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A droplet-based screen for wavelength-dependent lipid production in algae†

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We report a digital microfluidic system designed for droplet-based long-term culture and analysis of algae. The system includes unique innovations relative to standard devices including an active reservoir structure to maintain homogeneous cell density, a customized device layout capable of controlling a wide range of different droplet volumes, vertical interconnects to collect spent reagents, detection zones compatible with parallel-scale optical measurements using a standard multiwell plate reader, and optimized features for droplet dispensing in parallel. The method allows for automated, multiplexed analysis with significant reductions in human intervention, representing a decrease from 600 pipette steps (for a conventional screen in multiwell plates) to fewer than 20 (for the new microfluidic technique). The system was applied to screen conditions favourable for lipid generation in the widely used algal model for biofuel production, *Cyclotella cryptica*. A dependence on illumination wavelength was observed, with the best conditions (representing a four-fold increase relative to control) comprising an alternation between yellow (~580 nm) and blue (~450 nm) illumination wavelengths. These effects were observed for both micro- and macro-scale cultures, and are consistent with a putative mechanism involving photooxidative stress. We propose that the microfluidic system described here is an attractive new screening tool with potential advantages for applications in renewable energy, biotechnology, materials science, and beyond.

Broader context

Algae have been touted for many years as a renewable source of high-energy lipids for biofuel production. But current algae-lipid energy densities cannot compete with traditional fuels, meaning that new methods must be developed to discover conditions favourable for lipid production. In response to this challenge, we have developed a droplet-based microfluidic method capable of high-throughput screening of algal culture conditions with minimal human intervention. After evaluating hundreds of conditions, a new phenomenon was observed: incubation at yellow wavelengths (~580 nm) causes algae cells to increase production of energy-rich lipids two-fold relative to cells grown under standard conditions. Further, alternating exposure to blue wavelengths (~450 nm) and yellow wavelengths results in a four-fold increase, an effect that is observed on both the micro- and macro-scales. The dependence of lipid production on yellow illumination correlates with the generation of reactive oxygen species, which is consistent with potential mechanisms related to wavelength-dependent photooxidation. Follow-up work is needed, but these results suggest that culture-illumination wavelength will be a useful variable to explore in the quest to increase energy yields for algal-derived biofuels.

Introduction

There is great interest in the development of renewable sources of energy-bearing materials, such as biofuels, to eventually replace the limited supply of fossil fuels.[1–3] One system that has received attention as a potential source of biofuel is the energy-rich neutral lipids (often in the form of triacyl glycerols, TAG) that are produced by algae.[4] There are a number of reasons for this popularity, including: (1) the TAG content in algae can exceed 80% of the dry weight, which is greater than other precursors used to produce biofuel (*e.g.*, sugarcane, soybean), (2) the lipid yield per total culture area is the highest for algae compared to all other crops and therefore does not require a large cultivation area, (3) algae grows extremely rapidly, with biomass doubling times of 24 h or less, and (4) algae does not compete with crops grown for food consumption. Despite these favourable properties, biofuels generated from algae grown in standard conditions do not have the energy-density necessary to compete with traditional fuels and therefore they are not

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currently economically viable.[5] Thus, it is widely recognized that for algae-derived biofuel to be useful, new methods must be developed for harvesting increased densities of energy-rich molecules.

There have been intense research efforts aimed at increasing and modifying the accumulation of lipids in algae through biochemical and genetic approaches.[6,7] The biochemical approach entails controlling cultivation conditions[8–11] (*e.g.*, nutritional content, temperature, and pH); for example, the elimination of nitrogen, phosphorus, or silicon from algal culture medium stresses the cells, causing enhanced generation and accumulation of high-energy lipids, but at the cost of reduced algal replication. The genetic approach involves over- or under-expressing genes involved in lipid biosynthesis pathways;[12–16] for example, over-expression of Acetyl CoA Carboxylase (ACCase) has been found to increase neutral lipid production rates. In both types of systems (biochemical and genetic), algae are typically grown in 100 mL flasks with the results being analyzed in well-plates. In many cases, this process is not automated, and thus suffers from the tedium of repetitive pipetting.

It has long been recognized[17–20] that microfluidics, a technology that exploits fluid flow in enclosed micron-dimension channels, represents a potential boon for applications involving algae. Specifically, microfluidic systems have been developed for investigation of algal growth kinetics,[17] for electroporating algal cells,[18] for optimization of photosynthesis,[19] and for classification of populations of algal cells.[20] Recently, similar methods have been developed to evaluate of lipid production in algae,[21–25] either as a function of nutrient limitation,[21–23] single-cell sequestration,[24] or as a consequence of variations in light intensity.[25] These studies are exciting and innovative, but they largely reproduce what is known about algae;[8–11] namely, that culture conditions are important variables in biomass and lipid accumulation. As far as we are aware, microfluidics has never before been used as a discovery tool to identify new conditions that result in enhanced lipid production.

A potential alternative to microchannels for miniaturization and integration of methods related to algae culture and analysis is known as digital microfluidics[26,27] (DMF). In DMF, droplets are manipulated on an open array of electrodes by the application of electric fields.[28] The DMF format possesses unique features that are not present in channel-based microfluidics; for example, in DMF, each droplet is controlled individually without the need for pumps, valves, or mechanical mixers. DMF has been used previously for a number of applications related to mammalian cell-culture and analysis,[29–37] but there is only a single previous report of DMF applied to an application involving algae[38] (a study that simply confirmed that algae can be cultured on DMF devices).

