 2006, 2008a,b). At the core of all of these models is the use of sigmoidal feedback terms (mimicking allosteric regulation in biochemistry) to modulate the expression of facets of physiology. These functions have as inputs variables that report satiation for the nutrient in question (e.g., N-source acquisition potential is controlled by cellular N:C).

The models have been used across many organism types, with good fitting to data (e.g. Flynn et al. 2001; John & Flynn 2002; Flynn et al. 2005; Mitra & Flynn 2006).

In essence, then, SAPPM is well founded as a logical progression from a series of previous developments. The main advances achieved here are described in **Section A1.3**.

A1.3 Overview Description

SAPPM is an ODE-based system dynamics model capable of describing the growth and activities of contrasting protist plankton functional types of different allometries and stoichiometries, and displaying acclimation to changes in the environment. The model deploys an innovative approach (an Acquisition Potential Control Mechanism – APCM; **Section A1.4**) to describe and control the exploitation of different nutrient types. This brings together and harmonises the use of sigmoidal feedback terms used in previous models (see **Section A1.2**).

The functional types currently described are:

- Protozooplankton (protoZ; osmotrophic, phagotrophic)
- Phytoplankton, non-diatom (protoP; phototrophic, osmotrophic)
- Phytoplankton, diatom (phototrophic, osmotrophic)
- Constitutive mixoplankton (CM; phototrophic, osmotrophic, phagotrophic)

These types are selected for by assigning value to specific variables (constants) that de facto operate as switches.

The stoichiometric currencies used are C, N, P, plus for diatoms, Si.

The state variable requirements for the protist are:

- C
- N
- P
- Si (diatom only)
- Chl (phototrophic species only)
- Average growth rate
- Average gross photosynthetic rate (phototrophic species only)

The protists can be additionally described with respect to:

- Size (ESD) and motility (including changes in motility with satiation)
- Range of stoichiometry (C:N:P; for diatoms, C:N:P:Si)
- Variable (i.e., acclimative) Chl:C
- Exploitation potential for NH_4^+, NO_3^-, DIP, DOC, DFAA, all linked to nutritional status and scope for growth
- Exploitation of prey (food) particles of different sizes, motilities and different qualities (preference, palatability, toxicity, C:N:P), all linked to encounter rates and turbulence
- Obligatory need for photosynthate in phytoplankton or mixoplankton (affecting capacity for heterotrophic growth in darkness)

A1.4 Normalised Acquisition Potential Control Mechanism (nAPCM)

The following explains the functioning of the normalised APCM concept.

The nAPCM makes reference to normalised quotas of N:C, P:C etc, 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 equations are also simplified.

- Acquisition potential for different resources is controlled using a Goldilocks construct to simulate (de)repression of physiology. This permits complex multi-nutrient and multi-stressor interactions to be considered.
- 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 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.

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.

In total then, equations make reference to:

- nI normalised input (referenced to a variable, such as N:C)
- nOI normalised optimum 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 is shown in **Fig.A1**.

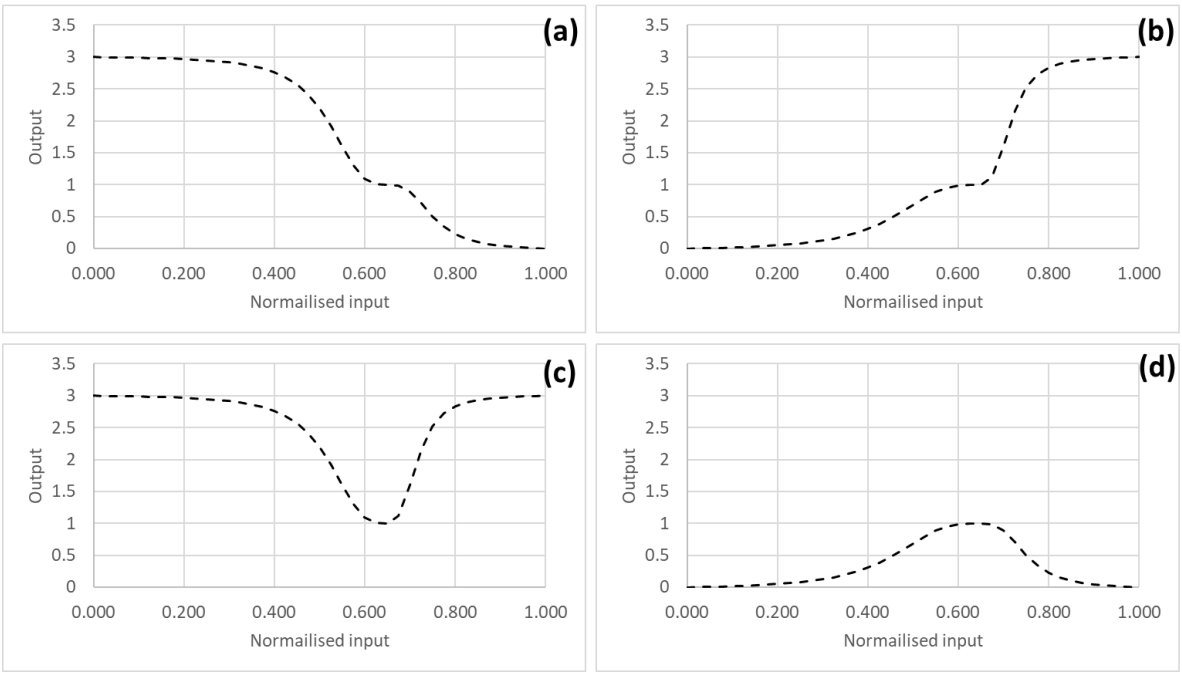


Fig.A1 Four different response curve configurations. In each instance the optimum is at 0.65, at which point the output is 1. Curve (a) could represent a de-repression of DIN transport as N:C decreases below the optimum and a repression above the optimum. Curve (b) could represent a de-repression of 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, 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.A1**, 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, JPR).

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.

The following Sections should be read cross-referenced with Appendix 2.

A1.5 Switching Between Protist Function Types

Switching is not achieved by a single switch (though processes described below could be coupled such that a "master switch" could be configured), but rather through setting several characteristics.

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

ChlCm : the maximum Chl:C ratio; this must be zero for protoZ as these are not phototrophic.

NCo and **PCo** : the minimum cellular N:C and P:C; these may (likely) be low for phototrophic organisms but will be closer to the optimal values (*NCopt*, *PCopt*) for non-phototrophic protists.

Optimal_CR : the proportion of encountered optimal prey that are captured; this may likely be 10-20% (i.e., 0.1 – 0.2). The value is 0 for *protP*.

RelminUmPS : the minimum proportion of growth to be supported by phototrophy; this is to account for the fact that many protists capable of phototrophy seem to have an absolute requirement for light and thence for phototrophy else they cannot grow. They may, however, be able to survive in darkness. If growth is possible in total darkness, this value must be zero.

RelPSm : the relative value of *PSmax* (which de facto is set in reality by the cellular enzyme activity of RuBisCO) compared to the maximum growth rate at the current temperature (*UmT*). 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 *UmT*.

RelUmNH4 : the relative growth rate compared to the maximum growth rate at the current temperature (*UmT*) that can be supported by growth using ammonium-N. Typically this would be 1. This value must be set as 0 if unable to use NH4.

RelUmNO3 : the relative growth rate compared to the maximum growth rate at the current temperature (*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 nitrate.

RelUmPS : the maximum relative rate of growth on phototrophy as described as a proportion of *UmRT*. For protozooplankton this must be set as 0. For pure phototrophs this may be less than 1 if *UmT* can only be attained by the support of osmotrophy (exploiting DOC, or DFAA).

sw_diat : the switch selecting for "diatom". This automatically disallows predation and stops motility, and also enables Si uptake.

sw_mot : the switch selecting for motility. 0 for no motility, and hence sedimentation; 1 for motility

UmRT : the maximum growth rate at the reference temperature. The actual maximum growth rate (*UmT*) depends on temperature. Protozooplankton and diatoms, in particular, can exceed a division per day (0.693 d^{-1}), but most non-diatom protP and mixoplankton will be 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.

A1.6 Nutrient Transport and Osmotrophy

The nutrients described in the model are:

- Ammonium
- Nitrate
- Phosphate
- Silicate (for diatoms)
- DOC
- DFAA

Of these, all but the silicate are described using a similar general construct that relates the acquisition potential (AP) to the nutrient status.

The Forrester diagram is shown in **Fig.A2**, with the auxiliary describing AP in cyan.

Ammonium and nitrate transports are controlled by reference to the N:C quota. When this declines the AP increases. AP for ammonium develops at higher N:C and develops more rapidly than for nitrate. This enables the description of the ammonium-nitrate interaction, with ammonium usage being preferred, and there being 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 is controlled by reference to the P:C quota. Like the control of ammonium and nitrate, reference to the quota uses the normalised quota construct of Flynn (2008b).

Silicate transport is different because the control and fate of this nutrient usage is different, related as it is to the cell-division cycle. The description of silicate uptake follows that of the short-cut version of Flynn & Martin-Jézéquel (2000), as per Flynn (2001).

DOC transport is similar to that for ammonium, in that it references N:C, but it operates at the opposite end of the N:C spectrum. Thus, while ammonium and nitrate AP is increased at low N:C and curtailed at high N:C, DOC AP increases as N:C increases (and hence when the cell is C-limited).

DFAA transport differs because amino acids comprise both a C and N source. DFAA AP is thus increased at both low and high N:C.

Depending on the settings for an absolute requirement for some proportion of C coming via phototrophy, growth can proceed even as far as the value of UmT on osmotrophy (using DOC+DIN, or DFAA, +DIP of course).

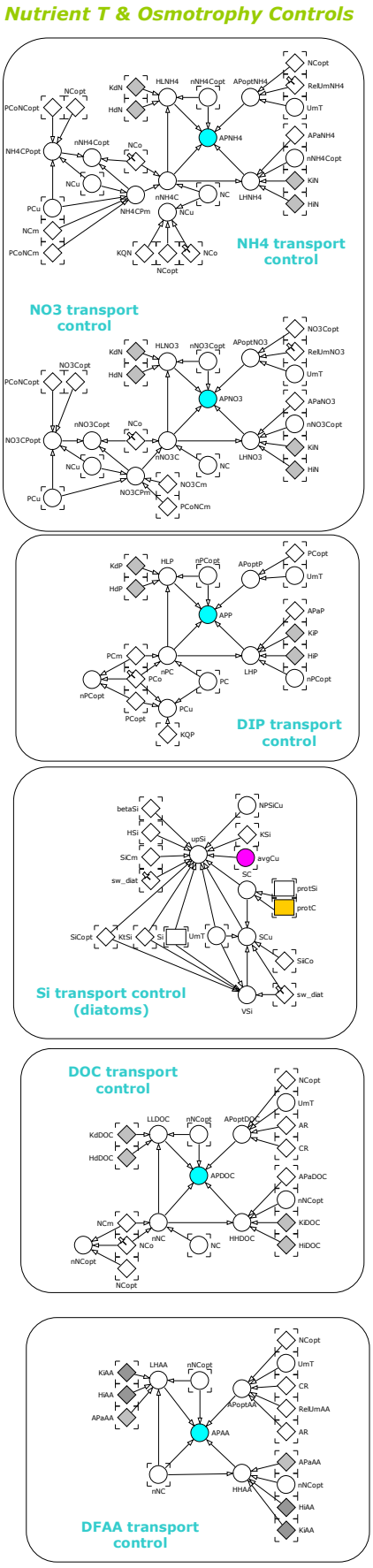


Fig.A2 Nutrient transport and osmotrophy.

A1.7 Phagotrophy

Phagotrophy is used by protozooplankton and mixoplankton. The Forrester diagram is shown in **Fig.A3**. Much of this follows the description in Flynn (2018) and can be developed further according to ideas in that text as well.

