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# Towards optimal inorganic carbon delivery to microalgae culture

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ABSTRACT

Microalgae offer the potential to sequestrate $CO_2$ and reduce the world's dependency on fossil fuels, thereby mitigating the greenhouse gas effect, which has resulted in climate change over time. Carbon is one of the critical nutrients for microalgae growth and product formation; the choice of carbon and pH control techniques has been identified as the key to improving the sustainability of microalgae cultivation. However, supplying $CO_2$ in the gas phase is not cost-effective and could be counterproductive as a high percentage could be lost back to the atmosphere due to low solubility in the medium. Additionally, using conventional pH control techniques such as acid, base, and buffers is expensive at scale. This study assessed freshwater *Chlorella sorokiniana* cultivation using $NaHCO_3$ solely as the inorganic carbon and for pH modulation. The investigation focuses on the effect of bicarbonate feed rates and culture starting pH in the acidic, neutral, and basic regimes on the pH modulation, biomass accumulation, and product yield in a fed-batch system. The final pH increased beyond the upper limit threshold for each pH regime, with the highest level of control achieved in the basic regime (7.50–8.48 ± 0.11). The maximum biomass accumulation reached 2.49 g/L and a doubling time of 49 h. Evaluation of the pigment showed a higher chlorophyll *a* content, 25.85 mg/L, similar to a $CO_2$-fed culture and higher than the bicarbonate culture reported. The lipid contents showed a fatty acid composition suitable for biodiesel production with a higher percentage of saturated fatty acids (SFAs) (62.93 %–90.86 %) across all conditions. The sole use of bicarbonate for simultaneous inorganic carbon supply and pH control can be a cost-effective measure without compromising biomass and lipid productivities.

## 1. Introduction

The effect of carbon emissions from fossil fuel combustion on the climate has been well reported. To mitigate its effect, carbon capture has been proffered as one of the solutions [1]. Microalgae are one of the organisms that can sequester $CO_2$ through photosynthesis to produce carbohydrates which may be used to synthesize different biomolecules such as lipids and proteins. Compared to plants, microalgae can sequester up to fifty times more $CO_2$ [2], with potential to produce low and high-value fuels and chemicals which can be used to offset other cost of production, such as nutrients and light.

Carbon is one of the essential elements required by microalgae for growth. Inorganic carbon delivery methods depend on the microalgae species and the mode of cultivation. The supply of $CO_2$ to microalgae culture promotes biomass production. Ferreira et al. [3] reported a 130 % increase in *Chlorella vulgaris* biomass formation when 5 % $CO_2$ was used compared to atmospheric air. The increased growth rate was attributed to the improved photosynthetic activity due to the up-regulation of the protein activities related to the tricarboxylic acid (TCA) cycle [4].

Compared to compressed $CO_2$, bicarbonate is the most commercially viable means of supplying inorganic carbon to large-scale raceway ponds, and PBRs [5]. Given the relative low-cost and higher utilisation efficiency of $NaHCO_3$, it has been researched previously as the source of inorganic carbon for microalgae cultivation. Optimal bicarbonate concentration can benefit microalgae biomass and lipid accumulation during culture. $NaHCO_3$ concentration of 0.6 g/L was reported to increase biomass density of *Scenedesmus obliquus* by 23 % compared to atmospheric air and obtained 20.91 % total lipid content [6]. Zhang et al. [7] reported increased RuBisCO and carboic anhydrase (CA) activity when 0.5 g/L $NaHCO_3$ was used to cultivate *Chlorella pyrenoidosa*. This led to a significant increase in the photosynthetic pigment of the microalgal cell.

However, the addition of bicarbonate raises the pH of the medium to alkaline, a suitable pH range for marine microalgae [8]. For freshwater

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microalgae, the increase in pH is usually controlled by either injection of $CO_2$, acid, or the use of buffer. Both approaches are not economically viable for large-scale cultivation, due to the cost and reduced $CO_2$ utilisation efficiency [9].

The inorganic carbon uptake potential of microalgae varies, and it is heavily dependent on the pH of the external medium. At lower pH, the inorganic carbon is in the form of free $CO_2$ and some microalgae cells rely on the diffusion of $CO_2$ as they are unable to uptake the $HCO_3^-$ and $CO_{2aq}$ [10]. Selective active uptake of $HCO_3^-$ are also restricted to neutral pH in some microalgae, with active $CO_{2aq}$ uptake lacking. The ability of some microalgae to optimally uptake $HCO_3^-$ than $CO_{2aq}$ at slightly alkaline pH of 8.2 has also been reported [11]. The ability of the cells to actively uptake both carbon forms at pH 8.2 was due to the reduction in the amount of dissolved $CO_2$ in the medium. Moazami-Goudarzi and Colman [10] reported a fifty times increase in the dissolved $CO_2$ when the pH was reduced from 8.2 to 7.5, which resulted in repression of both $HCO_3^-$ and $CO_2$ uptake [12].

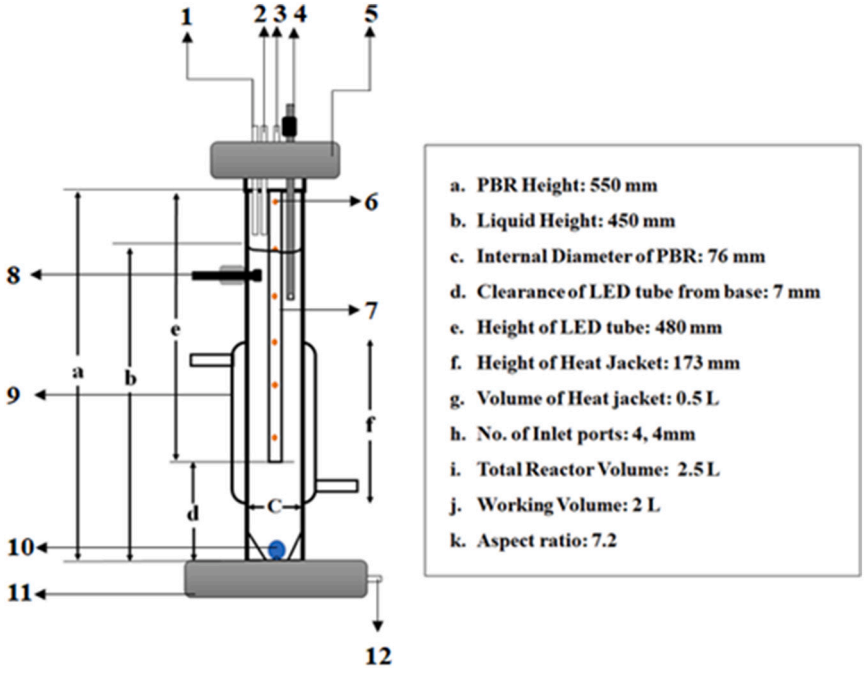
The effect of nitrogen sources on the pH modulation of inorganic carbon transport has been reported [13]. The species of nitrogen used in the culture media can affect the pH of the microalgae culture. The utilisation of $NH_3$-N leads to the release of protons which acidifies the culture medium, while the use of $NO_3$-N leads to $OH^-$ release, which results in medium alkalinisation. The pH drift is usually mitigated by including a chemical buffer in the media or controlling it with acid/base injection. The use of ammonia as a nitrogen source is preferred to other sources because its uptake requires less energy for metabolism [14,15].

With the aim of sustainably growing freshwater microalgae without the conventional pH control methods, $NH_3$-N will be primarily used as the nitrogen source, and the corresponding pH reduction can be modulated with pulse or intermittent bicarbonate feeding. Hence, the simultaneous control of the pH and supply of inorganic carbon solely in the form of bicarbonate to the freshwater microalgae, *Chlorella sorokiniana*, will be investigated. The impact of the different growth conditions on product (pigments, lipid, fatty acid) formation will also be investigated.

## 2. Materials and methods

### 2.1. Chemicals and microorganisms

The microalgae used in this study is the freshwater green *Chlorella sorokiniana* and was selected due to its robustness to wider ranges of process conditions and extensive characterization in the literature. The *C. sorokiniana* (UTEX 1230) strain was kindly provided by Professor Saul Purton from the Institute of Structural and Molecular Biology, University College London, London. The microalgae strain was stabbed on an agar slant and streaked on a plate prepared from Tris minimal media for long term (10 weeks) maintenance at 4 °C. For short term maintenance, 10 mL of the cells (inoculum) were inoculated in 90 mL of Tris minimal and incubated at ambient light, 28 °C, and agitated at 100 rpm. The cells




| Label | PBR Parts |
|---|---|
| 1, 2, 3 | Inlet ports |
| 4 | pH probe |
| 5 | Head plate |
| 6 | Led light |
| 7 | Internal led holder |
| 8 | Dissolved oxygen probe |
| 9 | Heat jacket |
| 10 | Air sparger |
| 11 | Air Inlet |
| 12 | Base |

**Fig. 1.** Laboratory-scaled photobioreactor design specifications.

were sub-cultured every two weeks to maintain viability [16].

### 2.2. Laboratory-scale photobioreactor (LPBR) set-up

Six 2.5 L LPBRs were designed, built, and used for this study. Fig. 1 shows the LPBR and its design specification. The cylindrical body of each reactor is 550 mm long, while the inlet tube that internally holds the LED light strip (Yunbo 2835-NP, China) is 480 mm long. The choice of internal lighting is for effective light dispersal in the reactor, to reduce the effect of dark shading with increased culture density [17], and to allow the external surface for temperature maintenance rather than support lighting. As illustrated in Fig. 1, a heat jacket was integrated for temperature control in the PBR; the surface area of the PBR in contact with the circulating water is 0.04 $m^2$. The total volume of the heat jacket is 0.5 L. 4 mm inlet ports were included on the head plate to pump in solutions, inoculate, sample, and probe attachment. The PBR was designed using polycarbonate due to its high light transmittance and heat resistance (softening temperature of 150 °C) properties as obtained from the datasheet [14].

