Algal Research 30 (2018) 149–161

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Review article

Microfluidic systems for microalgal biotechnology: A review

Hyun Soo Kim[a,b], Timothy P. Devarenne[c], Arum Han[a,d,*]

[a] Department of Electrical and Computer Engineering, Texas A&M University, College Station, TX 77843, USA
[b] Korea Institute of Machinery and Materials, Daegu Research Center for Medical Devices and Rehabilitation, Daegu 42994, South Korea
[c] Department of Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843, USA
[d] Department of Biomedical Engineering, Texas A&M University, College Station, TX 77843, USA

ARTICLE INFO

Keywords:
Microalgae
Cyanobacteria
Microfluidics
Lab-on-a-chip
Biofuel
Microfabricated systems

ABSTRACT

Microalgae have a demonstrated potential as producers of high-quality renewable biofuel feedstocks as well as other high-value chemicals. However, significant improvements from microalgal biology and strain development to downstream processing are required to achieve economically viable microalgae-derived biofuels and bioproducts. Mainstream techniques used in microalgal research are based on conventional cell culture and cell handling systems, which are bulky, labor-intensive, time-consuming, and also limited in throughput. Microfluidic lab-on-a-chip systems can offer cost- and time-efficient alternatives to advance microalgal biofuel and bioproduction research by providing high precision and high efficiency cell/reagent handling capabilities, enabling high-throughput assays in a fully automated fashion. Here, we review recent advances in the development and application of microfluidic lab-on-a-chip systems for microalgal biotechnology, especially microalgae-based biofuels, including microsystems for single-cell resolution high-throughput cell identification and separation, highly efficient cell transformation, high-throughput parallel cell cultivation, cell harvesting, and cell analysis applications. Other microfluidic applications such as microalgae-based fuel cells and microalgae-based biosensing platforms are also reviewed towards the end. We conclude by suggesting possible future directions on how microfluidic lab-on-a-chip systems can be utilized to overcome current challenges and improve the current status in microalgal biotechnology.

1. Introduction

Microalgae, photosynthetic microorganisms that are present in most water sources, have been traditionally used as feedstock producers for creating various high-value chemicals, such as biopharmaceuticals, cosmetics, nutraceuticals, and functional foods [1–3]. In the past two decades, emphasis on CO_2 reduction and production of sustainable biofuels due to concerns over climate change and limited reserves of fossil fuels have brought greater interest to microalgae as future sources of renewable biofuels. In addition, increasing interest in sustainable and eco-friendly manufacturing have brought more attention to microalgae-based bioproduction of specialty and high-value molecules. Thus, research and commercialization interests on microalgae have become increasingly diverse.

Microalgae are typically characterized by higher photosynthetic efficiency, faster growth rate, and higher oil content compared to other oil-producing crops, such as soybean and oil palm used in the production of second- and third-generation biofuels [1,4–6]. Additional advantages that make microalgae an attractive biofuel resource are: less water is required for microalgae cultivation, which reduces demand on freshwater sources [7]; no or minimal competition with food supply, land usage, and associated environmental impacts since microalgae can be cultured in brackish water on non-arable land [8]; microalgal species that are best suited for local environments can be found, as they exist in almost all ecosystems on Earth [2]; and CO_2 biofixation through photosynthesis, minimizing CO_2 footprint [9,10]. Despite these promising potentials, the production cost of current microalgae-based biofuels and bioproducts remains well above economic viability, and significant improvements are required throughout all steps of the microalgal biofuel/bioproduct production pipeline [11–13]. The key processes associated with the production pipeline are strain selection and development, cultivation, harvesting, lipid extraction, and conversion. However, conventional approaches utilized in many of these key processes, for example, strain selection and development, biomass analysis, lipid extraction and analysis, all mostly rely on bulky instruments and complicated procedures that are time-consuming, labor-intensive, high cost, and low throughput [14].

Microfabricated devices and microfluidic lab-on-a-chip systems can

* Corresponding author at: Department of Electrical and Computer Engineering & Department of Biomedical Engineering, Texas A&M University, 237 GERB & 309C WERC, TAMU 3128, College Station, TX 77843-3128, USA.
E-mail address: arum.han@ece.tamu.edu (A. Han).

https://doi.org/10.1016/j.algal.2017.11.020
Received 27 July 2017; Received in revised form 16 November 2017; Accepted 18 November 2017
Available online 29 December 2017

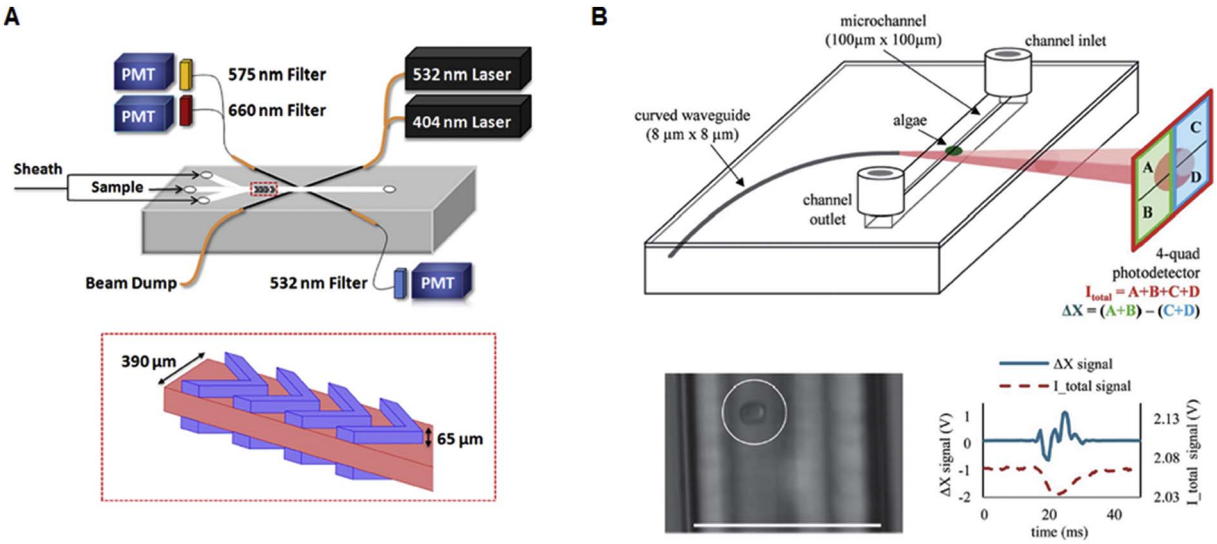

Fig. 1. Mcrofluidic devices for microalgae strain identification. (A) Optofluidic characterization of marine microalgae using a microflow cytometer. Reproduced, with permission, from [28]. (B) Optical classification of microalgae species with a glass-based lab-on-a-chip system. Reproduced, with permission, from [30].

offer attractive alternatives for current cell culture and handling technologies to advance the current state of microalgal biofuel/bioproduct research by providing several advantages over conventionally used systems [15]. Microsystems have the capability to precisely control, monitor, and manipulate tiny amounts of samples at nano- to pico-liter scale, process and analyze cells at single-cell resolution with high precision, and are able to conduct all of these procedures in a massively parallel format to achieve high-throughput assays at low cost [16–18]. These systems can also be made into a portable format for on-site *in situ* analysis of microalgal samples. In this review, we present recent developments in microfluidic platforms for microalgal biotechnology applications. First, we will report on microfluidic studies related to microalgal biofuel applications, such as strain selection and development (cell identification, separation, and transformation), cultivation, and downstream processing (cell harvesting and lipid extraction). In addition, other microfluidic applications related to microalgal biotechnologies, including microalgae-based microbial fuel cells (µMFCs) and microalgae-based biosensors, will be reviewed. Finally, we will conclude by suggesting possible future directions on how microfluidic lab-on-a-chip systems can be utilized to overcome current challenges and improve the current status in microalgal biotechnology. Our discussions will focus on green microalgae (Chlorophyta) and diatoms, but will also include examples using cyanobacteria, as many microsystems that can be applied to cyanobacteria can be very well directly applied to green microalgae as well.

2. Microsystems for strain selection and strain development

Estimates indicate that there are 200,000–800,000 microalgal species in the world, however, most of the on-going microalgae research has been conducted with only a small number of species [19,20]. Identifying better-performing microalgal strains (*e.g.*, higher productivity, stress tolerance) through bioprospecting can be one route to achieve commercially viable microalgae-based biofuel production or to obtain strains that show high productivity in a given local environment [11,12,21,22]. Microalgal strains discovered from the local environments in which microalgal productions will occur are preferred for production purposes in particular geographical locations, since they typically express superior adaptability to local environmental conditions [22,23]. This is also important in applications where native microalgae can be used as indicators for various environmental monitoring applications. Further developing these strains into highly efficient production strains through genetic and metabolic engineering, as well as through directed evolution, can result in strains that can produce desired high-value products efficiently, have phenotypes that native strains are unlikely to possess, while also being tolerant to biotic and abiotic stress (*e.g.*, temperature, salinity, pathogens) coming from the local environment [11,12].

2.1. Microalgal species identification

The most traditional method to identify microalgae is through light microscopy and molecular biology, where samples are collected from various environmental locations, brought to the laboratory, and analyzed with imaging, followed by genotyping. Although this method is reliable, it is time-consuming and labor-intensive. In recent years, flow cytometry, including imaging flow cytometry, has been used to automate this process [24,25], but this involves considerable cost due to the high cost of such instruments. In addition, as all of these methods rely on bulky instruments, on-site measurement of collected samples is not possible, and thus, the collected samples from bioprospecting have to be analyzed later in a laboratory setting.

Microfluidic flow cytometry offers potential as an alternative method, where the small and compact size of the device makes it portable, enabling on-site microalgae analysis and classification during bioprospecting. The first microfluidic cell cytometer to analyze and distinguish microalgal cells was developed by Benazzi et al. [26], which was capable of measuring chlorophyll autofluorescence of cells flowing through the optical detection zone in a microfluidic channel. In addition, an impedance spectroscopy electrode to measure cell sizes was integrated into the flow channel, allowing for simultaneous measurement of impedance signals and autofluorescence from cells passing through the detection region of the chip. An improved microflow cytometer featuring flow focusing in two dimensions was constructed by Hashemi et al. [27]. Here the sample flow was hydrodynamically focused both in horizontal and vertical directions using flow-guiding grooves on the top and bottom of the microchannel, resulting in two symmetric sheath streams to wrap around the central microalgae-laden sample stream. With a 488 nm laser excitation, side scattering and chlorophyll/phycoerythrin autofluorescence properties of three different microalgal species were measured, showing increased detection capabilities of samples as small as 1 µm in diameter. However, the 488 nm laser used in this device did not strongly excite chlorophyll *a*. Thus, their subsequent work utilized two excitation lasers, 404 nm and 532 nm, closer to the maximum excitation wavelengths of both chlorophyll and phycoerythrin (Fig. 1A) [28]. Four different species of