Here, we describe the first microfluidic system applied to discovery of algae culture conditions favourable for generating high-energy lipids. The new method, which relies on DMF, was used to make hundreds of measurements on algae exposed to different potential stressors, with very low levels of manual intervention (*i.e.*, 600 fewer pipette steps compared to traditional methods for screening in multiwell plates). More importantly, we report the discovery of a new phenomenon: incubation at yellow wavelengths (~580 nm) causes the cells studied here to produce significantly more energy-rich lipids than cells grown under standard conditions. In addition, it was found that an alternating exposure to blue wavelengths (~450 nm) and yellow wavelengths results in favourable cycling of algae growth and lipid production. Finally, we discuss potential mechanisms that may account for this effect, which may have important implications for future work to develop algal sources of biofuel.

Results and discussion

Digital microfluidic system

Cyclotella cryptica was used as a model system because these diatoms are used routinely for lipid production for biofuel research.[1,39] Screening for lipid production in algae requires careful accounting for neutral lipid amounts (which can be interrogated by fluorescence), as well as for algal cell density (which can be interrogated by absorbance). Traditional methods for multiplexed algae culture and multiplexed fluorescence and absorbance measurements are tedious and time-consuming, requiring many transfers between well plates, which led us to develop a new method relying on digital microfluidics.

Several design iterations were required to develop a device capable of long-term, multiplexed culture and analysis of algae. Numerous challenges were encountered in this process (summarized in Table S1 in the ESI†), including (a) the observation that algae settling during initial loading and seeding results in uneven distribution of cells into sub-droplets, (b) a need for simultaneous control over hundreds of droplets spanning a wide volume range (from 150 μL to 2 μL), (c) accumulation of excessive waste products over time, (d) a need for multiplexed absorbance and fluorescence detection, and (e) a requirement for reliable sub-droplet dispensing in parallel. The final device design, shown in Fig. 1, includes a number of unique innovations developed in response to these challenges, including (a) an "active" reservoir structure[38] to maintain homogeneous cell density, (b) a pattern of electrodes with varying sizes and shapes dedicated to controlling particular droplet volumes, some "bussed" (*i.e.*, electrically connected) together to reduce operational complexity, (c) a series of vertical through-holes to allow waste liquid to wick away from the device, (d) an array of detection zones (including transparent windows for transmission[38]) with 9 mm pitch compatible with absorbance and fluorescence measurements using a standard multiwell plate reader, and (e) dispenser-electrode modules with narrow central electrodes to facilitate necking during droplet dispensing.[40] The latter innovation was particularly important for reliable droplet dispensing in parallel on bussed electrodes, and a test to identify optimum geometries for best performance is summarized in Table S2 in the ESI.† The device was programmed and managed using an automated system (Fig. S1 in the ESI†) with impedance sensing and feedback control over droplet position,[33] which was critical for successful

