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Selection for fitness at the individual or population levels: modeling effects of genetic modifications in microalgae on productivity and environmental safety

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Abstract

A mechanistic model of microalgae is used to explore the implications of modifying microalgal chlorophyll content and photosynthetic efficiency with an aim to optimizing commercial biomass production. The models show the potential for a ten-fold increase in microalgae productivity in genetically modified versus unmodified configurations, while also enabling the use of bioreactors of greater optical depth operating at lower dilution rates. Analysis suggests that natural selection of a trait benefiting the individual (high Chl:C_{max}; i.e. high antennae size) conflicts with artificial selection of a trait (low Chl:C_{max}) of most benefit to production at the population level. The implication is that GM strains rather than strains selected from nature will be most beneficial for commercial algal biofuels production. Further, escaped GM algae populations may, depending on the specific nature of the modification, be quickly out-competed by the natural forms because individually a high Chl:C is beneficial in low light environments. However, it remains possible that changes in biochemical composition associated with genetic modification of photosystem competence, or with other selection processes to enhance commercial gain, may adversely affect the

value of such organisms as prey for zooplankton, leading to the unwanted generation of future harmful algae.

Keywords: biofuel; GM; nutrient-limitation; photoacclimation; phytoplankton; quota-model

1. Introduction

Growth of microalgae for commercial gain has for many decades been the subject of intense interest (Borowitzka and Borowitzka, 1988; Williams, 2007). Recent interest in microalgal derived biofuels has provided additional impetus, according to its pundits providing a potential ethical solution to global fuel demands and for sustainable chemicals production (Sheehan et al., 1998; Spolaore et al., 2006; Chisti, 2006, 2007; Huntley and Redalje, 2007). In theory at least, microalgae offer high productivity, high oil yields (20-70% dry weight, Hu et al., 2008), and can be grown on marginal land not competing with food crops (Williams, 2007). Deployment of genetically modified (GM), or transgenic, microalgae will inevitably be considered (Gressel, 2008). However, the use of GM algae extends concerns leveled at GM higher plants (e.g., Johnson et al., 2007); densities run to millions of organisms mL^{-1} , growing in 100's or potentially 1000's m^3 of a liquid that is easily leaked, while the organisms could be transported vast distances (e.g., on a bird, or as an aerosol). On the other hand, in open-pond systems GM clones enabling better performance in monocultures may risk being out-competed by natural forms.

Most developmental work on commercialization of microalgal growth is inevitably shrouded in secrecy, with the only airing of information in carefully controlled publicity displays which cannot easily be subjected to scientific judgment. The material that is in the scientific literature includes descriptions of manipulating algal physiology to enhance commercial production such as the optimization of fatty acid profiles (Navarro-Angulo and Robledo, 1999; Li et al., 2008; Chi et al., 2009) and enhancing productivity by manipulating the photosynthetic machinery (e.g., Melis, 2009). There are also some theoretical explorations using models to aid in the optimization of bioreactor design. These models, such as that of Fernández et al. (2000), place very simple

empirical models of biology within descriptions of bioreactors of different dimensions and dilution rates. These two features of bioreactor design, optical depth (maximum distance from illuminated surface of the water to the organism) and the dilution rate (input of new medium, and hence removal of medium containing biomass), are crucial for commercial success. The greater the optical depth of the reactor the less space is required for the installation, the lower diel temperature fluctuations and evaporation losses. The lower the dilution rate the lower the expense of growth media preparation and the higher the biomass density, so decreasing cell concentration and dewatering costs. On the down side, higher optical depths promote light limitation, while low dilution rates are linked to low growth (and hence low production) rates.