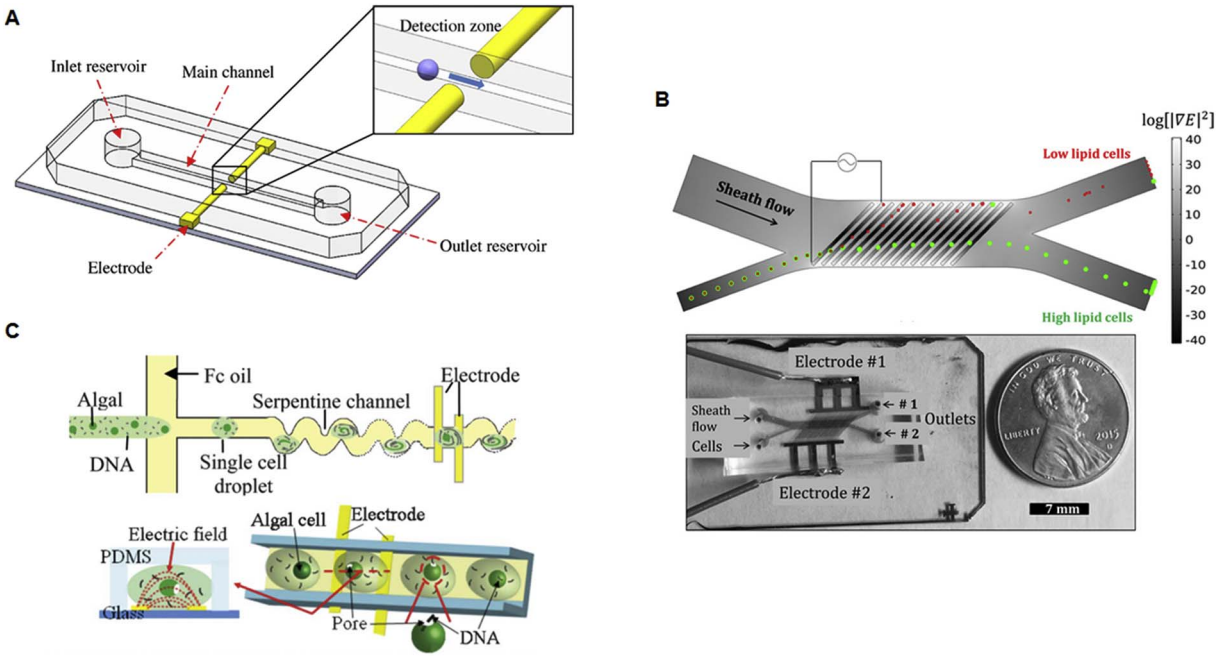

Fig. 2. Microfluidic devices for microalgae strain characterization and development. (A) Capacitive detection of living microalgae in a microfluidic chip. Reproduced, with permission, from [46]. (B) Continuous-flow sorting of microalgal cells based on their lipid content using high-frequency dielectrophoresis. Reproduced, with permission, from [57]. (C) Droplet electroporation in microfluidics for efficient cell transformation with or without cell wall removal. Reproduced, with permission, from [71].

microalgae having a wide size range (1–50 μm) and different amounts of intrinsic chlorophyll and phycoerythrin were successfully differentiated using this microsystem.

While the use of flow cytometry principles in microfluidic systems successfully demonstrated the identification of cells, they still require multiple laser sources, wavelength selection filters, and detectors, which make size and cost reduction of the integrated microfluidic flow cytometry systems difficult. Schaap et al. [29–31] developed an optofluidic device that could distinguish microalgae species with a simpler setup. A single laser source and a single quadrant-cell photodetector were utilized, where microalgae inside a microchannel were illuminated by the laser light, which was guided through a curved waveguide perpendicularly positioned next to the microfluidic channel, and distinctive wavelets associated with cell geometry and size were detected (Fig. 1B). Photodetector signals obtained from nine different microalgal species showed unique and different signatures from each other, which could then be used to discriminate each species.

2.2. Microalgae characterization based on cell morphology and physiology

Microfluidic devices for identifying cell characteristics such as lipid content, viability, and size have also been developed. A microfluidic flow cytometer, similar in structure to the ones used for microalgae species identification, was introduced to assess both photosynthetic characteristics and lipid accumulation of microalgal cells [32]. This microdevice utilized a 2D hydrodynamic cell focusing scheme, where a single target cell could pass through an optical detection region at a rate of 100 cells/s. By sequentially measuring chlorophyll autofluorescence intensity as well as Nile red stained lipid fluorescence of the flowing cells, photosynthetic efficiency and lipid accumulation were evaluated, respectively, at the single-cell level. Comparison of both parameters in unstressed and stressed cells of the diatom *Phaeodactylum tricornutum* revealed the diversity within populations, where a number of stressed cells retained photosynthetic efficiencies comparable to that of unstressed cells, yet exhibited substantial lipid accumulation. Another platform used for microalgal lipid analysis was a droplet microfluidics-based platform that can generate hydrogel droplets encapsulating a single cell inside [33]. Droplet microfluidics provides the capability to perform high-throughput assays, where large numbers of uniformly sized water-in-oil emulsion droplets or gel droplets, each encapsulating one or more cells, can be easily generated at speeds as high as 3.7×10^5 events/s [34]. Here each of these droplets functions as an individual pico-liter scale bioreactor that can be easily manipulated. These droplets can be individually cultured, transported, merged, and analyzed to enable massively parallel processing and experimentations to be achieved within a short period of time [35–40]. Using such a format, the heterogeneity in lipid content as well as viability of single microalgal cells encapsulated inside microcapsules have been analyzed through fluorescence microscopy [41,42].

Chlorophyll autofluorescence intensity, which is proportional to chlorophyll content in microalgae, is an indicator of photosynthetic capacity and activity in microalgae, and can also be used to evaluate cell viability. Based on this principle, several microfluidic platforms designed to characterize microalgae viability have emerged. In these systems, a laser diode or microscope light was used to illuminate the samples and the chlorophyll emission spectra were detected with a photodiode that converted the chlorophyll autofluorescence intensity into a corresponding output voltage. For example, a microfluidic biosensor developed by Wang et al. [43] distinguished live and dead cells of six different microalgal species using this method. Later, a more compact microfluidic platform was implemented to evaluate cell viability under different chemical treatments, where on-chip sample transport was controlled by electrokinetic flow [44]. In order to reduce the operation cost of such devices as well as to improve the portability towards *in situ* analysis, a microfluidic chip integrated with an organic light emitting diode (OLED) and an organic photodetector (OPD) was also developed [45]. In this work, a blue OLED, an OPD exhibiting a high quantum efficiency in the 680–700 nm wavelength region, and absorption filters with high transmittance and high attenuation of more than 80% and 40 dB, respectively, were fabricated, allowing for low-cost manufacturing of microchip systems as well as easy integration compared to commercial optical setups. This platform successfully characterized the viability and concentrations of *Chlamydomonas reinhardtii* cell populations. Cell viability can also be assessed by means of cellular capacitance analysis. It is known that living cells have intact plasma membranes while dead cells have broken ones. This damaged

membrane can change the dielectric properties of cells, which can be measured by analyzing the cells' capacitance. A three-dimensional capacitive microfluidic sensor was designed by placing two cylindrical copper wires across a detection microchannel [46]. The frequency-dependent capacitance changes of both live and dead *Dunaliella salina* cells were characterized, where a larger capacitive change was observed from live cells compared to that of dead cells (Fig. 2A).

Song et al. [47,48] employed a microfluidic differential resistive pulse sensor (RPS) to detect and count microalgal cells and to measure their sizes. The RPS is a method to electrically detect the impedance change across a sensing region when a cell passes this area [49]. Two microalgae species, *Chlorella vulgaris* and *Pseudokirchneriella subcapitata* (a.k.a. *Raphidocelis subcapitata*), which have different cell sizes, were successfully characterized and distinguished using this microsystem. In a subsequent work, a cell viability detection scheme using chlorophyll autofluorescence intensity detection described above [43] was combined with this microfluidic RPS technology, which could exclude interference from other particles or dead microalgal cells, eventually allowing for more accurate measurements.

2.3. Microalgae sorting by lipid content, viability, and size

In addition to just identifying microalgal cells, separating them based on their properties (*e.g.*, lipid content, viability, and size) is also of high interest. Cell sorting is typically carried out to separate cells having desired traits from a large population of cells. In microalgae-based biofuel applications, the interesting traits may include faster growth rate, higher lipid production, or tolerance to environmental stress [11,12,19,22]. Deng et al. [50] proposed a microfluidic device capable of separating microalgae having different lipid contents by dielectrophoresis (DEP). DEP is an electric field-based, label-free cell manipulation technique, where its force is dependent on the dielectric properties of cells and their surrounding medium [51,52]. Using an integrated electrode array in a microchannel, microalgae can be exposed to a non-uniform alternating current (AC) electric field that exposes cells to positive or negative DEP force depending on their lipid content, which moves cells either towards the electrodes (positive DEP) or away from the electrodes (negative DEP). For example, *C. vulgaris* cells with 11% and 45% lipid content were successfully separated using this device. Although the lipid content-based cell sorting capability was successfully demonstrated, the overall throughput of the device was extremely low as the separation had to occur when no flow was present. To improve the throughput, the authors further developed the DEP microfluidic device to perform continuous sorting of cells flowing through a microchannel [53]. Here, the sorting scheme was based on the relative strength of the hydrodynamic force and negative DEP force influencing the microalgal cells flowing through the device. All cells experienced the same hydrodynamic force but different magnitude of negative DEP force depending on their lipid content, resulting in different flow trajectories along the microchannel. Using this method, separation of *C. vulgaris* cells with 13% and 21% lipid content were successfully achieved. It is known that positive DEP force is typically stronger than negative DEP force in an interdigitated electrode design [54–56]. Based on this knowledge, a continuous-flow cell sorting device capable of sorting cells based on their lipid content using high-frequency DEP force was constructed [57]. At a high frequency of 50 MHz, DEP response of cells primarily depends on the dielectric properties of the cytoplasm, which is influenced by the amount of lipid accumulation inside cells. A clear separation between high- and low-lipid accumulating *C. reinhardtii* mutants was observed, where the low-lipid cells experienced positive DEP force and showed a zigzag trajectory, while high-lipid cells flowed along the hydrodynamic stream under the influence of negative DEP force (Fig. 2B).

In addition to lipid content, different characteristics of microalgae such as viability and size can also be assessed and used to separate cells using direct current-dielectrophoresis (DC-DEP) microfluidic platforms. DC-DEP employs microstructures such as pillars or hurdles made of insulating materials in a microchannel to create a spatially defined non-uniform electric field from an uniform DC electric field instead of having to use an AC field that is typically needed in DEP systems [58]. The DC-DEP systems have several advantages compared to AC-DEP systems, such as simple microfabrication (resulting from single-material microfabrication and no need for electrode patterning), reduced biofouling at the test region (due to remotely positioned electrodes), and higher throughput (as deeper microchannel capable of handling more samples can be employed). A microchannel comprising of an array of cylindrical insulating posts was utilized to selectively separate or concentrate a mixture of viable and non-viable microalgae [59]. Due to differences in cell membrane conductivity, live and dead microalgal cells experience different DEP responses, and thus, separation of viable cells can be achieved. Song et al. [60] designed an insulating microchannel that had a triangular hurdle in the middle to continuously separate microalgae by DC-DEP. Through this microdevice, *C. vulgaris* and *P. subcapitata*, which differ by only few micrometers in diameter, could be successfully separated.

To separate microalgae samples from bacterial contamination, inertial microfluidic devices have also been employed [61]. Inertial microfluidics relies on flow phenomena in an intermediate range of Reynolds number (Re) ($\sim 1 < Re < \sim 100$), where Re is a dimensionless parameter describing the ratio between inertial and viscous forces in a flow. In this regime, both the inertia and the viscosity of a fluid are finite, and can provide a deterministic nature, which allows for precise focusing and ordering of cells within a microchannel [62–65]. When a mixture of microalgae and bacteria were injected through two lateral inlets of a 3-inlet microchannel with a buffer flow through the center inlet, inertial flow confined the larger cells (microalgae) to the channel center due to inertial force, while the smaller cells (bacteria) remained in their hydrodynamic streamlines (away from the center flow stream). Using this device, *Coenochloris signiensis* cells were purified from bacteria with efficiencies of up to 99.8%.

2.4. Cell transformation

Much effort have been dedicated to transforming microalgae to have desired traits of interest [21,66]. To deliver the target gene constructs into cells, electroporation or vortexing with glass beads are conventionally used [67,68]. However, these traditional methods typically have very low gene delivery efficiencies due to the thick cell wall of microalgae. The transfer of exogenous DNA into microalgae is also mainly dependent on the random diffusion of target genes, which does not guarantee efficient gene delivery. In addition, the exogenous DNA being delivered into the cells can be damaged during electroporation or glass bead vortexing, resulting in inaccurate or undesired gene integration into the nucleus [69,70]. To overcome these limitations, Qu et al. [71] reported a continuous-flow electroporation method based on a droplet microfluidics platform. Droplet microfluidics was utilized here to encapsulate microalgal cells and target DNAs together into spatially confined droplets. In-droplet electroporation took place while the droplets containing both cells and DNAs flowed across 5 pairs of microelectrodes biased with a constant electroporation voltage (Fig. 2C). Transformation results of wild-type *C. reinhardtii* CC-124 cells using this process showed ~200 times higher transformation efficiency than the conventional bulk process.