Both prey and the protist predator are assigned a size (ESD) and speed of motility. Motility for the protist is decreased as the organism becomes satiated or it can be switched off; similarly, motility of the prey can be altered if required. Turbulence is also an input variable if required. Predator-prey encounter follows the usual calculations, making reference to the sizes of the organisms and their speeds. Care must be taken to ensure the equation references the fastest organism at the appropriate part of the calculations. There are then additional terms that determine whether the encounter results in a successful capture. These could be developed to include allometric and palatability/toxicity relationships (see Flynn 2018).

Having established the relative rates of potential prey capture for the different prey items, the actual rates of capture are then calculated using the SCEB approach (Flynn & Mitra 2016).

Prey assimilation makes reference to the total aggregate food quality with respect to the ingested C:N:P. In reality, prey of different qualities would likely be degraded differently. However, it is likely that if one prey is of good C:N:P then other prey growing in the same water body would also be of at least reasonable quality. Quality is handled through the values of *AEqual* and *stoich_con* (See **Appendix 2**). This model does not modify assimilation efficiency (*AE*) through reference to prey quantity, though this could be readily introduced, as per Flynn (2018).

There is an acquisition potential control for phagotrophy, which makes reference to concurrent levels of phototrophy for those protists with an obligatory need for some level of photosynthesis.

Emergent values of AE for C, N, P are computed.

As described here, only two prey types are described. The coder could elect to potentially allow any functional type to engage with all other functional types (and thus configure the phagotrophy controls to reference the total number of functional types, including self), or that no individual type will be able to consume more than a few functional types. For full flexibility the former route would be preferable, though this may make for very long equations if there are more than ca. 6 functional types. It is important to note that a functional type in this context includes allometric divisions; small medium and large diatoms would thus equate to 3 functional types.

The Forrester diagram for phagotrophy is shown in **Fig.A3**.

Phagotrophy Controls

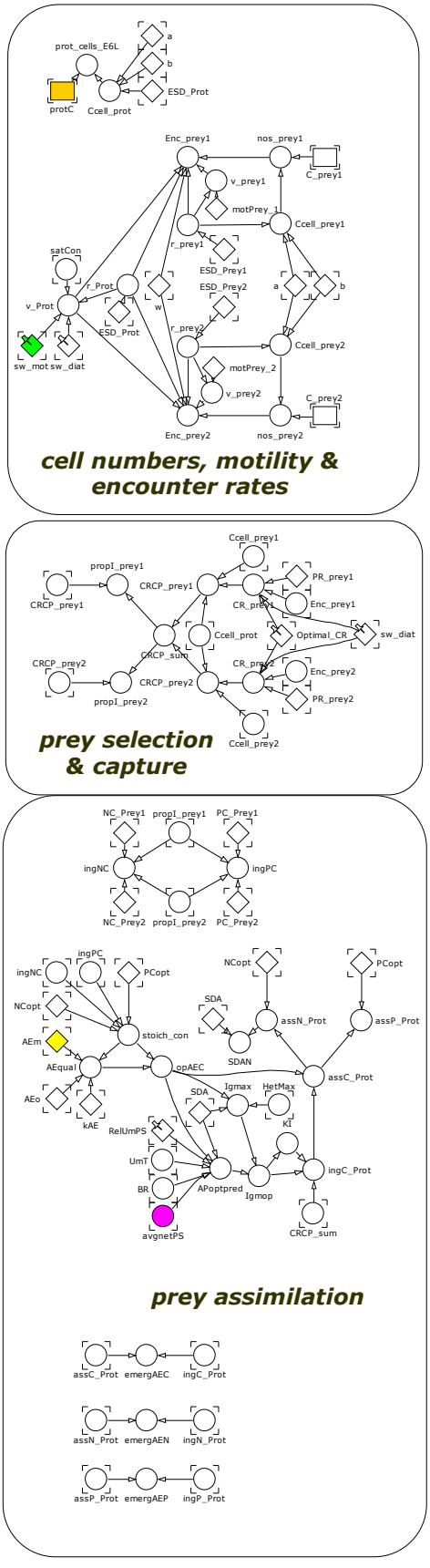


Fig.A3 Phagotrophy

A1.8 Phototrophy

The phototrophy description is developed from Flynn (2001), although it has the following modifications:

- A stated minimum Chl:C to prevent the value going too close to zero
- 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 is an important advance because it permits growth in L:D cycles to approach that in continuous light by increasing the rate of C-fixation during the L phase. However, this scope requires the addition of a state variable to record the average growth rate over the last day or so.
- Osmotrophy using DOC can depress the need for C-fixation
- Maximum growth may not be attainable by phototrophy alone (set by *RelPSm*)
- A critical minimum amount of C coming through phototrophy is set by *minPhotUm*.

Photosynthesis is computed as previously implemented (using the Smith equation; see Flynn 2018).

The Forrester diagram for phototrophy is shown in **Fig.A4**.

Phototrophy Controls

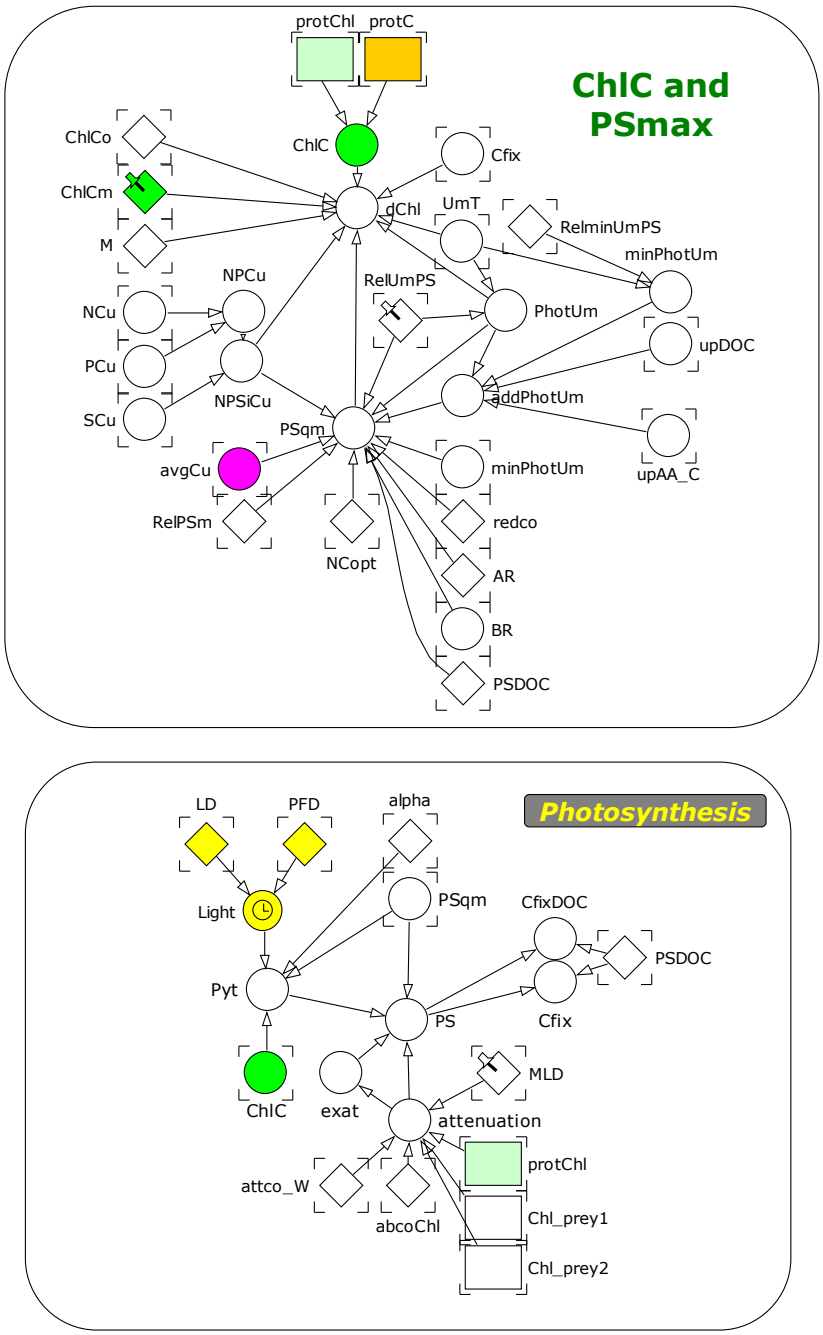


Fig.A4 Phototrophy

A1.9 Growth

Growth is the balance of all C-inputs and outputs. Outputs include respiration associated with anabolic and catabolic activities, nitrate reduction, and SDA.

The control of DFAA uptake, and of DOC uptake vs leakage are also handled within this block (see Forrester diagram in **Fig.A5**).

As part of growth regulation, and the control of phototrophy, the model makes reference to the moving average of net growth and net photosynthetic rate; these are coloured purple in the Forrester diagrams. The original Studio implementation made use of the "delay-pipeline" function to calculate the true moving average over the previous 1d. This requires the programme to hold the values of each of these rates for the previous 1 days-worth of timesteps. An alternative approach is available that at each timestep subtracts a fraction of the previous "average" and adds a similar proportion of the current timestep's value.

The average growth rate is also used to compute a satiation control (*satCon*) index, which is also used to modulate swimming movement of the otherwise motile protist.

Phagotrophy deploys an eat-to-live approach (Flynn 2018).

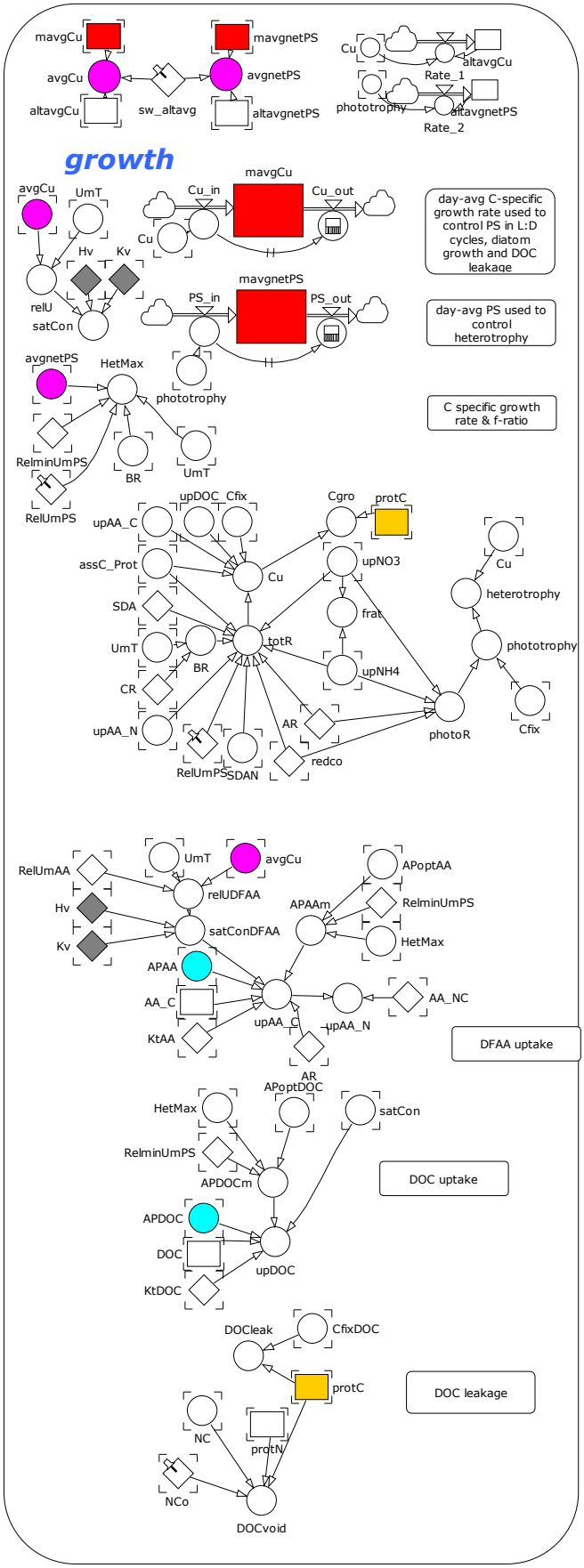


Fig.A5 Growth processes

A1.10 Temperature

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} (**Fig.A6**).

