Algal Research 44 (2019) 101657

Contents lists available at ScienceDirect

Algal Research

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

A droplet-based gradient microfluidic to monitor and evaluate the growth of Chlorella vulgaris under different levels of nitrogen and temperatures

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ARTICLEINFO

Keywords:
Chlorella vulgaris
Gradient microfluidics
Traditional batch cultures
Temperature
Nitrate
Growth kinetics

ABSTRACT

Algae are potential sources for different industrial products. However, biofuel production from algae still needs to reach valuable economical approaches. Select algae and optimize culture conditions considered main challenges for biofuel production. Traditional batch cultures' techniques are time and labor consuming. PDMS-based Microfluidics is time-and labor-saving technique with high throughput single cell analysis. We present gradient-based microfluidics as tools for studying algal growth under different cultivation conditions. Applying our device provides the opportunity to generate droplets with different concentrations of medium nutrients' in 200 s which keeps reagent and time for preparing such solutions using conventional techniques. Chlorella vulgaris was encapsulated inside uniformly droplets with different NO_{3-}-N concentrations of 2.46, 6.69, 10.91, and 15.14 mM/L. separated devices were exposed to different temperatures of 20, 27, and 37 °C. The photosynthetic energy conversion for each cell per droplet was calculated. It was observed that the higher the NO_{3-} concentration, the higher the growth kinetics. According to the presented results, the optimum nitrate and temperature conditions were 15.14 mM/L and 37 °C. Our device is considered the third effort for applying a gradient chip in algal biotechnology field. It is unique and simple in use with high competence results.

1. Introduction

Cultivation and selection of suitable microalgae considered challenges for biofuel production. However, several factors affect the optimum algal growth and lipid accumulation such as availability and concentration of nutrients, CO_2, temperature, pH, and light conditions [1]. Optimum cultivation and production conditions are species-specific [2]. Traditional batch cultures' techniques to select algae and optimize conditions is time and labor consuming.

Microfluidics are fabricated systems in which 10^{-9} to 10^{-18} L of fluids can flow through connected microchannels with dimensions of 10 to 100 μm [3]. Most fabrication process is done using poly dimethylsiloxane (PDMS) which is a biocompatible, transparent and inexpensive elastomer polymer [3,4]. PDMS has many advantages like gas permeability to prevent solution evaporation [5], and appropriate for analyses of biological and chemical samples with faster prototyping and possibility of electro sensing [6]. PDMS-based Microfluidics is basically consisting of a valve of a planar glass substrate and two layers of PDMS [7].

In 2005, the first three microfluidics for cell analysis were fabricated including chemostats and single-cell encapsulation where optical density, dissolved oxygen, and pH were monitored continuously [8-10].

The three-known microfluidics' subsets are flow-based microfluidic large-scale integration (MLSI), droplet microfluidics, and digital microfluidics (DMF) based on electrowetting [5]. Droplet microfluidics provide chemical separation [11], the single-cell scale which gaining a unique insight cell metabolism [12], manipulate fluids to control chemical and physical conditions [4], nutrient replenishment [13], media swapping [14], and Paralyzed detection of multiple droplets at once [15].

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https://doi.org/10.1016/j.algal.2019.101657
Received 20 May 2019; Received in revised form 13 July 2019; Accepted 4 September 2019
Available online 15 November 2019

Microfluidics are time and labor saving techniques with high throughput single cell analysis where > 100 droplets can be manipulated at once in a space < 4 × 4 cm (this number can be differentiated according to the design) in which each droplet is considered a separate batch culture [16]; [5] whereas it is impossible to accomplish by the traditional methods [17]. Microfluidics are one of the microtools which afford obvious economic benefits over conventional cell analysis techniques. Microfluidics use fractions of reagents and materials and maximize the dimensionally of the data produced by combining multiple measures together [17]. Money, space and time saving the capacity of integration and parallelization of these devices allowed researches to easily conduct different experiments [6]. Dealing with small volumes reduces preparation and detection times controls molecular concentrations and interactions, saves needed reagents and chemical wastes [18].

C. vulgaris is a eukaryotic unicellular alga with size range 3-5 μm. It has great attention due to its importance as food supplement [19], antioxidant [13,20,21], bioremediation [22], biofertilizer agent [23], and energy feedstock [24].

This study aims to develop a new droplet-based gradient microfluidic as a tool to enable high-throughput screening systems towards algal biofuel. This screening device was tested to automatically generate different concentrations of nitrate and study their effect on Chlorella vulgaris growth kinetics. Cells were encapsulated in uniform droplets with different nitrate concentrations. A different device was applied for each temperature. Applying this device will save the reagents preparation time. The growth was monitored by chlorophyll intensity per droplet which then correlated to quantum yield.