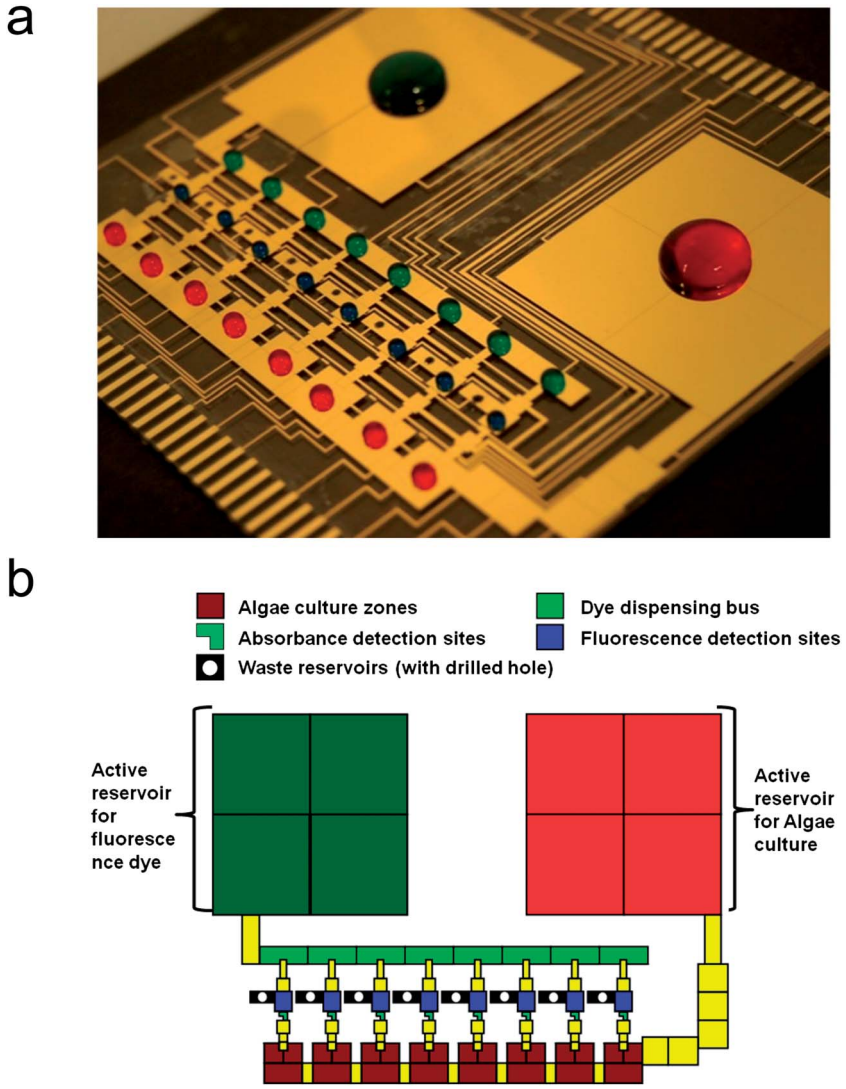

Fig. 1 Digital microfluidic device used for algae culture and multiplexed screening. (a) Picture (shown without top plate for clarity) and (b) schematic. The design includes two four-electrode active reservoirs, eight bussed dye dispensing electrodes, eight algae culture zone electrodes, eight absorbance detection electrodes, eight fluorescence detection electrodes, and eight waste reservoir electrodes bearing 1 mm-dia. through-holes. All dispensing regions feature narrow electrodes (a ratio of at least 0.5 : 1 relative to the destination electrode), which was found to be important for reliable parallel dispensing (as per ESI Table S2†).

completion of the complex sequences required for culture and screening.

The device shown in Fig. 1 was designed to generate arrays of droplets containing algae, which were (a) exposed to different culture conditions, and (b) interrogated with regular absorbance and fluorescence measurements. The method is summarized in Fig. 2 (and in a ESI movie file†), and described in detail in the methods section. Briefly, in step one, one aliquot each of algae suspension and LipidTOX (a fluorogenic reporter that fluoresces in the presence of neutral lipids) were loaded onto the device and continuously mixed to maintain homogeneity. In steps two and three, arrays of eight algae culture droplets and eight label-droplets were formed, and in steps four and five, absorbance and fluorescence measurements (after mixing with label droplets) were collected. Steps three-five were repeated multiple times after incubation under different conditions to screen for those that are favourable for neutral lipid production.

The device and method shown in Fig. 1 and 2 was designed to operate with minimal manual intervention. In typical experiments, two initial pipette steps were sufficient for a single device to run for 24 hours, generating 48 absorbance/fluorescence measurements on algae in 24 different states (*i.e.*, eight different culture conditions evaluated at three different time points). In practice, each state was evaluated in at least three replicates (on three different devices), which allowed for the generation of 144 data-points from a total of 6 pipette steps. For comparison, generating the same data using well plates and a single-channel pipette would require 168 pipetting steps.[41] In the work described below, these methods were applied to evaluate an array of different culture conditions, such that <20 pipette steps were sufficient to generate data that would

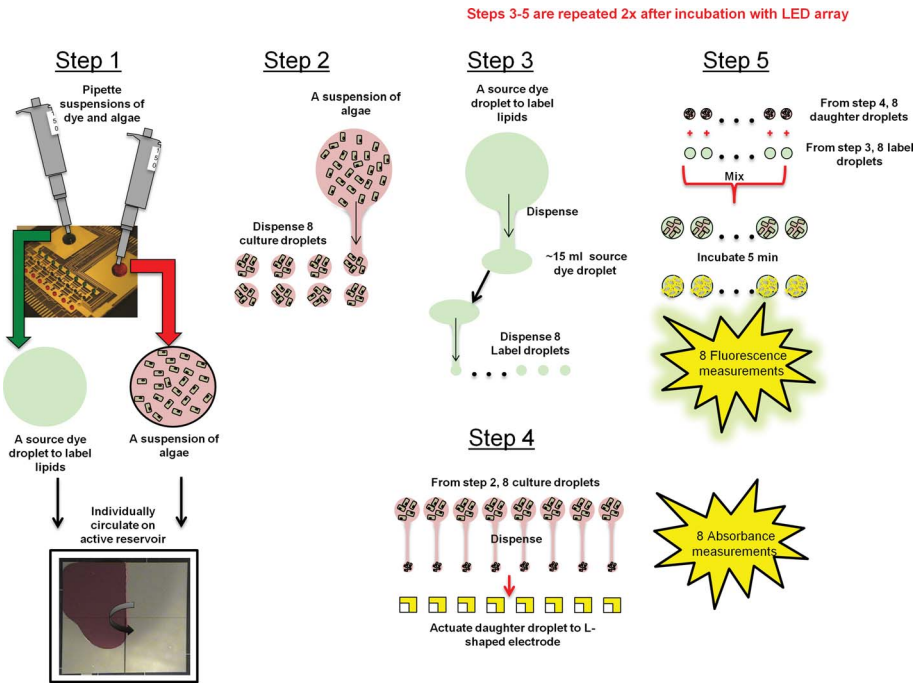

Fig. 2 DMF algae culture and screening scheme. The pictures are frames from a movie that can be found online as ESI.† In step 1, aliquots of algal cell suspension and dye solution are loaded and mixed to maintain homogeneity. In step 2, eight culture droplets are dispensed and actuated to the algae culture zones. In step 3, a dye source droplet is dispensed and actuated to the dye dispensing bus, forming eight label-droplets. In step 4, eight daughter algae culture droplets are dispensed and actuated to corresponding L-shaped electrodes for absorbance measurements. In step 5, eight daughter culture droplets and eight label fluorescent droplets are mixed and incubated for fluorescence measurements. Steps 3–5 are repeated multiple times after incubation under different illumination conditions.