Two features of microalgal photo-physiology obviously worthy of genetic modification are apparent: i) the amount of pigment present in the organism responsible for capturing photons; ii) the efficiency with which photosystems convert sunlight into fixed C (indicated by the initial slope of the photosynthesis-irradiance curve, α^{Chl}). At low light, microalgae acclimate by increasing their pigment content, with chlorophyll:C (Chl:C) increasing towards a maximum (Chl:C_{max}). Biochemically, a high Chl:C is associated with a large photosystem antenna. While the higher the value of Chl:C_{max} the greater the light harvesting capacity of the individual cell, at the population level the enhanced total light absorption leads to a rapid decrease in light availability as one moves further from the illuminated surface. This event is commonly seen in nature, with the shallowing of the mixed depth being a key determinate of the initiation of algal blooms. In commercial culture of microalgae the use of bioreactors, or open ponds, of high optical depth is desirable because it increases space utilization. Unfortunately, the trade off is in decreasing algal growth rates. Restricting Chl:C_{max}, and the synthesis of secondary pigments, through genetic modification would decrease light absorption within the water column, allowing organisms at greater optical depth to receive more light. There has been some considerable attention paid to such modifications, through decreasing the chlorophyll antennae size (e.g., Beckmann et al., 2009; Melis, 2009). The higher the value of α^{Chl} the more efficient is the conversion of light energy to ATP and reductant; for a given

value of Chl:C cells can then photosynthesise at a higher rate. Further, possession of a high α^{Chl} may mitigate against premature photoacclimation (increase in Chl:C) and hence premature self shading. Our understanding of the genetic underpinning of physiology now enables such possibilities to be actively explored (Mussgnug et al., 2007; Beckmann et al., 2009).

Here we apply a model of algal physiology of a type well described in the algal literature (e.g., Flynn, 2003), and applied to biogeochemical environmental investigations (e.g., Fasham et al., 2006), to consider reactor design and operation, and also the implications of genetic modification. To undertake the latter specifically requires the use of a mechanistic model of algal physiology in which physiological parameters can be altered with some level of confidence; our model provides such assurance. These models also allow a consideration of the implications of nutrient exhaustion (desired or otherwise) and of the self-shading that inevitably occurs during the growth of dense algal cultures. We explore the ramifications of modifying these two parameters ($\text{Chl:C}_{\text{max}}$ and α^{Chl}), consider the expected increases in yields that may be obtained from using GM algae, discuss the implications for the design and operation of cultivation system (shallow raceway or closed photobioreactor), and explore the effects of contamination of GM algae with non-GM algae, and vice versa.

2. Model description and assumptions

The mechanistic variable stoichiometric multi-nutrient model of microalgal growth developed by Flynn (2001) was used, equipped with the “GMFM” photoacclimation submodel described in Flynn et al. (2001). This model contains a dynamic linkage between photosynthesis and nutrient acquisition, describing changes in C-N-P biomass and Chl:C in response to growth under nutrient and/or light limitations. This model was developed in keeping with knowledge of algal physiology and has been fitted to various experimental data sets (e.g. Flynn 2001, John and Flynn, 2002; Flynn, 2008a). It has also been applied to oceanographic scenarios (Fasham et al., 2006; Mitra et al., 2007).

For the purpose at hand we have not sought to configure the model to describe any specific organism. The default configurations described in Flynn (2001) have been used except that the maximum growth rate ($\mu_{\max}$) was set at two divisions a day, consistent with the use of faster growing microalgae in culture systems. Alternative configurations for testing were the same with respect to maximum growth rate, respiration rate, C:N:P quota description, etc., except with respect to the maximum value of Chl:C ($\text{Chl:C}_{\max}$) and the initial slope of the photosynthesis-irradiance curve (α^{Chl}). The non-genetically modified (non-GM) configuration had $\text{ChlC}_{\max}$ set at 0.06 (g g^{-1}) and α^{Chl} at $7 \cdot 10^{-6} \text{ gC}^{-1} \text{ g}^{-1} \text{ Chl } \mu\text{mol}^{-1} \text{ photon m}^{-2}$, typical for these parameters (Geider et al., 1998; Flynn, 2001; Flynn et al., 2001 and references therein). Genetic modification of $\text{ChlC}_{\max}$ was considered to limit the maximum pigment content to 25% and 10% of non-GM levels. The value of α^{Chl} were considered at 2 and 5 times the non-GM values (Table 1). The uppermost value used ($35 \mu\text{gC}^{-1} \text{ g}^{-1} \text{ Chl } \mu\text{mol}^{-1} \text{ photon m}^{-2}$) describes a theoretical capability which, depending on the value assumed for the light absorbance coefficient, borders on or slightly exceeds the maximum theoretical value of $0.125 \text{ mol C (mol photon)}^{-1}$.