2. Materials and methods

2.1. Microfluidic design

The microfluidic device consists of three main compartments: the gradient generator in the top, the culturing chamber in the bottom, and the mixing part in the middle (Fig. 1). Inside the gradient generator, streams of two different solutions were combined then split into droplets where the oil was flow. The second part of the device consists of oscillating curved channels to mix the ingredients inside the droplets perfectly and form homogeneous encapsulated conditions all over each droplet. The third section of the microdevice is called a culture chamber and has several parallel channels to maintain droplets in the desired conditions to monitor the real-time results of the experiment. The height and the width of the channel was consistent all over the design; 160 and 250 μm, correspondingly. Consequently, the solutions were diluted, and a series of concentrations are produced. Up to our study, when standard and nitrogen-free medium were introduced into the gradient generator, the resulted nitrogen concentrations (%N) were applied according to the following equation [25]:

$$\%N = (F1 + F2)/F1 * 100$$

where F1; the flow rate (μl/min) of channel with standard medium, and F2; the flow rate (μl/min) of channel with nitrogen-free medium.

2.2. Fabrication process

The fabrication process of microfluidic static arrays device started with performing the design using SOLIDWORKS® software. The design was initially patterned on a photoresist mask. Then, using the mask and UV light, the silicon wafer already spin-coated with SU-8 photoresist (SU-82075, Microchem Inc., MA) was UV-crosslinked with the conventional photolithography process [26] and then coated with the surfactant (tridecafluoro-1,1,2,2-tetrahydrooctyl) trichlorosilane (United Chemical Technologies, Inc., PA) to facilitate poly dimethylsiloxane (PDMS) release from the master molds. Afterward, the fabrication process ended with PDMS molding. The PDMS kits were mixed with a ratio of 1:10 (w/w), degassed, poured on the master, then kept at 65 °C for 24 h. After cooling, the PDMS layer was gently peeled off from the master. PDMS layer with a thickness of 4 mm was bonded with a glass slide of 50.8 × 9 × 76.2 mm^3 using oxygen plasma treatment. After assembly, PDMS devices were loaded with Aquapel (Pittsburgh Glass Works, LLC) to generate hydrophobic properties inside channels' surfaces to stabilize droplets.

2.3. Species preparation

Chlorella vulgaris was purified from collected freshwater samples at Port Said, Egypt. Purification was done on solidified BG11 medium with

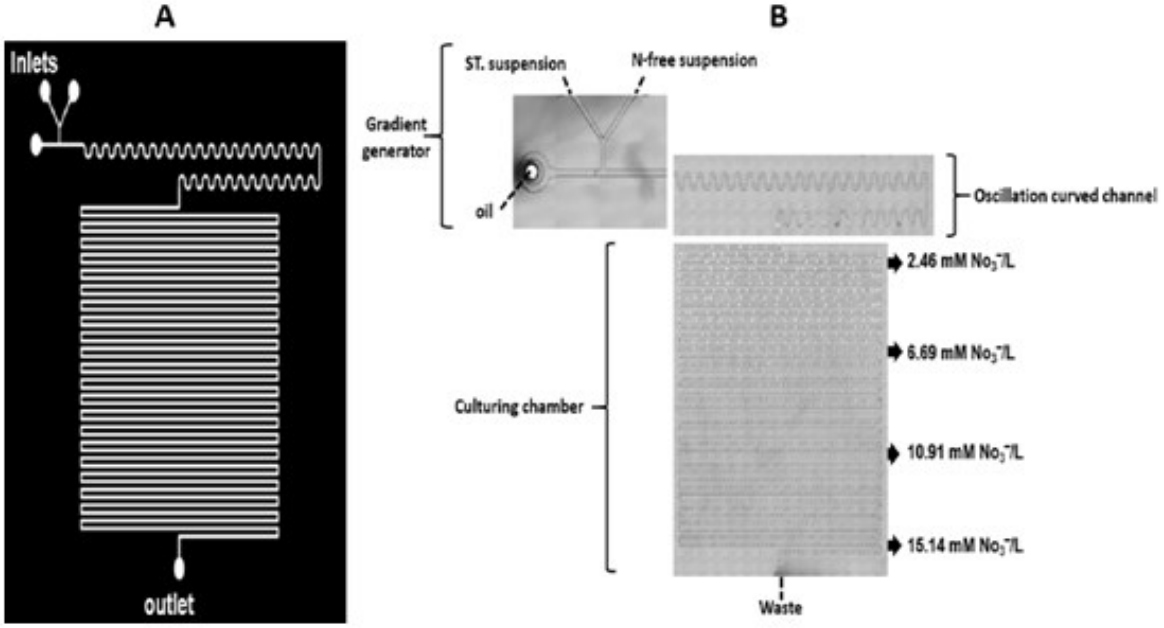

Fig. 1. The gradient-based microfluidic used for this experiment. A; The master's design of the microfluidic. B; gradient-based device parts showing the main areas; gradient generator where the two different solutions had been introduced, the oscillation curved channel where the ingredients were mixed inside the droplets perfectly to form homogeneous encapsulated conditions all over droplets, and the culturing chamber where the droplets were maintained for monitoring.

