Mathematics for streamlined biofuel production from unicellular algae

M. A. Bees[1,*] and O. A. Croze[2]

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Running Title: Mathematics for efficacious biofuel production from algae

1) Department of Mathematics, University of York, Heslington, York YO10 5DD, UK
2) Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, UK

*Corresponding author. **Phone:** + 44 (0) 1904 322038 **E-mail:** martin.bees@york.ac.uk

Martin A Bees[*1] & Ottavio A Croze[2]

One of the greatest challenges of this century is to employ Nature's resources to address the World's energy, food, water and chemical requirements without further unsettling the potentially precarious environmental balance in which we live. The recent resurgence of interest in green algae for biotechnological applications, such as bioenergy, carbon capture and pharmaceuticals, means it is vital that we understand the dynamics of suspensions of living cells. It is widely appreciated that mathematics can aid the optimization of the production of biofuels from algae. However, less obviously, mathematics can reveal mechanisms associated with the fact that many species of unicellular algae swim, and do so in preferred directions in response to environmental cues. Accumulations of cells can induce macroscale hydrodynamic instabilities due to their buoyancy, called bioconvection. There are immediate consequences for algal photobioreactor design, such as methods for cell harvesting, avoiding biofouling and understanding cellular dispersion in pipe flow.

Introduction

Algal biofuels

As the Earth's population rises, and mean energy demands per populace advance at a pace year on year, despite modern efficiencies, and the expensive extraction of Earth's finite greenhouse-warming fossil fuels engenders political and economic conflict, it would be very foolish not to explore every avenue for alternative means of energy production.

The sustainable production of biofuels from microorganisms has been an attractive alternative to fossil fuels for several decades. Recently, it has recently undergone a renaissance as a candidate to retard the increase of atmospheric carbon in the face of rising energy costs. Under optimal nutrient conditions microorganism populations can grow exponentially, yielding large quantities of biomass to make biodiesel [1, 2, 3] and commercially valuable by-products [4]. Microorganisms, such as green algae, can also be induced to produce hydrogen gas (see later) [5, 6].

Photosynthetic microorganisms, such as microalgae are particularly favoured, because of their ability to fix atmospheric carbon, at a faster rate than plants without physically displacing food crops. Favoured methods for intensive cultivation of algae for biofuel production are open raceway ponds, consisting of open-air recirculation channels, and closed bioreactors, with arrays of tubes or panels [1, 7, 8, 9, 10]. Current intensive schemes prefer fast growing species, such as *Chlorella spp.*, that produce oily compounds when stressed (e.g. by nutrient deprivation; [11]). A life cycle assessment of biodiesel production has shown raceway ponds to be most economical in terms of energy and CO_2 [3]. However, it may be better to culture fast-growing, salt-loving algae such as *Dunaliella salina* that are less susceptible to invasion by other species. These algae can be grown extensively in large unstirred ponds and yet, under the right conditions, can accumulate both lipids and β-carotene, albeit in smaller amounts than the most productive but vulnerable species [27]. For precise control, algae can be cultured intensively in tubular bioreactors.

Production of algal biodiesel involves three main phases: algal growth in open or closed photobioreactors, the application of stress (such as nitrogen deficiency) to increase the amount of lipid (fats and oils) per cell, harvesting and downstream processing. In processing, lipids are first extracted from the cells and reacted with alcohols (typically methanol or ethanol) to form biodiesel and glycerol (a process referred to as transesterification) [2]. Biohydrogen production from algae typically involves a two-stage process [12]: cells are first grown in a bioreactor, and then transferred to

sulphur-deficient media under anaerobic conditions where they undergo stress, resulting in the evolution of hydrogen gas. Hydrogen production can be sustained by cycling between sulphur-replete and sulphur-deficient media [13].

The production of high-value by-products of algal growth (such as the nutrient supplement β-carotene) has been profitable for some time, but unsolved bioengineering problems have held back microalgal biofuels from economic viability [1, 2, 3]. For commercial success, photobioreactors should be optimised within the practical constraints of engineering, and the fundamental limits of biology, chemistry and physics.

In recent years, much progress has been made in understanding the physics of microalgal suspensions, employing mathematical descriptions of biased swimming behaviour and subjecting the models to detailed mathematical analysis [6, 14, 15, 16, 17, 18], but these principles have not yet been harnessed to optimise the engineering of biofuel production. This is particularly true with regard to cell accumulation and hydrodynamic instabilities due to swimming behaviour.

Swimming algae

It is estimated that 90% of all harmful algal bloom (HAB) species in our oceans and lakes swim [19]. This startling statistic illustrates that there is a distinct biological advantage associated with swimming. Moreover, many microorganisms swim in preferred directions to improve their environmental conditions. For example, algae swim typically towards regions of weak light intensity and away from potentially damaging bright-light conditions (termed **phototaxis**). Even in the dark the typically negatively buoyant cells tend to swim upwards due to bottom-heaviness or sedimentary torques (**gravitaxis**), a strategy that may be advantageous in murky ponds or deep water. This behaviour is modified significantly in shear flow, leading cells to swim towards regions of downwelling fluid, a response called **gyrotaxis** [20]. This can lead to cell transport phenomena at rates that are much faster than swimming alone. Important swimming species, amongst others, include *Dunaliella*, *Hematococcus, Heterosigma* (a species associated with harmful algal blooms) and *Chlamydomonas*.

Key terms
Gravitaxis: directed swimming motion in response to gravity
Gyrotaxis: biased swimming motion due to a combination of gravitational and viscous torques, typically leading to cell focusing in downwelling regions of the fluid.
Phototaxis: directed swimming motion in response to light
Bioconvection: flow and patterns in suspensions of microorganisms due to biased swimming behaviour

However, the biased swimming behaviour of algae is mostly overlooked or thrown out as an insignificant complication in bioreactor design; bioreactors typically are stirred or bubbled in an attempt to remove heterogeneity. This may be a mistake. In this article we shall describe several ways in which the mathematical analysis of swimming could provide a step-change in the way that photobioreactors are designed and cells harvested.