A flexible and open-source Arduino microcontroller was used to monitor and control operating conditions in the LPBR The Arduino microcontroller is a versatile and inexpensive method for data acquisition and control [18]. The hardware architecture consists of the Arduino Mega (2560 Rev 3), pH, dissolved oxygen (DO), and K-type thermocouple sensors (397-1264, RS Components, UK). Two 12 V DC pumps (Yosoo pump, Amazon, China) were integrated for pumping solutions into the PBR, while the real-time data were acquired in CSV file format through the integrated micro-SD reader.

#### 2.2.1. Algae cultivation and maintenance

The seed culture was prepared from the stock microalgae culture by inoculating 180 mL of Tris Acetate Phosphate (TAP) medium in a 500 mL Erlenmeyer flask (at 10 % v/v) and incubated in an orbital shaker (MaxQ 4000, Thermoscientific, UK) at 28 °C and 140 rpm for three days.

#### 2.2.2. Cultivation in the laboratory-scale photobioreactor

1.8 L of autoclaved media and 0.2 L (10 % v/v) microalgae inoculum was aseptically pumped into the sterilised PBR. The autoclaved bicarbonate feed solution was connected to the PBR inlet tube on the headplate. The temperature was set to 32 °C, and mixing was provided by an air compressor (Pond Piston Air pump, ACQ-003, China) connected to a filter and regulated with a rotameter (LZM-6T, China). Periodic sampling was done by pumping out the desired sample volume through the outlet tube, which has a tube clip and filter at its end to protect the culture from contamination.

### 2.3. Experimental design

In this study, batch and fed-batch experiment were implemented. For the fed-batch system, bicarbonate was fed into the culture at varying intervals. The volume fed ($V_f$) was calculated based on the feeding intervals (h), desired final concentration of bicarbonate, and the length of the experiment. The pH regime was based on the equilibrium pH carbon species of acid (5.0–6.3), neutral (6.3–7.5), and basic (7.5–8.3) region. 11 g/L of 400 mL of sodium bicarbonate was used, giving 2.2 g/L final bicarbonate solution in the 2 L culture. The volume fed into the culture was calculated based on ten days of feeding which was based on the average days reported for cultivating freshwater *Chlorella* sp.

$$V_f(mL) = \frac{40\ mL}{24/F.I} \tag{1}$$

where $V_f$ (mL) is the volume fed per time into the culture, $F.I$ the Feeding Interval in h, and 40 mL is the total bicarbonate fed into the culture per day, totalling 400 mL in ten days. Table I summarises the experimental design. The effect of the feeding on pH change, growth kinetics, and product formation was analysed. The experiments were carried out under uniform light intensity of ~160 μmol $m^2$/s. All experiments were run in duplicates.

**Table I**
The fed-batch experimental design indicating the feeding intervals, volume, and pH regime.

| Feeding interval (h) | Feeding frequency/day | Volume (mL) | pH regime |
|---|---|---|---|
| 2 | 12 | 3.33 | 5.0 |
| 4 | 6 | 6.66 | 6.3 |
| 6 | 4 | 10.00 | 7.5 |

### 2.4. Analytical methods

#### 2.4.1. Biomass quantification

The biomass concentration was determined by sampling the culture at intervals (typically daily), the optical density (OD 750 nm) of the sample was measured using a spectrophotometer (Jenway 632621 Visible Spectrophotometer, UK). The dry cell weight (DCW) in g/L was obtained by drying a known sample volume on a pre-dried filter paper to a constant weight. A calibration plot was generated of the different biomass concentrations as respective OD; the equation of the plot was used to convert subsequent OD to DCW g/L. The specific growth rate, μ, was calculated using Eq. (2) [2] during the log phase.

$$\boldsymbol{\mu} = \frac{\boldsymbol{Ln}(\boldsymbol{X_2}/\boldsymbol{X_1})}{(\boldsymbol{t_2} - \boldsymbol{t_1})} \tag{2}$$

where $X_1$ and $X_2$ are the initial and final DCW concentration in g/L at time $t_1$ and $t_2$, respectively.

#### 2.4.2. Pigment quantification

2 mL culture was centrifuged at 10000 rpm for 2 min and the pellet obtained was resuspended in dimethylformamide (DMF) by shaking for 15 min at room temperature. The tubes were centrifuged for 2 min at 10000 rpm using the Eppendorf centrifuge (5418, USA). Using a DMF resistant cuvette (BRAND UV cuvettes, Germany), the OD of the supernatant was measured at 647 and 664 (Jenway Spectrophotometer) and converted to each pigment type using Eqs. (3) and (4) [19], where the subscripts a and b refer to the wavelength in (nm) and A the corresponding absorbance.

$$\boldsymbol{Chl\ a} = \mathbf{12.70}\boldsymbol{A_{664}} - \mathbf{2.79}\boldsymbol{A_{647}} \tag{3}$$

$$\boldsymbol{Chl\ b} = \mathbf{20.70}\mathbf{A_{647}} - \mathbf{4.62}\mathbf{A_{664}} \tag{4}$$

#### 2.4.3. Ammonia quantification

Megazyme® (K-AMIAR, Germany) was used to quantify ammonium concentration in the culture supernatant. 2 mL culture was spun down at 10000 rpm, for 15 min, and the supernatant was analysed using the enzymes provided. The final ammonium concentration was computed from the 340 nm absorbance.

#### 2.4.4. Measurement of $CO_2$ fixation rate

The $CO_2$ fixation rate was calculated by using Eq. (5) [20]:

$$\boldsymbol{R}\left(\frac{\boldsymbol{gCO_2}}{\boldsymbol{L}}\Big/\boldsymbol{day}\right) = \boldsymbol{C_{carbon}P}\,\frac{\boldsymbol{M_{CO_2}}}{\boldsymbol{M_C}} \tag{5}$$

where $C_{carbon}$ is the carbon content of the microalgae cell (%, w/w) measured with an elemental analyser (see below); $M_{CO2}$ is the molecular weight of $CO_2$, $M_c$ is the molecular weight of carbon, and P is the biomass productivity.

#### 2.4.5. Elemental analysis

The biomass's elemental analysis (C, H, N, S) was conducted on the Elementar Vario Micro Cube analyser, (UK). ~2 mg of the sample was

used with an average of two measurements.

#### 2.4.6. Total lipid extract (TLE)

The total lipid content was extracted from the microalgae cells using a Dionex model Accelerated Solvent Extractor (ASE350, USA). A known mass of the microalgae biomass was weighed into the ASE cells and placed in the ASE extractor. The lipid was extracted from the freeze-dried microalgal cells using dichloromethane (DCM) and methanol (MeOH) in the ratio (1:1, v:v) at high temperature and pressure.

The lipid residue was collected in pre-combusted 60 mL vials and transferred to pre-weighed 8 mL vials. The lipid extract was dried under nitrogen, and the mass of the total lipid extract is determined from the difference between the initial weight of the 8 mL vial and the final weight with the TLE after drying. The percentage of TLE was calculated using Eq. (6).

$$\%TLE = \frac{TLE\ Mass\ (g)}{Mass\ of\ Microalgae\ Biomass\ (g)} * 100 \quad (6)$$

#### 2.4.7. Total fatty acid methyl esters determinations

The lipid extract was resuspended in DCM and 10 mg of the TLE was separated into total neutral (TNF) and acid fraction (TAF) in a special silica gel (Supelclean LC-NH2 SPE Si-gel) column using DCM: Isopropyl alcohol (ISO) and 4 % acetic acid (v:v). The collected fractions were dried under nitrogen. The TAF fraction was methylated with MeOH and 12 % $BF_3$ and placed in an oven at 70 °C for 1 h. The methylated fraction was further cleaned up in a regular silica gel column using hexane for column cleaning and DCM for eluting the methylated acids. The separated FAMEs were dried under $N_2$ and loaded with 1.5 mL DCM into GC vials. The TNF fraction was extracted into two fractions of Aliphatic hydrocarbons and Ketone/Esters/Aromatics eluting with hexane and DCM respectively. The extracted samples were dried and loaded with DCM into GC vials.

Analysis of the FAMEs was carried out on Agilent GC–MS 59977A MSD and GC-FID 7890B GC System equipped with Restek rtx1 column (60 m × 250 μm × 0.25 μm) (Restek, USA). 1 μL injection volume in a split less mode, and at a flow rate of 1.2 mL/min was used. The initial temperature was set at 60 °C for 2 min and analysed at a ramp rate of 30 °C/min to 120 °C for 0 min and finally at a ramp rate of 5 °C/min till 330 °C and held for 15 min making a total time of 61 min. Peaks were quantified using a calibration curve, given in Supplementary material, from three different methyl ester of C16, C23, and C30. C16 was used for FAMEs with retention time before 25 min, C23 for 25–35 min, and C30 for FAMEs above 35 min.

The Cetane number (CN) was computed with Eqs. (7), (8), and (9) [21] using the fatty acid methyl ester dry wt% composition.

$$SCSF = \frac{1}{100}\sum(MW_i * wt\%of\ saturated\ methyl\ esters) \quad (7)$$

where *SCSF* is the straight chain saturated fatty acid, $MW_i$ is the molecular weight of individual saturated methyl esters. The degree of unsaturation ($DU_m$) is given by

$$DU_m = MUFA + (2 * PUFA\ Cn:2, wt\%) + (3 * PUFA\ Cn:3, wt\%) \quad (8)$$

where $DU_m$ is the degree of unsaturation, MUFA is the monounsaturated fatty acid, PUFA is the polyunsaturated fatty acid. The CN and higher heating value (HHV) is given by,

$$CN = 63.41 - (0.0728 * DU_m) + (0.03495 * SCSF) - (3.26 * 10^{-4} * DU_m * SCSF) \quad (9)$$

$$HHV = 3.55C^2 - 232C - 2230H + 51.2C * H + 131N + 20600 \quad (10)$$

#### 2.4.8. Thermogravimetric analysis, TGA

The thermogravimetric analysis was done using Mettler Analytical, TGA. ~10 mg of sample were analysed at a heating rate of 10 °C/min from room temperature to 900 °C. Nitrogen was used to maintain an inert atmosphere.