1.5% agar. The alga was examined using Olympus® microscopy. Samples were maintained by re-culturing in BG-11 medium. Cultures were kept at 23 ± 0.13 °C and continuous lighting of 80 μmol/m^2/s. All steps were done under aseptic conditions and tools were sterilized. Cells were inoculated in BG11 medium with replete and deplete nitrogen for four days before loading inside the device. The nitrogen-free medium was prepared by omitting the sodium nitrate.

To prepare inoculums for both cultures, certain volumes of maintained cultures were centrifuged and washed three times with the desired medium type. Samples were re-suspended in a new medium and diluted to 1151 ± 7.5 × 10^4 cells/mL.

2.4. Droplet generation

C. vulgaris diluted samples with replete and deplete N were loaded separately in 1 mL syringes and connected to Harvard pumps (70-4506, Holliston, MA). The Harvard pumps were linked with each other, with the Zeiss microscope, and with the LabVIEW program using USP cables. The carrier oil; 3M™ Fluorinert™ Liquid FC-40 oil (Maplewood, MN) with 2% 008 FluoroSurfactant, (Ran Biotechnologies, MA) was loaded to another 1 mL syringe and connected to a Chemyx pump (Fusion Touch 400, Chemyx Inc., US). Suspensions and the carrier oil were linked to different channel's inlets using 0.5 mm ID Tygon tubing (Cole-Parmer, Vernon Hills, IL). The medium suspensions' syringes were connected to the two arms of the Y-shaped part while the carrier oil connected to the perpendicular channel. N-replete suspension, N-deplete suspension, and oil were loaded with different flow rates of 240, 40, and 600, respectively. The LabVIEW program was set up to automatically change the flow rate every 10 s with a rate of 10 μL/h. As a result, the flow rate of both medium suspensions was gradually changed and ended with 40 and 240 μL/h for N-replete and N-deplete suspensions. In a total of 200 s, 660 droplets with N gradients were generated with a rate of 3 droplets/s. Afterward, the pumps were turned off and the culturing chamber was maintained as batch culture conditions. Five technical replicated were considered for each condition. A controlled device was loaded with Fluorescein-based dye (Thermo Fisher Scientific, CO., US) to ensure the automated gradual concentrations of the solutions (Fig. 2).

In a designed mixing on-chip platform growth of C. vulgaris observed inside 240 μm droplets with different NO_3^- conditions of 2.46, 6.69,10.91, and 15.14 mM $NaNO_3$/L and maintained in the culturing chamber with no merging issues. After loading each device, devices were presented to different temperatures of 20, 27, and 37 °C, at 80 μmol/m^2/s. Brightfield and fluorescent images were taken for the whole device using a digital camera connected to a Zeiss microscope.

2.5. Conventional batch cultures versus droplet-based devices

The growth of C. vulgaris was examined inside the two different systems of cultivation. The inoculate of 10^6 cells/ mL was added to 100 mL of sterilized BG11 media to represent the conventional batch techniques. Whereas to represent the on-chip technology, cells were encapsulated inside 240-μm droplets and maintained in a rail-road device. All cultures were incubated at 23 ± 0.13 °C and continuous lighting of 80 μmol/m^2/s.

The 240-μm droplets were generated inside a T-junction droplet generator Then transferred through connected tubing to the rail-road device. The droplet generator consisted of two perpendicular channels: a 200-μm wide channel for the carrier oil and a 160-μm wide channel for the cell suspension. The flow rates of the carrier oil and the cell suspension were 400 and 50 μL/h, correspondingly. The rail-road device was consisting of rail-road structure channel with width of 160-μm (Fig. 3).

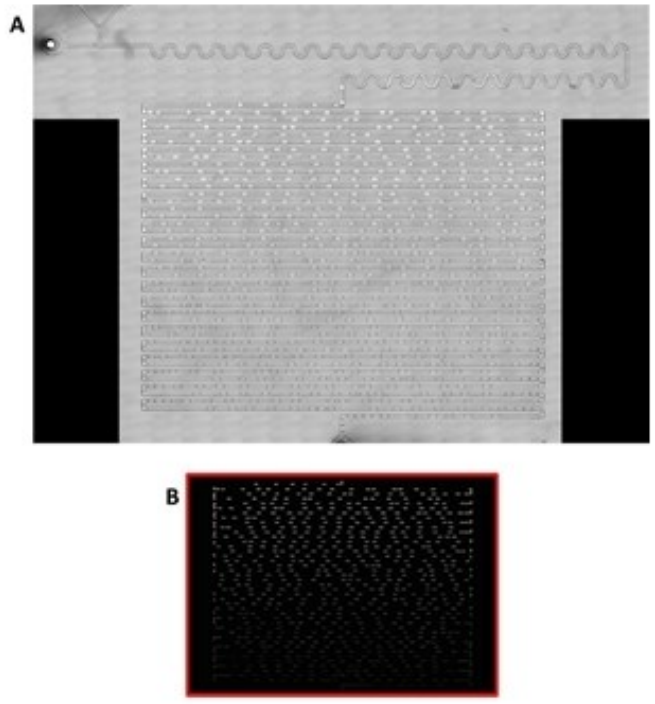

Fig. 2. A control device loaded with Fluorescein-based dye. A; The brightfield image of the whole microfluidic device. B; fluorescent image of the whole microfluidic device.