We assume no pleiotropic effects in our hypothetical GM algae. Such a situation is unlikely in practice; Mussgnug et al. (2007) modified algal photosystems essentially achieving a decreased $\text{ChlC}_{\max}$, but also causing a series of other changes associated with the restructuring of photosystems. Some of these may be advantageous while other not so. The absence of sufficient mechanistic understanding prevents us from further modifying the model structure.

While particular commercial exploitations of microalgal physiology may require induction of nutrient limitation (e.g., Shifrin and Chisholm, 1981), maximum biomass and growth rate requires initial growth to be non-nutrient-limited. The model describes the implications of nutrient limitation both directly upon growth rate, and also through impaired photosynthetic competency. Growth of the organism was simulated within media containing inorganic N and P nutrients at levels given by the classic f/2 medium of Guillard (1975) (12.35 mgN L^{-1} and 1.11 mgP L^{-1}), or supplied with ten times these nutrient concentrations (medium 5f). Phosphate levels much above

those in $f/2$ may precipitate in seawater, hence $f/2$ rather than f -medium is commonly used in marine algal culturing. Other nutrients (Si, Fe, CO_2) were assumed to be non-limiting in the simulations.

Cultivation was simulated in continuous flow (chemostat-style) systems at different dilution rates such that steady state growth rates equal dilution rates. Optical depths (Od) were considered over the range 0.01m to 1.5m. In closed-tubular bioreactors (Richmond, 2003) Od equates to a tube of diameter $2 \cdot \text{Od}$ assuming even illumination around the reactor tube. The larger Od values used in the simulations are most likely deliverable in raceway constructions, with light entering from the top only. Homogeneous distribution of cells was assumed within the growth vessel, as required for a chemostat-style system, and also as typically engineered in commercial reactors. Accordingly, depth integrated photosynthesis was computed as for oceanographic models (Fasham et al., 2006). Surface photon flux density (PFD) was set at $1000 \mu\text{mol photons m}^{-2} \cdot \text{s}^{-1}$, ca. half that on a cloud-free day.

In addition to simulating the growth of different microalgal configurations in different bioreactors, we also considered the implications of either a contamination of a bioreactor by a non-GM microalgal strain, or of a natural (non-GM) population by a GM strain. Again, we considered that all other physiological parameters were the same; only $\text{Chl:C}_{\text{max}}$ and/or α^{Chl} were varied. We also assume that there are no mutations of evolutionary value during the 50d period of the simulation; this matter is considered further in Discussion. For the simulated contamination shown here, the dilution rate was fixed at 0.693 d^{-1} (i.e., 50% μ_{max}); other rates do not affect the underlying results shown. Contaminations of GM-algae containing reactors operating at steady-state had the non-GM contaminant added at 1% of GM biomass. The consequences of leakage from a GM cultivation system into a watercourse of different optical depth was simulated by introduction of the GM algae into a non-GM population at 10% of the original non-GM biomass (Table 2).

Optimal commercial production requires maximizing both areal production ($\text{AP, gC m}^{-2} \text{ d}^{-1}$) and volumetric production ($\text{VP, gC m}^{-3} \text{ d}^{-1}$) rates, thus maximising land use, minimising water and

nutrient consumption, and minimising harvesting costs. A system with high VP would have a very small optical depth (assuming that light was not photoinhibitory), so that light was never limiting. Inevitably such a system would have a low AP. As optical depth is increased to enhance AP, so VP decreases, but that decrease is initially not so rapid as to compensate pro rata for the increase in depth because of photoacclimation by the microalgae. Initially then, as Od is increased so AP increases also. While the relative importance of these factors will vary from application to application, as a first approximation we have used $\sqrt{AP \cdot VP}$ ($\text{gC d}^{-1} \text{ m}^{-2.5}$) as an index of commercial production (commercial production index - CPI).