The need for more detailed mathematical modelling in algal biofuel technology

Mathematics is the unifying core language of the sciences. It is what allows us to quantify observations and place them within a mechanistic logical framework that symbolises, summarizes and allows us to test our understanding. It could be argued that all rational statements and descriptions of the mechanisms of a process are in essence mathematically, or logically, based, and that many scientific breakthroughs hinge on the success of mathematical descriptions. From a collection of assumptions we can formulate models, and by asking specific questions we can obtain exact answers. However, the answers are only as valid as the assumptions, and in many cases the uncertainty of the parameters and possible sensitive dependence of the non-linear models occasionally renders the predictions difficult to interpret.

Whilst mathematics is exact, mathematical modelling is more of an art; individuals from closely-linked subject areas approach modelling differently, emphasising dynamic, numerical, spatial and stochastic elements, and simplifying or complexifying to various degrees. It is essential to be able compare and contrast the approaches, and so there is a need for the kind of style and rigour often observed in studies in the more abstract areas of mathematical biology and theoretical biophysics. Furthermore, with the application of mathematical techniques to real-world problems comes the necessity for model simplification and the approximation of solutions. For complex problems, mathematicians generally employ a two pronged attack: asymptotic and numerical methods provide two distinct approximations that can together provide confidence in results. Some models can supply quantitative predictions, whereas others allow candidate mechanisms or hypotheses to be tested and are more qualitative in nature.

Modelling studies for algal biofuels are just starting to account for physical aspects of bioreactor design in growth dynamics [8, 9, 21]. Whilst the study of intracellular dynamics alone [6] can yield new insights in managing a well-mixed suspension of algae to maximize the product of interest, it is important to recognise the limitations of this approach. Spatial aspects may be incorporated in such descriptions implicitly, such as light absorption, but natural suspensions are inherently heterogeneous over a range of scales. In essence,

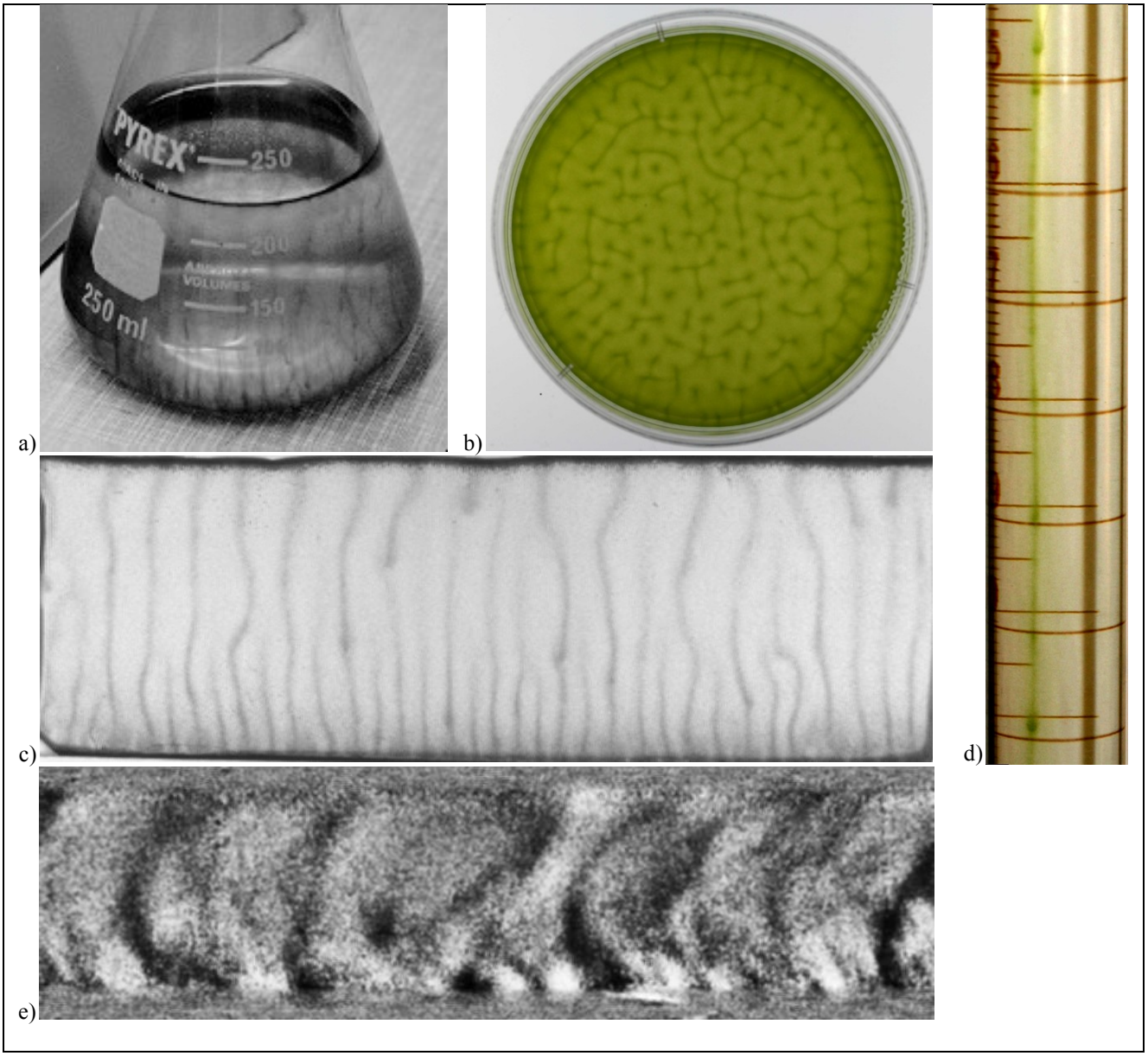

Fig. 1: Depictions of bioconvection in suspensions of gyrotactic swimming green algae, *Chlamydomonas augustae* (a, c, d & e; can produce H_2) and *Dunaliella salina* (b; biofuel candidate; used for production of β-carotene). a) Bioconvection plumes in a culture flask (<10^5 cells cm^{-3}). b) Bioconvection pattern from above in a shallow layer (10^6 cells cm^{-3}; depth 5 mm; diameter 5 cm; brightfield). c) Bioconvection plumes in a vertical Hele-Shaw cell between two glass slides (10^6 cells cm^{-3}; channel width 1 mm; height 2.5 cm; brightfield red light, no phototaxis; photo with J. O. Kessler). d) A gyrotactic plume in an almost vertical cylinder of diameter 2 cm displaying a secondary 'blip' instability (10^5 cells cm^{-3}). e) More complex bioconvection structures are found from the side in a horizontal tube of diameter 2 cm (2.2×10^6 cells cm^{-3}), even in the presence of a flow (mean flow speed is 8.3×10^{-3} cm s^{-1}) [16].