## 3. Results and discussion

#### 3.1.1. Effect of feeding intervals (F.I) and initial pH (I.pH) on the pH kinetics

The effect of the feeding intervals and initial pH on the culture's pH kinetics is shown in Fig. 2. For most of the fed-batch conditions investigated, the pH increased beyond the upper thresholds set irrespective of the pH regime. The pH regimes are representative of the acidic, basic, and neutral conditions of the culture and are directly linked to the form of bicarbonate group (aqueous $CO_2$, $HCO_3$, or $CO_3^{2-}$) present in the broth. The acidic region with initial pH of 5.0 had an average final pH of 8.40. There was a linear increase in the pH with bicarbonate feeding, with the maximum pH reaching 9.0. This trend is similar for most experimental conditions, albeit with different final pH, as shown in Table II. Based on the deviation of the final pH from the upper limit threshold for each pH regime, I.pH 7.5 at F·I 6 h provided the highest level of pH control. The final pH at the basic regime decreased (9.70, 9.12, 8.48) with an increase in the feeding interval. Initial pH 6.3 had a similar pattern to 7.5, even though it could not maintain the neutral region; however, it had a relatively lower final pH of 8.40 and 8.76 at the higher feeding interval of 4 and 6 h, respectively.

Another key observation is that due to the stepwise pH increase, the bulk of the biomass was produced at the desirable pH of 6 to 8.5, which usually falls between days 3 to 10 of the exponential phase. This can further be exploited as two-phase growth, where at higher pH and with depleted $NH_4^+$, the stress on the microalgae cells is channelled towards product formation such as lipid and carbohydrate [22].

The complex metabolism in microalgae means that the optimum pH condition is strain-dependent, while most freshwater generally have values between 6 and 7.5, acidic and alkalophilic grow optimally at pH 4 and 9.5, respectively [23]. Therefore, the rate of pH change in a microalgae culture must be well understood. While there is no direct comparison with this work, the effect of pH using different carbon forms on microalgae has been well studied; according to Zhang et al. [24], deviation from optimal pH can be detrimental to biomass formation and sometimes lead to erratic metabolite production. While this technique could not directly maintain the pH at each regime as designed, the initial stepwise increase in pH allows more control than the batch system (Supplementary material) where the pH increased abiotically and rapidly. The ability to sustainably reduce the rate of pH change in the culture is critical because less complex pH control is desired due to the algae's highly complex biochemical reaction [25]. Furthermore, it simplifies the move towards large-scale microalgae cultivation's use of natural nutrients and sole use of bicarbonate for microalgae growth, where fewer chemical nutrients are desired. The cost of pH control can be high depending on the method used; hence the sole use of bicarbonate for simultaneous inorganic carbon supply and pH control can be a cost-effective measure without compromising biomass and lipid productivity.

#### 3.1.2. Effect of culture conditions on growth kinetics

The effect of the initial pH on the growth kinetics is shown in Fig. 3. For all of the culture conditions there was a short lag phase, which is likely due to the inoculum being at the exponential phase, and this is quite critical as it also indicates the quick adaptation of the microalgae cells to the experimental and PBR conditions. All the conditions had linear growth profile except at I.pH 5.0, F.I 6 h and I.pH 7.5, F.I 4 h, which both had a linear growth until the sixth day followed by an exponential growth which lasted for <48 h before going into the stationary phase.

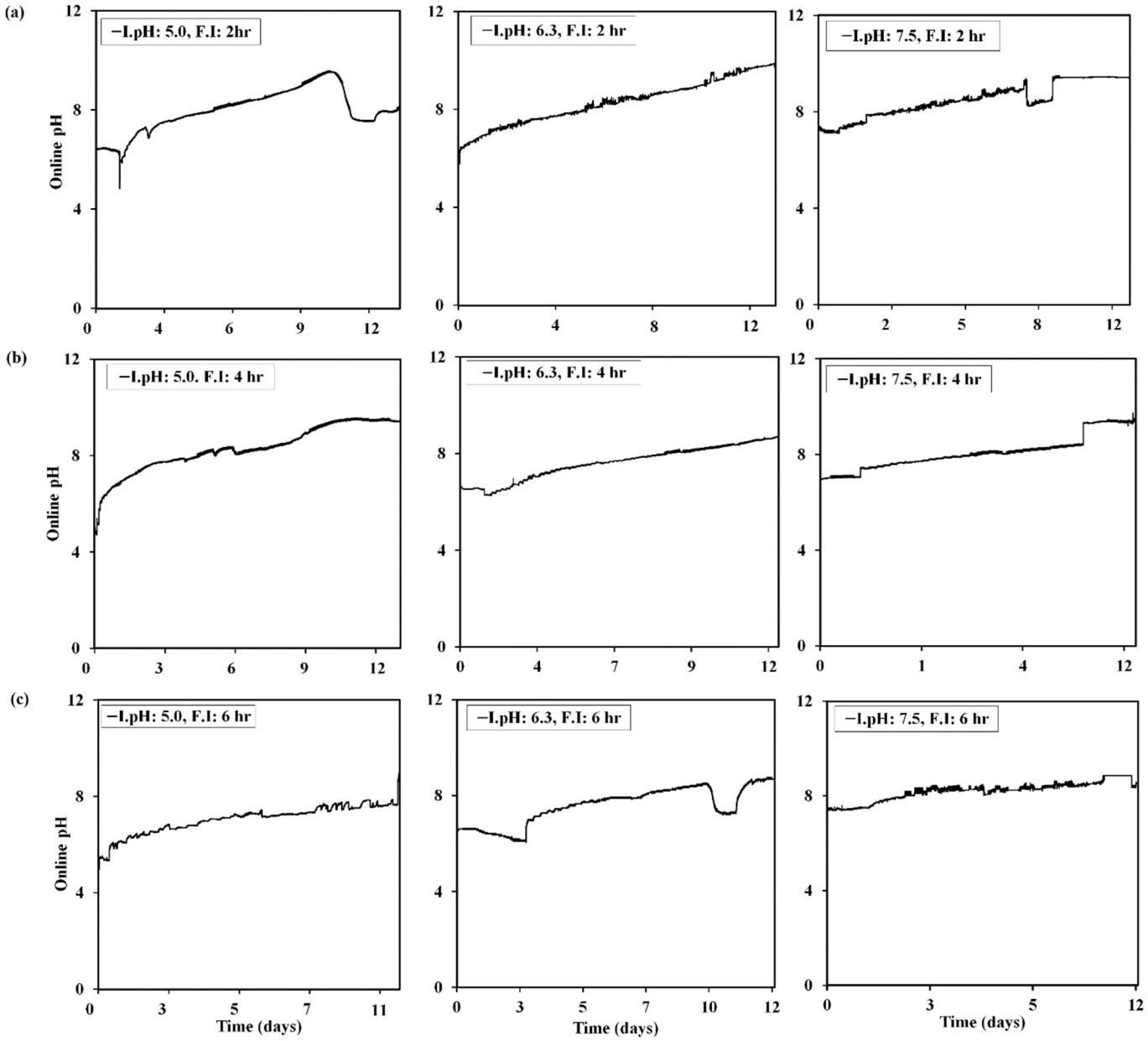



**Fig. 2.** pH kinetics of the fed-batch phototrophic cultivation of *C. sorokiniana* at different pH regimes, 5.0, 6.3, 7,5 and feeding Intervals of 2, 4 and 6 h. The data was obtained online with the Arduino set-up.

**Table II**
Table showing the summary of the growth kinetics of the fed-batch phototrophic culture of *C. sorokiniana* using $NaHCO_3$ solely as the inorganic carbon source and for pH modulation. Light intensity: 160 μmol/$m^2$s.

| Feed interval (h) | Initial pH | Final pH_1 | Final pH_2 | Max. biomass g/L | SGR (μ) /day | Doubling time | Bio. productivity g/L/d | Initial $NH_4^+$ g/L | Final $NH_4^+$ g/L | Yield x/c g/g | Carbon fixation g/L/d |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 2 | 5.0 | 8.43 | 8.37 | 1.70 | 0.11 | 145.38 | 0.19 | 0.14 | 0.027 | 0.72 | 0.36 |
| | 6.3 | 9.86 | 9.77 | 1.74 | 0.21 | 77.79 | 0.37 | 0.14 | 0.019 | 0.76 | 0.71 |
| | 7.5 | 9.18 | 10.22 | 1.68 | 0.14 | 114.94 | 0.24 | 0.14 | 0.012 | 0.72 | 0.43 |
| 4 | 5.0 | 9.09 | 9.04 | 1.53 | 0.15 | 109.73 | 0.23 | 0.14 | 0.008 | 0.66 | 0.42 |
| | 6.3 | 8.64 | 8.15 | 1.77 | 0.23 | 73.21 | 0.40 | 0.14 | 0.022 | 0.76 | 0.75 |
| | 7.5 | 9.45 | 8.79 | 2.49 | 0.34 | 49.19 | 0.84 | 0.14 | 0.031 | 1.05 | 1.55 |
| 6 | 5.0 | 8.75 | 8.62 | 2.24 | 0.12 | 137.82 | 0.27 | 0.14 | 0.023 | 1.09 | 0.49 |
| | 6.3 | 8.77 | 8.75 | 1.43 | 0.21 | 77.99 | 0.30 | 0.14 | 0.022 | 0.63 | 0.57 |
| | 7.5 | 8.55 | 8.40 | 1.67 | 0.16 | 106.74 | 0.26 | 0.14 | 0.031 | 0.75 | 0.48 |
| Batch | 6.3 | 9.96 | 9.94 | 0.74 | 0.03 | 110.63 | 0.11 | 0.14 | na | 0.44 | 0.20 |