2.6. Analyze algal growth inside droplets

Tracking the same droplets stored in the culture chamber allowed time-course growth analysis, where the growth of cells was characterized by chlorophyll autofluorescence intensity per unit area. Fluorescent and brightfield images were captured for whole devices and each droplet/ device using a Zeiss AxioObserver Z1 microscope (Carl Zeiss MicroImaging, LLC) equipped with a digital camera (Orca Flash 2.8, CMOS, Hamamatsu, Japan) and a filter set (excitation: 460-500 nm, emission > 600 nm). Images were analyzed using ImageJ [27]. Specific growth rates were calculated using Eq. (A.1) according to [28]. The results were presented out of 5 replicates and the standard deviation was considered.

$$\mu\,(d^{-1}) = \ln(N_2 - N_1)/T_2 - T_1 \quad \text{(A.1)}$$

where, N_2 and N_1 are the cell number at the times t_2 and t_1, respectively.

Then the difference between Initial and time-course chlorophyll autofluorescence (F_0 and F_t) was variable. That difference was normalized to F_t to represent the initial yield of quantum photosynthesis (F_m) according to the modified equation of Erickson and Jimenez [29],

$$F_m(\%) = ((F_0 - F_t)/F_0) \times 100 \quad \text{(B.1)}$$

2.7. Statistical analysis

Differences between resulted groups were compared using One-way ANOVA [30]. The standard deviation [31] for results out of 5 replicates were considered.

3. Results and discussion

3.1. Chlorella vulgaris inside gradient-based microfluidic chip

Testing the fabricated mixing device provided the opportunity to examine cell growth under different nutrient concentrations at the same

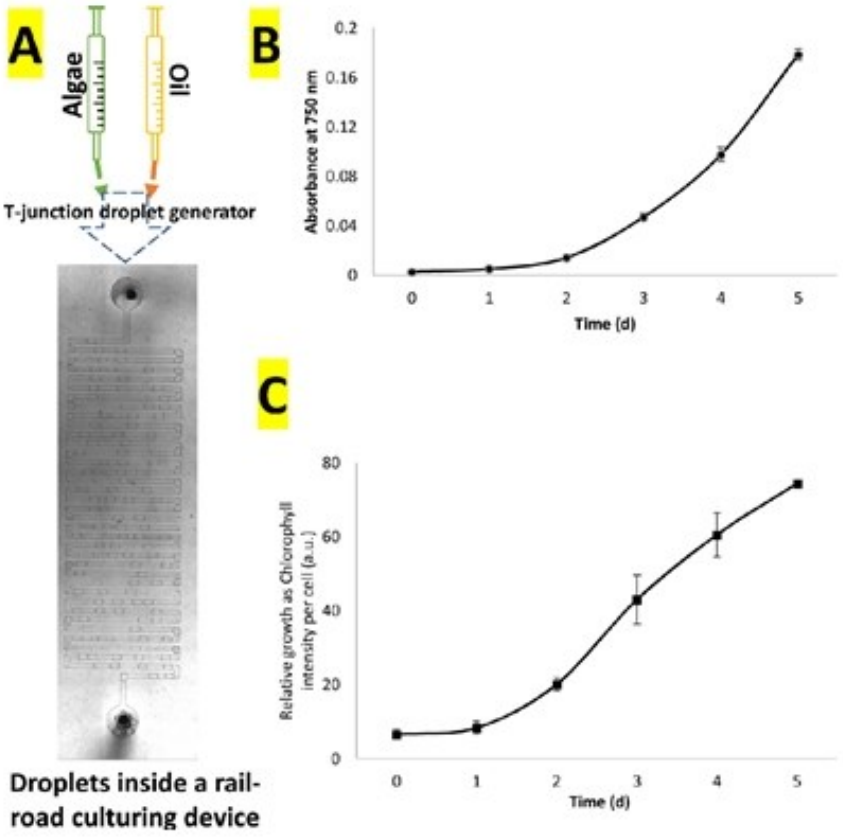

Fig. 3. Growth performance for Chlorella vulgaris cultivated in BG11 medium at 80 μmol/m^2/s and 20 °C. A: shows the droplet generation process and the culturing device where the droplets were maintained for further monitoring and analysis. B: the growth performance for Chlorella vulgaris cultivated in 100 mL BG11 medium at 80 μmol/m^2/s and 20 °C (n = 4). C: the growth performance for Chlorella vulgaris cultivated inside 240-μm droplets with BG11 medium at 80 μmol/m^2/s and 20 °C (n = 10).

device. Each droplet can be considered a separate culturing unit. Cells were trapped independently inside each droplet. The flow of each cell suspension was considered until all droplets were generated to confirm the mixing chances. The hydrostatic pressure used to generate droplets displayed a linear relationship between cell number, chlorophyll autofluorescence and occupied area per droplet.