Later, the authors developed a digital microfluidics-based electroporation system where an array of electrodes were utilized to handle droplets as well as to conduct gene transformation by applying electric potentials to the electrode patterns [72]. Two different electroporation mechanisms were realized in the system. First, when a droplet comes in contact with the two electrodes, a direct current from the applied voltage flows through the droplet, resulting in static droplet electroporation that showed an order of magnitude higher transgene expression over conventional bulk electroporation systems. Second, when only one

electrode comes in contact with the droplet at a time, the droplet moved across the electrode pattern and a tiny discrete charge transfer occurs, which induced electroporation (named "bouncing droplet electroporation"). Although this second mechanism had a lower transgene expression efficiency than the static droplet electroporation, this method showed promises due to being able to easily integrate with other droplet microfluidics functions, such as in-droplet cell culture. Using this system, droplets having much larger volume (1 μL) compared to the previous system (70 pL) could be manipulated, which increased the overall productivity.

Another microfluidic microalgae transformation platform was implemented to perform gene delivery by combining a nanowire array and a pneumatically actuated microvalve system [73]. When the integrated pneumatic valve was actuated, this force pushed the underlying microalgal cells against the gene-coated nanowires, which allowed the genes to be physically delivered into the cells due to nanowire penetration into the cells. Once the pneumatic valve was released, microalgae samples into which genes were delivered could be recovered. This gene delivery scheme was applied to transfer the hygromycin B resistance gene into *C. reinhardtii* cells, and the result showed more than 10,000 times higher transformation efficiency compared to conventional methods.

3. Microsystems for cultivation

Microalgae obtain their metabolic energy through photosynthesis, and their growth and lipid production are strongly dependent on culture conditions such as light, nutrient levels, temperature, CO_2 concentration, and media pH. Therefore, understanding how the various culture parameters influence microalgal growth and lipid accumulation is critical in measuring strain productivity, optimizing the cultivation processes, and improving the overall biofuel feedstock production efficiency. Various microfluidic microalgae culture platforms have been developed and utilized to investigate these relationships. These microfluidic platforms can be classified into three different types of microscale photobioreactors; continuous-flow microfluidics photobioreactors, droplet microfluidics photobioreactors, and digital microfluidics photobioreactors.

3.1. Continuous-flow microfluidics photobioreactors

Continuous-flow microfluidics photobioreactors are microalgae culture platforms that typically have arrays of miniaturized culture compartments, which are few tens to hundreds of micrometers in dimensions and have nano- to pico-liter scale volumes. In these devices, microalgae samples are loaded, cultured, and analyzed in cell culture compartments under continuous or periodic liquid flow through reagent-delivering microchannels. Among the various culture factors, light conditions such as light intensity, light cycle, and wavelength are some of the most important parameters that can affect microalgal growth and lipid production [19]. Chen et al. [74] constructed a 96-well microplate integrated with red light emitting diode (LED) arrays where the light intensity and cycle of each culture chamber could be controlled independently with each individual LED. This platform could provide light intensities at 128 different levels and light cycles as short as 10 μs, and was successfully used to analyze the light-dependent growth rates, photosynthetic efficiency, and lipid production efficiency of *Dunaliella tertiolecta*. Kim et al. [75] has developed a microfluidic light-controllable photobioreactor array capable of simultaneously investigating the effect of 64 different light exposure conditions on the growth and lipid production of the colony-forming microalgae *Botryococcus braunii* in one experimental run (Fig. 3A). The light intensity control layer utilized a microfluidic gradient generator that produced 8 different concentrations of black dye, and by shining light through this layer, resulted in 8 different light intensities to be applied to the underlying arrays of 54 nL volume photobioreactors. Eight different light-dark cycles in the platform were produced by filling a microchannel placed perpendicular to the light intensity control channels with either water (100% light transmission) or black dye (0% light transmission) to generate 8 different light cycles. By combining these two layers, 64 combinations of light conditions, each having different intensity and cycle, could be applied to the 64 underlying microfluidic microalgae culture chambers. Single microalgal colony-trapping microstructures inside each culture chamber allowed trapping of single *B. braunii* colonies for continuous tracking of growth and lipid production at single-colony resolution.

Although the platforms described above were able to control both light intensities and light cycles, the wavelength of the incident light for each culture chamber in an array could not be adjusted as a single light source was used. Thus a multiplexed pixel-based irradiance platform capable of controlling all light variables such as intensity, cycle, and wavelength has been developed (Fig. 3B) [76]. A programmable LCD screen with an LED array backlight could control all light variables for each culture chamber, where microalgal growth under 238 different light conditions could be carried out in parallel. Recently, Perin et al. [77] introduced a simple and flexible light-controllable photobioreactor with up to 9 different light intensities created by overlaying photo-filters on top of the platform. A microfluidic photobioreactor delivering the light to surface-bound microalgae through an evanescent field of an optically excited slab waveguide was also developed to explore an alternative way of illuminating photobioreactors [78]. In this platform, growth rates of cyanobacteria *Synechococcus elongatus* under different illumination intensities were successfully characterized. Since these slab waveguides are easily stackable, the authors proposed that this illuminating scheme could also be used in large-scale microalgae cultivation systems to overcome shading effects coming from dense cultures.

Microfluidic photobioreactors for examining other culture conditions, such as nutrient availability, CO_2, and pH, have also been developed and utilized. Holcomb et al. [79] developed a poly(dimethylsiloxane) (PDMS) microfluidic device to culture and isolate microalgae, and examine the effect of nitrogen deprivation on lipid production. A microfluidic photobioreator composed of 2 × 4 perfusion chamber arrays has also been employed to compare growth and lipid production under different nitrogen-stressed conditions [80]. Each perfusion chamber incorporated a pneumatic valve that enabled cell loading, chemical exchange, and cell recovery, while 2 μm-high micropillar structures kept cells within the perfusion chambers. Using this platform, phototaxis behavior in the absence of Ca^{2+} and the cytotoxic effect of herbicides on *C. reinhardtii* cells were also analyzed. A high-throughput single-cell screening platform capable of conducting selective sample retrieval after on-chip analysis has been reported by Kim et al. [81]. This photobioreactor was composed of 1024 single-cell trapping sites and was successfully utilized to monitor the growth of single *C. reinhardtii* cells under different nitrogen conditions and retrieve target single cells of interest (Fig. 3C).

To further compare the effects of different nutrient levels, microfluidic gradient generators that could produce a series of diluted nutrient concentrations were adopted. For example, Bae et al. [82] combined an array of C-shaped microalgae growth chambers with an on-chip gradient generator, and utilized it to examine the concentration of sodium acetate that could promote *C. reinhardtii* growth rate and lipid production. In addition, Zheng et al. [83] exploited a gradient generator with culture chambers containing a semi-porous membrane floor integrated on top. Once different concentrations of chemicals were generated in the bottom layer, the chemicals could diffuse into the culture layer above through the porous membrane. Using this platform, the growth of five different microalgal strains was characterized under different copper concentrations. A microfluidic culture platform with the capability of controlling any desired chemical microenvironment for dynamic stimulation of cells inside the culture chambers was also developed [84]. In this system, microalgae samples could be confined

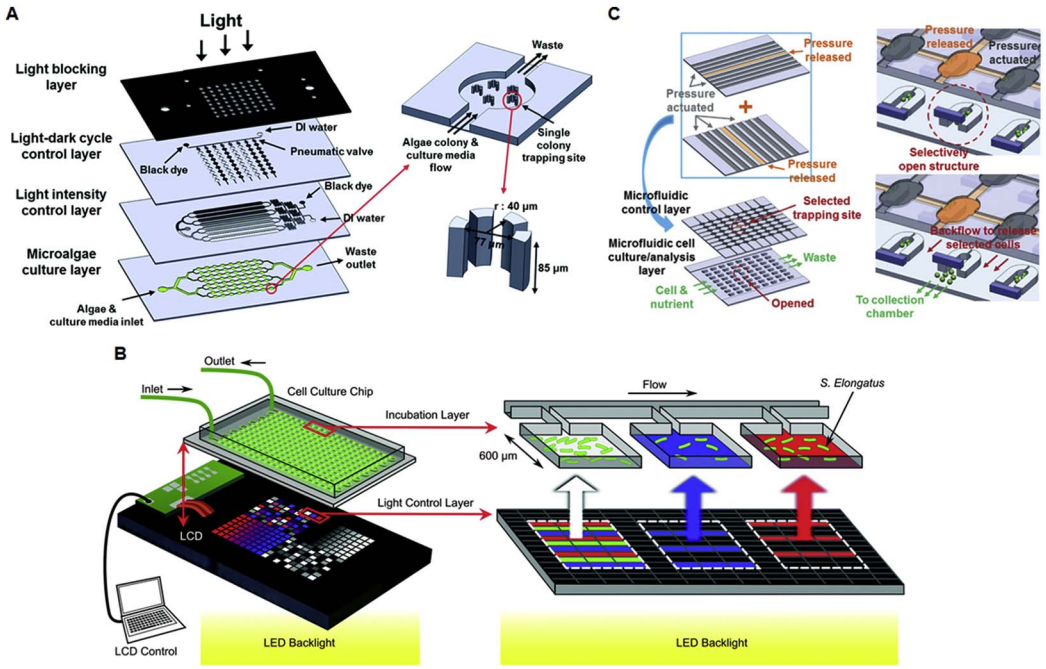

Fig. 3. High-throughput continuous-flow microfluidics photobioreactors for microalgae culture and analysis. (A) A microfluidic photobioreactor array for screening light conditions (intensity and cycle) impacting microalgal growth and lipid production. Reproduced, with permission, from [75]. (B) Microalgae on display: a microfluidic pixel-based irradiance platform as a photobioreactor. Reproduced, with permission, from [76]. (C) A high-throughput microfluidic single-cell screening platform capable of selective cell extraction. Reproduced, with permission, from [81].

within the culture chambers by designing the chamber height to be slightly lower than the cell size. The growth profile as well as changes in chlorophyll autofluorescence in *Chlorella sorokiniana* under fluctuating nitrogen conditions were characterized.

A microfluidic platform that can perform multiple processes, including culturing, analysis of lipid accumulation, and lipid extraction was developed [85]. The device was composed of two layers; a cell culture chamber with an output reservoir on the bottom layer, and a microchannel comprised of a square micropillar array on the top layer, where the micropillar array served as a physical filter to keep cells within the cell chamber during the lipid extraction process. The seed culture, nitrogen-deprived media, and solvent for extracting lipids were injected sequentially at different time periods to complete the entire process on chip. Different lipid productivities of various *C. reinhardtii* strains were successfully analyzed and compared. In this group's later work, the throughput of the platform was increased 8 times by integrating 8 units into a single device [86,87]. Using this platform, growth and lipid production of 8 different microalgal strains under nitrogen starvation, high temperature, as well as high salt concentrations were characterized.