algae have their own agenda of survival and proliferation; many species see fit to expend energy on swimming in preferred directions. For the economic viability of low-value products such as biofuels, bioreactor design must seek to minimize energy input. Industrial suspension mixing and cell harvesting require large amounts of energy input, and thus cost. Yet many swimming algae have their own mechanisms to induce suspension mixing (bioconvection; see later) and natural cell accumulations can be exploited for cell harvesting [22]. In order to employ such potentially cost-reducing phenomena it is desirable to understand mechanistically how the algae behave in a given flow, subject to nutrient and light conditions. For example, nutrient stress can lead cells to store protein and starch [12, 23] asymmetrically within the cell, which in turn affects their gyrotactic swimming behaviour, mixing and self-concentration. An understanding of the behaviour of one cell does not automatically lend itself to an understanding of many hydrodynamically and

Box 1. Modelling suspensions of swimming cells

A key non-dimensional parameter in fluid dynamics is the Reynolds number, Re, defined as a characteristic length scale, L, multiplied by a characteristic velocity, U, divided by the kinematic viscosity, v: Re=UL/v. It describes the relative strength of inertial to viscous effects. For microorganisms such as single-celled algae, diatoms and bacteria, Re is very small, indicating that inertia can, for the most part, be neglected; simple calculations reveal that if a bacterium stopped swimming it would come to rest over a distance less than 10^{-9} m in a time of 10^{-11} s. The consequence is that one need only consider a balance of forces and torques acting on a swimming cell. Disregarding here the typically complex swimming stroke of many microorganisms (but see [41] for simulations of swimming biflagellates in shear flows) we require a torque balance on each cell, accounting for rotation.

For gyrotactic cells (see main text), the key torques are viscous torques acting on the cell surface due to gradients in the flow, and gravitational torques such as the offset of the centre-of-mass from the centre-of buoyancy of the cell (or even sedimentary torques due to body asymmetry) [41, 32, 24]. However, cells tend to swim stochastically so one must also include these effects, which may be modelled using a 'Fokker-Planck description' [24] or a more complete description involving 'generalised Taylor dispersion theory' [43].

To cut a long and detailed story short, one can scale up from models of individuals to describe the mean cell swimming direction, **Q**, and diffusivity, **D**, of a blob of cells in a given flow. This then feeds into a continuum model for a dilute suspension of local concentration n that is subject to a macroscale fluid flow of velocity **u.** The cells drive the fluid flow, but gradients in the flow determine the direction in which cells swim and, ultimately, how they are distributed in the fluid. The fluid is assumed to be incompressible, such that

$$\nabla \cdot \mathbf{u} = 0.$$

Newton's second law, with mass per unit volume multiplied by acceleration on the left balancing the forces per unit volume on the right, provides the classical Navier-Stokes equations with a negative buoyancy term (2nd on the right) for cells:

$$\rho\left(\frac{\partial \mathbf{u}}{\partial t} + (\mathbf{u}\cdot\nabla)\mathbf{u}\right) = -\nabla p_e + nv\Delta\rho\mathbf{g} + \mu\nabla^2\mathbf{u},$$

where p_e is the excess pressure, ρ is fluid density, ρ+Δρ is the cell density, v is cell volume, **g** is the acceleration due to gravity and μ(= v ρ) is the fluid viscosity. The 1st term on the right is the force per unit volume due to pressure gradients, and the 3rd is the force per unit volume due to viscous effects. Finally, we require a cell conservation equation

$$\frac{\partial n}{\partial t} = -\nabla\cdot[n\,\mathbf{u} + nV_s\,\mathbf{Q}(\mathbf{u}) - \mathbf{D}(\mathbf{u})\cdot\nabla n],$$

which balances the rate-of-change of cell concentration with cell fluxes on the right-hand-side due to advection by the flow, mean cell swimming and swimming diffusion. Boundary conditions are required at the walls of particular vessels; typically stress-free or no-slip conditions are applied at free surfaces or solid boundaries, respectively, as well as zero cell-flux conditions at each boundary. This fully coupled continuum description can be used to explore pattern formation [34, 35] and the transport properties of the suspension. Other taxes, such as phototaxis, are readily incorporated into such a description [44].

photosynthetically coupled cells. However, mathematical descriptions have been developed that can scale up the behaviour of one cell to a continuum description of a living suspension of algae. The mathematical analyses of such descriptions have been particularly successful in describing pattern formation, such as bioconvection [24, 15], and the transport of living suspensions of algae in laminar and turbulent flows in bioreactors [18]. However, there are significant mathematical challenges remaining. For instance, it is not clear how best to combine the huge range of time and length scales necessary for applications in biofuels. Furthermore, the precise nature of the coupling between intracellular dynamics, through behaviour to the macroscale and back again via shading and photosynthesis (amongst other coupling) has yet to be determined, modelled and utilized.

Our aims in this article are twofold: we shall draw attention to the beneficial role that mathematicians and physicists can have in the development of biotechnological methods; and we shall highlight what we believe to be the undervalued, critical impact of swimming behaviour on the production of biofuel from algae.

▪ The limitations of this article

This article does not attempt to provide a full review of the literature, which is done elsewhere [15, 24, 25, 26]. Instead it describes some of the recent research interests of the authors and thus possibly puts undue weight on aspects of the work in which they have been involved. Previous reviews do not discuss the use of detailed mathematical descriptions involving fluid dynamics and biological behaviour to explore the impact of cell swimming and intracellular dynamics on biofuel production.

To illustrate the importance and impact of mathematical modelling on all aspects of biofuel production from algae we shall focus on four key results that would not exist without it.