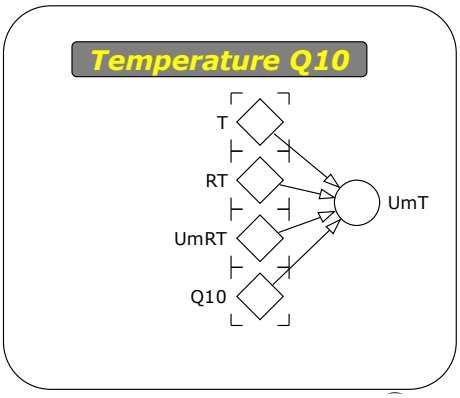


Fig.A6 Temperature

A1.11 Protist Biomass

Biomass is described as state variables (with units of g element m^{-3}), for C, N, P, and also for diatoms, Si. Chl also has a state variable. See the Forrester diagram in **Fig.A7**.

There are outputs for C (respiration and DOC), N (regeneration) and P (regeneration). These releases also prevent the stoichiometric ratio of N:C and P:C becoming too large.

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

P increases by phagotrophy or 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 protist.

Chl synthesis and degradation is described via *Chlgro* (positive or negative). Stoichiometric allocations to photosystems are not explicitly defined, so C,N,P associated with Chl and phototrophy are all included within the bulk protist C,N,P state variables.

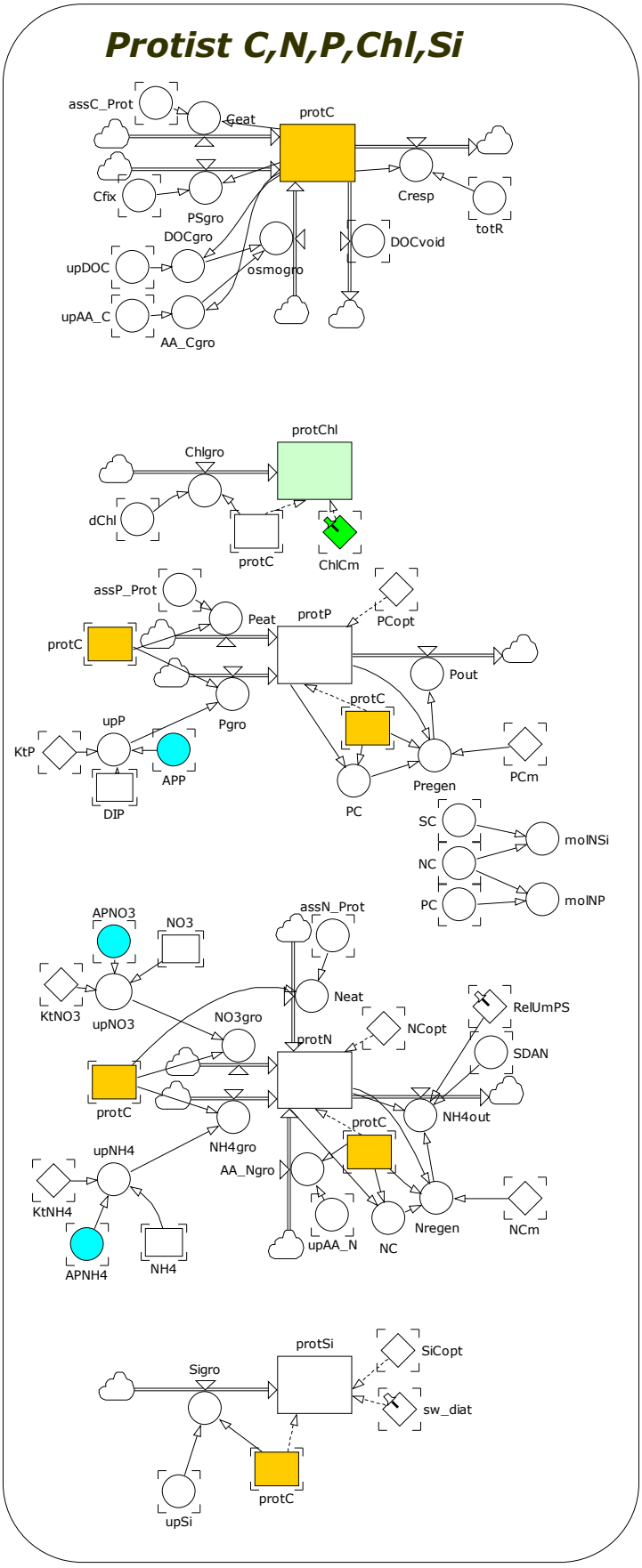


Fig.A7 Protist biomass

A1.12 Voided Biomass

Biomass ingested is part digested (according to AE for each of C, N, P) and the balance is voided (**Fig.A8**). With changes in the C:N:P of prey (food), the AE for each element differs and hence so does the N:C and P:C of the voided matter.

The model makes no reference to the reprocessing of voided matter (e.g., Flynn & Davidson 1993). To do so would require an additional "prey" type to be specified for phagotrophic selection and ingestion.

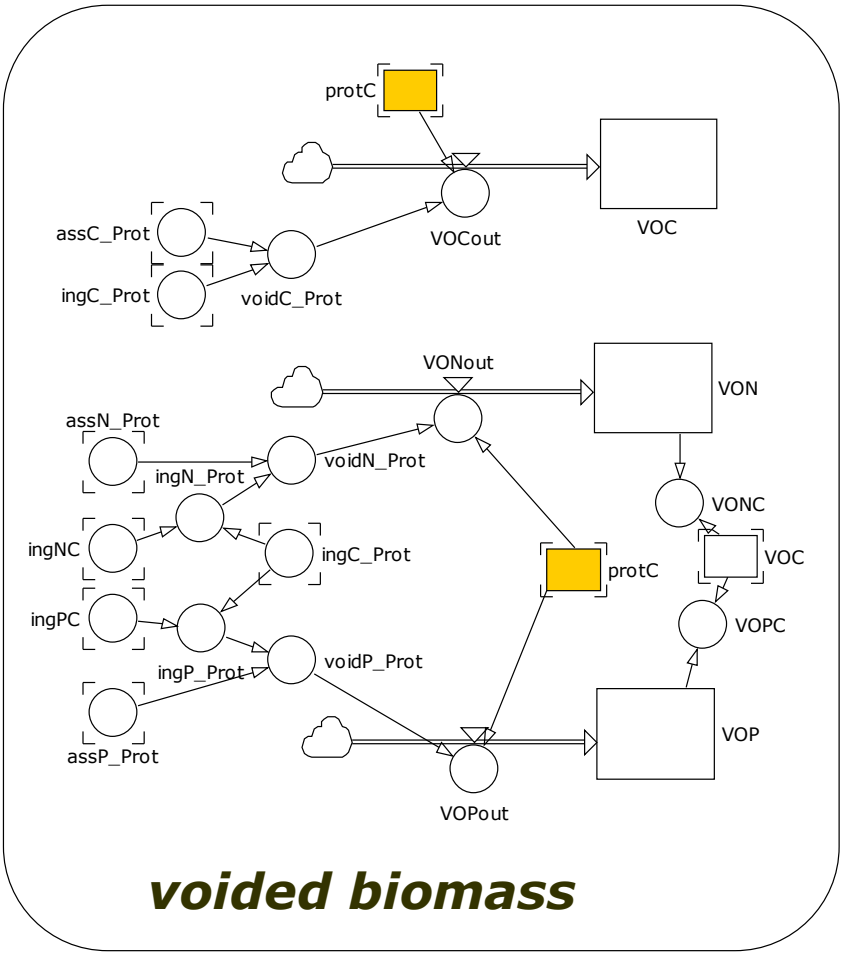


Fig.A8 Voided biomass

A1.13 External Nutrients

External nutrients include (**Fig.A9**):

- Nitrate (as gN m^{-3})
- Ammonium (as gN m^{-3})
- Phosphate (as gP m^{-3})
- Silicate (as gSi m^{-3})
- DOC (as gC m^{-3})
- DFAA (as gC m^{-3} and gN m^{-3})
- DIC (as gC m^{-3})

The N:C of the DFAA can be altered as required.

System C, N, P and Si are calculated.

DIC is included only as a balance check; there is no reference made to DIC-limitation (nor to pH which would change as DIC is consumed).

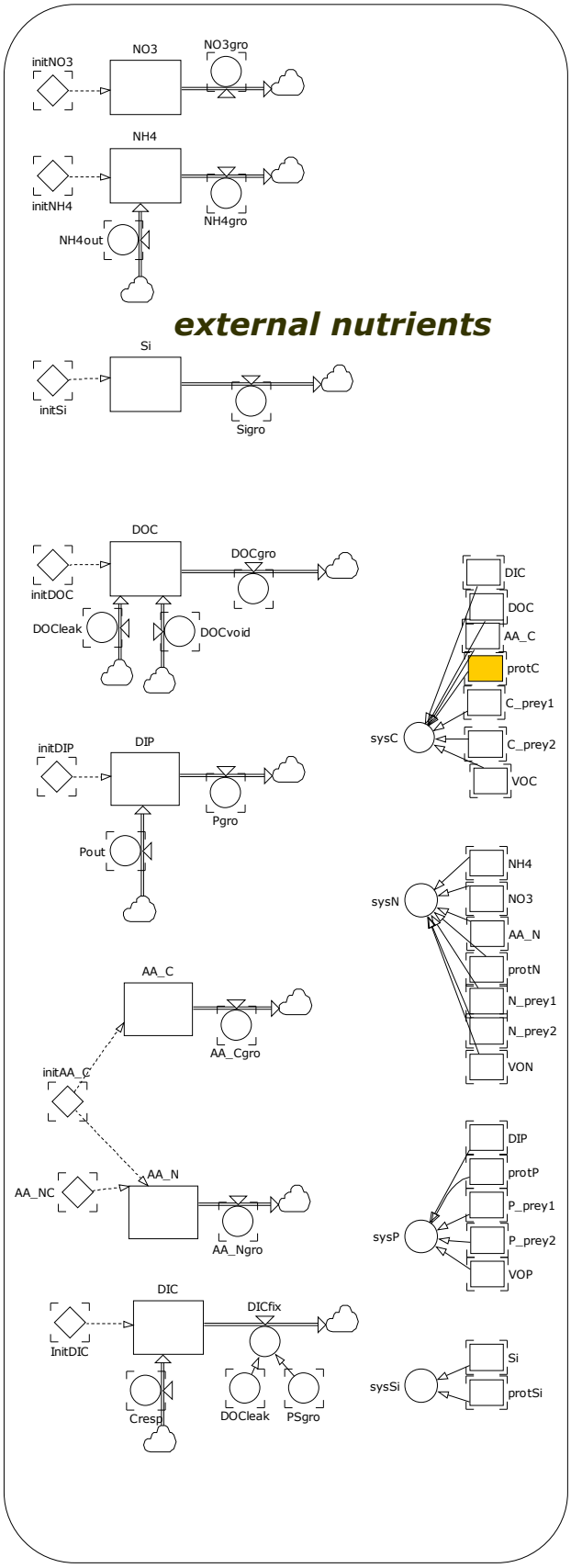


Fig.A9 External nutrients

A1.14 Prey

Prey are described (**Fig.A10**) in terms of C, N, P and Chl. C,N,P are involved in defining the biomass transfer during phagotrophy and the allied stoichiometric issues of palatability, AE and nutrient regeneration.

Prey Chl in this model has a function simply affecting light attenuation; in testing the protist configured as a phototroph it is important to thus set prey biomass as zero else there is a large light attenuation signal from the prey.

Prey also have attributes of allometry and the movement, as used in the prey encounter module.