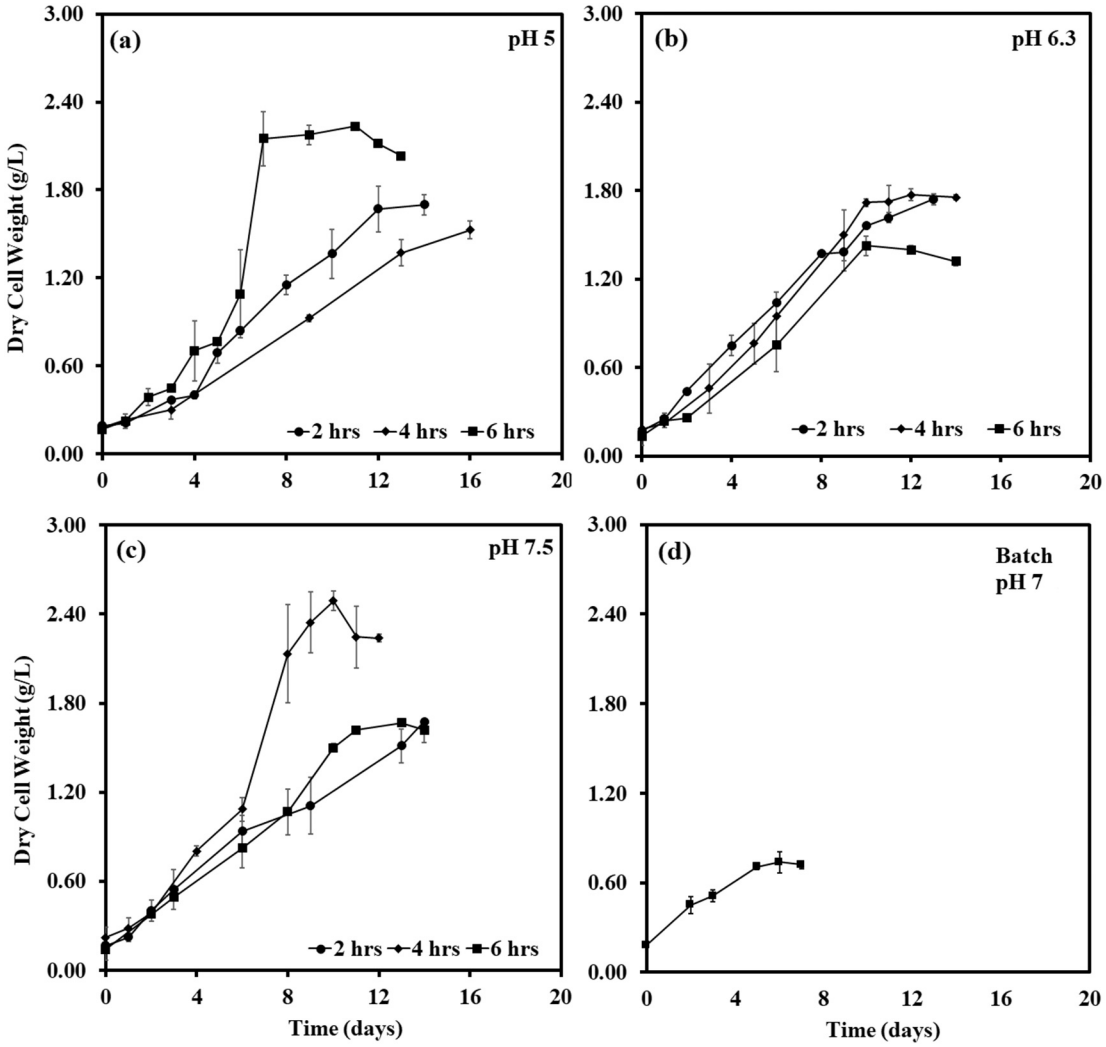



**Fig. 3.** Growth kinetics of *Chlorella sorokiniana* at different initial pH (I.pH) condition of 5, 6.3, and 7.5 and bicarbonate feeding time intervals of 2, 4 and 6 h. Total working volume: 2 L, Tris-base negative, Light intensity: 160 μmol $m^2s^{-1}$, initial pH (I.pH): 5, 6.3, and 7.5 Error bars represent the standard deviation of n = 2.

The effect of the experimental culture condition on the maximum biomass, g/L, see Table II, suggests that at the lower F.I of 2 h, the pH regime has no direct effect on the maximum biomass formed, however at F.I of 4 h, the maximum biomass formed increased with the pH regime, with the highest, 2.49 g/L, at the pH 7.5. Feeding interval of 6 h shows that the highest biomass is formed at pH 5, with pH 6.3 and 7.5 showing little difference between them. The maximum biomass of 2.49 g/L was obtained under F.I 4 h and I.pH 7.5, which indicates that the highest biomass was formed at higher feeding intervals of 4 and 6 h, with the initial pH being the difference. This difference was apparent in the doubling time, as a significantly longer time, 137 h was observed in the condition with the second-highest biomass.

The effect of F.I on the specific growth rate, μ, /day shows that, at 2 and 6 h, a similar growth rate pattern at the three pH regimes were observed, where pH 6.3 had the highest μ of 0.21/day, followed by pH 7.5 and 5.0 respectively. 4 h showed a linear increase in the growth rate with the pH regime. The doubling time showed an inverse trend compared to the values of μ, with the doubling time decreasing with an increase in growth rate. The doubling times are generally low due to slow bicarbonate metabolism and feeding pattern, with the fastest at 49 h; this is much lower than what is obtainable and reported in the literature where doubling times of 3.5–24 h are reported [14]. Assessment of the effect of the F·I and I.pH on the biomass productivity, Table II indicates that; at 4 h, the biomass productivity increased linearly with the pH regime as observed in both the specific growth rate and maximum biomass.

While there are conflicting reports regarding the maximum biomass obtained in *Chlorella* sp. culture due to the use of different culture systems, 1 to 3 g/L and high biomass productivity of 2.3 g/L/d has been generally reported [14,26] in a well-buffered system, with $CO_2$ as the primary carbon source or pH control. The average biomass productivity reported is in the region of 0.3 g/L/d [27] and is comparable to this work. Hence, the biomass obtained here is comparable, indicating that the culture methodology adopted in this work has no negative impact on the biomass formed, and an optimal bicarbonate feeding can be solely substituted for $CO_2$ while helping to manage the pH change in freshwater microalgae.

### 3.1.3. *Effect of $NH_4^+$ concentration on the final pH of the culture*

Nitrogen is one of the critical nutrients used by the cell for growth. Typically nitrate, urea of $NH_4Cl$ can be used, depending on the metabolic capability of the desired microalgae strain and the process design. During microalgae metabolism, assimilation of inorganic nitrogen and conversion to amino acids and proteins is energetically demanding; microalgae prefer $NH_4^+$ as the metabolic cost of reducing it to organic matter is relatively lower than the other forms of inorganic nitrogen [28].

$NH_4Cl$ is utilised in this work to leverage on proton released during

the utilisation of $NH_4^+$ for the control of pH. The relatively slower rate of ammonium consumption compared to [14,15] is likely due to the limitation as a result of the fed-batch process adopted. From the ammonium consumption, biomass growth kinetics, and rate of pH change, it is probable that the ammonium utilisation is coupled to the carbon supply and uptake, hence, decoupling both conditions and improving the ammonium utilisation while still maintaining the fed-batch methodology proposed might further help to reduce the rate of pH change. Fig. 4 describes the ammonium utilisation profiles during the culture. A linear decrease in the ammonium concentration was observed in all the culture conditions used, with final concentrations between 10 and 30 mg/L.

### 3.1.4. Carbon fixation, (g/L/d)

The carbon fixation was calculated from the elemental carbon using Eq. (5). The elemental carbon in the biomass were all between 48.22 and 51.82 %, which is consistent with 46–51 % reported in the literature [29]. With *Chlorella* needing a large amount of carbon to grow, the carbon utilisation rate in this study ranges from 0.36 to 1.55 g/L/d, as shown in Table III. *Chlorella* sp. has been reported to sequester up to 1 $gCO_2$/L/day in optimal condition and can rise to 6 $gCO_2$/L/day in a membrane-type reactor due to the high mass transfer rate and small bubbles which reduces the surface area of mass transfer [30]. The carbon fixed in this study is similar to the report of a similar specie, where between 0.1 and 1.8 $gCO_2$/L/day was reported [31]. The carbon fixation rate was significantly higher than that of a similar batch culture study where bicarbonate was the sole carbon source [20]. The low carbon fixation was attributed to the low biomass productivity due to the inability of the microalgae cell to fully utilise the bicarbonate in batch culture.