3.2. Droplet or digital microfluidics photobioreactors

Droplet-based microfluidics culture platforms can provide several orders of magnitude higher throughput compared to continuous-flow microfluidics platforms described above, since each pico-liter scale droplet that functions as an independent bioreactor can be generated, cultured, and manipulated at speeds of tens to thousands of operations per second. Various photobioreactors based on droplet microfluidics have been developed to examine growth kinetics and lipid production of microalgae as well as to perform high-throughput microalgal mutant screening assays. Growth kinetics of single or multiple *C. vulgaris* cells was studied using static droplet arrays [88]. The device contained arrays of hydrodynamic trapping structures, allowing cell-encapsulated droplets to be trapped, forming static arrays of droplets. Using this device, it was found that the growth kinetics of single microalgal cells are heterogeneous compared to that observed in bulk-scale experiments. Pan et al. [89] developed a droplet microfluidics platform comprised of two separate modules, a droplet generator and a droplet incubator, for quantitative tracking of microalgal growth. Growth kinetics of three different microalgal species were analyzed by varying droplet sizes or culture volume, initial number of cells inside the droplets, and nutrient conditions such as pH, nitrogen, and salinity. A similar droplet microfluidics photobioreactor was also reported by Sung et al. [90]. This device had an array of pillar structures integrated into a second droplet incubator to capture droplets for accurate monitoring of cell growth and to improve the transfer rate of CO_2 into droplets through gas-permeable PDMS. Growth of microalgae under different CO_2 concentrations as well as different light intensities was characterized using this device.

Although all of the above droplet microfluidics photobioreactors were able to provide growth analysis capability, molecules produced by cells within the droplets cannot be analyzed. Abalde-Cela et al. [91] reported a droplet screening platform to detect ethanol-producing cyanobacteria. In this work, the amount of ethanol produced inside droplets was analyzed by means of a two-step enzymatic assay that utilized H_2O_2 derived from ethanol oxidation to convert Amplex Red into the highly fluorescent compound resorufin. Using this method, droplets containing the cyanobacteria *Synechocystis* sp. engineered to produce ethanol were successfully distinguished from droplets having a wild-type strain. Kim et al. [92] have introduced a droplet microfluidics microalgae analysis platform that can provide both growth and lipid production screening capabilities (Fig. 4A). The key new feature in this platform was the on-chip fluorescent lipid staining capability, which allowed intracellular lipids to be stained and quantified by merging the cell-encapsulated droplets with droplets containing the neutral lipid staining fluorescent dye Nile red. Using this platform, differences in growth and lipid production of *C. reinhardtii* cells under different nitrogen culture conditions were successfully evaluated. To further improve the throughput and screening capability of the platform, an automated optical detection system and a microfluidic droplet sorting scheme were integrated in their subsequent work (Fig. 4B) [42]. This system allowed for easy and simultaneous quantification of cell growth and lipid production as cell-encapsulated droplets flowing through the optical detection zone were analyzed based on the chlorophyll autofluorescence intensity for assessing cell number as a growth indicator, and the fluorescence intensity of BODIPY-stained lipids for quantifying intracellular lipid amount. A hydraulic sorting function integrated into the platform enabled selective collection of droplets showing higher chlorophyll signal indicating faster growth, and higher BODIPY signal

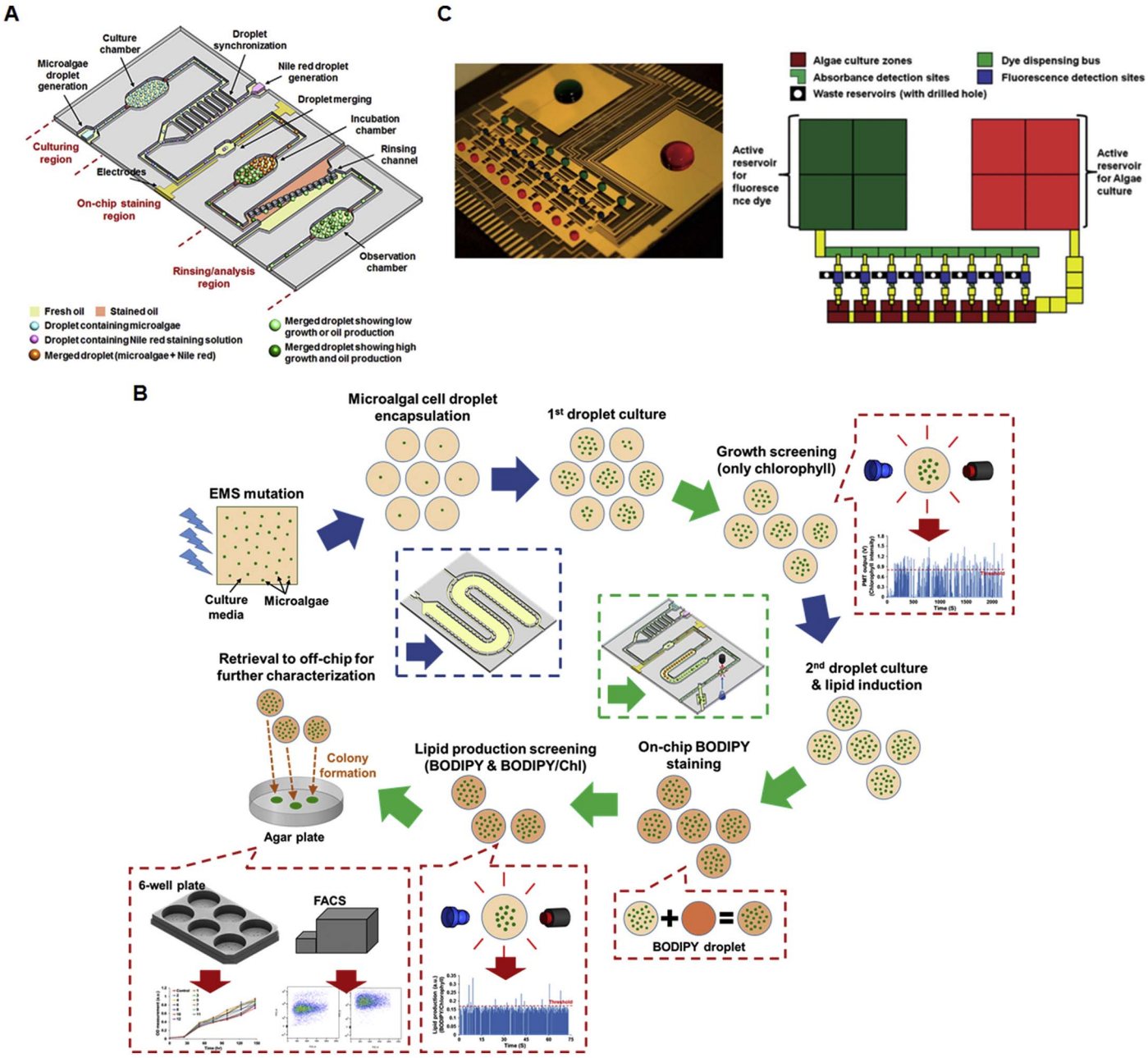

Fig. 4. High-throughput droplet or digital microfluidics photobioreactors for microalgae culture and analysis. (A) A droplet microfluidics platform for rapid microalgal growth and lipid production analysis. Reproduced, with permission, from [92]. (B) A screening strategy utilized in a high-throughput droplet microfluidics screening platform for selecting fast-growing and high-lipid-producing microalgae from a mutant library. The middle insets show microfluidic modules that were used in each screening steps (blue & green arrows). Reproduced, with permission, from [42]. (C) A digital microfluidics platform for analyzing wavelength-dependent lipid production in microalgae. Reproduced, with permission, from [100]. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

indicating increased lipid production. Using this platform, 8 potential microalgal variants having both higher growth and lipid production were successfully screened from a randomly mutated *C. reinhardtii* library of 200,000 cells. This is the first microfluidic platform that was actually utilized in a high-throughput microalgal mutant screening application with both on-chip growth and on-chip lipid production screening capabilities. A millifluidic droplet screening platform to characterize cell-to-cell heterogeneity in growth rate and cell division capabilities of isogenic *C. reinhardtii* cell populations was also developed [93]. The millifluidic droplets, in other words much larger droplets of 500 nL compared to typical microfluidic droplets of several tens of pL to a few nL, were used not only for analyzing growth kinetics over one week, but also to sort and collect droplets of interest containing viable and healthy cells for further testing.

Another type of droplet-based microfluidics platform is digital microfluidics (DMF). Like the emulsion droplet-based systems discussed above, DMF utilizes droplets as an independent cell culture vessel, however, has fundamental differences in how droplets are formed and manipulated. One of the most commonly used device configurations in DMF is a closed two-plate format, where droplets can be actuated using the electrowetting-on-dielectric (EWOD) principle. In this two-plate format, droplets are sandwiched between two electrode-patterned substrates. Typically, the top layer consists of transparent indium tin oxide (ITO) as a ground electrode with a hydrophobic coating, and the bottom layer has an array of actuation electrodes also coated with a hydrophobic insulator. Applying voltage to an electrode pair makes those electrodes effectively hydrophilic, drawing droplets onto the electrodes where voltage is applied. By having arrays of sandwiched

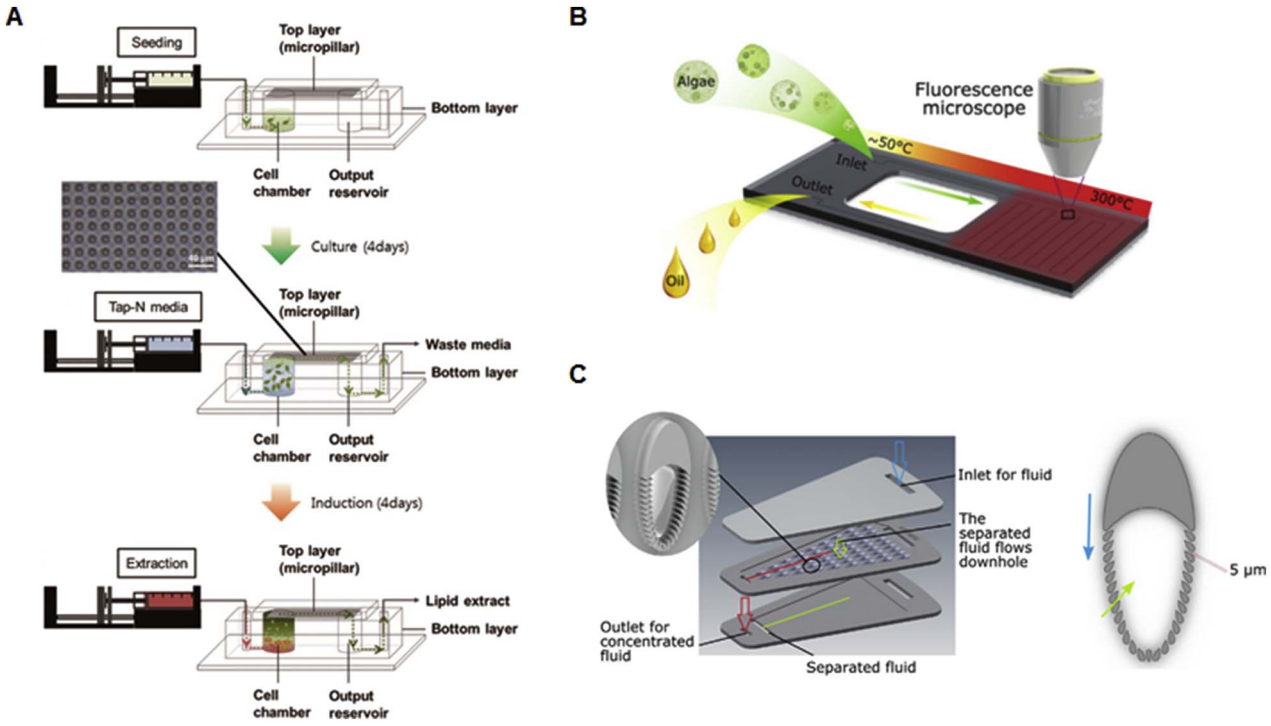

Fig. 5. Microfluidic devices that can be used for improving downstream processes. (A) An integrated microfluidic platform for conducting multiple post-processes, from microalgal culture to lipid extraction, on-chip. Reproduced, with permission, from [85]. (B) Biomass-to-biocrude conversion on a chip *via* hydrothermal liquefaction of microalgae. Reproduced, with permission, from [102]. (C) Continuous harvesting of microalgae by a microfluidic particle separation technique. Reproduced, with permission, from [104].

electrodes, droplets can be individually transported, mixed, split, dispensed, and analyzed by controlling the voltage applied to the arrays of electrode pairs [94–97]. A more detailed description of EWOD can be found in another review paper [98]. Applying this technology, Au et al. [99] constructed a microbioreactor for on-chip cell culture and cell density analysis. The platform consists of a reactor region with four electrodes in a 2 × 2 array format mated to a sample region. In the reactor region, the microalgae solution was dispensed to form a mother droplet, and culturing/mixing of microalgae was conducted by circulating the mother droplet periodically, followed by breaking off small daughter droplets for growth measurement. These daughter droplets were moved onto a region that included an opening for cell density measurement. The growth profiles in the microbioreactor experiment were comparable to that of a conventional large volume flask culture. Later, the authors developed a more advanced DMF system capable of conducting on-chip lipid staining and analysis, in addition to growth characterization (Fig. 4C) [100]. On-chip lipid quantification was achieved by introducing droplets that contained the neutral lipid staining reagent LipidTOX and merging them with microalgae-containing droplets. The effect of light wavelength and alternating illumination on microalgal growth and lipid production was also assessed by testing 8 different light conditions simultaneously. The most favorable light condition for the diatom *Cyclotella cryptica* was found to be cycling between a 15 hour blue light illumination and a 9 hour yellow light illumination, resulting in ~4-fold or ~2-fold higher lipid production compared to that with only blue or yellow light illumination, respectively.