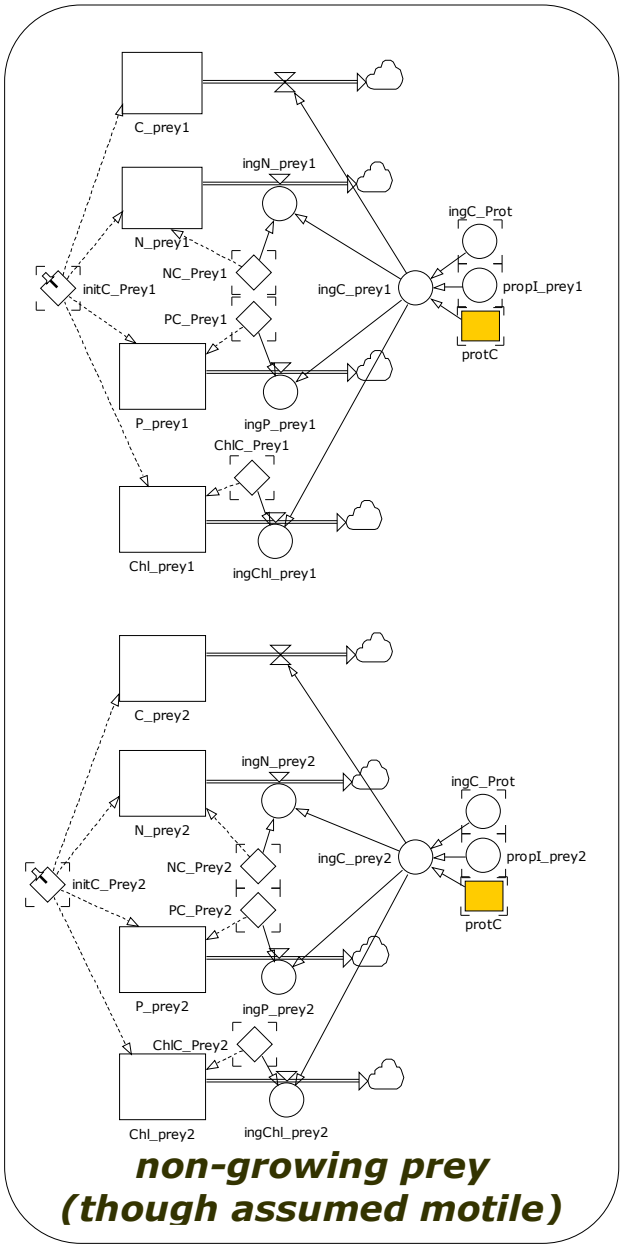


Fig.A10 Prey

A1.15 References

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Appendix 2. Equations

The model equations in this Appendix describing microalgal physiology (SAPPM) was developed within the EU-funded MixITiN project.

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Equations describing the culture systems, and allied linkages between the SAPPM model equations and those systems to form the DST was developed within the ERDF funded EMA project.

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This appendix provides the full equations used for the model described in **Chapters 10-12**. This is an arrayed model describing a single species of protist (which can be configured to describe various functional forms, see **Appendix 1**) growing in 3 photobioreactors (PBR). Accordingly, the equations are configured in an arrayed format with the array having dimension "PBR" where, here, that array size is 3 (i.e., 3 different PBR configurations are possible).

The model details are provided in three tables:

1. State variables, including flows in and out
2. Constants
3. Auxiliaries, which provide the equations themselves (including the equations for the flows for the state variables)

A word about the "constants" – there are very many constants but the vast bulk of these contain numbers that most modellers would have placed explicitly within equations as they control broad functionality. The constants that are of especial importance for modification to configure the model for different species of different PBR setups, are indicated in colour (see **Table.2** legend).

The equations are those specifically for the Powersim Studio-10 model. However, with some minor modification to take account of differences in syntax, these equations may be used in other platforms, including Fortran or GNU Octave.

For the state variables, flows are designated as "+" if entering the state variable (gain), and "-" if exiting (loss).

For further information on modelling using this approach, see Flynn (2018) and Akoglu & Flynn (2020).

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Table 1. State variables. All have dimensions of "PBR", set here to describe 3 different PBR configurations growing the same species of protist.

Name	Unit	Documentation	Initial value	Flows
AA_Cpbr	mgC	DFAA in PBR wrt C	initAA_C*V	+AACex+DFAA_Cleak -AA_Cgro
AA_Npbr	mgN	DFAA in PBR wrt N	initAA_C*AA_NC*V	+AANex +DFAA_Nleak -AA_Ngro
AlgC_pbr	mgC	C-biomass in the PBR	InitProtC*V	+Ceat +osmogro +PSgro -Cresp -DFAA_Cleak -DOCvoid_pbr -ProtCex
AlgChl_pbr	mgChl	Chl-biomass in the PBR	AlgC_pbr*ChlCm/3	+Chlgro -protChlex
AlgN_pbr	mgN	N-biomass in the PBR	AlgC_pbr*NCopt	+AA_Ngro +Neat +NH4gro +NO3gro -DFAA_Nleak -NH4out -protNex
AlgP_pbr	mgP	P-biomass in the PBR	AlgC_pbr*PCopt	+Peat +Pgro -Pout -protPex
AlgSi_pbr	mgSi	diatom Si-biomass in the PBR	IF(sw_diat=1,AlgC_pbr*SiCopt,0)	+Sigro -protSiex
altavgCu	gC gC^{-1} d^{-1}	alternative calculated day-average net growth rate	0	+altCu_in
altavgnetPS	gC gC^{-1} d^{-1}	alternative calculated day-average gross PS rate	0	+alpPS_in
C_prey1pbr	mgC	prey1 C-biomass in PBR	initC_Prey1/V	-C_prey1ex -ingC_prey1
C_prey2pbr	mgC	prey2 C-biomass in PBR	initC_Prey2*V	-C_prey2ex -ingC_prey2
Chl_prey1pbr	mgChl	prey1 Chl-biomass in PBR	initC_Prey1*ChlC_Prey1*V	-Chl_prey1ex -ingChl_prey1
Chl_prey2pbr	mgChl	prey2 Chl-biomass in PBR	initC_Prey2*ChlC_Prey2*V	-Chl_prey2ex -ingChl_prey2
Cum_gC_Algae	gC	cumulative protist C diluted out (harvested)	0	+gCex
Cum_gC_CO2	gC	cumulative gCO2 used	0	+gC_CO2
Cum_gC_DOC	gC	cumulative g DOC-C used	0	+gDOC_Cex
Cum_gChl_Algae	gChl	cumulative protist Chl diluted out (harvested)	0	+gChlex
Cum_gN_Algae	gN	cumulative protist N diluted out (harvested)	0	+gNex
Cum_gN_NH4	gN	cumulative g NH4-N used	0	+gNH4ex
Cum_gN_NO3	gN	cumulative g NO3-N used	0	+gNO3ex
Cum_gN_NO3_in	gN	cumulative g NO3-N into reactor	0	+gNO3in

Table 1/ cont

Name	Unit	Documentation	Initial value	Flows
Cum_gP_Algae	gP	cumulative protist P diluted out (harvested)	0	+gPex
Cum_gP_DIP	gP	cumulative g DIP-P used	0	+gDIPex
Cum_gProt_Algae	g protein	cumulative protist protein diluted out (harvested)	0	+Proteinex
Cum_gSi	gSi	cumulative g Si used	0	+gSiex
Cum_m3_H2O	m^3	cumulative H20 used	0	+V_out
DICpbr	mgC	DIC in PBR	InitDIC*V	+Co2_inject +Cresp +DICex -DICfix
DIPpbr	mgP	DIP nutrient in PBR	initDIP*V	+DIPin +Pout -DIPin -Pgro
DOCpbr	mgC	DOC nutrient in PBR (the form of DOC is not defined, but is assumed as labile, such as glucose)	initDOC*V	+DOCin +DOCleak +DOCvoid_pbr -DOCex -DOCgro
mavgCu	gC gC^{-1} d^{-1}	day-average net growth rate	1.00E-12	+Cu_in -Cu_out
mavgnetPS	gC gC^{-1} d^{-1}	day-average gross PS rate	0	+PS_in -PS_out
N_prey1pbr	mgN	prey1 N-biomass in PBR	initC_Prey1*NC_Prey1*V	-ingN_prey1 -N_prey1ex
N_prey2pbr	mgN	prey2 N-biomass in PBR	initC_Prey2*NC_Prey2*V	-ingN_prey2 -N_prey2ex
NH4pbr	mgN	NH4 nutrient in reactor	initNH4*V	+NH4in +NH4out -NH4ex -NH4gro
NO3pbr	mgN	NO3 nutrient in PBR	initNO3*V	+NO3in -NO3ex -NO3gro
P_prey1pbr	mgP	prey1 P-biomass in PBR	initC_Prey1*PC_Prey1*V	-ingP_prey1 -P_prey1ex
P_prey2pbr	mgP	prey2 P-biomass in PBR	initC_Prey2*PC_Prey2*V	-ingP_prey2 -P_prey2ex
Sipbr	mgSi	Si nutrient in PBR	initSi*V	+Siin -Siex -Sigro
V	m^3	reactor volume	Reactor_V	+V_in -V_out
VOCpbr	mgC	C-biomass as voided particulates in the PBR	0	+VOCout -VOCex
VONpbr	mgN	N-biomass as voided particulates in the PBR	0	+VONout -VONex
VOPpbr	mgP	P-biomass as voided particulates in the PBR	0	+VOPout -VOPex

Table 2 Constants. dl – no units. Some, as indicated, have dimensions of "PBR", set here to describe 3 different PBR configurations growing the same species of protist. Green names are especially important for configuring the protist; Blue for the PBR.

Name	Unit	Documentation	Value	Dimensions
a	nu	parameter for derivation of C-cell content for protist of a given volume	0.216	
AA_NC	gN gC^{-1}	N:C of DFAA	(1*14)/(6*12)	PBR
abcoChl	m2 $(mg\ Chl)^{-1}$	light absorbance coefficient for chlorophyll	0.02	
AEm	dl	maximum assimilation efficiency; 1-AE is thus the proportion ingested that is voided as particulates	0.6	
AEo	dl	minimum AE	0.3	
alpha	$(m^2\ g\text{-}1\ chl.a)$ *$(gC\ umol^{-1}$ photon)	alpha for photosynthesis in protist	7.00E-06	
alpha_Prey1	$(m^2\ g\text{-}1\ chl.a)$ *$(gC\ umol^{-1}$ photon)	alpha for photosynthesis in prey1	7.00E-06	
alpha_Prey2	$(m^2\ g\text{-}1\ chl.a)$ *$(gC\ umol^{-1}$ photon)	alpha for photosynthesis in prey2	3.00E-06	
APaAA	dl	multiplier for additional maximum acquisition of DFAA on upturn	3*0	
APaDOC	dl	multiplier for additional maximum acquisition of C on upturn	3	
APaNH4	dl	multiplier for additional maximum acquisition of NH4 on upturn	3	
APaNO3	dl	multiplier for additional maximum acquisition of NO3 on upturn	0	
APaP	dl	multiplier for additional maximum acquisition of DIP on upturn	10	
AR	gC gN^{-1} d^{-1}	anabolic respiration cost in terms of C for assimilation of internal NH4 (excluding that via NO3 reduction) into amino and nucleic acids.	1.5	
attco_W	m^{-1}	absorbance coefficient for growth medium (water)	0.01	PBR
b	dl	parameter for derivation of C-cell content for protist of a given volume	0.939	
betaSi	dl	control for Si (diatom) uptake	0.4	
C_drywt_con	g dry weight gC^{-1}	conversion factor from C to dry weight (exact value varies with organism and nutrient status)	3	
ChlC_Prey1	gChl gC^{-1}	prey1 cellular Chl:C ratio	0.06	
ChlC_Prey2	gChl gC^{-1}	prey2 cellular Chl:C ratio	0.03	
ChlCm	gChl gC^{-1}	maximum cellular Chl:C ratio	0.06	
ChlCo	gChl gC^{-1}	minimum Chl:C	0.001	
CR	dl	catabolic respiration quotient; the actual value is related to Umax as organisms with a higher Umax also "live faster" with a higher basal activity	0.05	
dil	d^{-1}	Background dilution rate	0	PBR