typically require more than 600 pipette steps, representing >30-fold reduction in manual intervention. The recent report of methods to form and operate 4096-element DMF devices[42] should eventually facilitate even greater reductions in manual intervention in the future (*e.g.*, >500-fold fewer pipette steps[43]), allowing for high-throughput screening (HTS) that is typically only available to researchers with access to expensive robotic platforms. In addition, given the recent report of inexpensive DMF device formation by inkjet printing on paper substrates,[44] devices similar to those used here may eventually be generated in any laboratory at very low cost. Thus, we propose that the devices and methods described here, which are free from moving parts, pumps, valves, and fittings and rely on off-the-shelf detection systems (*i.e.*, a plate reader), may eventually be useful to democratize high-throughput screening for applications ranging from systems biology[45] to materials discovery[46] to ionic liquid screening for biofuel production.[47]

Algae screening results

We used the system described above to evaluate the effects of algae culture illumination, motivated by two recent results in the literature,[48,49] both of which rely on standard well-plate methods. Wang *et al.*[48] reported that algae growth rates (with no evaluation of lipid production) are dependent upon illumination wavelength and intensity, and Chen *et al.*[49] reported that lipid generation was dependent upon illumination intensity and duty cycle at a single wavelength. Building on these studies, we hypothesized that a screen for different culture wavelengths and intensities might reveal optimized conditions for high-energy lipid production.

Custom illumination systems relying on arrays of light emitting diodes (LEDs) were developed (Fig. S2 in the ESI†) to screen for the effects of culture illumination wavelength and intensity in arrays of *C. cryptica* grown in DMF devices. In initial experiments, algae were exposed to a multicolour array of LEDs operating at two different intensities. As shown in Fig. 3a, the growth rate (measured by absorbance) of algae cultured under blue light is highest, resulting in the highest level of biomass accumulation. This is expected, as the *C. cryptica* used here have an absorbance spectrum (Fig. S3 in the ESI†) with maxima at blue (strong) and red (weak) wavelengths, which is consistent with that of the primary photosynthetic pigment, chlorophyll c.[50,51] In addition, algal cells cultured under high intensity (250 μmol m^{-2} s^{-1}, Fig. 3b) have greater biomass accumulation compared to cells cultured under low intensity (50 μmol m^{-2} s^{-1}, Fig. 3a). Intensity-dependent proliferation is well known[48] and is related to the number of electrons produced per photosynthetic unit. Reducing this number (by reducing the number of photons absorbed), reduces the rate of photosynthesis, which results in depressed growth rates.[7,52]

Algae growth rate (Fig. 3a and b) is only a part of the story for biofuel production. The production of neutral lipids can be directly interrogated using a fluorogenic probe;[53] Fig. 3b and c shows fluorescence data for algae cultured using DMF. As shown, algae cultured under yellow LEDs (followed closely by

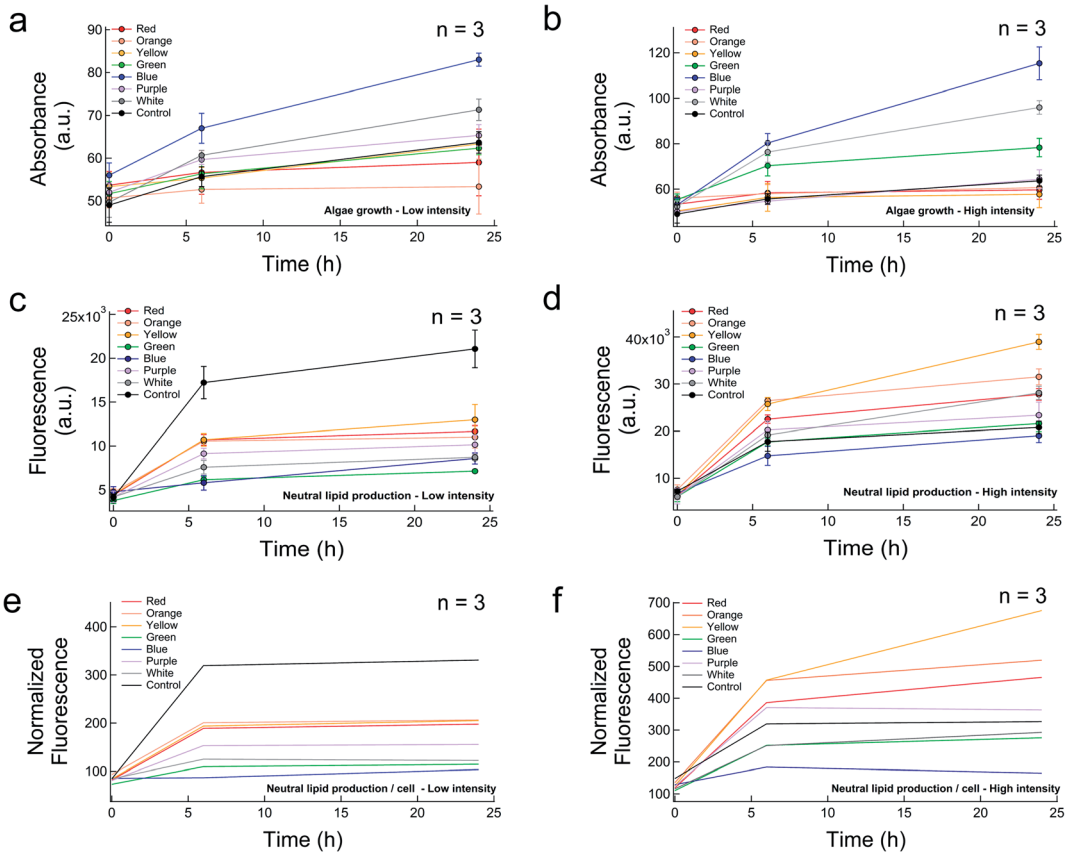

Fig. 3 Multicolour screening results. (a and b) Algal density (measured by absorbance), (c and d) neutral lipid production (measured by LipidTOX fluorescence), and (e and f) lipid production per algal cell (fluorescence normalized by absorbance) for *C. cryptica* cultured in droplets on DMF devices. Seven different illumination wavelengths are represented by colour plus a control with no illumination (black). The screen was repeated at low [50 μmol m^{-2} s^{-1}, (a, c and e)] and high [250 μmol m^{-2} s^{-1}, (b, d and f)] intensities. Error bars represent ± 1 S.D. from three replicates.