**Table III**
Elemental analysis and calorific values of *C. sorokiniana* grown in different experimental conditions. The higher heating value (HHV) were computed using Eq. (10). The data represents the average of two experimental repeats.

| I.pH | F.I (h) | Carbon (%) | Hydrogen (%) | Nitrogen (%) | Sulphur (%) | HHV (MJ/kg) |
|---|---|---|---|---|---|---|
| 5.0 | 2 | 50.47 | 7.68 | 9.08 | 1.03 | 21.84 |
| 5.0 | 4 | 49.40 | 7.57 | 8.95 | 0.68 | 21.24 |
| 5.0 | 6 | 49.47 | 6.72 | 9.75 | 0.62 | 21.12 |
| 6.3 | 2 | 51.82 | 7.08 | 9.35 | 0.61 | 22.33 |
| 6.3 | 4 | 50.83 | 7.29 | 9.32 | 0.90 | 21.91 |
| 6.3 | 6 | 51.21 | 7.25 | 9.52 | 0.90 | 22.12 |
| 7.5 | 2 | 48.22 | 7.53 | 7.89 | 0.85 | 20.50 |
| 7.5 | 4 | 50.38 | 6.71 | 9.82 | 0.64 | 21.55 |
| 7.5 | 6 | 49.69 | 7.55 | 9.26 | 0.63 | 21.42 |

I.pH: Initial pH, F.I: Feeding intervals, HHV: High heating value.

Importantly, the fed-batch technique in this study seem not to reduce the carbon fixation capacity of phototrophically grown *C. sorokiniana* as the carbon fixation values shown in Table III are similar to the literature as previously discussed. The difference in the carbon fixation efficiency from the results is likely due to the feeding interval, with the highest

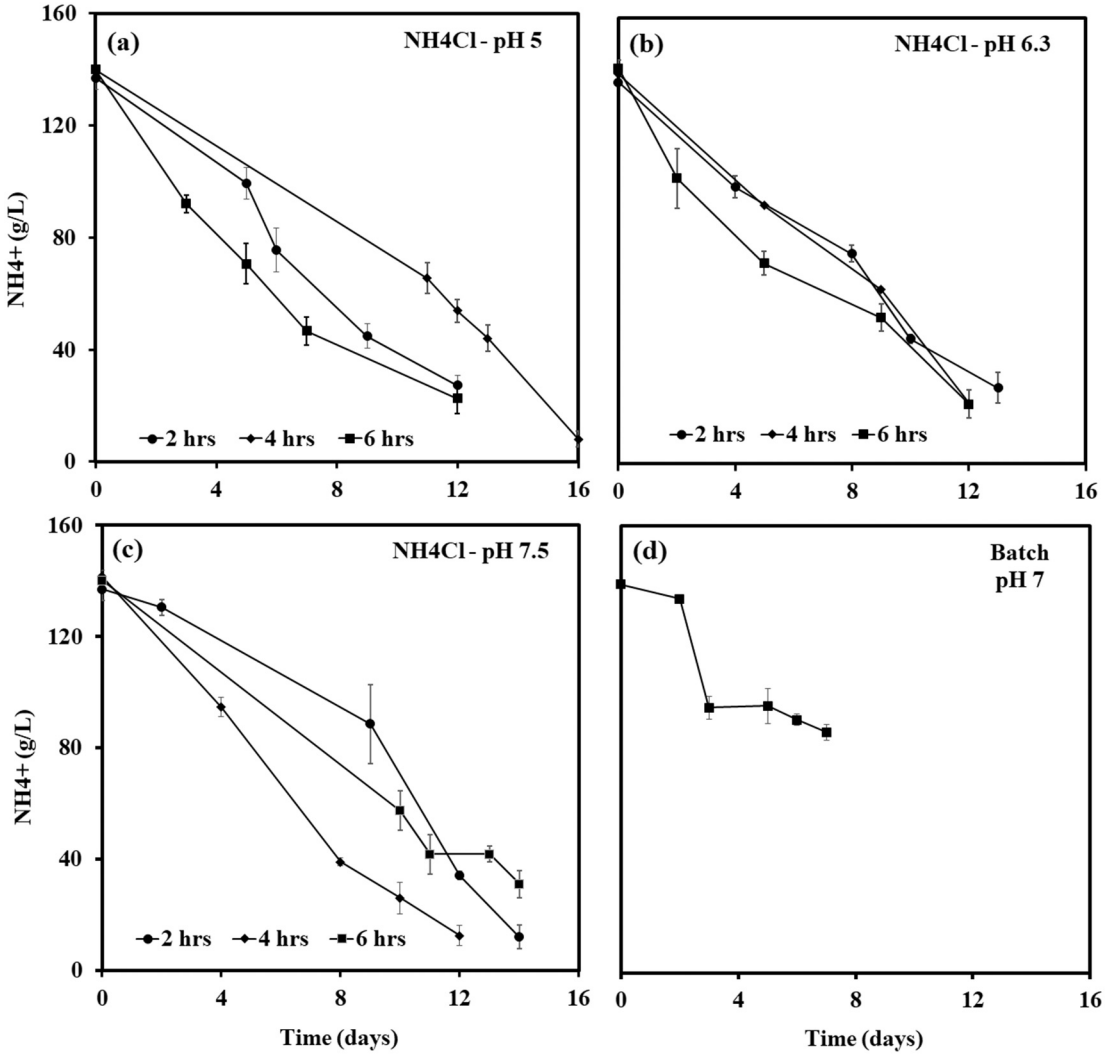



**Fig. 4.** Ammonium utilisation in the photoautotrophic culture of *C. sorokiniana* with bicarbonate as sole inorganic carbon source.

fixation rate achieved at the 4 h F.I. conditions. This indicates an optimal feeding rate of bicarbonate to the culture.

### 3.1.5. Effect of culture condition on the pigment content

Pigment is one of the important products that can be obtained from microalgae. Chlorophyll a, b and carotenoids make up the majority pigment in *Chlorella* sp. [32]. The maximum chl-a and b concentration is shown in Fig. 5. The highest chl-a concentration was 25.85 mg/L obtained in pH 6.3, F.I 6 h. Under pH 6.3, the chl-a content increased with the F.I. Under 4 h, the chl-a content increased with pH as observed earlier with other culture characteristics. With F.I 2 and 4 h, pH regime 7.5 had the highest chl-a, while 6 h had a significant increase from 5.0 to 6.3, followed by a drop in 7.5. Chl-b increased with pH in F.I 4 h, likewise in 2 h, albeit 6.3 and 7.5 pH regimes are not statistically different. As observed in chl-a, 6 h F.I showed an increase in chl-b content from 5.0 to 6.3 and drops in 7.5. The highest chl-a concentration of 25.85 mg/L is in similar range to that reported by Ojo et al. [14] with $CO_2$ as the carbon source and considerably higher than reported by Abinandan and Shanthakumar [32] when bicarbonate was used for batch culture of *Chlorella* sp. The relatively higher pigment concentration suggests that the methodology favours pigment production [33].

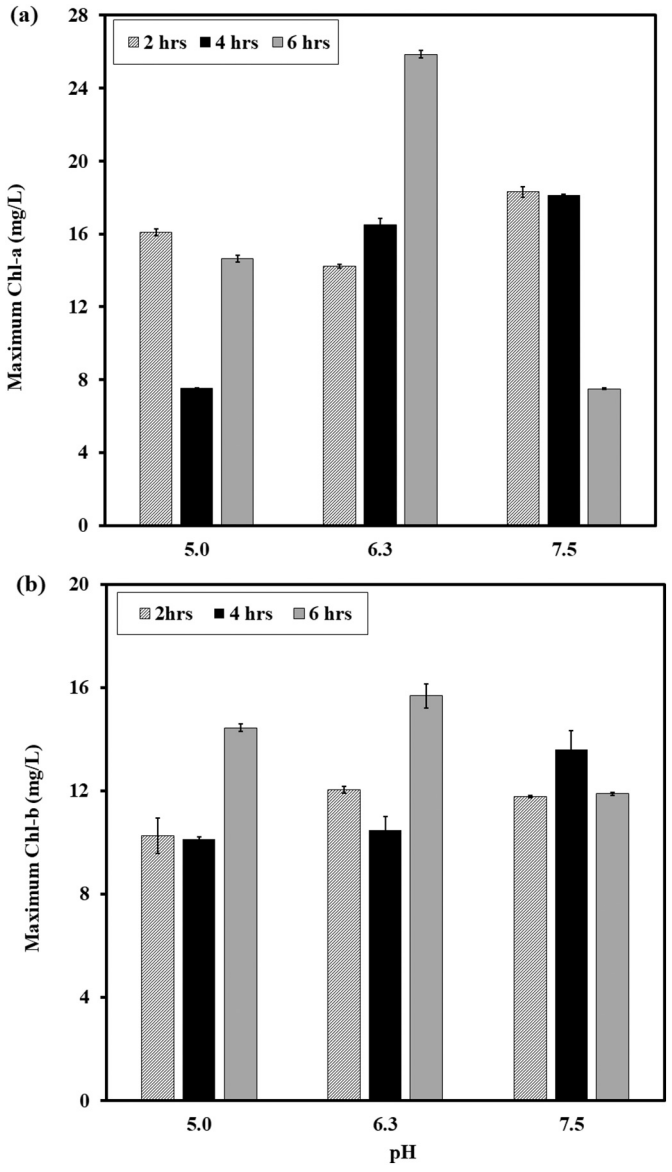



**Fig. 5.** Evaluation of the pigment formation at different feeding interval and initial pH. The pigment was extracted and quantified as described in Section 2.4.2. Error bars represent the standard deviation of n = 2.

### 3.1.6. Total lipid extract

The effect of the growth conditions on the total lipid extract is shown in Fig. 6a. The sample with the initial pH 5.0, feeding interval of 4 h had the highest average TLE of 16 % with no significant difference to 6 h. There was no notable difference in the TLE of all the feeding intervals at pH 6.3, with an average of 11 %. At pH 7.5, feeding intervals 2 and 6 h had the higher TLE with 4 h having the lowest TLE, 7 %, of all the experimental conditions. The batch culture had a TLE of 12 % which is comparable to the fed-batch culture.