The compared results of the maximum growth rates (2.4 ± 0.2 d^{-1}) for C. vulgaris cultured inside the batches and the microfluidics were consistence. Referring to our previous work [32], the comparison between the traditional batch cultures and the droplet-based devices to evaluate the growth of C. vulgaris under different irradiance and nitrate conditions, indicting the uniformity. As temperature and nitrogen affected cell growth and behavior; a mixing platform was developed to evaluate that effect because there are not many papers reported about algal growth under combined stress factors inside chips. C. vulgaris encapsulated for 3.5 days were maintained in BG11 medium with different nitrate concentrations—2.46, 6.69, 10.91, and 15.14 mM/L and different temperatures—20, 27, and 37 °C, at 80 μmol/m^2/s. The growth rate was calculated for each condition between 24 and 72 h (Table 1).

In 1931s, the chlorophyll autofluorescence was induced using pulse-amplitude-modulating fluorometry then correlated to the carbon dioxide assimilation [90]. It is based on the energy of the absorbed photon by the chlorophyll molecule. That energy will be either used to drive the electron transfer in photosystem II with opened reaction center or to excite the fluorescence under low light intensities. With increasing the light intensity, the reaction center of photosystem II will close, the electron transfer will cease, and the fluorescence will rise. Therefore, the observation of Chlorophyll autofluorescence's induction with its relationship to reactions' efficiency inside photosystem II provides a rapid and non-destructive method to quantify the quantum yield of photosynthesis [29].

Growth of C. vulgaris was tested inside different designs of chips under different cultivation conditions [33,34]. Kwak and coworkers

Table 1
Maximum growth rate for C. vulgaris suspended in BG11 medium with different nitrate concentrations of 2.46, 6.69, 10.91, and 15.14 mM $NaNO_3$/ L inside 240-μm droplets at 80 μmol/m^2/s and different temperatures of 20, 27, and 37 °C. Standard deviation was presented by positive and negative directions. (n = 5).

Temperature (°C)	Nitrogen condition (mM $NaNO_3$/L)	Growth rate (/h)
20	2.46	0.004
	6.69	0.007 ± 0.002
	10.91	0.03 ± 0.1
	15.14	0.04 ± 0.1
27	2.46	0.03 ± 0.04
	6.69	0.06 ± 0.09
	10.91	0.04 ± 0.2
	15.14	0.04 ± 0.02
37	2.46	0.04 ± 0.06
	6.69	0.09
	10.91	0.18
	15.14	0.18 ± 0.02

[35] screened the growth and lipids inside C. vulgaris under N-deplete TAP-C medium at 30 °C as high temperature and a high salt concentration of 200 mM NaCl. The growth rate of C. vulgaris encapsulated inside droplets in immobilized arrays was 0.55-1.52/d under different light cycles [36]. The maximum cell density and doubling time of C. vulgaris cultured in a simple droplet-based microfluidic system were 4.5 × 10^8 cell/mL and 12/h, respectively [37]. In case of testing C. vulgaris under different CO_2 concentrations of 1%, 2.5%, 5%, and 7.5%, and different light intensities of 35, 55, 100, 150, and 200 μmol/m^2/s in a droplet-based photobioreactor, the doubling time (/h) differed by conditions from 7.4 ± 1.9 to 9.2 ± 0.18/h [38]. Again, the growth kinetics of C. vulgaris maintained in BG11 medium inside a biochip using an electrostatic mechanism in controlled conditions was monitored [39].

Previous efforts regarding gradient-based microfluidics with

different designs had been published. For promoting rapid growth and high lipid content inside Chlamydomonas reinhardtii, cells were maintained under different nitrogen, phosphorus, and sodium acetate concentrations. The device has consisted of two PDMS layers; the top layer was included 40-unit C-shaped growth chambers and 8 microchannels for generating sodium acetate gradients with 5 chambers for repeatability. Whereas the top PDMS layer was included two inlet holes and one outlet hole. The dimensions of microchannels were 200 μm width, 30 μm height, and 260 μL volume. Cell growth and lipid expression were quantitively explored. The results were validated to flask-scale experiments [40]. Five marine species; Platymonas subcordiformis, P. helgolandica, Phaeodactylum tricornutum, Porphyridium cruentum, and Chlorella sp. were copper-stressed on a gradient-based microfluidic with a power-free valving system. The device has consisted of three layers; a bottom gradient generator layer, a semi-porous membrane, and a top diffusible culturing layer of 16 chambers. Solutions were dynamically mixed to generate eight dilutions which diffused into the chambers through the semi membrane layer. Cell viability under copper stressed conditions was estimated by chlorophyll autofluorescence and esterase activity [41]. According to the mentioned efforts our gradient chip can be considered unique and simple in use with high competence results.