4. Microsystems for harvesting, lipid extraction, and biomass processing

The downstream processes of microalgae harvesting, lipid extraction, and lipid conversion into biofuels and other co-products contribute to 60% of total production costs, thus further technological breakthroughs are required to reduce the overall cost of microalgae-based biofuels and bioproducts [101]. For cell harvesting, the small cell size and their similarity in density to water make this process challenging. Several techniques based on centrifugation, flocculation, filtration, and flotation have been developed and are conventionally utilized. Lipid extraction, along with biomass dewatering, are both energy-intensive processes. To increase the extraction efficiency, various extraction methods that involve cell rupturing techniques (e.g., autoclaving, homogenization, bead milling, chemical treatments with solvents, acids, and enzymes) and physical techniques (e.g., freezing, osmotic shock) have been utilized conventionally. After lipids are extracted, biofuels or other co-products are produced through a transesterification reaction with acidic or alkaline catalysts. This process is also a challenging task due to difficulties in product recovery as well as the generation of toxic chemical byproducts.

Microfluidic platforms have just begun to emerge as tools for investigating ways to further improve these downstream processes. As shown in Fig. 5A, a microfluidic platform that can conduct multiple post-processes on-chip, including lipid extraction (described above in 'Continuous-flow microfluidics-based photobioreactors'), was utilized to optimize lipid extraction conditions by varying the type, volume, and temperature of solvents used [85]. The most efficient condition was identified by measuring the amount of triacylglycerols (TAG) extracted from *C. reinhardtii* cells. Cheng et al. [102] developed a microfluidic reactor that could provide insights into, as well as control over the high temperature and high pressure cracking of biomass *via* hydrothermal liquefaction (HTL). Although HTL has recently received much attention as an attractive method for microalgal lipid conversion, the specific roles of various parameters, such as temperature, pressure, heating rate, and reaction time, still remain unclear. Here the developed microscale reactor was utilized to assess the effect of these parameters on HTL efficiency. The reactor was fabricated out of glass and silicon substrates to support the high pressures and high temperature needed in the HTL process. Microalgal cells were injected into the chip, flowed through an on-chip heating region, and then the converted oil could be collected through the outlet. This reactor allowed for *in situ* observation of the

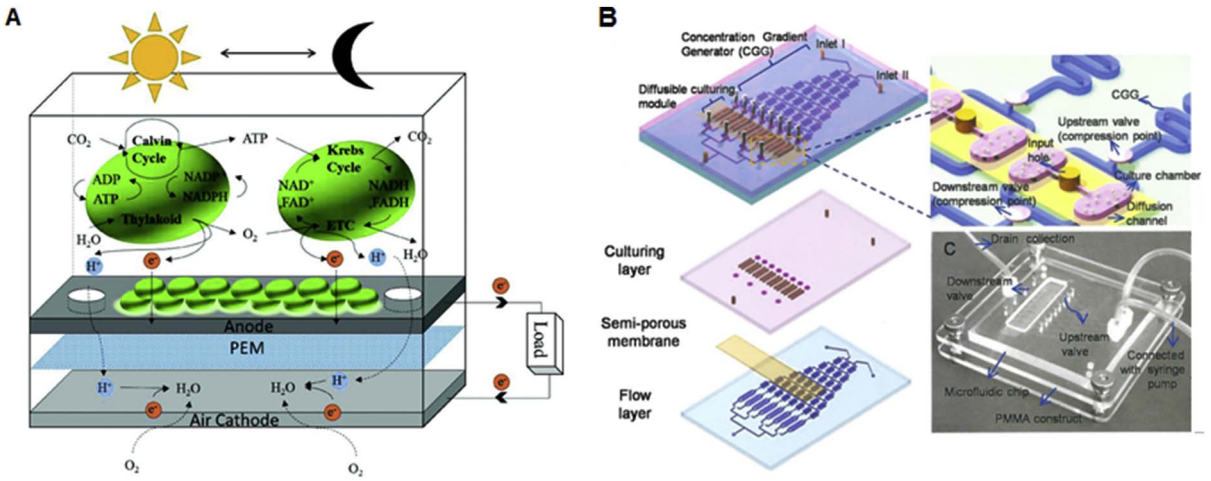

Fig. 6. Microfluidic microalgae platforms for miniaturized microbial fuel cells (μMFCs) and biosensor applications. (A) A micro-sized bio-solar cell for self-sustainable power generation. Reproduced, with permission, from [111]. (B) An integrated microfluidic device for toxicity screening applications using marine microalgae culture. Reproduced, with permission, from [83].

HTL reaction process using fluorescence microscopy by analyzing the microalgal slurry using chlorophyll autofluorescence at 675 nm and the crude oil for aromatic compounds at 510 nm (Fig. 5B).

One of the most promising approaches towards lowering microalgal harvesting cost is the optimization of the pre-concentration step before the de-watering process to minimize the amount of water that needs to be removed [103]. To address this issue, a microfluidic pre-concentration chip was designed for continuously concentrating microalgal cells [104]. This device contained an array of trilobite-shaped structures incorporating gaps of 5 μm in the blades around the structure (Fig. 5C). Particles larger than the gap size pass around these structures, while smaller particles and most of the carrier liquid flow through the gaps. By separately collecting the microalgal cells that are larger than the gap, pre-concentration of cells could be achieved in the device before moving to the de-watering step. Wang et al. [105] also developed a microfluidic cell concentrator for cyanobacteria *Synechocystis* sp. harvesting. In this platform, inertial forces generated from the curved microfluidic structure and geometry of microchannels focused cells to a known equilibrium position within the channel, followed by separation of the concentrated cyanobacteria stream from culture medium. Through this design, the original cell suspension could be concentrated as high as 3.28-fold, with maximum cell recovery efficiency of 98.5%.

5. Other microsystems utilizing microalgae

5.1. Miniaturized microbial fuel cells (μMFCs) utilizing cyanobacteria and microalgae

Along with renewable biofuel research where microalgae are utilized as a lipid source for biofuel production, microalgae have also been utilized in fuel cell applications [106–108]. A miniaturized photosynthetic electrochemical cell (μPEC) was implemented by utilizing cyanobacteria to produce electrons under light exposure [109]. The platform is composed of 5 different layers consisting of a glass support layer, an anode, a proton exchange membrane (PEM), a cathode, and an additional glass support layer. The glass layer on the anode side was utilized to permit light entry into the reaction chamber. Using the cyanobacteria *Anabaena sp.*, power density of 20–40 pW/cm^2 was obtained, but the energy conversion efficiency was very low (less than 1%). Yoon et al. [110] reported a microscale photosynthetic MFC that could significantly increase the power density by utilizing the cyanobacteria *Synechocystis* sp. in the anode as well as increasing the surface area-to-volume ratio, achieving a power density of 7.09 nW/cm^2. Despite this improvement, the device performance was still insufficient for any practical use and/or self-sustainable power generation. Later, the same group [111] developed a miniature microfluidics-based single-chamber device, which had both the anode and cathode in an upright configuration with an integrated bubble trap (Fig. 6A). Through the upright electrode design, solar energy capture could be maximized and carbon-based electrode materials could be utilized for the anode, which improved cyanobacterial attachment and electron coupling to the anode. The air bubbles in the system facilitated gas exchange for long-term sustainable operation. With this novel design, the power density was significantly increased to ~90 nW/cm^2. However, the authors claimed several challenges that need to be resolved for the μMFC to achieve comparable power density to heterotrophic MFCs, such as poor electron transfer to the anode and inefficient microbe-anode interactions. Shahparnia et al. [112] used *C. reinhardtii* as the photosynthetic microorganism for their Micro Photosynthetic Power Cell (μPSC). Here, cylindrical gold electrodes were patterned on two sides of the PEM instead of making the electrodes on the surface of the chambers, and a power density of 36 μW/cm^2 was obtained. Despite these efforts, due to the extremely low power density achieved, it is unlikely that miniaturized MFCs based on microalgae will have much practical use. Rather, the main contributions of such devices could be to better understand photosynthesis-based MFCs as well as testing and optimizing the various operation conditions.

5.2. Microalgae as biosensors

In recent years, microalgae have been utilized for toxicity assays due to their high sensitivity in metabolic activity changes upon exposure to toxins as well as reproducibility in such assays. Toxic substances tend to have a large impact on microalgal metabolic activities, and these changes can be translated into electrical or optical signals within microalgae-based biosensors. Based on this sensing mechanism, microalgae have been used to detect different pollutants such as herbicides, heavy metals, and volatile organic compounds [113]. This same sensing mechanism has also been employed in microfluidic microalgae-based biosensing platforms. Siebman et al. [114] implemented an AC dielectrophoretic microchip to capture *C. reinhardtii* cells to be exposed to several environmental pollutants such as mercury, methylmercury, copper, copper oxide nanoparticles, and diuron. One metabolic activity measured in response to exposure to these toxins was the increased generation of cellular reactive oxygen species (ROS) as measured by fluorescent intracellular oxidative stress indicators. Lefèvre et al. [115] developed a fluorescent biosensor capable of measuring the small variations in chlorophyll autofluorescence induced by

a herbicide pollutant. This biosensor exhibited 10 times higher sensitivity compared to a commercial fluorescence-based sensor. A microfluidic platform for environmental toxicity testing was also constructed, which had a concentration gradient generator that could dynamically establish 8 different chemical concentrations on-chip (Fig. 6B) [83]. The toxicity effects of different copper concentrations on cell division rate, cell viability, and metabolic activity were examined using 4 different marine microalgae, showing dose-related toxicity responses. In this group's later work, 4 concentration gradient generators were integrated on a single microdevice, where 4 parallel assays, including toxicity testing of heavy metals (Cu, Ni, Hg) and a chemical compound (phenol), as well as combined toxicity testing (Cu and phenol) could be performed simultaneously [116]. Motility response of two motile microalgae, *Platymonas subcordiformis* and *Platymonas helgolandica* var. *tsingtaoensis*, was used as a toxicity sensing signal under various concentrations of toxicants. The toxicity of copper chloride for microalgae was also studied using microfluid segments created using a seven-port manifold that allowed for cells, cultivation medium, and the copper chloride solution to be injected together into the carrier solution flow [117]. The computer-controlled seven-port manifold generated stepwise concentrations of copper chloride in the microfluidic segments. The toxicity effect on *C. vulgaris* growth was evaluated through two different optical measurements; transmission light intensity and chlorophyll autofluorescence intensity.