3. Results

Bioreactor operation

Figure 1 shows standing-stock biomass levels of systems supported by f/2 or 5f nutrient concentrations. Noting the different Z-axis (biomass) scales, while it is apparent that increasing nutrient availability 10 fold enables up to an order of magnitude increase in biomass, the detrimental effect of light limitation is particularly clear from the 5f series (Fig. 1 d-f). The effects of artificially decreasing $\text{Chl:C}_{\max}$ through genetic modification, as shown by the GM^{C1} and GM^{C2} configurations (see Table 1) can also be seen, with enhanced production at greater optical depths being achievable because of a lessening in self-shading. Sharp transients in plots, for example at dilution 0.4 d^{-1} and optical depth 0.1 m in Fig. 1a, represent switch points between light and nutrient limitations of microalgal growth.

Figure 2 shows volumetric and areal production rates for systems supported by f/2 nutrients (CF. Fig. 1a-c). Because nutrients are exhausted in low Od systems except at very high dilution rates, light limitation becomes increasingly important as Od is increased. In such high Od systems, GM variants with low $\text{Chl:C}_{\max}$ show *ca.* 3 fold enhanced AP (Figs. 2d-f). The coupled consequence of changes in VP and AP when employing GM configurations show a shift in peak CPI to lower dilution rates and greater Od (Fig. 2g-i). Thus, the use of the GM variant with

decreased $\text{Chl:C}_{\max}$ (GM^{C1} , GM^{C2} ; Table 1) would enhance commercial production by permitting the use of larger (deeper) bioreactors with a lower throughput of growth medium (less media to harvest and less wastage).

Employing 10 fold higher nutrient concentrations (Fig. 3) than in f/2 media (Fig. 2) minimises the likelihood of nutrient limitation but does not eliminate it. Areal production is increased in small Od systems (note the scale of the VP values in Fig. 2, compared with Fig. 3), but at high Od light limitation is more important than nutrient limitation (Fig. 2d vs Fig. 3d). Switches between light and nutrient limitation are apparent, for example, in the maximum non-GM microalgal production at $\text{Od}=0.01\text{m}$ and at 0.3d^{-1} dilution (Fig. 3d; also seen in Fig. 1d); nutrient limitation occurs at lower dilution rates, with light limitation at higher rates. This sharp transient occurs because nutrient-deprived cells contain less pigment, lessening light absorbance by the water column suspension, so increasing the likelihood of nutrient limitation further. Using a GM variant with low $\text{Chl:C}_{\max}$ (GM^{C1} , GM^{C2} ; Table 1) increases AP in low Od systems in 5f versus f/2 medium, but there is little difference at the greatest Od as these systems are severely light limited (e.g. Fig. 3f vs. 2f).

While the use of GM configuration with decreased $\text{Chl:C}_{\max}$ has a clear advantage, with a greatly increased volumetric and hence areal production over the nonGM counterpart (Fig. 3), deploying GM microalgae with elevated α^{chl} has far less impact (Fig. 4). Using elevated α^{chl} increases production greatly in low Od systems, in which light is less limiting. However, at high Od the advantage is most apparent at high dilution rates. The explanation is that this GM configuration ($\text{GM}^{\alpha 1}$, $\text{GM}^{\alpha 1}$) still has a high $\text{Chl:C}_{\max}$ (Table 1) and hence self-shading remains a limiting factor for population growth. As described above, sharp transients seen at some dilution rates reflect transitions from nutrient to light limitations. The values of CPI for these configurations (not shown) are high only within small Od systems (as would be deployed in tubular photobioreactors; Richmond, 2003).

Combining elevated α^{Chl} and decreased $\text{Chl:C}_{\text{max}}$ in a GM organism has the greatest potential for increasing production (Fig. 5), with production as much as 10 fold greater than when using the non-GM configuration over a wide range of dilution rates and optical depths (e.g., nonGM vs $\text{GM}^{\text{C2}\alpha 2}$). The improvement is most apparent from the CPI values for various microalgal configurations shown in Fig. 6. The commercial advantage is not simply with elevated production, but also because production costs would be decreased through the use of larger Od reactors operating at lower dilution rates and hence requiring less medium to prepare and subsequently to process.