1. We shall begin by considering the mechanisms responsible for generating bioconvection patterns. In so doing we shall briefly describe the motion of an individual biflagellate, including stochastic

aspects, before moving on up the scales to describe **continuum approaches**. We shall discuss applications of the models to predict the wavelength of bioconvection patterns and the implications for growth in single and mixed cultures. We shall discuss **using swimming cells to stir highly productive non-swimmers.**

2. We shall present recent results on modelling and **optimising hydrogen production** from sulphur-stressed suspensions of algae.

3. We shall show how the parameters in our models, such as the mean and standard deviation of swimming speeds, and the flagellar beat frequency, may be measured with ease using the technique of **Differential Dynamic Microscopy,** predicated on mathematical models of swimming behaviour.

4. We shall describe theory to predict the dispersion of biased swimming microorganisms in laminar and turbulent flows in tubes, and present the startling conclusion that **cells and nutrients separate as they travel down tubes**. And we shall suggest how **cell focusing may be employed in bioreactor design.**

Bioconvection and bioreactors

▪ Single species: taxes and bioconvection

There are, of course, other taxes for stimuli beyond the three taxes described above (phototaxis, gravitaxis and gyrotaxis; for instance many cells respond to chemical gradients and others can respond to magnetic fields). The biased swimming behaviour invariably results in cells accumulating in certain regions of the fluid. Furthermore, the cells typically have a different density to the fluid in which they swim, which can cause instabilites and drive fluid flow over time scales of tens of seconds and length scales of cm. For instance, gravitactic (or phototactic) cells tend to accumulate at the upper surface of a shallow layer, leading to overturning instabilities and bioconvection patterns [27, 28, 29, 30], as can be observed in Fig. 1b. Gyrotactic instabilities arise when small perturbations in the fluid flow leads cells to swim towards relatively downwelling regions forcing the fluid to move downwards even quicker owing to the cells added mass [20, 22, 31]. This feedback loop leads to finely focussed plumes of cells in non-mixed cultures (Fig. 1a), vertical Hele-Shaw vessels (Fig. 1c) and vertical tubes (Fig. 1d). If the flow is driven downwards by a pressure gradient, the algae form plumes down the centre of the tube, but for upwelling flow the cells are driven to the cell wall. In a horizontal tube (Fig. 1e) the bioconvection patterns are more complicated, even in the presence of a pressure gradient driving the flow [16].

Much theoretical work has been done to understand these bioconvection structures (e.g. [32, 33, 34, 35, 36, 37, 38, 39, 40]). Modelling involves the consideration of the swimming motion of individual swimmers [41], scaling up from individuals to a description of many cells in a small region [42, 43], and thence to equations for a continuum description of a suspension [24].

See Box 1 for more details of how suspensions of algae are modelled. See also the general reviews on bioconvection [15, 24], recent progress on modelling phyto-gyro-gravitactic bioconvection [44], modelling helical swimming trajectories [45], implications for the distribution of phytoplankton [46, 47], and bioconvection in a stratified environment [48].

▪ Bioreactors: biofouling and harvesting

Consider a tubular bioreactor with upwelling and downwelling components. In the downwelling regions, gyrotactic swimming cells will accumulate centrally, away from the walls. There are three consequences. Firstly, there will be very little biofouling of the tube walls. Secondly, the light transmittance will be significantly affected: non-mixed culture flasks of gyrotactic cells display plume structures, as in Fig. 1a, and allow light to penetrate deep into the suspension or collections of tubes, termed 'the cheese plant effect' [17]. Thirdly, the cells self-concentrate at precisely the centre of the tube, so this is the ideal location to harvest the cells, saving some of the considerable costs associated with harvesting algae. (Note that phototaxis and gravitaxis may also offer efficient methods for cell harvesting.) In regions with horizontal flow and upper boundaries, bioconvection will ensue, which may affect the form of the flow and mixing. In the upwelling laminar regions of the flow, the cells tend to focus at the walls. Therefore, it would be wise to aerate this component to facilitate gas exchange and prevent biofouling. (Typically, in bioreactors with airlifts it is indeed the 'upcomer' that is bubbled.) Even in turbulent flow, cells spend more time in locally downwelling regions, leading to significant transport effects (see later).

Light will also play an important role due to phototaxis, generating preferential swimming motion and bioconvection [30].

▪ Mixed species: stirring with swimmers

In a fully mixed suspension, the principle of competitive exclusion for two species competing for the same resource dictates that the least fit species will be driven to extinction [49]. However, the fittest species are unlikely to synthesise large

amounts of nonessential useful product, such as lipids.

Much is known about modelling single species algal growth in stirred culture (e.g. Fogg & Thakes 1987), although there are surprising subtleties (e.g. microalgal synchronisation; [50]). Additionally, many algae (half of 300 species in [51]) acquire vitamins such as B12 from symbiotic bacteria; under bioreactor conditions algae will always be mixed with bacteria. Recent interest in biofuels has stimulated a surge in growth studies. Nitrogen limitation, leads some species (such as *Chlorella* and *Scenedesmus spp.*) to amass triacylglycerides (esters used for biodiesel; [7]). Furthermore, shading-induced light limitation is extremely important in bioreactors, as is invasion by alien species.

Published experiments on growth in suspensions of algae have not considered the impact of bioconvection, which is in part due to the difficulty of describing both the short timescales of bioconvection and the longer timescales associated with growth. But mostly it is because bioconvection is obscured by standard culture protocols that incorporate mixing.

There are only a small number of studies on the growth of mixed species, but always in stirred cultures. Of these, several have focused on the effect of secreted chemicals (allelopathy; [52]), predator-prey interactions [53], or competition for nutrients [54]. Combinations of freshwater species have been studied [55]: no two species grew as well when mixed. Particular emphasis was on *Hematococcus pluvialis* and *Chlamydomonas reinhardtii*, where the latter was found to suppress the former by release of a fat-like extracellular substance. Furthermore, results for the combination of *C. reinhardtii* and *Chlorella vulgaris*, show suppressed growth (by 25%). Another significant study [56] analysed the effect of pH on the growth of *Chlamydomonas globosa* and *Chlorococcum ellipsoideum*. From total chlorophyll it was inferred that the combined growth for unbuffered media provided a yield smaller than that of *C. globosa* grown in isolation, and bigger than that of *C. ellipsoideum*, as the latter species alters the pH. However, for buffered media, the growth of both species was enhanced. Nutrient competition and allelopathy have been modelled for *C. vulgaris* and *Pseudokirchneriella subcapitata* and compared with batch and chemostat experiments [52]. The effect of light has been recognised as important for mixed species [56] particularly with reference to shading, but has not been incorporated in the published studies and models.