6. Conclusion and perspective

In the past decade, various microfluidic platforms for microalgal biotechnology applications have emerged. Increasing interest in microalgae as a source of bioenergy and other high-value chemicals have drawn more researchers into this field. With this increasing interest, it is ever more critical to have technologies that can rapidly accelerate such efforts to improve efficiency and lower cost, an area where microfluidic systems for microalgal biotechnology can have a significant impact. Microfluidic systems possess many advantages, such as fast analysis, high sensitivity, portability, and capability of integrating multiple functions onto a single chip, and thus is an ideal technology platform to overcome many bottlenecks encountered in microalgal biotechnology [15].

First, the development of microfluidic systems in which microalgae with desired traits can be generated more efficiently through genetic and metabolic engineering, and high-throughput platforms that can screen through the vast microalgal libraries generated, are expected to be of significant interest. Current automation for gene assembly relies on robotic technology, which requires considerable operation cost. In contrast, modest infrastructure and inexpensive microfluidic devices can be suitable for widespread use due to their potential for higher throughput and lower cost. Lin et al. [118] reported the use of DMF for DNA ligation with single DNA fragment insertions. Shih et al. [119] also reported a versatile platform that combined digital and droplet microfluidics to conduct more efficient DNA assembly based on commonly used DNA assembly protocols. These platforms can be integrated with previously developed high-efficiency microfluidic transformation techniques such as electroporation and gene insertion with nanowires, where all procedures, from gene preparation (DNA assembly) to the transformation processes, can be conducted on a single device [71–73]. At the same time, other microfluidic techniques that have been successfully utilized for mammalian cell transformations, for example, combination of electric field and ultrasonic wave (*i.e.*, electrosonoporation) developed by Longsine-Parker et al. [120], can be applied to further improve the transformation efficiency. Microfluidic systems described to date for microorganisms, in general, also have greater capacity for massively parallel analysis over conventional approaches. For example, Ingham et al. [121] implemented a high-throughput culture and screening chip containing 10^6 individual microbial cultivation wells. In addition, diverse high-throughput screening assays have been conducted using droplet microfluidics platforms. However, many of these high-throughput microfluidic analysis and screening platforms have not yet been applied to microalgal biotechnology research. So far, only few studies performing high-throughput microalgae screening have been reported [42,81,91,92,122], but as many microfluidic platforms are becoming more mature and easy to use, we expect that more research will be carried out using such microfluidic platforms, which could rapidly advance the field.

Second, the development of novel microscale analytical tools, or interfaces that can integrate microfluidic platforms with extremely powerful conventional analytical instruments in a high-throughput fashion, is another area that can benefit from further development. The most commonly used analysis method, particularly for lipid analysis in microalgae, is based on neutral lipid-straining fluorescent dyes such as Nile red and BODIPY. Although this fluorescent staining method can provide a convenient and easy way of quantifying lipid production in microalgae, and thus is most commonly utilized in microfluidic platforms for microalgal biotechnology, it requires a labeling step, and consequently is more suitable for end-point measurements instead of time-course analysis. In addition, these dyes require an additional cell staining step to be conducted on the microfluidic chip, making its design and operation significantly more complicated. More importantly, since the dye stains all neutral lipids, the method does not provide any information on the types and composition of lipids produced by the cells. If the scope is broadened to other high-value biomolecules beyond just lipid production, fluorescent dyes that can specifically stain such bioproducts typically do not exist (beyond some enzymatic assays). Contrary to this, other conventional analytical methods such as gas chromatography – mass spectrometry (GC–MS) can provide accurate and quantifiable analyses of complicated lipid compositions. However such analytical techniques are typically not compatible with microfluidic systems because larger sample amounts are usually required, and interfacing such analytical methods to microfluidic platforms is not easy. Thus, it is desirable to have non-invasive, label-free, and *in-situ* sensing capabilities for a variety of biomolecules produced by microalgae, which can also be easily integrated with microfluidic platforms. Such techniques already exist, including Raman spectroscopy, which relies on utilizing vibrational frequencies specific to molecular structures and thus, provides both molecular specificity and quantification capabilities. Such analysis has been utilized for *in situ* microalgal lipid analysis in recent years [123–126]. Efforts are now ongoing to integrate this powerful analytical technique into microfluidic platforms. Kim et al. [127] recently reported a method that allows the use of Raman spectroscopy with PDMS-based microfluidic devices to perform on-chip, droplet-based *in vivo* microalgal lipid analysis with single-cell resolution. The time-course tracking and analysis of lipid accumulation in *C. reinhardtii* cells under 8 different culture conditions was successfully conducted, demonstrating the potential for Raman-microfluidics based lipidomics. Zhang et al. [128] applied a single-cell laser-trapping Raman spectroscopy approach for *in vivo* lipid profiling of several microalgal species. Integration of this approach, as well as other label-free analytical techniques, into high-throughput microfluidic platforms will significantly broaden the capabilities of state of the art microfluidic platforms for microalgal biotechnology, and can enable many new applications.

Finally, several areas of future microfluidics applications for microalgae should address the challenge of advancing towards cost-effective microalgal cultivation that can lead to successful commercialization. For example, one likely avenue of using microalgae is the production of high-value molecules by several different species and/or strains of microalgae, rather than single strains, which will require a large number of strain combinations to be tested, an aspect that could be optimized using the high-throughput nature of microfluidics. When using open-pond culture systems as the primary growth platforms for microalgae, issues of contamination and variations in environmental

conditions, both of which can reduce microalgae productivity, will need to be addressed. Microfluidic bioreactors can be utilized to simulate these changing environmental conditions and their effects on microalgal growth and biomolecule production. Also, studies have shown that culturing several microalgal species together, each with different optimal growth parameters, can help maintain productivity when environmental conditions (*e.g.*, temperature, light intensity) change [129]. Thus, simulating how such a mixed-culture performs under varying conditions should be an area where arrays of microfluidic bioreactors can be utilized. Additionally, co-cultivation of microalgae with beneficial bacteria can help fight off contamination from other harmful bacteria or other microorganisms [130], and therefore, testing varieties of co-culture combinations through microfluidic systems would be of high interest. One final issue that must be addressed is the need to study and understand how the data generated from microfluidic systems translate to results from larger lab-scale and pilot-scale culture systems (and ultimately industrial-scale culture systems).

Acknowledgements

The authors would like to thank the support from the National Science Foundation (NSF) Emerging Frontiers in Research and Innovation (EFRI) [grant number 1240478] to A.H. and T.P.D. and the NSF [grant number 1532188] to A.H. Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the NSF.

Author contributions

All authors (H.S.K., T.P.D., and A.H.) conceived the idea and designed the current review article. H.S.K. and A.H. contributed to the initial drafting of the article. Subsequent revisions were made by all authors. All authors read and approved the final manuscript.

Competing financial interests

The authors declare no competing financial interests.

Statement of informed consent, human/animal rights

No conflicts, informed consent, human or animal rights applicable.

References

[1] L. Zhu, Biorefinery as a promising approach to promote microalgae industry: an innovative framework, Renew. Sust. Energ. Rev. 41 (2015) 1376–1384.
[2] T.M. Mata, A.A. Martins, N.S. Caetano, Microalgae for biodiesel production and other applications: a review, Renew. Sust. Energ. Rev. 14 (2010) 217–232.
[3] L. Brennan, P. Owende, Biofuels from microalgae—a review of technologies for production, processing, and extractions of biofuels and co-products, Renew. Sust. Energ. Rev. 14 (2010) 557–577.
[4] S. Chu, A. Majumdar, Opportunities and challenges for a sustainable energy future, Nature 488 (2012) 294–303.
[5] C. Sheridan, Big oil turns on biofuels, Nat. Biotechnol. 31 (2013) 870–873.
[6] R.H. Wijffels, M.J. Barbosa, An outlook on microalgal biofuels, Science 329 (2010) 796–799.
[7] G.C. Dismukes, D. Carrieri, N. Bennette, G.M. Ananyev, M.C. Posewitz, Aquatic phototrophs: efficient alternatives to land-based crops for biofuels, Curr. Opin. Biotechnol. 19 (2008) 235–240.
[8] T. Searchinger, R. Heimlich, R.A. Houghton, F. Dong, A. Elobeid, J. Fabiosa, S. Tokgoz, D. Hayes, T.H. Yu, Use of US croplands for biofuels increases greenhouse gases through emissions from land-use change, Science 319 (2008) 1238.
[9] B. Wang, Y. Li, N. Wu, C.Q. Lan, CO_2 bio-mitigation using microalgae, Appl. Microbiol. Biotechnol. 79 (2008) 707–718.
[10] Y. Chisti, Biodiesel from microalgae, Biotechnol. Adv. 25 (2007) 294–306.
[11] D.R. Georgianna, S.P. Mayfield, Exploiting diversity and synthetic biology for the production of algal biofuels, Nature 488 (2012) 329–335.
[12] Y. Chisti, Constraints to commercialization of algal fuels, J. Biotechnol. 167 (2013) 201–214.
[13] S.A. Scott, M.P. Davey, J.S. Dennis, I. Horst, C.J. Howe, D.J. Lea-Smith, A.G. Smith, Biodiesel from algae: challenges and prospects, Curr. Opin. Biotechnol. 21 (2010) 277–286.
[14] T. Mutanda, D. Ramesh, S. Karthikeyan, S. Kumari, A. Anandraj, F. Bux, Bioprospecting for hyper-lipid producing microalgal strains for sustainable biofuel production, Bioresour. Technol. 102 (2011) 57–70.
[15] A. Han, H. Hou, L. Li, H.S. Kim, P. de Figueiredo, Microfabricated devices in microbial bioenergy sciences, Trends Biotechnol. 31 (2013) 225–232.
[16] G.M. Whitesides, What comes next? Lab Chip 11 (2011) 191–193.
[17] G.B. Salieb-Beugelaar, G. Simone, A. Arora, A. Philippi, A. Manz, Latest developments in microfluidic cell biology and analysis systems, Anal. Chem. 82 (2010) 4848–4864.
[18] A. Grünberger, W. Wiechert, D. Kohlheyer, Single-cell microfluidics: opportunity for bioprocess development, Curr. Opin. Biotechnol. 29 (2014) 15–23.
[19] S.F. Han, W.B. Jin, R.J. Tu, W.M. Wu, Biofuel production from microalgae as feedstock: current status and potential, Crit. Rev. Biotechnol. 35 (2015) 255–268.
[20] Q. Hu, M. Sommerfeld, E. Jarvis, M. Ghirardi, M. Posewitz, M. Seibert, A. Darzins, Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances, Plant J. 54 (2008) 621–639.
[21] F. Guiheneuf, A. Khan, L.S. Tran, Genetic engineering: a promising tool to engender physiological, biochemical, and molecular stress resilience in green microalgae, Front. Plant Sci. 7 (2016) 400.
[22] A. Ghosh, S. Khanra, M. Mondal, G. Halder, O.N. Tiwari, S. Saini, T.K. Bhowmick, K. Gayen, Progress toward isolation of strains and genetically engineered strains of microalgae for production of biofuel and other value added chemicals: a review, Energy Convers. Manag. 113 (2016) 104–118.
[23] V.T. Duong, Y. Li, E. Nowak, P.M. Schenk, Microalgae isolation and selection for prospective biodiesel production, Energies 5 (2012) 1835–1849.
[24] P. Neofotis, A. Huang, K. Sury, W. Chang, F. Joseph, A. Gabr, S. Twary, W. Qiu, O. Holguin, J.E.W. Polle, Characterization and classification of highly productive microalgae strains discovered for biofuel and bioproduct generation, Algal Res. 15 (2016) 164–178.
[25] P. Neofotis, A. Huang, W. Chang, F. Joseph, J.E. Polle, Microalgae strain isolation, screening, and identification for biofuels and high-value products, Microalgal Production for Biomass and High-Value Products, 2016, pp. 63–89.
[26] G. Benazzi, D. Holmes, T. Sun, M.C. Mowlem, H. Morgan, Discrimination and analysis of phytoplankton using a microfluidic cytometer, IET Nanobiotechnol. 1 (2007) 94–101.
[27] N. Hashemi, J.S. Erickson, J.P. Golden, K.M. Jackson, F.S. Ligler, Microflow cytometer for optical analysis of phytoplankton, Biosens. Bioelectron. 26 (2011) 4263–4269.
[28] N. Hashemi, J.S. Erickson, J.P. Golden, F.S. Ligler, Optofluidic characterization of marine algae using a microflow cytometer, Biomicrofluidics 5 (2011) 32009–320099.
[29] A. Schaap, Y. Bellouard, T. Rohrlack, Optofluidic lab-on-a-chip for rapid algae population screening, Biomed. Opt. Express 2 (2011) 658–664.
[30] A. Schaap, T. Rohrlack, Y. Bellouard, Optical classification of algae species with a glass lab-on-a-chip, Lab Chip 12 (2012) 1527–1532.
[31] A. Schaap, T. Rohrlack, Y. Bellouard, Lab on a chip technologies for algae detection: a review, J. Biophotonics 5 (2012) 661–672.
[32] R.A. Erickson, R. Jimenez, Microfluidic cytometer for high-throughput measurement of photosynthetic characteristics and lipid accumulation in individual algal cells, Lab Chip 13 (2013) 2893–2901.
[33] D.H. Lee, C.Y. Bae, J.I. Han, J.K. Park, In situ analysis of heterogeneity in the lipid content of single green microalgae in alginate hydrogel microcapsules, Anal. Chem. 85 (2013) 8749–8756.
[34] D. Bardin, M.R. Kendall, P.A. Dayton, A.P. Lee, Parallel generation of uniform fine droplets at hundreds of kilohertz in a flow-focusing module, Biomicrofluidics 7 (2013) 34112.
[35] S.L. Anna, Droplets and bubbles in microfluidic devices, Annu. Rev. Fluid Mech. 48 (2016) 285–309.
[36] A.R. Guzman, H.S. Kim, P. de Figueiredo, A. Han, A three-dimensional electrode for highly efficient electrocoalescence-based droplet merging, Biomed. Microdevices 17 (2015) 35.
[37] T.P. Lagus, J.F. Edd, A review of the theory, methods and recent applications of high-throughput single-cell droplet microfluidics, J. Phys. D. Appl. Phys. 46 (2013) 114005.
[38] M.T. Guo, A. Rotem, J.A. Heyman, D.A. Weitz, Droplet microfluidics for high-throughput biological assays, Lab Chip 12 (2012) 2146–2155.
[39] J. Dai, H.S. Kim, A.R. Guzman, W.-B. Shim, A. Han, A large-scale on-chip droplet incubation chamber enables equal microbial culture time, RSC Adv. 6 (2016) 20516–20519.
[40] S.-I. Han, H.S. Kim, A. Han, In-droplet cell concentration using dielectrophoresis, Biosens. Bioelectron. 97 (2017) 41–45.
[41] R.J. Best, J.J. Lyczakowski, S. Abalde-Cela, Z. Yu, C. Abell, A.G. Smith, Label-free analysis and sorting of microalgae and cyanobacteria in microdroplets by intrinsic chlorophyll fluorescence for the identification of fast growing strains, Anal. Chem. 88 (2016) 10445–10451.
[42] H.S. Kim, S.-C. Hsu, S.-I. Han, H.R. Thapa, A.R. Guzman, D.R. Browne, M. Tatli, T.P. Devarenne, D.B. Stern, A. Han, High-throughput droplet microfluidics screening platform for selecting fast-growing and high lipid-producing microalgae from a mutant library, Plant Direct 1 (2017) 1–13.
[43] J. Wang, J. Sun, Y. Song, Y. Xu, X. Pan, Y. Sun, D. Li, A label-free microfluidic biosensor for activity detection of single microalgae cells based on chlorophyll fluorescence, Sensors 13 (2013) 16075–16089.
[44] J. Wang, Y. Song, M.M. Maw, Y. Song, X. Pan, Y. Sun, D. Li, Detection of size spectrum of microalgae cells in an integrated underwater microfluidic device, J. Exp. Mar. Biol. Ecol. 473 (2015) 129–137.
[45] F. Lefèvre, P. Juneau, R. Izquierdo, Integration of fluorescence sensors using