Contamination simulations

Contamination of a GM system by a native (non-GM) variant results in the elimination of GM variants with low $\text{Chl:C}_{\text{max}}$ (Fig. 7a). This event is most rapid in a high Od bioreactor, where the high $\text{Chl:C}_{\text{max}}$ of the non-GM clone enables them to make better use of available light, while simultaneously shading out the GM clone. In contrast, contamination of a GM clone exhibiting a high α^{Chl} by the non-GM clone has no impact on the GM clone (Fig. 7b); here the non-GM contaminant is eliminated because for a given photon flux density at the cell surface, GM^{α} converts more energy to growth. Contamination of a GM clone exhibiting low $\text{Chl:C}_{\text{max}}$ plus high α^{Chl} (clone $\text{GM}^{\text{C2}\alpha 2}$) by the non-GM variant is likely only at high Od (Fig. 7c). However, as this GM configuration allows the commercial exploitation of such high Od bioreactors, the threat is potentially important.

Simulations of the implications of the release of GM algae into watercourses of different depths were considered at a lower nutrient regime (see Table 2), commensurate with the likely nutrient load in such waters. Escape of a GM organism with a low $\text{Chl:C}_{\text{max}}$ appears of no danger as it is out-competed by the natural strain (Fig. 7d). A GM hybrid, with both low $\text{Chl:C}_{\text{max}}$ and high α^{Chl} , a form of most commercial value, also appears to be of minimal danger to a natural population (Fig. 7f). This is especially so as in most natural habitats Od would be greater than in cultivation systems and so the presence of a high $\text{Chl:C}_{\text{max}}$ in the natural strain would prove to be of greatest

importance in competing for light absorbance. In contrast, escape of a GM organism with an elevated α^{Chl} results in the success of the GM strain at the expense of the non-GM form (Fig. 7e).

4. Discussion

To our knowledge this is the first application of a full physiologically based mechanistic model of microalgal to the subject of the commercial exploitation of these organisms. Not only does it reveal some facets of the potential of GM microalgae for commercial exploitation, and of the associated risks, but it also shows an interesting feature of evolution.

Potential for enhanced production using GM microalgae

Genetic manipulation is thought to be essential to realise maximum commercial microalgal production (Chisti, 2007; Rosenberg et al., 2008). However, there has been little reported in the literature on modification of organisms to enhance productivity (e.g. for lipid production, Dunahay et al., 1996), other than for photosynthesis (e.g., Beckmann et al., 2009) most likely due to commercial sensitivities. Though others have produced models for algal productivity in photobioreactors (e.g. Acién Fernández et al., 1998; Camacho Rubio, 2002), ours is the first to consider the effects of genetic modification. Our simulations indicate optimum production for non-GM algae at a dilution rate of *ca.* 50% of maximum growth rate, depending on whether nutrients become exhausted. A potential 10 fold enhancement in microalgal production through genetic modification of photosynthetic parameters is simulated (Figs. 1-3, 5, 6), with deployment of clones with a lower $\text{Chl:C}_{\text{max}}$ decreasing the optimum dilution rate and increasing the optimum Od (e.g., Fig. 2). These operational changes are highly desirable, providing greater areal production (needing less land), and less use of water (also requiring less effort to harvest the microalgae).

It is worth noting that the levels of production simulated here assumes that dissolved inorganic carbon (DIC; primarily HCO_3^- and $\text{CO}_2(\text{aq})$) is non-limiting. Freshwater contains little DIC, and even seawater contains only 2mM. Marine phytoplankton growth becomes limited by DIC at values below *ca.* 0.2mM (Clark and Flynn, 2000). To support the consumption of the N from 5f

medium (123.5 g N m^{-3}) requires the input of ca. 740 g C as DIC per cubic metre. For the optimal dilution rate indicated by the simulations (ca. 0.5 d^{-1}), this equates to a need to dissolve into the growth medium ca. $700 \text{ L CO}_2 \text{ m}^{-3} \text{ d}^{-1}$ while retaining a constant medium pH, and temperature.