Table 2/ cont

Name	Unit	Documentation	Value	Dimensions
ESD_Prey1	µm	ESD of prey1 cell	6	
ESD_Prey2	µm	ESD of prey2 cell	8	
ESD_Prot	µm	ESD of nutrient replete protist cell	20	
har_f	d	Frequency of harvesting	200	PBR
har_pc	dl	Proportion harvested at frequency of har_f	0	PBR
HAv	dl	Hill number to control DFAA leakage	4	
HdAA	dl	H for decrease (downturn) in DFAA transport potential	2	
HdDOC	dl	H for decrease (downturn) in C transport potential	2	
HdN	dl	H for decrease (downturn) in DIN transport potential	4	
HdP	dl	H for decrease (downturn) in DIP transport potential	4	
HiAA	dl	H for increase (upturn) in DFAA transport potential	2	
HiDOC	dl	H for increase (upturn) in C transport potential	2	
HiN	dl	H for increase (upturn) in DIN transport potential	2	
HiP	dl	H for increase (upturn) in DIP transport potential	4	
HSi	dl	control for Si (diatom) uptake	2	
Hv	dl	Hill number for controlling satiation-modulated motility	2	
initAA_C	mgC m^{-3}	initial DFAA concentration	0.1*(12*6)	PBR
initC_Prey1	mgC m^{-3}	initial prey1 concentration	12*0	
initC_Prey2	mgC m^{-3}	initial prey2 concentration	12*0	
InitDIC	mgC m^{-3}	DIC	12*2000	PBR
initDIP	mgP m^{-3}	initial external DIP	31*32	PBR
initDOC	mgC m^{-3}	initial DOC concentration	0*(6*12)	PBR
initNH4	mgN m^{-3}	initial external NH4	14*10	PBR
initNO3	mgN m^{-3}	initial external NO3	14*880	PBR
InitProtC	mgC	initial protist C biomass inoculated into PBR	1000	
initSi	mgSi m^{-3}	initial external Si	28*50	PBR
kAE	dl	Constant for control of AE in response to prey quality	1.00E+03	
KAv	dl	K value to control DFAA leakage	2	
KdAA	dl	K for decrease (downturn) in DFAA transport potential	1	
KdDOC	dl	K for decrease (downturn) in C transport potential	1	
KdN	dl	K for decrease (downturn) in DIN transport potential	0.5	
KdP	dl	K for decrease (downturn) in DIP transport potential	1	
KgDIC	mgC m^{-3}	Kg for using DIC (see Clark & Flynn 2000)	1200	
KiAA	dl	K for increase (upturn) in DFAA transport potential	0.2	
KiDOC	dl	K for increase (upturn) in C transport potential	0.2	
KiN	dl	K for increase (upturn) in DIN transport potential	0.1	
KiP	dl	K for increase (upturn) in DIP transport potential	0.25	

Table 2/ cont

Name	Unit	Documentation	Value	Dimensions
KQN	dl	control constant of quota curve for N:C	10	
KQP	dl	control constant of quota curve for P:C	0.1	
KSi	dl	control for Si (diatom) uptake	0.001	
KtAA	mgC m^{-3}	Kt for transport of DFAA	(12*6)*1	
KtDOC	mgC m^{-3}	Kt for transport of DOC	1*(6*12)	
KtNH4	mgN m^{-3}	Kt for NH4 transport	14	
KtNO3	mgN m^{-3}	Kt for NO3 transport	14	
KtP	mgP m^{-3}	Kt for DIP transport	31	
KtSi	mgSi m^{-3}	Kt for Si transport	1*28	
Kv	dl	K for controlling satiation-modulated motility	0.05	
LD	dl	fraction of day as light	0.7	PBR
M	dl	scalar for controlling photoacclimation rate	2	
maxpcDFAA	dl	maximum % of incoming N that is leaked as DFAA. This is affected by the value of NCu via an exponential function.	0.1	
motPrey_1	dl	motility of prey_1 (1 if motile; 0 if not motile)	1	
motPrey_2	dl	motility of prey_2 (1 if motile; 0 if not motile)	1	
N_Protein_con	g protein weight gN^{-1}	conversion factor from N to protein (exact value varies with organism and nutrient status)	6	
NC_Prey1	gN gC^{-1}	N:C of prey1	0.15	
NC_Prey2	gN gC^{-1}	N:C of prey2	0.2	
NCm	gN gC^{-1}	N:C that totally represses NH4 transport	0.2	
NCo	gN gC^{-1}	minimum N-quota	0.05	
NCopt	gN gC^{-1}	N:C for growth under optimal conditions	0.15	
NO3Cm	gN gC^{-1}	N:C that totally represses NO3 transport	0.16	
NO3Copt	gN gC^{-1}	N:C for growth on NO3 under optimal conditions	0.14	
Optimal_CR	dl	proportion of prey of optimal characteristics captured by starved Zoo	0.1	
Oz	m	optical depth	0.1	PBR
pauseT	d	simulation pause frequency	100	
PC_Prey1	gP gC^{-1}	P:C of prey1	0.05	
PC_Prey2	gP gC^{-1}	P:C of prey2	0.03	
PCm	gP gC^{-1}	PC maximum quota	0.05	
PCo	gP gC^{-1}	PC minimum quota	0.005	
PCoNCm	gN gC^{-1}	maximum NC when PC is minimum (i.e. when PCu = 0)	0.12	
PCoNCopt	gN gC^{-1}	optimum NC when PC is minimum (PCu = 0)	0.1	
PCopt	gP gC^{-1}	PC optimum quota	0.024	

Table 2/ cont

Name	Unit	Documentation	Value	Dimensions
PFD	µmol photons m^{-2} s^{-1}	PFD (PAR)	{10,20,500}	PBR
PR_prey1	dl	handling index for prey1 (likelihood of capture)	0.1	
PR_prey2	dl	handling index for prey2 (likelihood of capture)	0.2	
PSDOC	dl	proportion of current PS being leaked as DOC	0.1	
Q10	dl	Q_{10} for UmRT	1.8	
Reactor_V	m^3	Reactor volume	{1,2,10}	PBR
redco	gC gN^{-1}	C respired to support nitrate reduction through to intracellular ammonium	1.71	
RelminUmPS	dl	minimum proportion of Umax to come via PS	0.05	
RelPSm	dl	relative PSmax value to the value required to support Umax on phototrophy; RelPSm*RelUmPS*Umax gives the maximum net PE curve plateau value. NOTE, this value could be less than Umax (i.e. <1) if Umax can only be attained by mixotrophy	4	
RelUmAA	dl	maximum growth rate supported by DFAA relative to Umax	1	
RelUmNH4	dl	maximum growth rate supported by NH4-N relative to Umax	0.9	
RelUmNO3	dl	maximum growth rate supported by NO3-N relative to Umax	0.8	
RelUmPS	dl	maximum growth rate supported by PS relative to Umax (if <1, the Umax can only be attained by mixotrophy)	1	
RT	°C	reference temperature for UmRT	10	
SDA	dl	specific dynamic action (proportion of ingested biomass that is respired, lost, during assimilation into consumer biomass)	0.3	
SiCm	gSi gC^{-1}	absolute maximum Si:C (diatom)	0.2	
SiCo	gSi gC^{-1}	minimum Si:C (diatom)	0.02	
SiCopt	gSi gC^{-1}	optimum Si:C for (diatom) growth	0.1	
sw_altavg	dl	0 if using original (full) moving average; 1 if using short-cut	0	
sw_CO2	dl	switch to maintain DIC constant at the incoming DIC concentration (1 if yes)	1	PBR
sw_diat	dl	switch for selecting "diatom"; 1 if diatom	0	
sw_DOCup	dl	switch enabling DOC uptake; 0 for no, 1 for yes	1	
sw_mot	dl	switch for motility; 0 if non-motile, 1 if motile	1	
T	°C	temperature	15	PBR
UmRT	d^{-1}	maximum growth rate using NH4-N at reference T	0.7	
w	m s^{-1}	turbulence	0.003	

Table 3 Auxiliaries dl – no units. Some, as indicated, have dimensions of "PBR", set here to describe 3 different PBR configurations growing the same species of protist.

Name	Unit	Documentation	Equation	Dimensions
AA_C	mgC m^{-3}	DFAA concentration as C	AA_Cpbr/V	PBR
AA_Cgro	mgC d^{-1}	uptake of DFAA with respect to C (noting that DFAA is C,N)	AlgC_pbr*upAA_C	PBR
AA_N	mgN m^{-3}	DFAA concentration as N	AA_Npbr/V	PBR
AA_Ngro	mgN d^{-1}	uptake of DFAA with respect to N (noting that DFAA is C,N)	AlgC_pbr*upAA_N	PBR
AACex	mgC d^{-1}	exchange of amino acid C in/out of PBR	(initAA_C*V)*inD - (IF(AA_Cpbr>0,AA_Cpbr*outD,0))	PBR
AANex	mgN d^{-1}	exchange of amino acid N in/out of PBR	AACex*AA_NC	PBR
addPhotUm	gC gC^{-1} d^{-1}	additional need for PS after accounting for the minimum need, and C input from upDOC and upDFAA	MAX(PhotUm-minPhotUm-upDOC-upAA_C,0)	PBR
AEqual	dl	efficiency parameter for assimilation	AEo+(AEm-AEo)*stoich_con/(stoich_con+kAE)*(1+kAE)	PBR
altCu_in	gC gC^{-1} d^{-1}	input to alternative day-averaging of growth rate	(Cu-altavgCu)/TIMESTEP/10	PBR
altPS_in	gC gC^{-1} d^{-1}	input to alternative day-averaging of C-fixation rate	(phototrophy-altavgnetPS)/TIMESTEP/10	PBR
APAA	gC gC^{-1} d^{-1}	potential acquisition rate for DFAA in terms of C (noting that DFAA is C,N)	APoptAA*(IF(nNC<nNCopt,LHAA,HHAA))	PBR
APAAm	gC gC^{-1} d^{-1}	acquisition potential for DFAA controlled (if appropriate by RelminUmPS>0) by HetMax or for a non-phototroph by APoptAA	IF(RelminUmPS>0,HetMax,APoptAA)	PBR
APDOC	gC gC^{-1} d^{-1}	acquisition potential for DOC	IF(sw_DOCup=1,APoptDOC*(IF (nNC<nNCopt,LLDOC,HHDOC)),0)	PBR
APDOCm	gC gC^{-1} d^{-1}	maximum acquisition potential for DOC, depending on average PS and BR; only applicable if there is a need for PS to contribute to C acquisition to support growth	IF(RelminUmPS>0,HetMax,APoptDOC)	PBR
APNH4	gN gC^{-1} d^{-1}	acquisition potential for NH4	APoptNH4*(IF (nNH4C<nNH4Copt,LHNH4,HLNH4))	PBR
APNO3	gN gC^{-1} d^{-1}	acquisition potential for NO3	APoptNO3*(IF (nNO3C<nNO3Copt,LHNO3,HLNO3))	PBR
APoptAA	gC gC^{-1} d^{-1}	uptake rate of DFAA to support the stated growth rate.	UmT*RelUmAA*(1+CR+AR*NCopt)	PBR
APoptDOC	gC gC^{-1} d^{-1}	acquisition potential for DOC at NC=NH4Copt to match Umax against respiratory costs	UmT*(1+CR+AR*NCopt)	PBR
APoptNH4	gN gC^{-1} d^{-1}	acquisition potential for NH4 at NC=NH4Copt to match UmaxNH4	UmT*RelUmNH4*NCopt	PBR
APoptNO3	gN gC^{-1} d^{-1}	acquisition potential for NO3 at NC=NO3Copt to match UmaxNO3	(UmT*RelUmNO3)*NO3Copt	PBR