The percentage TLE obtained is comparable to the literature as shown in Fig. 6a, with the percentage lipid content all ranging between 5 and 34 % [34]. Higher TLE has been reported [35], however, the cells were stressed or had $CO_2$ as the carbon source. While the percentage lipid was relatively low, the productivity was higher indicating more efficient lipid production.

### 3.1.7. Effect of bicarbonate and pH regime on the fatty acid methyl esters (FAME)

Biodiesel quality depends on the fatty acid profile, structural features of the various FAMEs, carbon chain length, degree of unsaturation, and number of double bonds [26]. The fatty acids vary with microalgae strain and the growth conditions such as the pH, carbon source, stage of harvest, and inorganic nutrient concentration [36].

Given the rate of pH change and the sole use of bicarbonate as the inorganic carbon source, it was essential to determine the effect of these conditions on the fatty acids (FAs) to establish the biodiesel suitability and nutraceutical properties. Eighteen FAs detected across all of the growth conditions were listed in Table IV. Eight of the FAMEs achieved >1 % relative composition in >80 % of the culture conditions and are regarded as major, while the other ten FAMEs with <1 % relative composition were referred to as minor. The eight major FAMEs are palmitic acid (C16:0), palmitoleic acid (C16:1), hexadecadienoic acid (C16:2), n-heptadecanoic acid (C17:1), linoleic acid (C18:2), stearic acid (C18:0), Elaidic acid (C18:1n9t), behenic acid (C22:0). The minor FAs are myristic acid (C14:0), pentadecanoic acid (C15:0), heptadecanoic acid (C17:0), Oleic acid (C18:1n9c), nonadecanoic acid (C19:0), arachidic acid (C20:0), heneicosylic acid (C21:0), tricosylic acid (C23:0), Lignoceric acid (C24:0), pentacosanoic acid (C25:0).

In general, a higher percentage of SFAs (62.93 %–90.86 %), was accumulated by the cells across all the growth conditions with the highest of 90.86 % under the initial pH 7.5, feeding interval 4 h, and the lowest of 62.93 % under the condition I.pH 6.3, F.I 4 h. C16:0 majorly contributed to the SFAs in all the growth conditions ranging from 45.28 %–73.41 %, except for I.pH 7.5, F.I 4 h which had C22:0 as the highest SFA. While it is quite common to have the UFAs as the highest % of FAs in microalgae [14,26,35], on the contrary SFA had the highest % in this study. Similar % SFA was reported by Kumar et al. [37] where ~80 % SFA was obtained in 5 % $CO_2$ compared to 49.35 % in flue gas, and this was attributed to the stress imposed by the flue gas on the microalgae cell which increases the degree of unsaturation. However, the higher % SFA in this study is likely due to less stress imposed on the cell, as a result of the low C:N ratio in the microalgae culture media [37]. The relative low carbon in the culture per time, might also lead to increased dissolved $O_2$ concentration which has been reported to also reduce the degree of unsaturation in the microalgae cell [20].

The percentage unsaturated fatty acids (UFAs) are relatively lower than most reported in the literature as the UFAs mostly has a higher ratio than the SFAs [26]. The lowest and highest UFAs (9.01 %–35.53 %) were obtained under pH 7.5 and 6.3 at feeding interval of 2 h, with the highest contributor being the C16:1. The minor FAs were mainly SFAs with longer chain carbon. High levels of SFAs and MUFAs have been reported to be desirable for biodiesel production due to its increase oxidative and thermal stability [35]. Reports have also suggested that FAMEs with a

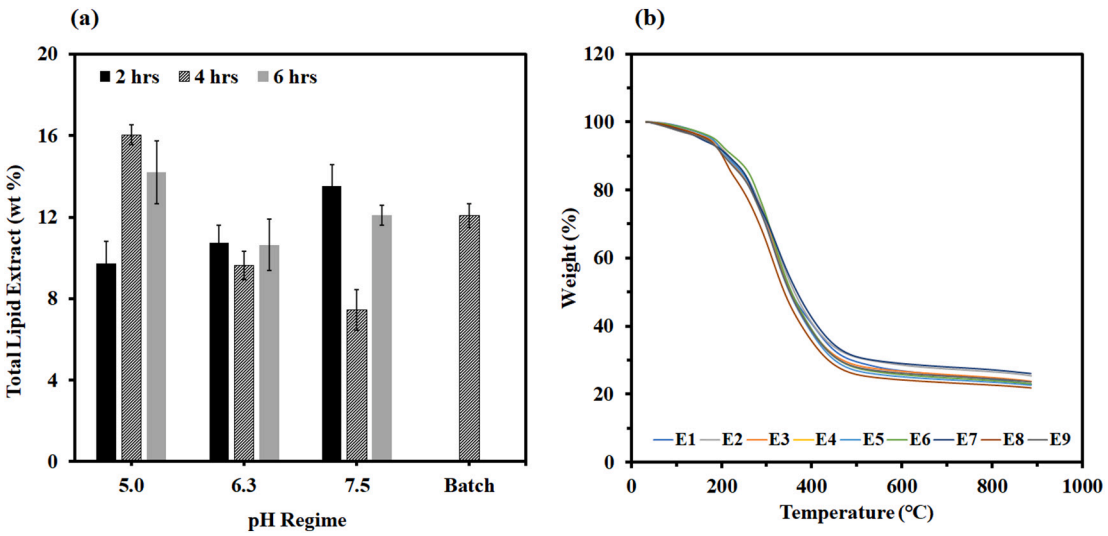



**Fig. 6.** (a) The total lipid extract, wt% at different pH regime in the phototrophic cultivation of *Chlorella sorokiniana* with bicarbonate. (b) thermogravimetric anlysis curve of the thermal degradation of a phototrophically grown *C. sorokiniana* at different culture conditions. E1 to E9 represents the culture condition at each pH and feeding intervals. E1: pH 5.0, F.I 2 h; E2: pH 5.0, F.I 4 h, E3: pH 5, F.I 6 h, E4: pH 6.3, F.I 2 h, E5: pH 6.3, F.I 4 h, E6: pH 6.3, F.I 6 h, E7: pH 7.5, F.I 2 h, E8: pH 7.5, F.I 4 h, E9: pH 7.5, F.I 6 h.

**Table IV**
Comparison of the fatty acid methyl ester (dry wt%) profile of the batch and fed-batch culture of *C. sorokiniana* using bicarbonate as the sole inorganic carbon. Experiments performed as described in Section 2.4.7. Error bar represents the standard deviation of n = 2.

| FAMEs | Structural formulae | pH 5.0 | | | pH 6.3 | | | pH 7.5 | | | Batch |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 2 | 4 | 6 | 2 | 4 | 6 | 2 | 4 | 6 | |
| Myristic acid | C14:0 | 1.02 ± 0.6 | 0.66 ± 0.0 | 0.80 ± 0.4 | 0.68 ± 0.3 | 0.93 ± 0.0 | 0.99 ± 0.0 | 0.88 ± 0.0 | 0.50 ± 0.0 | 1.14 ± 0.2 | 0.88 ± 0.0 |
| Pentadecanoic acid | C15:0 | 0.33 ± 0.0 | 0.22 ± 0.0 | 0.22 ± 0.0 | 0.30 ± 0.0 | 0.29 ± 0.0 | 0.27 ± 0.0 | 0.30 ± 0.0 | 0.12 ± 0.0 | 0.39 ± 0.0 | 0.26 ± 0.0 |
| Hexadecadienoic acid | C16:2 | 2.25 ± 0.2 | 1.62 ± 0.2 | 1.08 ± 0.0 | 1.32 ± 0.1 | 4.72 ± 0.3 | 2.15 ± 0.0 | 2.53 ± 0.1 | 0.50 ± 0.0 | 1.92 ± 0.2 | 0.80 ± 0.1 |
| Palmitoleic acid | C16:1 | 11.63 ± 0.2 | 11.31 ± 0.5 | 7.70 ± 0.2 | 8.66 ± 0.6 | 10.28 ± 1.2 | 10.06 ± 0.5 | 12.21 ± 1.2 | 3.76 ± 0.2 | 10.60 ± 0.6 | 8.33 ± 0.8 |
| Palmitic acid | C16:0 | 63.65 ± 3.4 | 59.07 ± 1.6 | 61.57 ± 2.6 | 45.28 ± 1.8 | 58.16 ± 2.3 | 65.62 ± 1.8 | 60.98 ± 3.4 | 32.59 ± 1.1 | 66.18 ± 3.1 | 73.41 ± 2.9 |
| n-Heptadecanoic acid | C17:1 | 8.66 ± 0.8 | 6.13 ± 0.4 | 6.21 ± 0.6 | 6.65 ± 0.2 | 9.28 ± 0.9 | 8.39 ± 1.2 | 8.41 ± 0.5 | 3.12 ± 0.8 | 9.12 ± 0.3 | 7.30 ± 0.0 |
| Heptadecanoic acid | C17:0 | 0.56 ± 0.0 | 0.60 ± 0.0 | 0.07 ± 0.0 | 0.62 ± 0.0 | 0.44 ± 0.0 | 0.19 ± 0.0 | 0.60 ± 0.0 | 0.18 ± 0.0 | 0.45 ± 0.0 | 0.54 ± 0.0 |
| Linoleic acid | C18:2n6c | 4.34 ± 0.2 | 3.53 ± 0.3 | 1.18 ± 0.0 | 1.78 ± 0.0 | 9.27 ± 0.5 | 5.29 ± 1.9 | 4.84 ± 1.1 | 0.81 ± 0.0 | 2.76 ± 0.0 | 1.17 ± 0.1 |
| Oleic acid | C18:1n9c | nd | nd | 1.18 ± 0.1 | nd | nd | nd | nd | 0.81 ± 0.0 | nd | nd |
| Elaidic acid | C18:1n9t | nd | 1.38 ± 0.0 | nd | 1.07 ± 0.0 | 1.98 ± 0.2 | 2.51 ± 0.2 | 2.20 ± 0.2 | nd | 1.14 ± 0.0 | 1.47 ± 0.1 |
| Stearic acid | C18:0 | 1.58 ± 0.4 | 1.84 ± 0.0 | 2.27 ± 0.1 | 1.34 ± 0.1 | 1.42 ± 0.0 | 1.26 ± 0.7 | 2.24 ± 0.3 | 1.35 ± 0.2 | 1.45 ± 0.1 | 1.43 ± 0.0 |
| Nonadecanoic acid | C19:0 | 0.07 ± 0.0 | nd | nd | 0.06 ± 0.0 | 0.06 ± 0.0 | nd | 0.12 ± 0.0 | nd | nd | nd |
| Arachidic acid | C20:0 | 0.36 ± 0.1 | 0.51 ± 0.0 | 0.81 ± 0.0 | 0.20 ± 0.0 | 0.31 ± 0.0 | 0.48 ± 0.0 | 0.95 ± 0.2 | 0.33 ± 0.0 | 0.45 ± 0.0 | 0.94 ± 0.1 |
| Heneicosylic acid | C21:0 | nd | nd | 0.12 ± 0.0 | nd | nd | nd | 0.20 ± 0.0 | nd | nd | nd |
| Behenic acid | C22:0 | 3.92 ± 0.6 | 9.75 ± 0.2 | 15.08 ± 0.3 | 29.64 ± 1.2 | 0.67 ± 0.0 | 1.07 ± 0.0 | 1.65 ± 0.1 | 55.79 ± 0.7 | 0.45 ± 0.0 | 1.06 ± 0.2 |
| Tricosylic acid | C23:0 | 0.40 ± 0.0 | 0.19 ± 0.0 | nd | 0.31 ± 0.0 | 0.31 ± 0.0 | 0.32 ± 0.0 | 0.82 ± 0.0 | nd | 0.28 ± 0.0 | 0.31 ± 0.0 |
| Lignoceric acid | C24:0 | 0.31 ± 0.0 | 0.32 ± 0.0 | nd | 0.25 ± 0.0 | 0.28 ± 0.0 | 0.39 ± 0.0 | 0.58 ± 0.0 | nd | 0.25 ± 0.0 | 1.54 ± 0.1 |
| Pentacosanoic acid | C25:0 | 0.78 ± 0.0 | 2.29 ± 0.1 | nd | 1.07 ± 0.1 | 0.06 ± 0.0 | nd | nd | nd | nd | nd |
| Saturated FAs | % SFA | 73.00 | 75.45 | 80.92 | 79.75 | 62.93 | 70.59 | 69.32 | 90.86 | 71.05 | 80.38 |
| Unsaturated FAs | % UFA | 26.88 | 23.97 | 17.35 | 19.48 | 35.53 | 28.39 | 30.19 | 9.01 | 25.55 | 19.07 |
| Active biofuel components | % C16–C18 | 90.43 | 83.86 | 80.17 | 65.40 | 90.84 | 93.32 | 91.47 | 42.63 | 91.72 | 93.65 |