organic optoelectronic components for microfluidic platform, Sensors Actuators B Chem. 221 (2015) 1314–1320.
[46] Y. Song, M. Li, J. Yang, J. Wang, X. Pan, Y. Sun, D. Li, Capacitive detection of living microalgae in a microfluidic chip, Sensors Actuators B Chem. 194 (2014) 164–172.
[47] Y. Song, J. Wang, J. Yang, Y. Wu, N. Li, N. Gong, X. Pan, Y. Sun, D. Li, Algae detection and ship's ballast water analysis by a microfluidic lab-on-chip device, Instrum. Sci. Technol. 40 (2012) 305–315.
[48] Y. Song, R. Peng, J. Wang, X. Pan, Y. Sun, D. Li, Automatic particle detection and sorting in an electrokinetic microfluidic chip, Electrophoresis 34 (2013) 684–690.
[49] H. Bayley, C.R. Martin, Resistive-pulse sensing from microbes to molecules, Chem. Rev. 100 (2000) 2575–2594.
[50] Y.L. Deng, J.S. Chang, Y.J. Juang, Separation of microalgae with different lipid contents by dielectrophoresis, Bioresour. Technol. 135 (2013) 137–141.
[51] R. Pethig, Dielectrophoresis: status of the theory, technology, and applications, Biomicrofluidics 4 (2010) 022811.
[52] B. Çetin, D. Li, Dielectrophoresis in microfluidics technology, Electrophoresis 32 (2011) 2410–2427.
[53] Y.L. Deng, M.Y. Kuo, Y.J. Juang, Development of flow through dielectrophoresis microfluidic chips for biofuel production: sorting and detection of microalgae with different lipid contents, Biomicrofluidics 8 (2014) 064120.
[54] Z.R. Gagnon, Cellular dielectrophoresis: applications to the characterization, manipulation, separation and patterning of cells, Electrophoresis 32 (2011) 2466–2487.
[55] K.A. Michael, S.R. Hiibel, E.J. Geiger, Dependence of the dielectrophoretic upper crossover frequency on the lipid content of microalgal cells, Algal Res. 6 (2014) 17–21.
[56] H. Song, J.M. Rosano, Y. Wang, C.J. Garson, B. Prabhakarpandian, K. Pant, G.J. Klarmann, A. Perantoni, L.M. Alvarez, E. Lai, Continuous-flow sorting of stem cells and differentiation products based on dielectrophoresis, Lab Chip 15 (2015) 1320–1328.
[57] E.J. Geiger, H. Hadady, D. Redelman, S.R. Hiibel, Continuous-flow sorting of microalgae cells based on lipid content by high frequency dielectrophoresis, AIMS Biophys. 3 (2016) 398–414.
[58] S.K. Srivastava, A. Gencoglu, A.R. Minerick, DC insulator dielectrophoretic applications in microdevice technology: a review, Anal. Bioanal. Chem. 399 (2011) 301–321.
[59] R.C. Gallo-Villanueva, N.M. Jesús-Pérez, J.I. Martínez-López, A. Pacheco, B.H. Lapizco-Encinas, Assessment of microalgae viability employing insulator-based dielectrophoresis, Microfluid. Nanofluid. 10 (2011) 1305–1315.
[60] Y. Song, J. Yang, X. Shi, H. Jiang, Y. Wu, R. Peng, Q. Wang, N. Gong, X. Pan, Y. Sun, D. Li, DC dielectrophoresis separation of marine algae and particles in a microfluidic chip, SCIENCE CHINA Chem. 55 (2012) 524–530.
[61] N. Godino, F. Jorde, D. Lawlor, M. Jaeger, C. Duschl, Purification of microalgae from bacterial contamination using a disposable inertia-based microfluidic device, J. Micromech. Microeng. 25 (2015) 084002.
[62] D. Di Carlo, Inertial microfluidics, Lab Chip 9 (2009) 3038–3046.
[63] H. Amini, W. Lee, D. Di Carlo, Inertial microfluidic physics, Lab Chip 14 (2014) 2739–2761.
[64] J.M. Martel, M. Toner, Inertial focusing in microfluidics, Annu. Rev. Biomed. Eng. 16 (2014) 371–396.
[65] J. Zhang, S. Yan, D. Yuan, G. Alici, N.T. Nguyen, M. Ebrahimi Warkiani, W. Li, Fundamentals and applications of inertial microfluidics: a review, Lab Chip 16 (2016) 10–34.
[66] M. Hlavova, Z. Turoczy, K. Bisova, Improving microalgae for biotechnology–from genetics to synthetic biology, Biotechnol. Adv. 33 (2015) 1194–1203.
[67] K.L. Kindle, High-frequency nuclear transformation of *Chlamydomonas reinhardtii*, Proc. Natl. Acad. Sci. 87 (1990) 1228–1232.
[68] K. Shimogawara, S. Fujiwara, A. Grossman, H. Usuda, High-efficiency transformation of *Chlamydomonas reinhardtii* by electroporation, Genetics 148 (1998) 1821–1828.
[69] P. Berthold, R. Schmitt, W. Mages, An engineered *Streptomyces hygroscopicus* aph 7″ gene mediates dominant resistance against hygromycin B in *Chlamydomonas reinhardtii*, Protist 153 (2002) 401–412.
[70] M.S. Parker, T. Mock, E.V. Armbrust, Genomic insights into marine microalgae, Annu. Rev. Genet. 42 (2008) 619–645.
[71] B. Qu, Y.J. Eu, W.J. Jeong, D.P. Kim, Droplet electroporation in microfluidics for efficient cell transformation with or without cell wall removal, Lab Chip 12 (2012) 4483–4488.
[72] D.J. Im, S.N. Jeong, B.S. Yoo, B. Kim, D.P. Kim, W.J. Jeong, I.S. Kang, Digital microfluidic approach for efficient electroporation with high productivity: transgene expression of microalgae without cell wall removal, Anal. Chem. 87 (2015) 6592–6599.
[73] S. Bae, S. Park, J. Kim, J.S. Choi, K.H. Kim, D. Kwon, E. Jin, I. Park, H. Kim, Do, T.S. Seo, exogenous gene integration for microalgal cell transformation using a nanowire-incorporated microdevice, ACS Appl. Mater. Interfaces 7 (2015) 27554–27561.
[74] M. Chen, T. Mertiri, T. Holland, A.S. Basu, Optical microplates for high-throughput screening of photosynthesis in lipid-producing algae, Lab Chip 12 (2012) 3870–3874.
[75] H.S. Kim, T.L. Weiss, H.R. Thapa, T.P. Devarenne, A. Han, A microfluidic photobioreactor array demonstrating high-throughput screening for microalgal oil production, Lab Chip 14 (2014) 1415–1425.
[76] P.J. Graham, J. Riordon, D. Sinton, Microalgae on display: a microfluidic pixel-based irradiance assay for photosynthetic growth, Lab Chip 15 (2015) 3116–3124.
[77] G. Perin, E. Cimetta, F. Monetti, T. Morosinotto, F. Bezzo, Novel micro-photobioreactor design and monitoring method for assessing microalgae response to light intensity, Algal Res. 19 (2016) 69–76.
[78] E.E. Jung, M. Kalontarov, D.F. Doud, M.D. Ooms, L.T. Angenent, D. Sinton, D. Erickson, Slab waveguide photobioreactors for microalgae based biofuel production, Lab Chip 12 (2012) 3740–3745.
[79] R.E. Holcomb, L.J. Mason, K.F. Reardon, D.M. Cropek, C.S. Henry, Culturing and investigation of stress-induced lipid accumulation in microalgae using a microfluidic device, Anal. Bioanal. Chem. 400 (2011) 245–253.
[80] Y.J. Eu, H.S. Park, D.P. Kim, J. Wook Hong, A microfluidic perfusion platform for cultivation and screening study of motile microalgal cells, Biomicrofluidics 8 (2014) 024113.
[81] H.S. Kim, T.P. Devarenne, A. Han, A high-throughput microfluidic single-cell screening platform capable of selective cell extraction, Lab Chip 15 (2015) 2467–2475.
[82] S. Bae, C.W. Kim, J.S. Choi, J.W. Yang, T.S. Seo, An integrated microfluidic device for the high-throughput screening of microalgal cell culture conditions that induce high growth rate and lipid content, Anal. Bioanal. Chem. 405 (2013) 9365–9374.
[83] G. Zheng, Y. Wang, Z. Wang, W. Zhong, H. Wang, Y. Li, An integrated microfluidic device in marine microalgae culture for toxicity screening application, Mar. Pollut. Bull. 72 (2013) 231–243.
[84] C.S. Luke, J. Selimkhanov, L. Baumgart, S.E. Cohen, S.S. Golden, N.A. Cookson, J. Hasty, A ficrofluidic platform for long-term monitoring of algae in a dynamic environment, ACS Synth. Biol. 5 (2016) 8–14.
[85] H.S. Lim, J.Y. Kim, H.S. Kwak, S.J. Sim, Integrated microfluidic platform for multiple processes from microalgal culture to lipid extraction, Anal. Chem. 86 (2014) 8585–8592.
[86] H.S. Kwak, J.Y. Kim, S.C. Na, N.L. Jeon, S.J. Sim, Multiplex microfluidic system integrating sequential operations of microalgal lipid production, Analyst 141 (2016) 1218–1225.
[87] H.S. Kwak, J.Y.H. Kim, H.M. Woo, E. Jin, B.K. Min, S.J. Sim, Synergistic effect of multiple stress conditions for improving microalgal lipid production, Algal Res. 19 (2016) 215–224.
[88] A. Dewan, J. Kim, R.H. McLean, S.A. Vanapalli, M.N. Karim, Growth kinetics of microalgae in microfluidic static droplet arrays, Biotechnol. Bioeng. 109 (2012) 2987–2996.
[89] J. Pan, A.L. Stephenson, E. Kazamia, W.T. Huck, J.S. Dennis, A.G. Smith, C. Abell, Quantitative tracking of the growth of individual algal cells in microdroplet compartments, Integr. Biol. 3 (2011) 1043–1051.
[90] Y.J. Sung, J.Y. Kim, K.W. Bong, S.J. Sim, Microdroplet photobioreactor for the photoautotrophic culture of microalgal cells, Analyst 141 (2016) 989–998.
[91] S. Abalde-Cela, A. Gould, X. Liu, E. Kazamia, A.G. Smith, C. Abell, High-throughput detection of ethanol-producing cyanobacteria in a microdroplet platform, J. R. Soc. Interf. R. Soc. 12 (2015).
[92] H.S. Kim, A.R. Guzman, H.R. Thapa, T.P. Devarenne, A. Han, A droplet microfluidics platform for rapid microalgal growth and oil production analysis, Biotechnol. Bioeng. 113 (2016) 1691–1701.
[93] S.P. Damodaran, S. Eberhard, L. Boitard, J.G. Rodriguez, Y. Wang, N. Bremond, J. Baudry, J. Bibette, F.A. Wollman, A millifluidic study of cell-to-cell heterogeneity in growth-rate and cell-division capability in populations of isogenic cells of *Chlamydomonas reinhardtii*, PLoS One 10 (2015) e0118987.
[94] M. Abdelgawad, A.R. Wheeler, The digital revolution: a new paradigm for microfluidics, Adv. Mater. 21 (2009) 920–925.
[95] M.J. Jebrail, A.R. Wheeler, Let's get digital: digitizing chemical biology with microfluidics, Curr. Opin. Chem. Biol. 14 (2010) 574–581.
[96] K. Choi, A.H. Ng, R. Fobel, A.R. Wheeler, Digital microfluidics, Annu. Rev. Anal. Chem. 5 (2012) 413–440.
[97] E. Samiei, M. Tabrizian, M. Hoorfar, A review of digital microfluidics as portable platforms for lab-on a-chip applications, Lab Chip 16 (2016) 2376–2396.
[98] F. Mugele, J.-C. Baret, Electrowetting: from basics to applications, J. Phys. Condens. Matter 17 (2005) R705–R774.
[99] S.H. Au, S.C. Shih, A.R. Wheeler, Integrated microbioreactor for culture and analysis of bacteria, algae and yeast, Biomed. Microdevices 13 (2011) 41–50.
[100] S.C.C. Shih, N.S. Mufti, M.D. Chamberlain, J. Kim, A.R. Wheeler, A droplet-based screen for wavelength-dependent lipid production in algae, Energy Environ. Sci. 7 (2014) 2366.
[101] J. Kim, G. Yoo, H. Lee, J. Lim, K. Kim, C.W. Kim, M.S. Park, J.W. Yang, Methods of downstream processing for the production of biodiesel from microalgae, Biotechnol. Adv. 31 (2013) 862–876.
[102] X. Cheng, M.D. Ooms, D. Sinton, Biomass-to-biocrude on a chip via hydrothermal liquefaction of algae, Lab Chip 16 (2016) 256–260.
[103] A.I. Barros, A.L. Gonçalves, M. Simões, J.C.M. Pires, Harvesting techniques applied to microalgae: a review, Renew. Sust. Energ. Rev. 41 (2015) 1489–1500.
[104] B.K. Honsvall, D. Altin, L.J. Robertson, Continuous harvesting of microalgae by new microfluidic technology for particle separation, Bioresour. Technol. 200 (2016) 360–365.
[105] L. Wang, D.S. Dandy, A microfluidic concentrator for cyanobacteria harvesting, Algal Res. 26 (2017) 481–489.
[106] S.B. Velasquez-Orta, T.P. Curtis, B.E. Logan, Energy from algae using microbial fuel cells, Biotechnol. Bioeng. 103 (2009) 1068–1076.
[107] D.P. Strik, H. Terlouw, H.V. Hamelers, C.J. Buisman, Renewable sustainable biocatalyzed electricity production in a photosynthetic algal microbial fuel cell (PAMFC), Appl. Microbiol. Biotechnol. 81 (2008) 659–668.
[108] S. Choi, Microscale microbial fuel cells: advances and challenges, Biosens. Bioelectron. 69 (2015) 8–25.
[109] M. Chiao, K.B. Lam, L. Lin, Micromachined microbial and photosynthetic fuel cells, J. Micromech. Microeng. 16 (2006) 2547–2553.