Table 3 cont./

Name	Unit	Documentation	Equation	Dimensions
APoptP	gP gC^{-1} d^{-1}	acquisition potential for P at PC=PCopt to match Umax	UmT*PCopt	PBR
APoptpred	gC gC^{-1} d^{-1}	predation rate to support the stated growth rate (ingCmax is previous models).	IF(RelUmPS>0 AND avgnetPS>0 AND avgnetPS<UmT,(1-avgnetPS/UmT),1)*((UmT+BR)/(1-SDA))/opAEC	PBR
APP	gP gC^{-1} d^{-1}	acqusiton potential for DIP	APoptP*(IF (nPC<nPCopt,LHP,HLP))	PBR
assC_Prot	gC gC^{-1} d^{-1}	assimilation rate of prey C (all sources) into protist	IF(opAEC>0,ingC_Prot*opAEC)	PBR
assN_Prot	gN gC^{-1} d^{-1}	assimilation rate of prey N (all sources) into protist	assC_Prot*NCopt	PBR
assP_Prot	gP gC^{-1} d^{-1}	assimilation rate of prey P (all sources) into protist	assC_Prot*PCopt	PBR
attenuation	dl	attenuation of light by water and by all sources of Chl	Oz*(attco_W+abcoChl*((AlgChl_pbr+Chl_prey1pbr+Chl_prey2pbr)/V))	PBR
avgCu	gC gC^{-1} d^{-1}	average C-specific growth rate; choice of two calculation options	IF(sw_altavg=0,mavgCu,altavgCu)	PBR
avgnetPS	gC gC^{-1} d^{-1}	average C-specific C-fixation rate; choice of two calculation options	IF(sw_altavg=0,mavgnetPS,altavgnetPS)	PBR
BR	gC gC^{-1} d^{-1}	basal respiration rate	UmT*CR	PBR
C_prey1	mgC m^{-3}	prey1 C-biomass concentration	C_prey1pbr/V	PBR
C_prey1ex	mgC d^{-1}	exchange of prey1 C in/out of PBR	(initC_Prey1*V)*inD - C_prey1pbr*outD	PBR
C_prey2	mgC m^{-3}	prey2 C-biomass concentration	C_prey2pbr/V	PBR
C_prey2ex	mgC d^{-1}	exchange of prey2 C in/out of PBR	(initC_Prey2*V)*inD - C_prey2pbr*outD	PBR
Ccell_prey1	pgC $cell^{-1}$	C content of prey1 cell	a*(4/3*PI*(r_prey1)^3)^b	
Ccell_prey2	pgC $cell^{-1}$	C content of prey2 cell	a*(4/3*PI*(r_prey2)^3)^b	
Ccell_prot	pgC $cell^{-1}$	C content of protist cell	a*(4/3*PI*(ESD_Prot/2)^3)^b	
Ceat	mgC d^{-1}	assimilation of C from prey	AlgC_pbr*assC_Prot	PBR
Cfix	gC gC^{-1} d^{-1}	gross photosynthesis rate retained for physiology	PS*(1-PSDOC)	PBR
CfixDOC	gC gC^{-1} d^{-1}	gross Cfix that is lost as DOC	PS*PSDOC	PBR
Cgro	mgC d^{-1}	population biomass growth rate	AlgC_pbr*Cu	PBR
Chl_prey1	mgChl m^{-3}	prey1 Chl-biomass concentration	Chl_prey1pbr/V	PBR
Chl_prey1ex	mgChl d^{-1}	exchange of prey1 Chl in/out of PBR	C_prey1ex*ChlC_Prey1	PBR
Chl_prey2	mgChl m^{-3}	prey2 Chl-biomass concentration	Chl_prey2pbr/V	PBR
Chl_prey2ex	mgChl d^{-1}	exchange of prey2 Chl in/out of PBR	C_prey2ex*ChlC_Prey2	PBR
ChlC	gChl gC^{-1}	protist cellular Chl:C ratio	MAX(AlgChl_pbr/AlgC_pbr,0)	PBR

Table 3 cont./

Name	Unit	Documentation	Equation	Dimensions
Chlgro	mgChl d^{-1}	population Chl rate of change	AlgC_pbr*dChl	PBR
CO2_inject	mgC d^{-1}	injection of CO2 into PBR	IF(DIC<InitDIC AND sw_CO2=1, (InitDIC-DIC)*V/TIMESTEP,0)	PBR
CR_prey1	prey $Prot^{-1}$ d^{-1}	potential capture of prey1 taking into account all factors	IF(sw_diat=0,Enc_prey1*PR_prey1*Optimal_CR)	PBR
CR_prey2	prey $Prot^{-1}$ d^{-1}	potential capture of prey2 taking into account all factors	IF(sw_diat=0,Enc_prey2*PR_prey2*Optimal_CR)	PBR
CRCP_prey1	gC gC^{-1} d^{-1}	potential C-specific ingestion of prey1	CR_prey1*Ccell_prey1/Ccell_prot	PBR
CRCP_prey2	gC gC^{-1} d^{-1}	potential C-specific ingestion of prey2	CR_prey2*Ccell_prey2/Ccell_prot	PBR
CRCP_sum	gC gC^{-1} d^{-1}	sum of potential C-specific ingestions of all prey types	CRCP_prey1+CRCP_prey2	PBR
Cresp	mgC m^{-3} d^{-1}	total respiration rate	AlgC_pbr*totR	PBR
Cu	gC gC^{-1} d^{-1}	instantaneous C-specific growth rate	Cfix+upDOC+upAA_C+assC_Prot-totR	PBR
Cu_in	gC gC^{-1} d^{-1}	input to day-averaging of growth rate	Cu	PBR
Cu_out	gC gC^{-1} d^{-1}	output to day-averaging of growth rate	FOR(A=FIRST(PBR).. LAST(PBR)|DELAYPPL(Cu_in[A],1,0))	FIRST(PBR) .. LAST(PBR)
Cum_gC_FAstar	g FA-C	cumulative protist FA-C diluted out (harvested)	Cum_gC_Algae-(Cum_gN_Algae/NCm)	PBR
Cum_gdrywt	g dry weight	cumulative protist dry weight diluted out (harvested)	C_drywt_con*Cum_gC_Algae	PBR
D	d^{-1}	total volume-specific dilution rate	dil+har_dil	PBR
dChl	gChl gC^{-1} d^{-1}	rate of change in Chl:C (synthesis and degradation)	IF(ChlC>ChlCo,ChlCm*PhotUm*NPSiCu*M*(1-Cfix/PSqm)*(1-ChlC/ChlCm)/(1-ChlC/ChlCm+0.05)-(IF(ChlC>ChlCo,ChlC*UmT*(1-NPSiCu))),0)	PBR
DFAA_C_out	gC gC^{-1} d^{-1}	leakage of DFAA-C	DFAA_N_out/AA_NC	PBR
DFAA_Cleak	mgC d^{-1}	leakage of C as DFAA	DFAA_C_out*AlgC_pbr	PBR
DFAA_N_out	gN gC^{-1} d^{-1}	DFAA leakage	upNtot*maxpcDFAA*IF(NCu<=1,(1+KAv^HAv)*NCu^HAv/(NCu^HAv+KAv^HAv),1)	PBR
DFAA_Nleak	mgN d^{-1}	leakage of N as DFAA	DFAA_N_out*AlgC_pbr	PBR
DIC	mgC m^{-3}	DIC	DICpbr/V	PBR
DICex	mgC d^{-1}	exchange of DIC-C in/out of PBR	(InitDIC*V)*inD - DICpbr*outD	PBR
DICfix	mgC m^{-3} d^{-1}	total usage of DIC in community Cfixation supporting protist growth and DOC release	PSgro+DOCleak	PBR

Table 3 cont./

Name	Unit	Documentation	Equation	Dimensions
DIP	mgP m^{-3}	DIP nutrient concentration	DIPpbr/V	PBR
DIPex	mgP d^{-1}	DIP lost from reactor	IF(DIPpbr>0,DIPpbr*outD,0)	PBR
DIPin	mgP d^{-1}	inflow of DIP to PBR	(initDIP*V)*inD	PBR
DOC	mgC m^{-3}	DOC nutrient concentration (the form of DOC is not defined, but is assumed as labile, such as glucose)	DOCpbr/V	PBR
DOCex	mgC d^{-1}	flow of DOC out of PBR	IF(DOCpbr>0,DOCpbr*outD, 0)	PBR
DOCgro	mgC d^{-1}	population uptake of DOC	AlgC_pbr*upDOC	PBR
DOCin	mgC d^{-1}	flow of DOC into PBR	(initDOC*V)*inD	PBR
DOCleak	mgC m^{-3} d^{-1}	release of DOC	AlgC_pbr*(CfixDOC)	PBR
DOCvoid_pbr	mgC d^{-1}	voiding of C as DOC if NC falls below NCo	IF(NC<NCo,(AlgC_pbr-AlgN_pbr/NCo)/TIMESTEP,0)	PBR
emergAEC	dl	emergent AE for C	IF(ingC_Prot>0,assC_Prot/ingC_Prot)	PBR
emergAEN	dl	emergent AE for N	IF(ingN_Prot>0,assN_Prot/ingN_Prot)	PBR
emergAEP	dl	emergent AE for P	IF(ingP_Prot>0,assP_Prot/ingP_Prot)	PBR
Enc_prey1	prey predator^{-1} d^{-1}	cell-specific encounter rate between protist and prey1	(24*60*60)*PI*(r_prey1/1E6 +r_Prot/1E6)^2*nos_prey1* (IF(v_prey1<v_Prot, (v_prey1^2+3*v_Prot^2+4* w^2)*((v_Prot^2+w^2)^-0.5), (v_Prot^2+3*v_prey1^2+4* w^2)*((v_prey1^2+w^2)^-0.5)))*3^-1	PBR
Enc_prey2	prey predator^{-1} d^{-1}	cell-specific encounter rate between protist and prey2	(24*60*60)*PI*(r_prey2/1E6 +r_Prot/1E6)^2*nos_prey2* (IF(v_prey2<v_Prot,(v_prey2 ^2+3*v_Prot^2+4*w^2)*((v_ Prot^2+w^2)^-0.5),(v_Prot^2+3*v_prey2^2 +4*w^2)*((v_prey2^2+w^2) ^-0.5)))*3^-1	PBR
exat	dl	-ve exponent of attenuation	EXP(-attenuation)	PBR
frat	dl	f-ratio	upNO3/(upNO3+upNH4+1e-12)	PBR
gC_CO2	gC d^{-1}	injection of CO2 into PBR (note this excludes over-aeration!)	CO2_inject/1e3	PBR
gC_PBR	gC PBR^{-1}	g of algal biomass-C in the bioreactor	AlgC_pbr/1e3	PBR
gCex	gC d^{-1}	protist C diluted out (harvested)	ProtCex/1e3	PBR
gChl_PBR	gChl PBR^{-1}	g of algal Chl in the bioreactor	AlgChl_pbr/1e3	PBR
gChlex	gChl d^{-1}	protist Chl diluted out (harvested)	protChlex/1e3	PBR
gDIPex	gP d^{-1}	DIP-P diluted out	IF(DIPex>0,DIPex/1e3)	PBR