Many of the species in the above experiments swim and generate structure (e.g. *Hematococcus*, *Chlamydomonas*, *Ceratium spp.*), but there are no data on the effect of swimming and bioconvection on growth. We hypothesize that in a non-stirred mixed culture a biased swimming species (such as *Chlamydomonas augustae*) can effectively mix a non-swimming species that is much valued in its ability to produce biofuels (such as *Chlorella vulgaris*). We base this hypothesis on preliminary experiments where we compare stirred and non-stirred cultures of single and mixed species: the useful biofuel species appears to do better in unstirred mixed cultures than in stirred monoculture (see Future Perspectives). As well as further experiments, mathematical modelling and analysis will play an essential role in testing this and other related hypotheses under experimentally realizable conditions. A convincing mathematical description needs to include information about the biased swimming behaviour of individual cells as well as entrainment in bioconvective flows, which may promote gas exchange and light penetration, of benefit to both species, coupled to dynamical models of growth. Such studies are underway by the authors. In this manner, it may be possible to model and thus assemble productive communities 'alga by alga', which should also incorporate models of interactions with bacteria [51].

Optimizing hydrogen production from algae

Mathematical models can also explore the mechanisms involved in the production of biofuels *per se*, and can aid the optimisation of key intracellular processes. There is much recent interest in modelling molecular interaction dynamics, including photosynthetic electron transport and carbon fixation. Here, we restrict ourselves to describing one example of modelling intracellular dynamics applied to biofuel production from algae that models the subtle interplay between photosynthesis, sulphur uptake, respiration, catabolism and hydrogen production via iron-hydrogenase [6].

Suspensions of unicellular, anaerobic, sulphur-stressed green algae, such as *C. reinhardtii*, have long been known to photosynthetically produce hydrogen gas [57] (biohydrogen, a biofuel of considerable potential) via the action of an iron-hydrogenase enzyme on the thylakoid membrane internal to the cell. Unfortunately, the iron-hydrogenase is inhibited by oxygen that is also produced during photosynthesis, rendering the two processes incompatible. However, recently, a two-stage process has been unveiled to temporally separate the two processes [12]: in the first stage, cells are grown under normal nutrient conditions, and then, in a second stage, the cells are transferred to media deficient in sulphur, which has the effect of partially deactivating the oxygen-evolving photosystem II (PSII). (In PSII, sulphur deprivation leads to deactivation of essential reaction-centre D1 protein biosynthesis.) Activity of photosystem I (PSI) and aerobic respiration are not directly affected by the absence of sulphur [12, 58, 59].

Box 2. Modelling H_2 production by algae

The biological details of the system to be modelled are described in the main text. The model consists of a set of mass balance equations modelling an asynchronous population of cells in a sealed container, with light from both sides. The following variables are modelled: cell volume fraction, $0 \leq \Lambda \leq 1$; concentrations (in μM) of external and internal sulphur, S and s, respectively, oxygen, ω, endogenous substrate, e, and protein, p; and hydrogen gas, h (in mL/L). The variables s, e and p are intracellular concentrations, called quota [67], and S is an extracellular concentration. The model consists of the following equations, which we write for brevity in words. For explicit details, consult [6]. The functional forms for sulphur uptake by the cell are empirically derived from independent experiments, such that

$$\frac{dS}{dt} = \text{sulphur system input} - \text{sulphur uptake by cell},$$

$$\frac{ds}{dt} = \text{sulphur uptake by cell} - \text{PSII repair} + \text{protein breakdown} - \text{protein production} - \text{growth/decay},$$

where 'PSII repair' indicates internal sulphur continuously required to repair photosystem II under illuminated conditions. The equation for protein is

$$\frac{dp}{dt} = -\text{protein breakdown} + \text{protein production} - \text{growth/decay},$$

and for oxygen is

$$\frac{d\omega}{dt} = \text{photosynthesis} - \text{respiration} - \text{supersaturation loss}.$$

Growth is modelled via

$$\frac{d\Lambda}{dt} = \Lambda \times (\text{growth or decay factors}),$$

and is dependent on light availability, $L(\Lambda)$, within the fully stirred suspension via p. Finally, hydrogen is produced at a rate specified by and in response to appropriate ranges of values of the other variables, such that

$$\frac{dh}{dt} = \Lambda \times (O_2 \text{ sensitivity}) \times (\text{PSII dependent} + \text{PSII independent}) \times (e^- \text{ pathway}).$$

The parameters are taken or ranges of estimates obtained from independent experimental measurements, where possible. Good qualitative and quantitative agreement with experiments is obtained, both in terms of hydrogen yield and production onset time, without resorting to fitting the model output to data. More importantly, the full system allows for systematic optimization of hydrogen production within the two-stage scheme [12] (sulphur-replete followed by anaerobic sulphur-deficient conditions) as a function of the initial sulphur concentration and the light intensity. For instance, the model indicates that optimization depends on whether the maximum H_2 production rate or the maximum yield is required, and predicts optimal values of the light intensity and non-zero initial sulphur concentration, that are in line with experiments. There are conflicting experimental results in the literature concerning rates of production per cell; the model provides support for a relatively constant H_2 production rate per cell. Furthermore, the real value of the model is that it can be used to design new schemes for controlling hydrogen production that move beyond the confines of the two-stage scheme.