Natural selection versus GM

The fundamental basis of life on this planet is thought to have been fixed some 2Ga ago (Shi et al., 2005), with natural selection then optimising the packaging of these key biochemical processes. These ancient so-called frozen metabolic accidents essentially fixed the molecular structure of key functional biomolecules, including processes such as the functionality of the photosynthetic Z-scheme that set α^{Chl} at the biochemical level (although the overall cellular packaging of the processes and presence of accessory pigments affect the expression of α^{Chl} at the cellular level). To alter the products of genes coding for such structures to enhance efficiency may be expected now to require a wholesale rebuilding of the functional proteins and support structures, rather than a relatively simple series of minor changes. This situation could be likened to modifying a helicopter to a jet fighter; both fly but the structures are so different that one could not possibly convert one to the other by substitution of components. While mutation to improve existing structures has almost certainly gone as far as it can, if it was possible to start with a synthetic alternative, then the chances of constructive mutation would again be opened.

On the other hand, expression (synthesis) and regulation of photosystems, which is in part reflected by changes in Chl:C during photoacclimation, does represent a characteristic more amenable to natural selection, and also to genetic modification (e.g., see Mussgnug et al. 2007). Indeed, there has been considerable interest in altering the size of the Chl antennae directed towards decreasing the wastage of energy by cells with a high Chl content when exposed to high light (reviewed by Melis, 2009). Beckmann et al. (2009) have undertaken this type of work, and indeed report the types of advantages that we see with our simulated microalgae, with a increased growth in dense suspensions due to an increased light penetration with less self-shading within the population.

For biofuel applications, microalgae grown in high density suspensions are subjected to alternating very high light and near darkness as they move through the optical depth. In nature, the optical depth is much greater, the biomass densities much lower, and cells develop a high Chl content when subjected to non-saturating light. This is a dynamic process that occurs over day-scales of time; this type of event in nutrient-replete phytoplankton can be seen in the data of Anning et al., (2000) which has been simulated using the model deployed here (Flynn et al., 2001). It is interesting to compare the areal production values projected in Fig. 2d and 3d, for nonGM configured microalgae growing in f/2 and 5f media, with those in highly productive oceanic waters (Sarmineto and Gruber, 2006). These values, ca. $5 \text{ g C m}^{-2} \text{ d}^{-1}$ ($\equiv 18250 \text{ kg C hectare}^{-1} \text{ yr}^{-1}$) under shallow water high nutrient conditions in simulated bioreactors compare well with productions of around $100 \text{ mol C m}^{-2} \text{ yr}^{-1}$ ($\equiv 3.29 \text{ g C m}^{-2} \text{ d}^{-1}$) for oceanic up-welling zones which are low nutrient, light limited zones of 10's metres depth. This suggests that even if we could fertilize the worlds ocean to enhance carbon-drawdown (e.g., through addition of iron; Buesseler et al., 2004), or indeed to enhance production in other ways, the current physiological configuration of microalgae itself will limit areal carbon fixation.

Light is the most fundamental “nutrient” likely to limit microalgal growth, especially in most water bodies in which the mixing depth may approach or exceed the critical depth (D_{crit} , where depth and day integrated photosynthesis equals respiration). For the individual microalga the capture of light is critical, and selection (all else being equal) would be expected to favour cells with a high Chl: C_{max} . However, for the population, the self-shading that accompanies the development of cells of high Chl:C is itself self limiting. That is why the genetic modification of photoacclimation to limit Chl: C_{max} results in greater production (Figs. 1-3), and is why there have been attempts to capitalise on such an approach (Melis, 2009). The use of genetically modified microalgae was suggested and explored over a decade ago (e.g. for lipid production - Dunahay et al., 1996). Indeed it is considered that the use of GM microalgae, like the deployment of GM higher plants, may be essential (Gressel, 2008) in order to boost production levels.