algae cultured under red and orange LEDs) produce the highest number of neutral lipids, and algae cultured under blue and green LEDs produce the lowest number of neutral lipids. This trend is particularly apparent at high intensity illumination (Fig. 3d), and also when evaluated as a function of neutral lipid production per cell (*i.e.*, fluorescence normalized to absorbance, Fig. 3e and f). As far as we are aware, this is the first report of a dependence of algal neutral lipid production on culture wavelength, which may be an important new finding for biofuel research. The mechanism for this effect is not known; experiments described below indicate that it correlates with oxidative stress.

The observation that yellow illumination is favourable for neutral lipid accumulation in algae is interesting (Fig. 3c–f), but illumination under yellow wavelengths (and others that are close) results in slow or negligible algae growth (Fig. 3a and b). An optimized system for biofuel production would be characterized by both high efficiency lipid production and high growth rates. With this in mind, we hypothesized that combinations of yellow (for high lipid production) and blue (for high growth rate) illumination might be best for overall energy production. An illumination system relying on combinations of blue and yellow LEDs was used to screen for growth rates and lipid production of algae with timed variations of yellow and blue illumination.

The absorbance data for the blue-yellow illumination experiments (representing growth rates) is shown in Fig. 4a. As expected, the growth rate is the highest for algae exposed to only blue illumination and lowest for algae exposed to only yellow illumination. Interestingly, for conditions in which algae were exposed to at least 12 h of blue illumination followed by exposure to yellow illumination (*i.e.*, 12 h blue/12 h yellow, 15 h blue/9 h yellow, 20 h blue/4 h yellow), growth rates remained high – in fact, higher than the case for any single colour other than blue (Fig. 3a and b). When the same conditions are evaluated by fluorescence (Fig. 4b), the intermediate cases (12 h blue/12 h yellow, 15 h blue/9 h yellow, 20 h blue/4 h yellow) result in the highest level of lipid production, presumably because the exposure to blue light allowed for the algae to proliferate rapidly, and the yellow light allowed for accumulation of lipids. The condition most favourable for lipid production was 15 h blue illumination followed by 9 h yellow illumination. After 24 h, this condition resulted in ~4-fold and ~2-fold greater lipid production than exposure to blue or yellow LEDs alone.

A problem with microfluidic screening experiments is the potential for "hits" that do not translate to experiments performed at conventional scales.[23] To test for this phenomenon in the results reported here, a large LED illumination system was applied to algae cultured in 200 mL flasks. The best condition

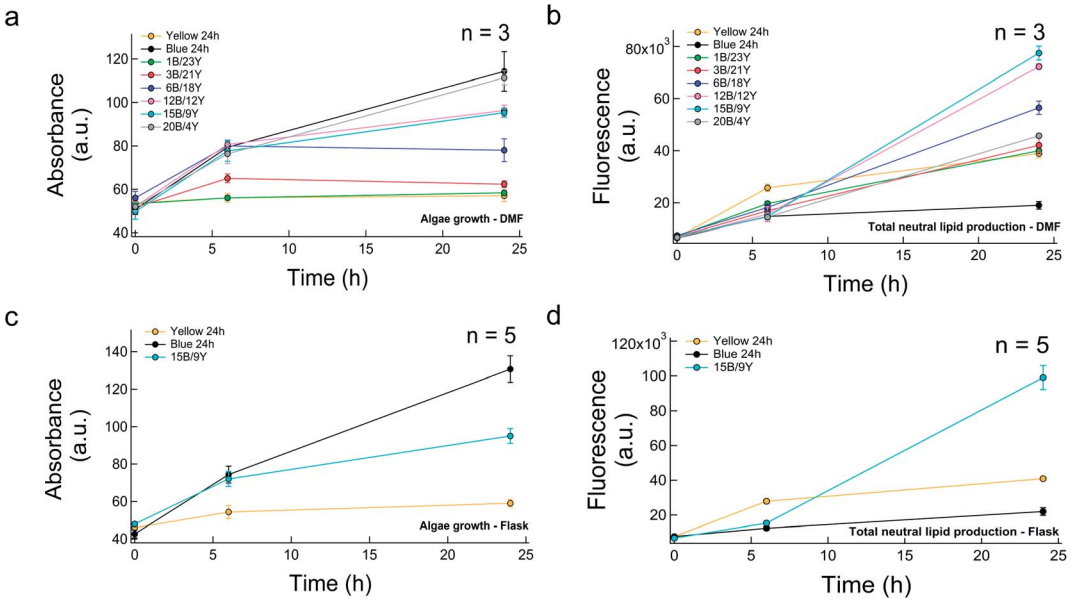

Fig. 4 Blue/yellow screening results. (a) Algal density (measured by absorbance) and (b) neutral lipid production (measured by LipidTOX fluorescence) for *C. cryptica* cultured in droplets on DMF devices incubated under eight different combinations of blue and yellow illumination for 24 h. Corresponding algal density (c) and lipid production (d) for three of the illumination conditions (24 h blue, 24 h yellow, and 15 h blue followed by 9 h yellow) implemented for *C. cryptica* cultured in 200 mL flasks. Error bars represent ± 1 S.D. from three replicates for DMF experiments and five replicates for flasks.