After 24 hours under illumination the rate of oxygen production from photosynthesis is less than that required by aerobic respiration [60, 12, 23, 59]. In the light, the oxygen-sensitive iron-hydrogenase is activated to act as a life-saving sink, to remove potentially damaging electrons produced from the partially deactivated PSII pathway (water splitting; 80%) and fermentation (20%), which yields hydrogen gas for approximately 100 hours [12, 61, 23, 62, 63]. After which activity ceases due to depletion of options for catabolism. Re-suspension of the cells in sulphur-sufficient media allows them to reset to the first stage and the process may be repeated [60].

The process has been demonstrated under solar light [64], although the optimal conditions are far from certain. The light conditions and suspension mixing had a large effect. Furthermore, the switching necessary between sulphur-deprived and sulphur-sufficient media is burdensome.

In order to optimize hydrogen gas production using the two-stage process, and to go beyond this framework to one of continuous sulphur and hydrogen control, a simple mechanistic model description was designed that contains the essential feedback loops and encompasses both sulphur-deprived and sufficient conditions [6]. The description builds upon previous partial descriptions (particularly [21, 65]).

The formulation of the model consists of a set of ordinary differential equations driven by time-dependent culture conditions. Naturally, the system is complex and incorporates active sulphur transport across the cell wall [66], cellular growth (a modified Droop model; [67]), sulphur dependent photosynthesis (subject to shading by other cells; [68]), protein and starch storage and breakdown, oxygen production and use, and hydrogen production. Naturally, there are quite a few parameters. However, the values of many of the parameters are either known or can be inferred from independent experiments (e.g. such as for active sulphur transport across cell walls). Importantly, the mechanistic approach avoided having to fit numerical solutions of the model to the data that it is required to reproduce. See Box 2 for a brief description of the model.

The upshot of this modelling approach is a robust description that can be probed to reveal optimal conditions. The optimization can be shackled to the two-stage process or less strongly constrained. The authors found good qualitative

Box 3. Measuring mean swimming parameters quickly and in bulk: DDM

Differential Dynamic Microscopy measures simultaneously the swimming characteristics of many microorganisms, such as bacteria driven by helical flagellar bundles inducing body wobble, or biflagellate algae with back-and-forth trajectories [74].

First, spatiotemporal fluctuations of the image intensity, $I(\mathbf{r},t)$, at position $\mathbf{r}$ and time t, are analysed via the differential image correlation function (DICF). The DICF, g, is the power spectrum of the fluctuation as a function of the time-delay, such that

$$g(q,\tau) = \langle |\, I(\mathbf{q}, t+\tau) - I(\mathbf{q}, t)|^2 \rangle,$$

where q is the magnitude of $\mathbf{q}$ the wavenumber, τ is the time delay between images, and the angled brackets indicate that we have averaged over time and orientation. Assuming that differences in image intensity are proportional to differences in cell concentration, one can show that the DICF is related to the intermediate scattering function (ISF), $f(q,\tau)$, via

$$g(q,\tau) = A(q)[\,1 - f(q,\tau)\,] + B(q),$$

where A(q) encodes the optics, particle shape and mutual arrangement, and B(q) captures the camera noise [72].

Second, for independent swimming cells the ISF can be constructed from

$$f(q,\tau) = \langle \exp\left(\mathbf{q}\cdot\Delta\mathbf{r}_j(\tau)\right)\rangle_j,$$

where $\Delta\mathbf{r}_j$ is the j-th particle displacement and the angle bracket here indicate an average over all particles [75]. Hence, the ISF can be constructed by modelling the behaviour of each swimming microorganism. Consider, for instance, the swimming biflagellate *Chamydomonas reinhardtii*, which swim with a breast stroke in back-and-forth manner at 50 Hz. Additionally, cells swim such that their swimming-induced axis of rotation is misaligned with their swimming direction, leading to helical trajectories with a frequency of 2 Hz. For example, a simple breast stroke model of swimming of the form

$$\Delta r = v\tau + A_0 \sin(2\pi f_0 \tau + \psi),$$

where v is the velocity from a Schulz distribution with standard deviation σ, and A_0, f_0 and ψ are the amplitude, frequency and random phase of the back-and-forth motion, respectively, provides a mathematical expression for the ISF of a suspension that can be fitted to the ISF from the image data found above. The process applied to images of 10^4 cells is able to determine the parameters and can clearly demonstrate the length and time scales over which the model could be improved, such as by incorporating helical motion. The analysis establishes that *C. reinhardtii* swims with a mean velocity v_0 = 89.6±2.8 μm/s, σ_0 = 24.9±4.6 μm/s, A_0 = 0.98±0.06 μm and f_0 = 48.6±0.6 Hz, in excellent agreement with cell tracking experiments [74].

agreement with published experiments for trends in hydrogen yield and initiation time. The optimal external sulphur and illumination conditions for hydrogen production were determined and found to differ for either the overall hydrogen production or its rate [6]. The question of designing a continuous control approach to intelligently stress the cells is postponed for a subsequent publication.

Practical high-throughput parameter measurement

If swimming behaviour were to be employed, or at least taken into account, in bioreactor design, it would be necessary to characterize each candidate species of microorganism. The values of the parameters feed in to the model descriptions.

Much information can be gained by the microscopic observation of individual swimmers. The location of the cells can be tracked from which data on swimming velocities can be extracted. Typically, a sequence of images is acquired, bright features are located, and the features are joined into the most likely trajectories, allowing for close approaches and missing data [69, 70]. Furthermore, observations of changes in orientation can provide information on the mechanisms for directional bias, such as measurements of the centre-of-mass offset. However, there are some drawbacks. For standard microscopy, tracking is limited to cells transiting the focal plane so, typically, long tracks are rare. This typically limits observations to the order of 100 cells. 3D tracking is also possible but requires specialist equipment [71].

Key terms
DDM: differential dynamic microscopy
DLS: dynamic light scattering; a technique that explores size structure of objects in suspension by measuring how light is scattered
ISF: intermediate scattering function; used in DLS
DICF: differential image correlation function

Differential Dynamic Microscopy (DDM) is a new method [72] that has been developed for the high-throughput analysis of motility, measuring mean attributes of 10^4 cells from a simple low-magnification movie in just a few minutes [73, 74]. In essence the method is analogous to and yields the same information as dynamic light scattering [75], the intermediate scattering function (ISF), $f(q,\tau)$, but is performed *in silico* and can gain better access to the length scales relevant to microorganism motility. See Box 3 for a brief description of the DDM method.