3.2. Temperature effects on cell growth kinetics

Moderate temperature is positively impacted photosynthetic efficiency, cell division, and enzymatic activity inside cells [42]. Rubisco activity and Calvin cycle is affected by temperature [43]. Presenting calculated results verified that 37 °C is the optimum temperature for growing C. vulgaris under different nitrogen conditions (Fig. 4). our results are consistent with [54] where the activity of Rubisco was increased inside C. vulgaris were grown in high CO_2 concentration at 20-38 °C. Because the growth can be enhanced by rising temperature according to the Arrhenius low [42], the temperature is a species-specific character [44], and some Chlorella species were identified as thermophilic species which they can grow in a range of 38-42 °C [45], we concede that our tested species that was isolated from a hot country is a thermophilic one.

Whereas, cells at 27, and 37 °C did not follow the principle of the linear proportion between nitrate and cell growth. However, the growth of normalized data charts at 37 °C showed higher growth than 27 °C, the calculated growth rates indicated the opposite; which is accepted with the literature [42,46]; the higher the temperature the lower the growth rate.

Serra-Maia and coworkers [47] had verified that C. vulgaris viability was increased from 20 °C to 28 °C and decreased by increasing temperature over 28 °C. According to their investigation, the optimal growth rate of C. vulgaris was 23 °C and the growth was inhibited at 28-30 °C. Kessler had indicated that the range of 26-30 °C is optimal for growing 14 Chlorella species [48]. Also, 25-30 °C was the temperature range preferred for C. vulgaris [46]. Although, Chlorella vulgaris showed higher growth rates at high temperatures 30-35 °C [49,50]. C. vulgaris were grown with maximum growth rate 1.24 ± 0.05/d at a temperature range 20-35 °C [51]. C. vulgaris growth rates were decreased from 0.14 to −0.01 d^{-1} in response to temperature range 25-38 °C [52]. As reported by Chinnasamy and coworkers [53], the growth of Chlorella vulgaris was increased at 25-30 °C and the optimal temperature was 30 °C. High temperatures (> 35 °C) were noticed as heat stress for Chlorella vulgaris [54,55]. The growth rate of C. vulgaris were cultivated under M-8 and urea growth media at 25, 30, and 35 °C was ranged from 0.01 and 0.02 h^{-1} and were not significantly different, respectively [46]. In other experiments, the maximum biomass productivity of C. vulgaris was reported at 30 °C and the growth become lower with increasing temperature to 35 °C [56,57].

The temperature has a great impact on microalgae growth. Microalgae can grow and behave differently within a temperature range of 15-35 °C [58]. Temperature effects on gases (CO_2/O_2) solubility in the medium [59], cell membrane fluidity system [60], enzymatic reactions and protein degradation inside cells leading to cell mortality in case of high temperatures [47]. According to species, cell mortality can happen at a range of temperatures between 20 and 45 °C [46]. Therefore, it is a key important to unravel the effect of temperature on cell growth and productivity of microalgae. The logic of the Arrhenius low states that at moderate temperatures, growth will be enhanced by raising temperatures [42] until reaching optimum temperature then the growth will be irreversibly correlated with high temperature [46]. Low temperatures lead to limit cell growth by reducing the fluidity of the cell membranes and increasing the proportion of unsaturated fatty acids [61]. According to Sorokin and Myers, [44] and Sorokin [62], the temperature is a species-specific character. Two types of Chlorella; thermophilic species are documented. An upper limit temperature species as C. sorokiniana which grow in a range of 38-42 °C [45] and a low-temperature species which usually grow around 25 °C.

3.3. Nitrate effects on cell growth kinetics

Nitrogen is available for phytoplankton in different oxidation cases—NO_3, NO_2, NH_4, and NH_3 [63]. Nitrate assimilation by microalgae is affected by different factors e.g., nitrate reductase activity, CO_2 availability, pH, temperature, and light [64-66]. Usually, researchers focused on nitrogen limitation tests to consider lipid production inside C. vulgaris where lipids induced by limiting nitrogen [67,68]. Nitrogen source affecting medium pH as nitrate fluctuated medium pH to 7.2 and ammonium reduced medium pH to 3 when C. vulgaris cultivated under mixotrophic conditions [69].

C. vulgaris is characterized by 4 different metabolism pathways; autotrophic, heterotrophic, mixotrophic, and photoheterotrophic [70] (Fig. 5). The autotrophic pathway is represented by photosynthesis where inorganic carbon converted into organic energy forms in the presence of light [71]. That is opposite to heterotrophic pathway which needs organic carbon to feed in dark [72]. Where the mixotrophic pathway means cells can grow as possible as food sources available without been restricted with autotrophic or heterotrophic metabolism [73]. The photoheterotrophic pathway takes place in the presence of light and organic carbon [71]. Heterotrophic metabolism has resulted in a higher growth rate than autotrophic one [74]. Different studies indicated mixotrophic metabolism as the best solution to obtain maximum biomass and lipid productivities from C. vulgaris [75]. Temperature drastically affects the growth of cells under autotrophic and heterotrophic pathways [76,77].