identified in the DMF screen (15 h blue followed by 9 h yellow) was evaluated, as well as yellow-only and blue-only controls. As shown in Fig. 4c and d, the trends observed in the macroscale are nearly the same as those identified in the microscale, which suggests that the effect scales from micro- to macro-scale. Future work will be required to test whether these effects scale to production-sized culture facilities,[1] but the conditions identified here are likely just the beginning – future screens might probe finer wavelength resolution, blue/yellow duty cycles with more than one cycle or higher frequencies, varying blue/yellow intensities applied at the same time, combination with dietary stresses, or the results for long-term culture with passaging. We propose that the DMF method described here may be uniquely well suited for these future experiments.

Potential mechanisms

It is well known that *C. cryptica* and related species increase the rate of biosynthesis of neutral lipids such as TAG in response to stresses[8–11] (*e.g.*, a lack of key dietary elements such as silicon). The resulting lipids are often stored in organelles known as lipid bodies; this is believed to be a convenient mechanism for converting photoassimilates (ATP and NADPH) to energy storage, as lipids can be readily catabolized for other processes when the period of stress has ended.[4,54] But the observation that exposure to yellow wavelengths results in lipid accumulation has not been reported before, and its mechanism is unknown. As a first test, we probed the nature of intracellular lipid storage in cells exposed to yellow and blue light. Fluorescence microscopy (Fig. 5a and b) of cells labeled with LipidTOX revealed that cells cultured under yellow illumination form large, brightly stained lipid bodies, particularly when compared to cells cultured under blue light. This suggests that illumination with yellow light, like nutrient-deprivation stress,[8–11] induces algae cells to store lipids in a format convenient for future use.[4,54] This begs the question: 'why?'

One hypothesis is that because yellow illumination likely results in low (or no) photosynthesis, algal cells may synthesize and store lipids as a counter-measure to photostarvation. The control data in Fig. 3 (black lines, representing culture in the dark) serve as a baseline for this effect – as shown, these cells exhibit modest increases in lipid production over the course of 24 h (Fig. 3d). In comparison, cells exposed to yellow light generate significantly greater (>2×) lipid levels than control cells (Fig. 3d), an effect that is increased when normalized per cell number (Fig. 3e). Thus, we propose that photostarvation-stress does not (fully) explain the observed effects of yellow-illumination on algae cells.

A second hypothesis for the mechanism of wavelength-dependent lipid accumulation is that it is related to oxidative stress, which is known to play a role in some other forms of lipid accumulation in algae.[4,54] To test this hypothesis, algae cultured under normal conditions (12 h white light/12 h dark), algae cultured under yellow (24 h) or blue light (24 h), and algae cultured under yellow light with 50 μg mL^{-1} ascorbate were evaluated for reactive oxygen species (ROS) production using DCFDA, a reporter that fluoresces in the presence of ROS. As a positive control, algae cultured under normal conditions without a dietary source of silicon (known to induce ROS) were also evaluated. As shown in Fig. 5c, the ROS levels for algae cultured under yellow light and under silicon-deprivation stress are greater than those for cells cultured in normal conditions and in blue light. In addition, the antioxidant ascorbate reverses the