Table 3 cont./

Name	Unit	Documentation	Equation	Dimensions
gDOCex	gC d^{-1}	DOC diluted out (harvested)	IF(DOCex>0,DOCex/1e3)	PBR
gN_PBR	gN PBR^{-1}	g of algal biomass-N in the bioreactor	AlgN_pbr/1e3	PBR
gNex	gN d^{-1}	protist N diluted out (harvested)	protNex/1e3	PBR
gNH4ex	gN d^{-1}	NH4-N diluted out	IF(NH4ex>0,NH4ex/1e3)	PBR
gNO3ex	gN d^{-1}	NO3-N diluted out	IF (NO3ex>0,NO3ex/1e3)	PBR
gNO3in	gN d^{-1}	flow of NO3-N into PBR	NO3in/1e3	PBR
gP_PBR	gP PBR^{-1}	g of algal biomass-P in the bioreactor	AlgP_pbr/1e3	PBR
gPex	gP d^{-1}	protist P diluted out (harvested)	protPex/1e3	PBR
gProt_PBR	g protein PBR^{-1}	g of algal protein in the bioreactor	gN_PBR*N_Protein_con	PBR
gSiex	gSi d^{-1}	Si diluted out	Siex/1e3	PBR
har_dil	d^{-1}	Harvesting dilution rate	IF((TIME>0), 1, 0)*IF((FRAC(TIME/har_f)=0), 1, 0)*har_pc/TIMESTEP	PBR
Harvest_NC	gN gC^{-1}	N:C of harvest	Cum_gN_Algae/Cum_gC_Algae	PBR
Harvest_NP	gN gP^{-1}	N:P of harvest	Harvest_NC/Harvest_PC	PBR
Harvest_PC	gP gC^{-1}	P:C of harvest	Cum_gP_Algae/Cum_gC_Algae	PBR
heterotrophy	gC gC^{-1} d^{-1}	rate of heterotrophy	Cu-phototrophy	PBR
HetMax	gC gC^{-1} d^{-1}	maximum heterotrophic C uptake when a proportion of C must come via PS; heterotrophy in darkness only coverings BR	IF(RelUmPS>0,IF(avgnetPS>0,avgnetPS/RelminUmPS+BR,BR),UmT+BR)	PBR
HHAA	dl	increase in acquisition potential for DFAA when NC is high	1+APaAA*(1+KiAA^HiAA)*(((nNC-nNCopt)/(1-nNCopt))^HiAA)/((((nNC-nNCopt)/(1-nNCopt))^HiAA)+KiAA^HiAA)	PBR
HHDOC	dl	increase in acquisition potential for DOC	1+APaDOC*(1+KiDOC^HiDOC)*(((nNC-nNCopt)/(1-nNCopt))^HiDOC)/((((nNC-nNCopt)/(1-nNCopt))^HiDOC)+KiDOC^HiDOC)	PBR
HLNH4	dl	decrease in acquisition potential for NH4	IF(nNH4C>nNH4Copt,1-(1+KdN^HdN)*(((nNH4C-nNH4Copt)/(1-nNH4Copt))^HdN)/((((nNH4C-nNH4Copt)/(1-nNH4Copt))^HdN)+KdN^HdN),0)	PBR
HLNO3	dl	decrease in acquisition potential for NO3	IF(nNO3C>nNO3Copt,1-(1+KdN^HdN)*(((nNO3C-nNO3Copt)/(1-nNO3Copt))^HdN)/((((nNO3C-nNO3Copt)/(1-nNO3Copt))^HdN)+KdN^HdN),0)	PBR

Table 3 cont./

Name	Unit	Documentation	Equation	Dimensions
HLP	dl	decrease in acquisition potential for DIP	1-(1+KdP^HdP)*(((nPC-nPCopt)/(1-nPCopt))^HdP)/((((nPC-nPCopt)/(1-nPCopt))^HdP)+KdP^HdP)	PBR
Igmax	gC gC^{-1} d^{-1}	maximum ingestion linked to the maximum rate of heterotrophy	(HetMax/(1-SDA))/opAEC	PBR
Igmop	gC gC^{-1} d^{-1}	operational maximum ingestion rate	MIN(APoptpred,Igmax)	PBR
inD	d^{-1}	volume-specific washin	V_in/V	PBR
ingC_prey1	mgC d^{-1}	ingestion rate of C from prey1	AlgC_pbr*(ingC_Prot*propI_prey1)	PBR
ingC_prey2	mgC d^{-1}	ingestion rate of C from prey2	AlgC_pbr*(ingC_Prot*propI_prey2)	PBR
ingC_Prot	gC gC^{-1} d^{-1}	Ingestion rate of prey-C	MIN(Igmop*CRCP_sum/(CRCP_sum+KI), CRCP_sum)	PBR
ingChl_prey1	mgChl d^{-1}	ingestion rate of Chl from prey1	ingC_prey1*ChlC_Prey1	PBR
ingChl_prey2	mgChl d^{-1}	ingestion rate of Chl originating from prey2	ingC_prey2*ChlC_Prey2	PBR
ingN_prey1	mgN d^{-1}	ingestion rate of N from prey1	ingC_prey1*NC_Prey1	PBR
ingN_prey2	mgN d^{-1}	ingestion rate of N originating from prey2	ingC_prey2*NC_Prey2	PBR
ingN_Prot	gN gC^{-1} d^{-1}	Ingestion rate of prey-N	ingC_Prot*ingNC	PBR
ingNC	gN gC^{-1}	ingestate N:C	(propI_prey1*NC_Prey1+propI_prey2*NC_Prey2)	PBR
ingP_prey1	mgP d^{-1}	ingestion rate of P originating from prey1	ingC_prey1*PC_Prey1	PBR
ingP_prey2	mgP d^{-1}	ingestion rate of P originating from prey2	ingC_prey2*PC_Prey2	PBR
ingP_Prot	gP gC^{-1} d^{-1}	Ingestion rate of prey-P	ingC_Prot*ingPC	PBR
ingPC	gP gC^{-1}	ingestate P:C	(propI_prey1*PC_Prey1+propI_prey2*PC_Prey2)	PBR
KI	gC gC^{-1} d^{-1}	satiation control constant	Igmop/4	PBR
LHAA	dl	increase in acqusition potential for DFAA when NC is low (driven by need for N)	1+APaAA*(1+KiAA^HiAA)*(((nNCopt-nNC)/nNCopt)^HiAA)/((((nNCopt-nNC)/nNCopt)^HiAA)+KiAA^HiAA)	PBR
LHNH4	dl	increase in acquisition potential for NH4 with normalised quotas	1+APaNH4*(1+KiN^HiN)*(((nNH4Copt-nNH4C)/nNH4Copt)^HiN)/((((nNH4Copt-nNH4C)/nNH4Copt)^HiN)+KiN^HiN)	PBR
LHNO3	dl	increase in acquisition potential for NO3	1+APaNO3*(1+KiN^HiN)*(((nNO3Copt-nNO3C)/nNO3Copt)^HiN)/((((nNO3Copt-nNO3C)/nNO3Copt)^HiN)+KiN^HiN)	PBR
LHP	dl	increase in acquisition potential for DIP	1+APaP*(1+KiP^HiP)*(((nPCopt-nPC)/nPCopt)^HiP)/((((nPCopt-nPC)/nPCopt)^HiP)+KiP^HiP)	PBR
Light	µmol photons m^{-2} s^{-1}	PFD applied	IF (FRAC(TIME)<LD,PFD,0)	PBR

Table 3 cont./

Name	Unit	Documentation	Equation	Dimensions
LLDOC	dl	decrease in acquisition potential for DOC	1-(1+KdDOC^HdDOC)*(((nNCopt-nNC)/nNCopt)^HdDOC)/((((nNCopt-nNC)/nNCopt)^HdDOC)+KdDOC^HdDOC)	PBR
minPhotUm	gC gC^{-1} d^{-1}	minimum Umax via PS only	UmT*RelminUmPS	PBR
molNP	molN $molP^{-1}$	molar biomass N:P	(NC/14)/(PC/31)	PBR
molNSi	molN $molSi^{-1}$	molar biomass N:Si (diatom)	(NC/14)/(SC/28)	PBR
N_prey1	mgN m^{-3}	prey1 N-biomass concentration	N_prey1pbr/V	PBR
N_prey1ex	mgN d^{-1}	exchange of prey1 N in/out of PBR	C_prey1ex*NC_Prey1	PBR
N_prey2	mgN m^{-3}	prey2 N-biomass concentration	N_prey2pbr/V	PBR
N_prey2ex	mgN d^{-1}	exchange of prey2 N in/out of PBR	C_prey2ex*NC_Prey2	PBR
NC	gN gC^{-1}	algal N:C	AlgN_pbr/AlgC_pbr	PBR
NCu	dl	N:C status of cell; 1 is maximum (good)	IF((NC<=NCopt), 1, 0)*IF((NC>=NCo), 1, 0)*(1+KQN)*(NC-NCo)/((NC-NCo)+KQN*(NCopt-NCo))+IF((NC>NCopt), 1, 0)	PBR
Neat	mgN m^{-3} d^{-1}	assimilation of N from prey	AlgC_pbr*assN_Prot	PBR
NH4	mgN m^{-3}	NH4 nutrient concentration	NH4pbr/V	PBR
NH4CPm	gN gC^{-1}	maximum N:C controlling NH4 transport, affected by PC status	PCoNCm+IF((PCu<NCu),PCu, 1)*(NCm-PCoNCm)	PBR
NH4CPopt	gN gC^{-1}	optimum N:C controlling NH4 transport, affected by PC status	PCoNCopt+IF((PCu<NCu),PCu,1)*(NCopt-PCoNCopt)	PBR
NH4ex	mgN d^{-1}	loss of NH4-N from reactor	IF(NH4pbr>0,NH4pbr*outD, 0)	PBR
NH4gro	mgN d^{-1}	uptake of NH4 into algal biomass	AlgC_pbr*upNH4	PBR
NH4in	mgN d^{-1}	flow of NH4-N into PBR	(initNH4*V)*inD	PBR
NH4out	mgN d^{-1}	NH4 release by regeneration	Nregen+AlgN_pbr*(IF(RelUmPS=0,SDAN))	PBR
nNC	dl	normalised quota for N:C	(NC-NCo)/(NCm-NCo)	PBR
nNCopt	dl	normalised optimal quota for N:C	(NCopt-NCo)/(NCm-NCo)	
nNH4C	dl	normalised optimal quota for N:C controlling NH4 transport	IF(NC<NH4CPm,(NC-NCo)/(NH4CPm-NCo),1)	PBR
nNH4Copt	dl	normalised NCopt for NH4 transport	(NH4CPopt-NCo)/(NH4CPm-NCo)	PBR
nNO3C	dl	normalised optimal quota for N:C controlling NO3 transport	IF(NC<NO3CPm,(NC-NCo)/(NO3CPm-NCo),1)	PBR
nNO3Copt	dl	normalised NCopt for NO3 transport	(NO3CPopt-NCo)/(NO3CPm-NCo)	PBR
NO3	mgN m^{-3}	NO3 concentration	NO3pbr/V	PBR

Table 3 cont./

Name	Unit	Documentation	Equation	Dimensions
NO3CPm	gN gC^{-1}	maximum N:C controlling NO3 transport, affected by PC status	PCoNCm+IF((PCu<NCu),PCu, 1)*(NO3Cm-PCoNCm)	PBR
NO3CPopt	gN gC^{-1}	optimum N:C controlling NO3 transport, affected by PC status	PCoNCopt+IF((PCu<NCu),PC u,1)*(NO3Copt-PCoNCopt)	PBR
NO3ex	mgN d^{-1}	loss of NO3-N from reactor	NO3pbr*outD	PBR
NO3gro	mgN d^{-1}	uptake of NO3 into algal biomass	AlgC_pbr*upNO3	PBR
NO3in	mgN d^{-1}	flow of NO3-N into PBR	(initNO3*V)*inD	PBR
nos_prey1	nos m^{-3}	cell abundance of prey1; Ccell is pgC/cell, C_prey1 is mgC/m3; transform betwen mg and pg is 10^9	IF(C_prey1pbr>0,10^9 *(C_prey1pbr/V)/Ccell_prey 1,0)	PBR
nos_prey2	nos m^{-3}	cell abundance of prey2; Ccell is pgC/cell, C_prey1 is mgC/m3; transform betwen mg and pg is 10^9	IF(C_prey2pbr>0,10^9 *(C_prey2pbr/V)/Ccell_prey 2,0)	PBR
nPC	dl	normalised quota for P:C	(PC-PCo)/(PCm-PCo)	PBR
nPCopt	dl	normalised optimal quota for P:C	(PCopt-PCo)/(PCm-PCo)	
NPCu	dl	nutrient status (assumes Liebig-like selection)	MIN(NCu,PCu)	PBR
NPSiCu	dl	minimum of N-P-Si limitation; Liebig-style limitation of growth	MIN(NPCu,SCu)	PBR
Nregen	mgN d^{-1}	if NC exceeds NCmax (actually the maximum that halts NH4 usage) then this excess is voided	IF(NC>NCm,(AlgN_pbr-AlgC_pbr*NCm)/TIMESTEP,0)	PBR
opAEC	dl	Operational AE for C	stoich_con*AEqual+1e-20	PBR
osmogro	mgC d^{-1}	total contribution to biomass growth from osmotrophy	AA_Cgro+DOCgro	PBR
outD	d^{-1}	volume-specific washout	V_out/V	PBR
P_prey1	mgP m^{-3}	prey1 P-biomass concentration	P_prey1pbr/V	PBR
P_prey1ex	mgP d^{-1}	exchange of prey1 P in/out of PBR	C_prey1ex*PC_Prey1	PBR
P_prey2	mgP m^{-3}	prey2 P-biomass concentration	P_prey2pbr/V	PBR
P_prey2ex	mgP d^{-1}	exchange of prey2 P in/out of PBR	C_prey2ex*PC_Prey2	PBR
PauseCon	dl	pause control of simulation; pauses simulation every multiple of simulation TIME defined by pause T	PAUSEIF(FRAC(TIME/pauseT) =0)	
PC	gP gC^{-1}	protist P:C	AlgP_pbr/AlgC_pbr	PBR
PCu	dl	P:C status of cell; 1 is maximum (good)	IF((PC<=PCopt), 1, 0)*IF((PC>=PCo), 1, 0)*(1+KQP)*(PC-PCo)/((PC-PCo)+KQP*(PCopt-PCo))+IF((PC>PCopt), 1, 0)	PBR
Peat	mgP d^{-1}	assimilation of P from prey	AlgC_pbr*assP_Prot	PBR
Pgro	mgC d^{-1}	increase rate in C-biomass	AlgC_pbr*upP	PBR
photoR	gC gC^{-1} d^{-1}	rate of phototrophy-related respiration. NOTE: this does not include the cost for assimilating amino acid N, nor of recovering SDA-NH4	(redco*upNO3)+AR*(upNH4 +upNO3)	PBR