Generally, it was obvious that lowering available nitrogen concentration will increase lipid content and decrease the growth rate [70,78,79]. This argument is consistent with our results. The growth was increased by increasing nitrate concentration in all temperature conditions. Nitrate had a high observed impact on C. vulgaris cells. At all temperatures, growth kinetics were directly proportioned to nitrate concentration. In addition, we agreed with [80], the higher the nitrate concentration from 2 to 6 mM NO_3^-/L, the higher the growth rate. And the higher the nitrate level from 29 to 97 mM NO_3^-/L, the lower the growth were recorded. C. vulgaris observed to grow at high nitrate concentration as 97 mM NO_3^-/L with no toxic reported effect and level of nitrate uptake increased with increasing medium nitrate concentration [80].

Our results were inconsistent with Converti and coworkers [52] who had reported no significant decrease in C. vulgaris growth rate with decreasing nitrate concentration from full to half and quarter amounts. Besides, higher biomass and lipid productivities were recorded together in case of cultivated Chlorella vulgaris under low nitrogen mode [75].

Chlorella preferred ammonium as a nitrogen source than nitrate whereas nitrate reductase level was decreased by ammonium addition while ammonium assimilation did not affect nitrate uptake [81]. Nitrate was investigated as the best nitrogen source to cultivate C. vulgaris in mixotrophic conditions with a specific growth rate of 0.87/d [69].

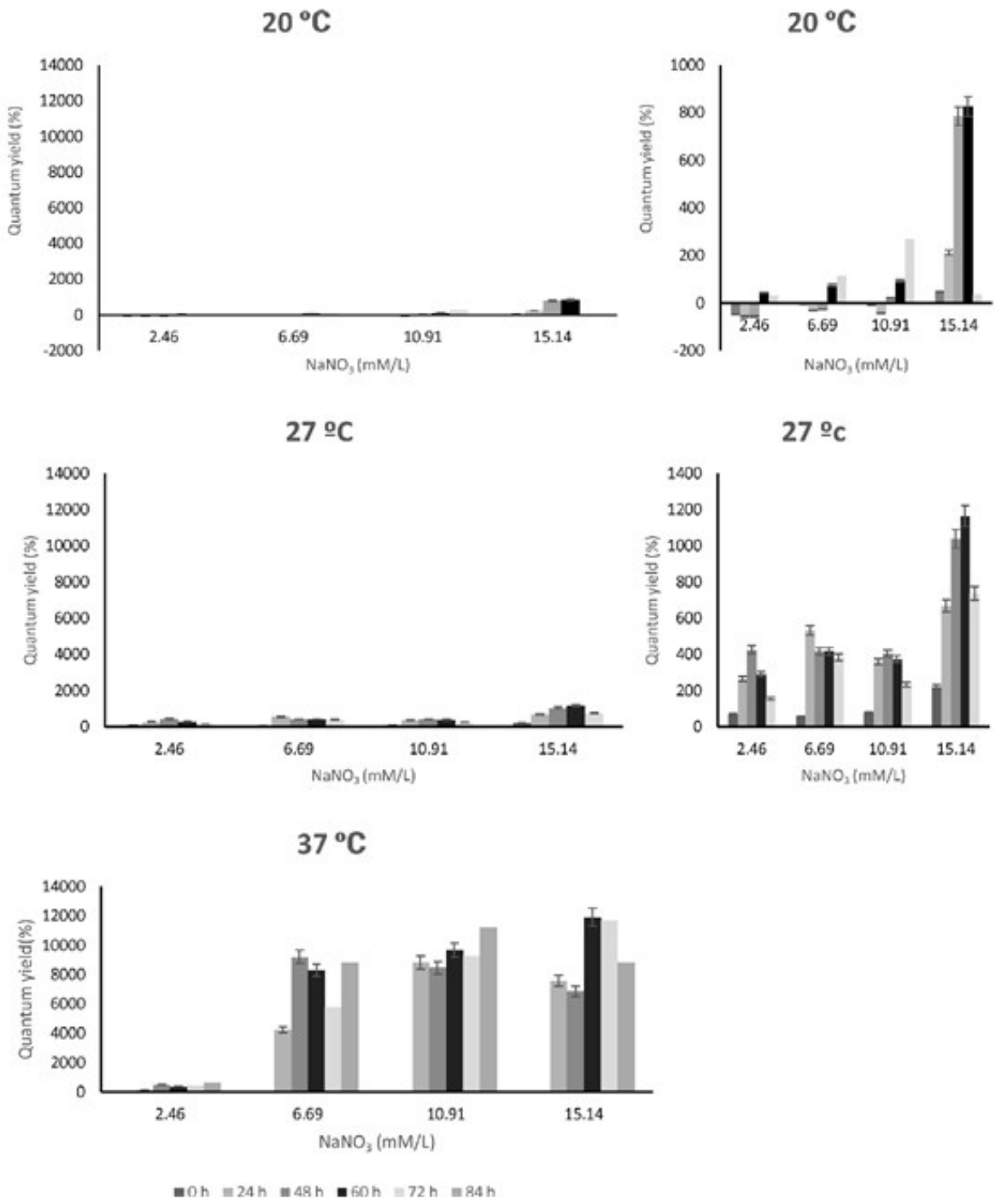

Fig. 4. Quantum yield (%) for C. vulgaris suspended in BG11 medium with different nitrate concentrations of 2.46, 6.69, 10.91, and 15.14 mM $NaNO_3$/L inside 240-μm droplets at 80 μmol/m^2/s and different temperatures of 20, 27, and 37 °C. Standard deviation was identified by positive and negative directions. (n = 5).