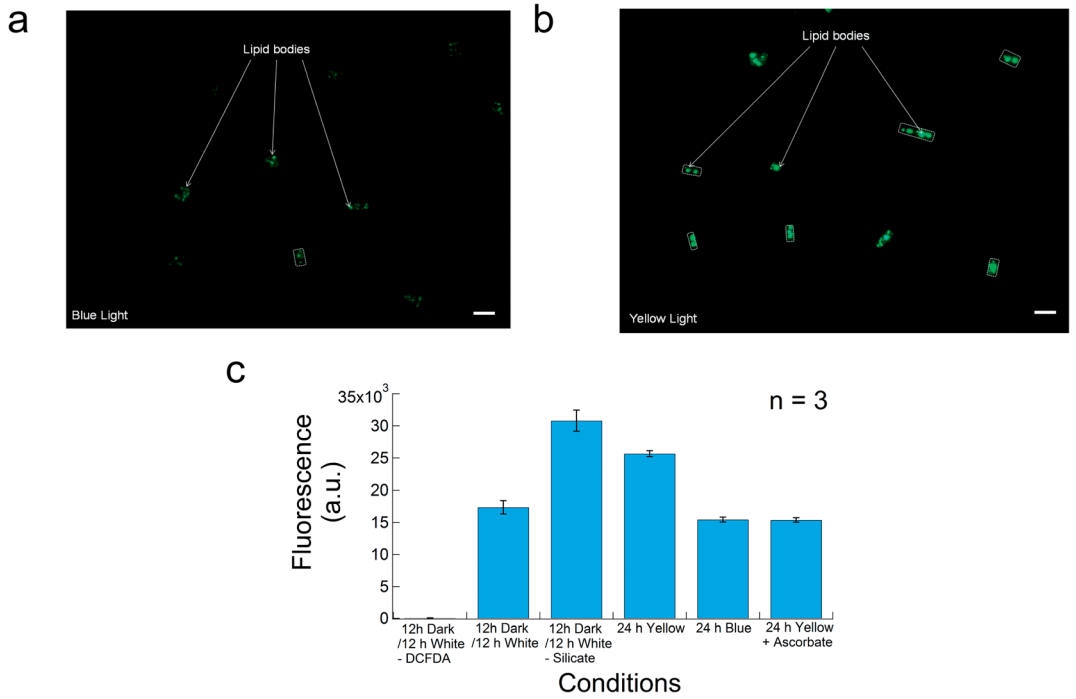

Fig. 5 Investigation into the mechanism of wavelength-dependent lipid production. Fluorescent micrographs of LipidTOX labeled *C. cryptica* cultured for 24 h in blue (a) or yellow (b) illumination. The membranes of some cells are indicated with dotted white lines to illustrate their size. Scale bars represent 10 μm. (c) DCFDA fluorescence intensities representing the concentration of reactive oxygen species (ROS). Error bars represent ± 1 S.D. from three replicates.

effect – cells cultured under yellow light with ascorbate have significant reductions in ROS levels relative to cells cultured without ascorbate. These data support the hypothesis that yellow light results in increased levels of algal photooxidative stress, while exposure to blue light does not. The general phenomenon of photooxidative stress is well known: when photosynthesis cannot keep up with the rate of photon absorption, the concentrations of excited singlet (^{1}Chl*) and triplet-state chlorophyll (^{3}Chl*) increase, which leads to the formation of excited, singlet-state oxygen ($^1O_2^*$) and other ROS. But it is unclear why photooxidative stress should be wavelength-dependent.

The primary mechanism used by photosynthetic organisms to combat photooxidative stress is up-regulation of stress-response genes involved in antioxidant synthesis.[55,56] In diatomic algae, there is some evidence that this response is related to wavelength; for example, Coesel *et al.*[57] recently reported that the genes encoding for enzymes associated with synthesis of potent antioxidant xanthophylls (including ZEP, VDE, and others) are preferentially expressed in response to exposure to illumination under blue light (relative to other wavelengths). We propose that this effect may explain the observations described here. If cells exposed to yellow illumination are unable to synthesize natural antioxidants, they may revert to lipid-production as means of coping until the stress has ended (as is the case for dietary stresses). This mechanism is conjecture, but it is likely one worth examining in some detail in the future, as a better understanding of wavelength-dependent lipid accumulation in algae would be useful in the quest to increase energy yields for algal-derived biofuels.

Conclusions

We have developed the first microfluidic method for discovery of new algae culture conditions favourable for generating high-energy lipids. In the new method, algae are divided into aliquots and exposed to varying conditions, with regular recordings of absorbance as a marker for cell density, and fluorescence of a marker for lipid accumulation. The screen led to the discovery of a new phenomenon: incubation at yellow wavelengths (~580 nm) causes algae to produce significantly more energy-rich lipids than algae grown under other conditions. Moreover, alternating exposure to blue wavelengths (~450 nm) and yellow wavelengths results in favourable cycling of algae growth and lipid production. Finally, we demonstrate that this effect correlates with an increase in reactive oxygen species, an observation that may be useful for elucidating the mechanism of wavelength-dependent stress. We anticipate that these findings may have important implications for future work to develop algal sources of biofuel. More generally, we propose that the digital microfluidic method described here is an attractive new screening tool with potential advantages for applications in renewable energy, biotechnology, materials science, and beyond.

Materials and methods

Reagents and materials

Unless otherwise specified, general-use reagents were purchased from Sigma Chemical (Oakville, ON, Canada) or Fisher Scientific Canada (Ottawa, ON, Canada). *Cyclotella*

cryptica (CCMP 332) algae and associated culture reagents were purchased from the National Center for Marine Algae and Microbiota (NCMA, East Boothbay, ME). HCS LipidTOX™ green neutral lipid stain was purchased from Life Technologies (Burlington, ON, Canada). DCFDA cellular reactive oxygen species detection assay kits (ab113851) were purchased from Abcam (Cambridge, MA).

DMF devices and experimental setup

DMF device and LED array design, fabrication, and operation are described in the ESI.†

Algae culture

Algae was grown in 50 mL aliquots in complete culture media formed from f/2 medium (NCMA, Maine, NE) supplemented with biotin and cyanocobalamin (2 nM final concentration ea., NCMA) and 0.02% w/v Pluronic F68 in Pyrex media bottles (max vol. 200 mL) at 20 °C under an alternating 12 h white light/dark cycle. Algae were maintained by weekly subculture at a 1 : 10 ratio translating to an inoculation density of $\sim 9.0 \times 10^5$ cells per mL to ensure that the algae is in exponential growth phase. The density was confirmed *via* regular absorbance measurements in 200 μL aliquots of algae suspension in clear-bottomed 96-well plates at 660 nm using a plate reader. To obtain the absorbance spectrum, a 1 mL aliquot of the media (no cells) was used as a reference and 1 mL aliquot of the culture was pipetted into a cuvette and measured using a UV/Vis spectrophotometer (Eppendorf, Westbury, NY).