It should be emphasised that DDM is complementary to tracking, in the sense that an accurate model of each swimmer is required in order to generate an ISF for the suspension. However, there is potential to construct a DDM signature for each species and even to explore biased swimming behaviour and the ISF of suspensions of two or more species. The main advantages of DDM are the speed with which one can obtain measurements from large numbers of individuals, the understanding obtained by the isolation of particular

Box 4. Flow in a tube, Taylor dispersion and the dispersion of biased swimming cells

For laminar flow in a tube with impermeable walls the flow adopts a parabolic profile called Poiseuille flow: the flow **u**=(u,v,w) in a vertical tube with no-slip boundary conditions is given by

$$w = -\frac{1}{4\mu}\frac{\partial p}{\partial z}(R^2 - r^2),$$

where $\frac{\partial p}{\partial z}$ is the pressure gradient in the z direction along the tube, R is the tube radius, r is the radial position and μ is the fluid viscosity. For turbulent flow the fluid velocity is more complicated, but the mean velocity profile is relatively simple: there is a relatively flat mean profile across the central region of the tube which decreases rapidly to zero near the tube walls. The flow in a channel is similar (see [18]). Passive tracer placed in the flow (e.g. a blob of dye or nutrient) will be sheared by the flow and initially adopts a parabolic profile. For long times, diffusion across streamlines in the flow allows the tracer particles to experience different flow velocities on the streamlines, leading to a Normal distribution in the axial direction. This is called Taylor dispersion (or Taylor-Aris dispersion) [77, 79], which states that the tracer will drift along the tube but will spread out in the axial direction with an effective diffusivity, D_e, of

$$D_e = D_m\left(1 + \frac{Pe^2}{48}\right); \quad Pe = \frac{UR}{D_m},$$

where D_m is the molecular diffusivity, U is the velocity at the centre of the tube and Pe is a non-dimensional quantity called the Peclet number. (Note, surprisingly, that with the definition of Pe substituted the effective axial diffusivity is made up of the molecular diffusivity plus a term proportional to one over the molecular diffusivity.) Similar results can be obtained for channel and turbulent flows [78, 76].

In contrast, biased swimming cells actively swim across streamlines, so classical Taylor theory does not apply. A new theory, based on the method of moments, allows for the prediction of the dispersion of biased swimming cells in suspension in a flow in a tube. First, the flow in the tube needs to be calculated. This is not necessarily Poiseuille flow as the negative buoyancy of the cells can alter the profile, but this can be calculated from the continuum model introduced in Box 1. Second, the cell conservation equation can then be expanded using the method of moments to reveal how the cells disperse: how they drift relative to the mean flow and how they diffuse in the axial direction [17, 80]. Closed form expressions for both drift and effective diffusivity have been found for general biased motion, but they are long and involved so are not repeated here [17]. To use these expressions, one only needs to insert the form of the biased motion to calculate the drift and diffusivity. The fact that biased swimming cells accumulate in different parts of the flow means that they drift down the tubes at a rate that is different to the mean flow (unlike nutrients), and they diffuse less than nutrients. **The significant conclusion is that biased swimming cells (with any form of taxis) disperse very differently to dye, nutrients or tracer particles (see main text) in both laminar and turbulent regimes in a flow in a tube or channel [18].**

length and time scales, and the relative simplicity of the apparatus, allowing measurements to be obtained easily in the field.

Cells and nutrients move at different rates in flows in tubes

Many closed algal photobioreactors are tubular. Such controlled environments are particularly important for species that are easily displaced in competition with more robust, faster growing but less-useful species. **A natural question to ask is how do the cells in suspension travel through the bioreactor relative to a given flow that is driven by a pressure gradient.** Of course, flow profiles in a tube with impermeable walls are well known, and must accommodate the fact that the fluid velocity is exactly zero at the wall (see Box 4).

A starting point might be to assume that swimming is negligible, or even that the cells are equally likely to swim in all directions, changing direction in some stochastic manner. With this assumption, one might come to the conclusion that the cells in a still fluid will diffuse. In which case, in a flow in a tube a process called Taylor dispersion (or Taylor-Aris dispersion; [77, 78, 79]) adequately describes how the centre of a blob of dye (or tracer particles) moves at the mean flow speed with the blob purely diffusing in the axial direction with a rate given by a simple formula that has been experimentally verified (see Box 4). This effective axial diffusivity is a curious result in that there is a term proportional to the molecular diffusivity plus another proportional to the reciprocal of molecular diffusivity. This is due to dye or tracer particles diffusing across streamlines in the flow and being rapidly separated by different flow velocities on those streamlines.

However, a suspension of biased swimming cells behaves very differently: the cells actively swim in preferred directions across the streamlines. The result is that cells accumulate in particular regions of the flow (e.g. gyrotactic cells accumulate in downwelling zones), drift relative to the mean flow and diffuse rather differently. This behaviour can be captured using mathematical analysis for both laminar [17, 80, 18] and turbulent flow regimes [18] (see Box 4). There are significant effects even with turbulent flow.

One of the main implications is that if cells and nutrients are inserted into a tubular bioreactor at the same point, they will separate rapidly. For instance, in a downwelling flow in a

vertical tube nutrients will be transported on average with the mean flow whereas gyrotactic algae will drift at close to twice this velocity and will diffuse much less. Similarly, chemotactic or phototactic microorganisms may be attracted towards particular boundaries, impeding drift. Furthermore, as indicated previously, many cells are negatively buoyant. Accumulations of cells can either drive fluid flow or significantly affect the flow profiles (see Fig 1). To a lesser extent, even non-swimming cells can be transported at a different rate than that of the mean flow due to buoyancy effects (e.g. [81, 82]).

Conclusions

The fossil fuels industry has employed mathematics for many decades to advance complex methods of extraction and processing. That there is much to be gained from mathematical modelling and analysis in these areas is evidenced by the heavy investment from government and private sources and the wealth of multidisciplinary literature. In contrast, few challenging mathematical results are available for the emerging biofuels industry. This is particularly true for biofuels from microorganisms.