The results from our analysis suggest that the greatest commercial production from microalgae is to be achieved not by selection from amongst naturally occurring strains, but through genetic modification of their photosynthetic capability. The reason for this is that natural selection has worked in a direction totally contrary to that required to optimise microalgal bioreactor production. Microalgae with higher $\text{Chl:C}_{\max}$ may have a poorer production as a population, but they are fitter individually; a low $\text{Chl:C}_{\max}$ is only advantageous in clonal microalgal blooms. Here, fitness is characterised by an ability to out-grow competitors under various conditions of light and nutrient availability. In traditional chemostats, fitness (R^*) can be defined as $R^* = \frac{D \cdot K_{\mu}}{\mu_{\max} - D}$, where D is dilution rate, $\mu_{\max}$ the maximum growth rate, and K_{μ} is the half saturation constant for growth (Grover, 1997). However, outside of a single-nutrient limited chemostat, as in an algal race-way, K_{μ} is not a constant, but (because it is a function of total cell physiology) varies according to the interrelationship between nutrient and light limitations. The importance of this interaction can be seen in the break-points in the 3D plots where nutrient rather than light limitation becomes important, not least because at that point the pigment level in the cell declines affecting light levels in the whole system.

It seems reasonable to expect that in nature, best advantage for the individual is given by a high α^{Chl} together with a high $\text{Chl:C}_{\max}$, a mix that in bioreactors pays little dividend as the advantage in having a higher α^{Chl} is rather lost if $\text{Chl:C}_{\max}$ also remains high (Fig. 4). The exponential rate of light absorbance through the water column rapidly removes the benefit in photochemical efficiency. In any case, because the value of α^{Chl} is a function of photosystem molecular structure, genetic modification of α^{Chl} presents a severe challenge. Fortunately from the point of view of employing genetic modification to enhance commercial microalgal production (as explained above), modifying (decreasing) $\text{Chl:C}_{\max}$, rather than maximising α^{Chl} , shows most promise for enhancing microalgal productivity. As the packaging of photosystems within a microalga also occupies much space within the cell, the setting of a decreased $\text{Chl:C}_{\max}$ has

implications also for increasing the cellular content of commercially useful chemicals. How all this may interact with cell size, given that light-limited cells are usually smaller (Falkowski and Raven, 1997), is not clear.

Environmental safety: competition between non-GM and GM strains

Given all the above, it is all the more fortuitous that while the escape of a GM clone with enhanced α^{Chl} appears dangerous (Fig. 7e), GM clones with lowered $\text{Chl:C}_{\text{max}}$, while being excellent in a cultivation system, are disadvantaged in competition with their non-GM counterparts (Fig. 7d, f). Taken at face value this could be taken to indicate that such genetic modification would pose no threat to nature; at least the basic tenet of genetically modifying microalgae with respect to the extent of photoacclimation (decreasing $\text{Chl:C}_{\text{max}}$) appears to pose minimal risk. Indeed, the greater problem appears to be the risk of contamination of such GM-containing cultivation system with naturally selected microalgae in open reactors (Fig. 7a, c). However, there are several important caveats for the above assumptions.

Firstly, it assumes that there is no useful mutation within the GM algae over the period of the interaction and hence that competition is more important than microevolution. This assumption is not unreasonable over the simulated period of 2 month duration. Secondly, it is most unlikely that the only feature of a microalga to be altered in genetic modification would be features affecting photosynthesis, such as the antenna size (affecting $\text{Chl:C}_{\text{max}}$). It is likely that other features affecting the biochemical composition would be altered for commercial gain, most notably the fatty acid content (Dunahay et al., 1996), which is also a target for modification to enhance biofuel production. Such changes will likely alter the value of the organism as food for the zooplankton that in nature control algal bloom potential through top-down regulation. It may also affect allelopathic interactions between species of microalgae. Indeed, the modification of factors other than those leading to enhanced photosynthesis are probably most dangerous, not least because it would appear that an advantageous mutation within a GM strain originally constructed to minimise self-shading (i.e. low $\text{Chl:C}_{\text{max}}$) would be to increase $\text{Chl:C}_{\text{max}}$ back towards values in the original strain. It is

notable that many environmentally successful microalgae are not those that outwardly seem best configured for growth in competition with other microalgae (Mitra and Flynn, 2005). Surviving grazing pressure has proven to be the most important facet affecting the success of alien plant species (e.g. Kimball and Schiffman, 2003; Vavra et al., 2007); we may yet tamper with the genetic configuration of microalgae at our peril, unwittingly producing new strains of harmful algae.