Illumination screening experiments

Prior to use, DMF devices were sterilized by incubating in 70% ethanol for 10 min. An algal cell suspension of 1.5×10^6 cells per mL in complete media and a working solution of LipidTOX reagent (stock soln diluted 200× in complete media) were formed and illumination screens were implemented in five steps (Fig. 2). In step one, two stock reagents were loaded into the appropriate active reservoirs: a 150 μL aliquot of algal cell suspension, and a 120 μL aliquot of LipidTOX solution. Each of these aliquots was continuously mixed in a circular pattern on the active electrodes until use. In step two, eight 12 μL culture-droplets of algae suspension were dispensed and driven to vacant algae culture zones, in series. In step three, a ∼15 μL source-droplet of LipidTOX solution was dispensed and driven along the dye dispensing bus, where an array of eight 2 μL label-droplets were dispensed. (If the volume of the source-droplet was not sufficient to dispense eight label-droplets, a second source-droplet was dispensed from the reservoir to supplement.) In step four, an array of eight 2 μL daughter-culture-droplets were dispensed and driven to the L-shaped electrodes The device was then positioned on a transparent 384 well-plate and was inserted into a PHERAstar microplate reader (BMG Labtech, Durham, NC) for absorbance measurements at 660 nm using a well-scanning program with scan matrix = 15 × 15, scan width = 3 mm, and a focal height = 8.7 mm. In step five, the eight daughter-culture-droplets and the eight label-droplets were merged, mixed and driven to the fluorescence detection sites, and incubated for 5 min. Finally, the device was positioned onto a black 384 well-plate and inserted into the microplate reader for fluorescence measurement at 488 nm excitation and 520 nm emission with a focal height = 8.7 mm, gain = 550 using the well-scanning program (20 × 20 scan matrix and 3 mm scan width). After measuring, the eight mixed droplets were driven to the waste reservoirs, where the fluids spontaneously wetted the through-holes and were absorbed onto a tissue below the device. After completion of steps one through five, the device was positioned under an LED array (Fig. S2 in the ESI†) and incubated for 6 hours, after which steps three through five were repeated. The device was then incubated under the LED array again for 18 hours, after which steps three through five were repeated again. Thus, a total of 48 measurements (24 absorbance and 24 fluorescence) were generated from two pipette steps and a single device.

The method described above was applied to two different families of DMF screening experiments (described in the ESI†): "multicolour" and "blue/yellow." Multicolour experiments were conducted with an array of seven LEDs with different peak wavelengths running at low or high intensity (photon flux of 50 or 250 μmol m^{-2} s^{-1}), and one non-LED control. All LEDs were in the "on" state for 24 h, and each condition was evaluated in triplicate. Blue/yellow experiments were all conducted at high intensity (250 μmol m^{-2} s^{-1}), with eight timed variations for pairs of blue and yellow LEDs (in each case, only one LED was "on" for a given duration): (1) 24 h yellow, (2) 24 h blue, (3) 1 h blue followed by 23 h yellow, (4) 3 h blue followed by 21 h yellow, (5) 6 h blue followed by 18 h yellow, (6) 12 h blue followed by 12 h yellow, (7) 15 h blue followed by 9 h yellow, and (8) 20 h blue followed by 4 h yellow. Each condition was evaluated three times on three separate devices.

Macroscale illumination experiments were conducted using freshly inoculated algae in 200 mL Pyrex flasks, exposed to: (1) 24 h yellow, (2) 24 h blue and (3) 15 h blue followed by 9 h yellow using the system described in the ESI.† Two 200 μL aliquots were collected after 0, 6, 24 h incubation and were placed in transparent- (for absorbance) or black- (for fluorescence) bottom 96-well-plates for measurements using the instrument and settings described above. For fluorescence measurements, the culture was supplemented with a 1 μL aliquot of a 1 : 20 dilution (in algae culture media) of the LipidTOX solution stock. The combined solution was aspirated three or four times and covered with aluminum foil for 10 min at room temperature prior to measurement. Each condition was evaluated five times in five different culture flasks. For microscopy, ∼1 μL aliquots LipidTOX-labeled algae were loaded onto a glass slide and covered by a coverslip. Images were obtained using a Leica DM2000 microscope (Leica Microsystems Inc., Concord, ON, Canada) with a 49002 EGFP/FITC/Cy2 filter set from Chroma Technologies (Chroma Technologies, Bellows Falls, VT).

Reactive oxygen species measurements

Algae cells were grown as above, inoculated at 5.0×10^6 cells per mL in 200 mL Pyrex flasks, and then grown under standard conditions (12 h light/dark cycle) for at least 1.5 weeks to ensure

low initial levels of stress. Each flask's contents were then centrifuged (3000 rpm, 5 min), resuspended in 50 mL complete media, complete media without sodium silicate, or complete media with 50 μg mL^{-1} ascorbic acid. Resuspended algae subjected to one of three illumination conditions: (1) 12 h light followed by 12 h dark, (2) 24 h yellow light, or (3) 24 h blue light. The cells were then evaluated using a 2′,7′-dichlorofluorescein diacetate (DCFDA) kit as per the manufacturer's instructions. Briefly, 5 mL aliquots of cells exposed to each condition were centrifuged (3000 rpm for 5 min), washed in 1× buffer solution (from the kit), centrifuged again, re-suspended in 500 μL DCFDA solution (from the kit) or 500 μL complete medium containing no dye (as controls), and incubated in the dark at 37 °C for 30 min. Each sample was then centrifuged, washed in the 1× buffer solution, then centrifuged again and resuspended in 1× buffer solution again. 150 μL aliquots from each sample were placed in transparent- (for absorbance, in samples not containing dye) or black- (for fluorescence, in samples containing DCFDA) bottom 96-well-plates for measurements using the instrument and settings described above. Each condition was evaluated five times in five different culture flasks.

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