Table 3 cont./

Name	Unit	Documentation	Equation	Dimensions
phototrophy	gC gC^{-1} d^{-1}	rate of (positive) net phototrophy	IF(Cfix>photoR,Cfix-photoR)	PBR
PhotUm	gC gC-1 d-1	maximum growth by phototrophy	UmT*RelUmPS	PBR
Pout	mgP d^{-1}	DIP release by regeneration	Pregen	PBR
Pregen	mgP d^{-1}	if PC exceeds PCmax then this excess is voided	IF(PC>PCm,(AlgP_pbr-AlgC_pbr*PCm)/TIMESTEP,0)	PBR
propI_prey1	dl	proportion of prey 1 in the diet	IF(CRCP_sum>0,CRCP_prey1/CRCP_sum)	PBR
propI_prey2	dl	proportion of prey 2 in the diet	IF(CRCP_sum>0,CRCP_prey2/CRCP_sum)	PBR
prot_cells_E6L	10^6 cells L^{-1}	millions of cells per L assuming a given pgC/cell	((AlgC_pbr/V)/Ccell_prot)	PBR
ProtCex	mgC d^{-1}	protist C diluted out from PBR	AlgC_pbr*outD	PBR
protChlex	mgChl d^{-1}	protist Chl diluted out (harvested)	AlgChl_pbr*outD	PBR
Proteinex	g protein d^{-1}	protist protein diluted out (harvested)	N_Protein_con*gNex	PBR
protNex	mgN d^{-1}	protist N diluted out (harvested)	AlgN_pbr*outD	PBR
protPex	mgP d^{-1}	protist P diluted out (harvested)	AlgP_pbr*outD	PBR
protSiex	mgSi d^{-1}	protist Si diluted out (harvested)	AlgSi_pbr*outD	PBR
PS	gC gC^{-1} d^{-1}	gross photosynthesis rate	PSqm*DIC/(DIC+KgDIC)*(LN(Pyt+SQRT(1+Pyt^2))-LN(Pyt*exat+SQRT(1+(Pyt*exat)^2)))/attenuation	PBR
PS_in	gC gC^{-1} d^{-1}	input into avgnet phototrophy calculation	phototrophy	PBR
PS_out	gC gC^{-1} d^{-1}	output from avgPS calculation	FOR(A=FIRST(PBR).. LAST(PBR)|DELAYPPL(PS_in[A],1,0))	FIRST(PBR) .. LAST(PBR)
PSgro	mgC d^{-1}	total contribution to biomass growth from C-fixation	AlgC_pbr*Cfix	PBR
PSqm	gC gC^{-1} d^{-1}	maximum photosyntheic rate required to support the highest growth rate (assumed to be NH4-supported); plateau of the gross PE curve.	IF(RelUmPS>0,1,0)*((1+PSDOC)*(minPhotUm+addPhotUm)*(IF(avgCu>0,MIN(RelPSm,PhotUm/avgCu),RelPSm))*(1+NCopt*(redco+AR))*NPSiCu+BR)+1e-6	PBR
Pyt	dl	intermediate in depth-integrated photosynthesis calculation according to the Smith equation	(alpha*ChlC*Light*24*60*60)/PSqm	PBR
r_prey1	µm	radius of prey1	ESD_Prey1/2	
r_prey2	µm	radius of prey2	ESD_Prey2/2	
r_Prot	µm	radius of nutrient replete cell	ESD_Prot/2	

Table 3 cont./

Name	Unit	Documentation	Equation	Dimensions
relU	dl	growth rate (day-averaged) relative to maximum	IF(avgCu>0,avgCu/UmT)	PBR
relUDFAA	dl	ratio of avgCu to potential for maximum growth rate using DFAA. This value will >1 if avgCu is already higher than the potential for DFAA support	IF(avgCu>0,avgCu/(UmT*RelUmAA),0.99)	PBR
satCon	dl	satiation control related to rate of growth (relU) via a sigmoidal function with Hill number Hv and K Kv; value 1 of unsatiated, 0 if fully satiated	IF(relU<1,(1+Kv^Hv)*(1-relU)^Hv/((1-relU)^Hv+Kv^Hv),0)	PBR
satConDFAA	dl	transinhibition control of DFAA uptake (saturation control)	IF(relUDFAA<1 AND relUDFAA>0,(1+Kv^Hv)*(1-relUDFAA)^Hv/((1-relUDFAA)^Hv+Kv^Hv),0)	PBR
SC	gSi gC^{-1}	diatom Si:C	AlgSi_pbr/AlgC_pbr	PBR
SCu	dl	Si:C status of the diatom cell; 1 is maximum (good)	IF(sw_diat=1,(IF(SC>SiCo,(IF(VSi>=UmT*SiCo,1,VSi/(UmT*SiCo))),0)),1)	PBR
SDAN	gN gC^{-1} d^{-1}	loss of assimilated N via SDA (if mixotrophic, this is then recovered, but at an additional C cost.	assN_Prot*SDA	PBR
Si	mgSi m^{-3}	Si nutrient concentration	Sipbr/V	PBR
Siex	mgSi d^{-1}	loss of Si from reactor	IF(Sipbr>0,Sipbr*outD,0)	PBR
Sigro	mgSi d^{-1}	increase in cell (diatom) Si-biomass	AlgC_pbr*upSi	PBR
Siin	mgSi d^{-1}	flow of Si into PBR	(initSi*V)*inD	PBR
stoich_con	dl	stoichiometric control used to regulate AE	MIN(ingNC/NCopt,ingPC/PCopt,1)	PBR
sysC	mgC m^{-3}	system-C	AA_Cpbr+DICpbr+DOCpbr+AlgC_pbr+C_prey1pbr+C_prey2pbr+VOCpbr	PBR
sysN	mgN m^{-3}	system-N	NH4pbr+NO3pbr+AlgN_pbr+AA_Npbr+N_prey1pbr+N_prey2pbr+VONpbr	PBR
sysP	mgP m^{-3}	system-P	DIPpbr+AlgP_pbr+P_prey1pbr+P_prey2pbr+VOPpbr	PBR
sysSi	mgSi m^{-3}	system-Si	Sipbr+AlgSi_pbr	PBR
totR	gC gC^{-1} d^{-1}	total respiration rate	(redco*upNO3)+AR*(upAA_N+upNH4+upNO3+(IF(RelUmPS>0,SDAN)))+(assC_Prot*SDA)+BR	PBR
UmT	gC gC^{-1} d^{-1}	Umax at current temperature	UmRT*Q10^((T-RT)/10)	PBR
upAA_C	gC gC^{-1} d^{-1}	DFAA uptake in terms of C (C-specific)	IF(AA_C>0,MIN(APAA,APAAm),0)*AR*satConDFAA*AA_C/(AA_C+KtAA)	PBR
upAA_N	gN gC^{-1} d^{-1}	DFAA uptake in terms of N (C-specific)	upAA_C*AA_NC	PBR

Table 3 cont./

Name	Unit	Documentation	Equation	Dimensions
upDOC	gC gC^{-1} d^{-1}	DOC uptake rate; if C-supply is fully satiated (satCon) shuts down this function. Note upDOC also interacts with PS.	IF(DOC>0,MIN(APDOC,APDOCm),0) *satCon*DOC/(DOC+KtDOC)	PBR
upNH4	gN gC^{-1} d^{-1}	NH4 uptake rate	IF(NH4>0,APNH4*NH4/(NH4+KtNH4),0)	PBR
upNO3	gN gC^{-1} d^{-1}	NO3 uptake rate	IF(NO3>0,APNO3*NO3/(NO3+KtNO3),0)	PBR
upNtot	gN gC^{-1} d^{-1}	total incoming N which would then pass through internbal amino acid pools and thus be possibly leaked	assN_Prot+upAA_N+upNH4+upNO3	PBR
upP	gP gC^{-1} d^{-1}	DIP uptake rate	IF(DIP>0,APP*DIP/(DIP+KtP),0)	PBR
upSi	gSi gC^{-1} d^{-1}	Si uptake rate	IF(sw_diat=1 AND Si>0 AND (SC<SiCm-0.01),1,0)*IF((SCu>NPSiCu OR SCu=1),IF(avgCu>0,(avgCu/UmT)^betaSi,0),1)*UmT*SiCopt*Si/(Si+KtSi)*(1-SC/SiCm)^HSi/((1-SC/SiCm)^HSi+KSi)	PBR
V_in	m3 d^{-1}	washin of medium	V_out	PBR
V_out	m3 d^{-1}	washout of medium	D*V	PBR
v_prey1	m s^{-1}	motility of prey1 (assumed to be a flagellate)	motPrey_1*(10^-6)*(38.542*(r_prey1*2)^0.5424)	
v_prey2	m s^{-1}	motility of prey2 (assumed to be a flagellate)	motPrey_2*(10^-6)*(38.542*(r_prey2*2)^0.5424)	
v_Prot	m s^{-1}	motility speed of protist, adjusted for its relative level of satiation	IF(sw_diat=0 AND sw_mot=1,satCon*((10^-6)*(38.542*(r_Prot*2)^0.5424))+1e-12, 1e-12)	PBR
VOCex	mgC d^{-1}	rate of washing out of voided C as particulates	VOCpbr*outD	PBR
VOCout	mgC d^{-1}	rate of voiding of C as particulates	AlgC_pbr*voidC_Prot	PBR
voidC_Prot	gC gC^{-1} d^{-1}	C-specific rate of C voiding as particulates	ingC_Prot-assC_Prot	PBR
voidN_Prot	gN gC^{-1} d^{-1}	C-specific rate of N voiding as particulates	ingN_Prot-assN_Prot	PBR
voidP_Prot	gP gC^{-1} d^{-1}	C-specific rate of P voiding as particulates	ingP_Prot-assP_Prot	PBR
VONC	gN gC^{-1}	N:C of voided material	VONpbr/VOCpbr	PBR
VONex	mgN d^{-1}	rate of washing out of voided N as particulates	VONpbr*outD	PBR
VONout	mgN d^{-1}	rate of voiding of N as particulates	AlgC_pbr*voidN_Prot	PBR
VOPC	gP gC^{-1}	P:C of voided material	VOPpbr/VOCpbr	PBR
VOPex	mgP d^{-1}	rate of washing out of voided P as particulates	VOPpbr*outD	PBR
VOPout	mgP d^{-1}	rate of voiding of P as particulates	AlgC_pbr*voidP_Prot	PBR
VSi	gSi gC^{-1} d^{-1}	potential transport rate of Si to compute SCu	IF(sw_diat=1,UmT*SiCopt*Si/(Si+KtSi),0)	PBR

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