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Legends

Table 1. Differences between the configurations of microalgae employed in this work. Other than setting the maximum growth rate at 1.386 d^{-1} (i.e., 2 divisions a day) all other parameters were as described in Flynn (2001) and Flynn et al. (2001). $\text{Chl:C}_{\max}$ ($\text{g Chl g}^{-1} \text{C}$) is the maximum chlorophyll content in nutrient replete cells. α^{Chl} ($\mu\text{gC}^{-1} \text{g}^{-1} \text{Chl } \mu\text{mol}^{-1} \text{photon m}^{-2}$) is the initial slope of the photosynthesis-irradiance curve.

Table 2. Configuration of contamination systems. For descriptions of the microalgae configurations, see Table 1. The macro nutrients N and P were as defined in the f growth medium described by Guillard (1975); N at 24.7 g m^{-3} ; P at 2.22 g m^{-3} . All other nutrients assumed to be in excess.

Fig. 1. Biomass standing stock for systems supported by f/2 nutrients (panels a-c), or by 5f medium (panels d-f), simulating growth of nonGM, GM^{C1} or GM^{C2} microalgae (see Table 1 for organism configurations).

Fig. 2. Volumetric (VP) and areal (AP) production in the f/2 systems. See Fig.1 (a-c) for standing-stock densities. Note the direction of the optical depth scales. Also shown is the commercial production index ($\text{CPI} = \sqrt{\text{AP} \cdot \text{VP}}$).

Fig. 3. Volumetric (VP) and areal (AP) production in the 5f systems. See Fig.1 (d-f) for standing-stock densities.

Fig. 4. Volumetric production (VP) for systems grown on 5f nutrients, simulating growth of nonGM, $\text{GM}^{\alpha1}$ or $\text{GM}^{\alpha2}$ microalgae (see Table 1 for algal configurations). Cf. Fig. 3 a-c; Fig. 3b and c show production with GM configurations with limited $\text{Chl:C}_{\max}$, while panels (b) and (c) here show the implications of enhancing α^{Chl} .

Fig. 5. Volumetric (VP) and areal (AP) production in 5f nutrient systems containing nonGM or GM configured microalgae with both decreased $\text{Chl:C}_{\max}$ and enhanced α^{Chl} . (See also Table 1). Note the direction of the optical depth scales.

Fig. 6. Commercial production index ($CPI = \sqrt{AP \cdot VP}$) for various microalgal configurations supported by 5f nutrients.

Fig. 7. Changes in percentage biomass of either GM contaminated by nonGM (a-c), or of nonGM contaminated by GM configured microalgae (d-f) over time following the simulated contamination event. See also Table 2.

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 5

FIG. 6

FIG. 7

Table 1.

Organism designation	Chl:C_{max}	α^{Chl}
nonGM	0.06	7
GM ^{C1}	0.015	7
GM ^{C2}	0.006	7
GM ^{α1}	0.06	14
GM ^{α2}	0.06	35
GM ^{C1α1}	0.015	14
GM ^{C2α2}	0.006	35

Table 2.

Original organism	Contaminating organism and additions as % original biomass	nutrient concentrations relative to f-medium
GM ^{C2}	nonGM at 1%	5f
GM ^{α2}	nonGM at 1%	5f
GM ^{C2α2}	nonGM at 1%	5f
nonGM	GM ^{C2} at 10%	f/0.2
nonGM	GM ^{α2} at 10%	f/0.2
nonGM	GM ^{C2α2} at 10%	f/0.2