nd: not detected, n = 2.

lesser number of double bonds are relatively more stable to oxidation than PUFAs [38]. Consequently, the fatty acid profile obtained in this study is desirable for biodiesel and further substantiated by the CN.

The percentage active biofuel component (C16–C18) in the FAs was between 42.63 % to 93.32 %, and as seen in Table IV, there was no notable difference between them at pH 5.0, while at 6.3, they increased with the feeding interval. At the highest pH of 7.5, feeding intervals 2 and 6 h had the highest biofuel component (91.47 and 91.72 % respectively), with the 4 h feeding interval being the lowest (42.63 %). The biodiesel properties are typically evaluated using several parameters such as the cetane number, iodine absorption value, long-chain saturated factor, degree of unsaturation ($DU_m$), cold filter plugging point [35] with CN being the prime indicator of the biodiesel quality [26,38]. Consequently, the CN was computed according to Mishra et al. [21], who reported a more robust equation for calculating the CN from the FAMEs. The SCSF and the modified $DU_m$ were used to estimate the CN. The CN was generally between 61.37 and 72.26, which satisfy the EN14214 value of >51 for biodiesel, and the higher the value, the better the ignition quality of the fuel. The CN at initial pH 5 increased with an increase in the feeding intervals, while at pH 6.30, F.I 2 h had the highest CN followed by 6 and 4 h, respectively. pH 7.5 had the highest CN at 4 h with a slight difference between the 2 and 6 h.

#### 3.1.8. *Effect of the pH regime on the thermogravimetric analysis of C. sorokiniana*

To further characterize and to understand the thermal decomposition pattern of the microalgae biomass at the different growth conditions, the thermogravimetric (TGA) analysis was carried out at a constant heating rate of 10 °C/min. The low heating rate was chosen as this reduced diffusion side effects during thermal degradation as a result of the small size, ~10 μm, of the microalgae [39]. The mass loss profiles are presented in Fig. 6b. There were different steps and stages of combustion; as with a typical thermal profile, the mass loss occurred with increased temperature. Three stages of devolatilisation (dehydration, devolatilisation of proteins, carbohydrates and lipids, and decomposition of the solid residue) appeared during the samples' thermal decomposition.

As shown in Fig. 6b, mass loss patterns at all of the culture conditions all have a similar shape and are comparable to the literature, where similar residue mass was obtained [40]. This indicates that the culture conditions - pH and feeding interval - do not affect the thermal degradation resistance of the *Chlorella sorokiniana* biomass. The average mass loss of over 75 % was relatively high compared to some microalgae species such as *Chroococcus*, which had a residual mass of 40–60 % [39,41] after treatment. There were generally 2 to 4 reaction steps for all of the samples, despite being pre-treated the same way (oven-dried at 45 °C) before the TGA experiment. Apart from I.pH 6.3, which had predominantly two steps at all the F.I (2, 4 and 6 h), I.pH 5.0 and 7.5 both have three steps at F.I of 2 and 6 h. The highest step of four was obtained under I.pH 5.0, F.I 4 h.

Initial weight loss below the average temperature of 137 °C, indicates the loss of water molecules and lightweight hydrocarbons [41,42], was observed in I.pH 5 and 7.5. For I.pH 6.3, the onset temperature of the initial reaction steps was 159.57, 157.22, 158.31 °C for 2, 4 and 6 h; the only possible reason could have been the absence of lighter hydrocarbons, as the moisture content of the microalgae was assumed to be similar because of the similar pre-treatment. The significant weight loss which is attributed to the devolatilisation of the carbohydrate, protein, and lipid in the biomass – and sometimes just the carbohydrate and proteins – as reported by [41] occurred at the average peak temperature of 315 °C across all of the samples. This agrees with the report of [43], where a significant weight loss occurred in the temperature range of 200–350 °C.

The thermal decomposition of lipid occurs between 250 and 600 °C, with a peak value of 400 °C [44]. This peak was mainly visible in pH 5.0 and feeding interval of 2 h where the onset, peak and end temperature was 397, 404 and 498 °C, respectively. For all other culture conditions, the peak was small and was lumped together at this phase. This likely indicates a lower lipid composition of the biomass at the culture conditions.

## 4. Conclusion

The results presented in this study demonstrated the potential of solely utilising bicarbonate as the inorganic source and pH modulation in a phototrophic *C. sorokiniana* fed-batch culture. It was shown that the highest initial pH of 7.5 and feeding intervals of 4 and 6 h provided the highest level of pH control. The initial stepwise pH increase allowed more pH control and higher biomass and product yields than the batch system, where the pH increased abiotically and rapidly. The optimal growth kinetics obtained is comparable to $CO_2$ culture with conventional pH control. The maximum lipid content was similar to the literature, and the pigment was relatively higher than $CO_2$ cultures. The high SFAs and MUFAs obtained makes the method suitable for biodiesel production.

On this basis, this method is considered suitable for the freshwater phototrophic culture while maintaining most conventional growth kinetics. While the scope of this study was restrained to studying the effect of pulse feeding at a constant rate and pH regimes, it would be interesting to explore the scalability of the methodology and the effect of other metabolic bicarbonates.

## Abbreviations

| | |
|---|---|
| ASE | accelerated solvent extractor |
| CA | carbonic anhydrase |
| CN | cetane number |
| DCW | dry cell weight |
| DMF | dimethylformamide |
| F.I | feeding intervals |
| FA | fatty acids |
| FAME | fatty acid methyl esters |
| HHV | higher heating values |
| LPBR | laboratory-scale photobioreactor |
| MeOH | methanol |
| MUFA | monounsaturated fatty acid |
| OD | optical density |
| PBR | photobioreactor |
| PUFA | polyunsaturated fatty acid |
| SCSF | straight chain saturated factor |
| SFA | saturated fatty acids |
| TAP | Tris Acetate Phosphate |
| TCA | tricarboxylic acid |
| TGA | thermogravimetric analysis |
| TLE | total lipid extract |
| TNF | total neutral fraction |
| UFA | unsaturated fatty acids |

## CRediT authorship contribution statement

Tolulope Victor Agbebi: Conceptualisation, Methodology, Investigation, Writing -Original draft preparation. Ian A. Watson: Conceptualisation, Supervision, Writing - Review and Editing. Ojo Ebenezer: Writing - Review and Editing.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.algal.2022.102841.