Nitrate assimilation depends on the activity of nitrate reductase (NR). NR is simulated by increasing nitrate concentration in the medium. Ammonia and nitrite accumulate, and growth inhibited as results of inducing NR in high nitrate concentrations. Further accumulation of ammonia leads to inhibition of NR [65,82-86] (Fig. 6).

Although the United States Environmental Protection Agency (EPA) and World Health Organization (WHO) were accepted the amount of lower than 10 mg/L NO_3-N in drinking water [87], the nitrate concentration in industrial wastewater always > 200 mg/L NO_3-N [88]. C. vulgaris is one of the most important species which used in bioremediation of water that has high concentrations of nitrate. C. vulgaris exhibited maximum growth and nitrate removal rates of 3.6 g/L and 16.4 mg/L/h at BG11 with 2400 mg NO_3–/L [89].

4. Conclusion

Chlorella vulgaris is a highly regarded microalgae for bioproduction as its favorable biological characteristics. A new gradient-based microdevice was fabricated to understand the adaptation of Chlorella species at different temperatures and nitrogen conditions and carried out by assessing growth kinetics. The device was perfect for generating different concentrations of fluids automatically and testing at the same time. The growth of cells was maximized at the optimal temperature. Cells were grown healthy with higher nitrate concentration. However,

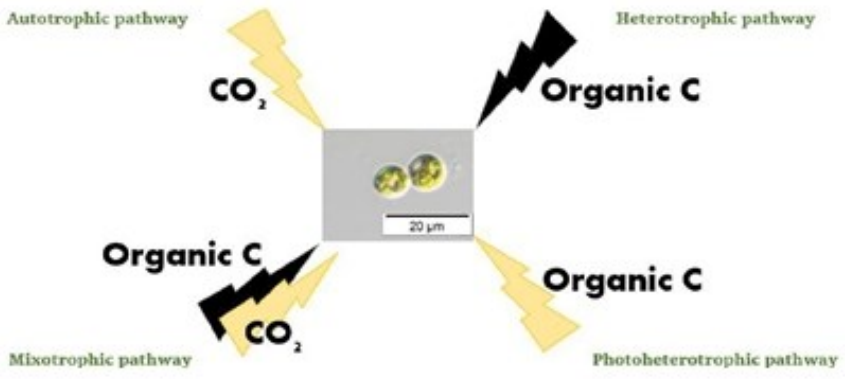

Fig. 5. Different metabolism pathways for Chlorella vulgaris.

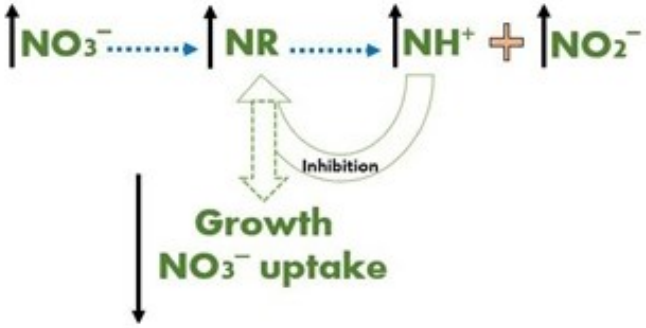

Fig. 6. Assimilation of nitrate uptake inside algal cells. Where, NO_{3-}; nitrate, NR; nitrate reductase, NH^{+}; ammonium, NO_{3-}; nitrite.

combining temperature and nitrate factors had a high impact on cell growth different than growth law in some cases. Finally, our results confirm feasibility of microfluidics for screening, and time- and labor-saving technique to study algae growth to encourage on-chip technology for microbial biotechnology. There are few papers about serving on-chip in microbial biotechnology fields especially, for gradient mechanism; it is only two previous work applied in algal biotechnology field.

Author contributions

All authors had substantial contributions to the conception and design of the study, and final approval of the version to be submitted. H.M.S., M.G.S.; Conceptualization and original draft preparation, D.N.; genetic identification, A.S.; design the microfluidic chip, M.S.Z., L.M., M.B., revising the manuscript critically for important intellectual content, N.S.D. and M.G.S.; analysis and interpretation of the data, and submission process.

Funding source

This work was supported by the Egyptian Cultural affairs as a part of a collaborated research between Port Said University, Port Said, Ministry of higher education, Cairo, Egypt and Texas A&M University, College Station, Texas, United States.

Acknowledgments

We are grateful to professor Arum Han for facilitating the on-chip work. Also, we acknowledge the helpful discussion with Dr. Schonna Manning.

Declaration of competing interest

The authors declare No conflicts, informed consent, human or animal rights applicable.

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