[110] S. Yoon, H. Lee, A. Fraiwan, C. Dai, S. Choi, A. Microsized Microbial, Solar cell: a demonstration of photosynthetic bacterial electrogenic capabilities, IEEE Nanotechnol. Mag. 8 (2014) 24–29.
[111] H. Lee, S. Choi, A micro-sized bio-solar cell for self-sustaining power generation, Lab Chip 15 (2015) 391–398.
[112] M. Shahparnia, M. Packirisamy, P. Juneau, V. Zazubovich, Micro photosynthetic power cell for power generation from photosynthesis of algae, Technology 3 (2015) 119–126.
[113] R. Brayner, A. Coute, J. Livage, C. Perrette, C. Sicard, Micro-algal biosensors, Anal. Bioanal. Chem. 401 (2011) 581–597.
[114] C. Siebman, O.D. Velev, V.I. Slaveykova, Two-dimensional algal collection and assembly by combining AC-dielectrophoresis with fluorescence detection for contaminant-induced oxidative stress sensing, Biosensors 5 (2015) 319–336.
[115] F. Lefevre, A. Chalifour, L. Yu, V. Chodavarapu, P. Juneau, R. Izquierdo, Algal fluorescence sensor integrated into a microfluidic chip for water pollutant detection, Lab Chip 12 (2012) 787–793.
[116] G.X. Zheng, Y.J. Li, L.L. Qi, X.M. Liu, H. Wang, S.P. Yu, Y.H. Wang, Marine phytoplankton motility sensor integrated into a microfluidic chip for high-throughput pollutant toxicity assessment, Mar. Pollut. Bull. 84 (2014) 147–154.
[117] D. Kürsten, J. Cao, A. Funfak, P. Müller, J.M. Köhler, Cultivation of *Chlorella vulgaris* in microfluid segments and microtoxicological determination of their sensitivity against $CuCl_2$ in the nanoliter range, Eng. Life Sci. 11 (2011) 580–587.
[118] H.-C. Lin, Y.-J. Liu, D.-J. Yao, Core–shell droplets for parallel DNA ligation of an ultra-micro volume using an EWOD microfluidic system, J. Assoc. Lab. Autom. 15 (2010) 210–215.
[119] S.C. Shih, G. Goyal, P.W. Kim, N. Koutsoubelis, J.D. Keasling, P.D. Adams, N.J. Hillson, A.K. Singh, A versatile microfluidic device for automating synthetic biology, ACS Synth. Biol. 4 (2015) 1151–1164.
[120] W. Longsine-Parker, H. Wang, C. Koo, J. Kim, B. Kim, A. Jayaraman, A. Han, Microfluidic electro-sonoporation: a multi-modal cell poration methodology through simultaneous application of electric field and ultrasonic wave, Lab Chip 13 (2013) 2144–2152.
[121] C.J. Ingham, A. Sprenkels, J. Bomer, D. Molenaar, A. van den Berg, J.E. van Hylckama Vlieg, W.M. de Vos, The micro-Petri dish, a million-well growth chip for the culture and high-throughput screening of microorganisms, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 18217–18222.
[122] J.Y. Kim, H.S. Kwak, Y.J. Sung, H.I. Choi, M.E. Hong, H.S. Lim, J.H. Lee, S.Y. Lee, S.J. Sim, Microfluidic high-throughput selection of microalgal strains with superior photosynthetic productivity using competitive phototaxis, Sci. Rep. 6 (2016) 21155.
[123] T.L. Weiss, H.J. Chun, S. Okada, S. Vitha, A. Holzenburg, J. Laane, T.P. Devarenne, Raman spectroscopy analysis of botryococcene hydrocarbons from the green microalga *Botryococcus braunii*, J. Biol. Chem. 285 (2010) 32458–32466.
[124] O. Samek, P. Zemánek, A. Jonáš, H.H. Telle, Characterization of oil-producing microalgae using Raman spectroscopy, Laser Phys. Lett. 8 (2011) 701–709.
[125] S.R. Fagerer, T. Schmid, A.J. Ibanez, M. Pabst, R. Steinhoff, K. Jefimovs, P.L. Urban, R. Zenobi, Analysis of single algal cells by combining mass spectrometry with Raman and fluorescence mapping, Analyst 138 (2013) 6732–6736.
[126] H. Wu, J.V. Volponi, A.E. Oliver, A.N. Parikh, B.A. Simmons, S. Singh, In vivo lipidomics using single-cell Raman spectroscopy, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 3809–3814.
[127] H.S. Kim, S.C. Waqued, D.T. Nodurft, T.P. Devarenne, V.V. Yakovlev, A. Han, Raman spectroscopy compatible PDMS droplet microfluidic culture and analysis platform towards on-chip lipidomics, Analyst 142 (2017) 1054–1060.
[128] P. Zhang, L. Ren, X. Zhang, Y. Shan, Y. Wang, Y. Ji, H. Yin, W.E. Huang, J. Xu, B. Ma, Raman-activated cell sorting based on dielectrophoretic single-cell trap and release, Anal. Chem. 87 (2015) 2282–2289.
[129] K.R. Johnson, W. Admassu, Mixed algae cultures for low cost environmental compensation in cultures grown for lipid production and wastewater remediation, J. Chem. Technol. Biotechnol. 88 (2013) 992–998.
[130] T.A. Bell, B. Prithiviraj, B.D. Wahlen, M.W. Fields, B.M. Peyton, A lipid-accumulating alga maintains growth in outdoor, alkaliphilic raceway pond with mixed microbial communities, Front. Microbiol. 6 (2015) 1480.