In this article, we present some academically inspired old results and some application-driven new results, which we believe to be of real interest to the biofuels community.

For instance, we discuss how the self-focusing nature of biased swimming cells may be exploited to harvest the cells or prevent biofouling. We explore the implications of biased swimming in suspensions of single and mixed species, and discuss the potential to mix suspensions of productive non-swimmers with swimming species (see below). We present a concrete example for the optimization of hydrogen gas from algae, which whilst simple to analyse mathematically is complex in its structure. We describe a new mathematical-experimental technique, differential dynamic microscopy, that can be used to rapidly and accurately measure the swimming characteristics of biofuel candidate algae using relatively simple apparatus. And finally, we show how an in-depth mathematical description of the swimming behaviour of algae can reveal the extent to which small biased motion in prescribed flow can lead to anomalous transport phenomena. In particular, biased swimming cells can be rapidly segregated from non-swimmers and nutrients. These results should be of significant value to those involved with the design of efficacious algal photobioreactors. In particular, the directionally biased swimming behaviour of algae should not be neglected; rather, it should influence bioreactor design and have a critical impact on the production of algal biofuels.

The bio-engineering community has yet to take stock of the old and new theoretical results described above. This is in part due to poor communication between the applied mathematics, physics and bioengineering communities. There is a distinct lack of overlap in expertise, motivation, perspective and language. It is important to note that some of the mathematics described is far removed from the experience of those at the frontline of biofuel development. Furthermore, many mathematicians and physicists have little idea as to where they should focus their effort to be of practical use. Here, we have attempted to bridge this gap by describing a few select results in accessible terms, but providing appropriate reference to the literature. Many of the results described herein can and should be developed further in a multidisciplinary setting.

For the greatest benefit to the biofuels industry, we call for greater and closer collaboration between the mathematical and bioengineering camps.

Future perspective

Current biofuel production methods from algae require a dramatic increase in performance to become competitive with fossil fuels. We believe this step change will be realised only if mathematical analysis, not just modelling and computation, is employed to guide engineering, as has been the case in the oil industry for many decades. Moreover, mathematicians and physicists should be engaged to bring forward and develop new techniques and analyses for application to the hard problems associated with the large range of spatial and temporal scales, and the complexity resulting from such living suspensions.

A better use of mathematical modelling and analysis has the potential to revolutionise biofuel production engineering, from culture growth to downstream processing. For example, our vision based on current modelling is of bioreactors for swimming cells with minimal fouling, which concentrate and harvest cells efficiently using tailored combinations of flows (gyrotaxis) and light (phototaxis). Swimming statistics (obtained automatically via differential dynamic microscopy) could be used to monitor the accumulation of valued products (e.g. lipids) and the health of a growing population (e.g. to manage hydrogen production).

In order for such systems to be developed, interdisciplinary collaboration is necessary; a tighter working relationship needs to be developed between physical and mathematical biologists, biofuel engineers and biologists. This needs to occur both within and between academia and industry, which will allow key bottlenecks to be identified and resolved.

In these respects, our own experience suggests that researcher exchanges can be extremely beneficial: we recently received an EPSRC mobility grant for OAC to move for one year from a mathematical biology group to a plant metabolism group. The project has been invaluable for two-way knowledge exchange and a source of important new problems (coupling swimming, bioreactors and symbiosis). Furthermore, there are examples of new training networks that attempt to bridge the divide between quantitative and engineering disciplines, such as the EU funded Initial Training Network 'Acclimation of Photosynthesis'.

Future bioreactor research should address
A) buoyancy effects of non-swimming species in bioreactor geometries;
B) lipid dynamics at the single cell level;
C) coupling biology and swimming mechanics in bioreactors, including i) up-scaling of growth and swimming dynamics, ii) heterogeneous distributions of algae in flows, iii) new bioreactor geometries, and iv) assembly of stable symbiotic communities tailored to enhance productivity; and
D) efficient self-concentrating systems for pond and tubular reactors using taxes.

In the context of C) iv), preliminary laboratory experiments show that the growth rate of *Chlorella vulgaris* (a lipid-productive non-swimming species) in unstirred co-culture of *Chlamydomonas augustae* (a non-productive swimming species) is greater than that in a stirred monoculture. It would seem that bioconvection due to *C. augustae* is quite able to match if not better the benefits of a mechanical stirrer. However, the experiments need to be repeated to provide statistically significant results, and we are engaged in modelling bioconvective and photosynthetic aspects of the system. The notion that the productivity of algal co-cultures might be greater than the productivity of monocultures because of swimming is a promising and unexplored avenue for biofuels research.

Financial & competing interest disclosure: none.

Acknowledgements

OAC and MAB gratefully acknowledge support from the EPSRC (EP/D073398/1) and the Carnegie Trust.

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Executive Summary

Introduction

- This article describes why, in our view, mathematical modelling is implicit to the biofuels industry and, in particular, for the efficacious deign of algal photobioreactors. Examples are provided.

Bioconvection & Bioreactors

- Many algae swim in biased directions. Swimming may be used to help harvest cells, allow greater light penetration in dense suspensions and prevent biofouling.
- The biased swimming of algae in suspension can result in bioconvection patterns over timescales of tens of seconds and length scales of centimetres.
- In mixed species suspensions, theory and preliminary experiments suggest that swimming cells can stir suspensions of highly productive non-swimmers. The non-swimmers may grow quicker in mixed suspensions than in single species mechanically stirred cultures.

Optimizing biohydrogen production from algae

- The production of hydrogen from algae may be optimized through the use of mechanistic mathematical models.

Practical high-throughput measurement of parameters

- A new mathematical-experimental technique, differential dynamic microscopy (DDM) can rapidly measure the characteristics of large numbers of swimmers in suspension with relatively simple apparatus.

Cells and nutrients move at different rates in flows in tubes

- In tubular bioreactors, biased swimming cells disperse very differently to nutrients or tracker particles: swimming cells and nutrients rapidly drift apart due to active swimming across streamlines and fluid flow gradients in the tube.

Conclusion

- We call for greater collaboration between the mathematical and bioengineering camps to help solve hard problems associated with biofuel production.

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