## References

[1] Q. Zheng, X. Xu, G.J.O. Martin, S.E. Kentish, Critical review of strategies for CO2 delivery to large-scale microalgae cultures, Chin. J. Chem. Eng. 26 (2018) 2219–2228, https://doi.org/10.1016/j.cjche.2018.07.013.
[2] V. Bhola, F. Swalaha, R. Ranjith Kumar, M. Singh, F. Bux, Overview of the potential of microalgae for CO2 sequestration, Int. J. Environ. Sci. Technol. 11 (2014) 2103–2118, https://doi.org/10.1007/s13762-013-0487-6.
[3] G. Ferreira, L.F. Ríos Pinto, R. Maciel Filho, L.V. Fregolente, A review on lipid production from microalgae: association between cultivation using waste streams and fatty acid profiles, Renew. Sust. Energ. Rev. 109 (2019) 448–466, https://doi.org/10.1016/j.rser.2019.04.052.
[4] B. Zhu, G. Chen, X. Cao, D. Wei, Molecular characterization of CO2 sequestration and assimilation in microalgae and its biotechnological applications, Bioresour. Technol. 244 (2017) 1207–1215, https://doi.org/10.1016/j.biortech.2017.05.199.
[5] C. Zhu, S. Chen, Y. Ji, U. Schwaneberg, Z. Chi, Progress toward a bicarbonate-based microalgae production system, Trends Biotechnol. 40 (2022) 180–193, https://doi.org/10.1016/j.tibtech.2021.06.005.
[6] I. Pancha, K. Chokshi, T. Ghosh, C. Paliwal, R. Maurya, S. Mishra, Bicarbonate supplementation enhanced biofuel production potential as well as nutritional stress mitigation in the microalgae scenedesmus sp. CCNM 1077, Bioresour. Technol. 193 (2015) 315–323, https://doi.org/10.1016/j.biortech.2015.06.107.
[7] L. Zhang, R. Guo, H. Li, Q. Du, J. Lu, Y. Huang, Z. Yan, J. Chen, Mechanism analysis for the process-dependent driven mode of NaHCO3 in algal antibiotic removal: efficiency, degradation pathway and metabolic response, J. Hazard. Mater. 394 (2020), 122531, https://doi.org/10.1016/j.jhazmat.2020.122531.
[8] C. Zhu, R. Zhang, L. Cheng, Z. Chi, A recycling culture of neochloris oleoabundans in a bicarbonate-based integrated carbon capture and algae production system with harvesting by auto-flocculation, Biotechnol. Biofuels 11 (2018) 204, https://doi.org/10.1186/s13068-018-1197-6.
[9] J. Lv, F. Zhao, J. Feng, Q. Liu, F. Nan, X. Liu, S. Xie, Transcriptomic analysis reveals the mechanism on the response of Chlorococcum sp. GD to glucose concentration in mixotrophic cultivation, Bioresour. Technol. 288 (2019), 121568, https://doi.org/10.1016/j.biortech.2019.121568.
[10] M. Moazami-Goudarzi, B. Colman, Changes in carbon uptake mechanisms in two green marine algae by reduced seawater pH, J. Exp. Mar. Bio. Ecol. (2012), https://doi.org/10.1016/j.jembe.2011.11.017.
[11] Y. Matsuda, T.G. Williams, B. Colman, Quantification of the rate of CO2 formation in the periplasmic space of microalgae during photosynthesis. A comparison of whole-cell rate constants for CO2 and HCO3- uptake among three species of the green alga Chlorella, Plant Cell Environ. 22 (1999) 397–405, https://doi.org/10.1046/j.1365-3040.1999.00399.x.
[12] J.V. Moroney, A. Somanchi, How do algae concentrate CO2 to increase the efficiency of photosynthetic carbon fixation? Plant Physiol. (1999) https://doi.org/10.1104/pp.119.1.9.
[13] S. Kim, Y. Lee, S.-J. Hwang, Removal of nitrogen and phosphorus by Chlorella sorokiniana cultured heterotrophically in ammonia and nitrate, Int. Biodeterior. Biodegradation 85 (2013) 511–516, https://doi.org/10.1016/j.ibiod.2013.05.025.
[14] E.O. Ojo, H. Auta, F. Baganz, G.J. Lye, Design and parallelisation of a miniature photobioreactor platform for microalgal culture evaluation and optimisation, Biochem. Eng. J. 103 (2015) 93–102, https://doi.org/10.1016/j.bej.2015.07.006.
[15] N. Poddar, Utilisation of Glycerol as a Carbon Source for Mixotrophic Growth and Lipid Accumulation in Marine Microalgae, The University of Melbourne, 2019.
[16] R.A. Andersen, The microalgal cell, in: Handbook of Microalgal Culture: Applied Phycology and Biotechnology, 2013, https://doi.org/10.1002/9781118567166.ch1.
[17] M. Heining, R. Buchholz, Photobioreactors with internal illumination - a survey and comparison, Biotechnol. J. 10 (2015) 1131–1137, https://doi.org/10.1002/biot.201400572.
[18] P. Kamble, Z. Khan, S. Capper, J. Sharp, I. Watson, Improving downdraft gasifier stability by robust instrumentation and control systems, in: Energy Procedia, Elsevier, 2017, pp. 2214–2217, https://doi.org/10.1016/j.egypro.2017.12.591.
[19] A.R. Wellburn, The spectral determination of chlorophylls a and b, as well as Total carotenoids, using various solvents with spectrophotometers of different resolution, J. Plant Physiol. 144 (1994) 307–313, https://doi.org/10.1016/S0176-1617(11)81192-2.
[20] M.K. Lam, K.T. Lee, Effect of carbon source towards the growth of Chlorella vulgaris for CO2 bio-mitigation and biodiesel production, Int. J. Greenh. Gas Control 14 (2013) 169–176, https://doi.org/10.1016/j.ijggc.2013.01.016.
[21] S. Mishra, K. Anand, P.S. Mehta, Predicting the cetane number of biodiesel fuels from their fatty acid methyl ester composition, Energy Fuel 30 (2016) 10425–10434, https://doi.org/10.1021/acs.energyfuels.6b01343.
[22] A.K. Minhas, P. Hodgson, C.J. Barrow, A. Adholeya, A review on the assessment of stress conditions for simultaneous production of microalgal lipids and carotenoids, Front. Microbiol. 7 (2016) 546, https://doi.org/10.3389/fmicb.2016.00546.
[23] R. Qiu, S. Gao, P.A. Lopez, K.L. Ogden, Effects of pH on cell growth, lipid production and CO2 addition of microalgae Chlorella sorokiniana, Algal Res. 28 (2017) 192–199, https://doi.org/10.1016/j.algal.2017.11.004.
[24] Yonggang Zhang, L. Ren, H. Chu, X. Zhou, T. Yao, Yalei Zhang, Optimization for Scenedesmus obliquus cultivation: the effects of temperature, light intensity and pH on growth and biochemical composition, Microbiol. Biotechnol. Lett. 47 (2019) 614–620, https://doi.org/10.4014/mbl.1906.06005.
[25] H. Sun, W. Zhao, X. Mao, Y. Li, T. Wu, F. Chen, High-value biomass from microalgae production platforms: strategies and progress based on carbon metabolism and energy conversion, Biotechnol. Biofuels 11 (2018) 227, https://doi.org/10.1186/S13068-018-1225-6.
[26] H. Auta, Ultra Scale-down Process Synthesis of Microalgae Primary Recovery Operations, University College London, 2017.
[27] R.K. Bharti, D.W. Dhar, R. Prasanna, A.K. Saxena, Assessment of biomass and lipid productivity and biodiesel quality of an indigenous microalga Chlorella sorokiniana MIC-G5, Int. J. Green Energy 15 (2018) 45–52, https://doi.org/10.1080/15435075.2017.1351368.
[28] G. Salbitani, S. Carfagna, Ammonium utilization in microalgae: a sustainable method for wastewater treatment, Sustainability 13 (2021) 956, https://doi.org/10.3390/su13020956.
[29] Y. Gao, A. Tahmasebi, J. Dou, J. Yu, Combustion characteristics and air pollutant formation during oxy-fuel co-combustion of microalgae and lignite, Bioresour. Technol. 207 (2016) 276–284, https://doi.org/10.1016/J.BIORTECH.2016.02.031.
[30] B. Wang, Y. Li, N. Wu, C.Q. Lan, CO2 bio-mitigation using microalgae, Appl. Microbiol. Biotechnol. (2008), https://doi.org/10.1007/s00253-008-1518-y.
[31] M. Adamczyk, J. Lasek, A. Skawińska, CO2 biofixation and growth kinetics of Chlorella vulgaris and Nannochloropsis gaditana, Appl. Biochem. Biotechnol. 179 (2016) 1248–1261, https://doi.org/10.1007/s12010-016-2062-3.
[32] S. Abinandan, S. Shanthakumar, Evaluation of photosynthetic efficacy and CO2 removal of microalgae grown in an enriched bicarbonate medium, 3Biotech 6 (2016) 9, https://doi.org/10.1007/s13205-015-0314-5.
[33] X. Ma, Y. Mi, C. Zhao, Q. Wei, A comprehensive review on carbon source effect of microalgae lipid accumulation for biofuel production, Sci. Total Environ. 151387 (2021), https://doi.org/10.1016/J.SCITOTENV.2021.151387.
[34] T. Luangpipat, Y. Chisti, Biomass and oil production by Chlorella vulgaris and four other microalgae — effects of salinity and other factors, J. Biotechnol. 257 (2017) 47–57, https://doi.org/10.1016/j.jbiotec.2016.11.029.
[35] C.L. Wan Afifudeen, S.H. Loh, A. Aziz, K. Takahashi, A.W.M. Effendy, T.S. Cha, Double-high in palmitic and oleic acids accumulation in a non-model green microalga, messastrum gracile SE-MC4 under nitrate-repletion and -starvation cultivations, Sci. Rep. 11 (2021) 381, https://doi.org/10.1038/s41598-020-79711-2.
[36] M. Blasio, S. Balzano, Fatty acids derivatives from eukaryotic microalgae, pathways and potential applications, Front. Microbiol. 12 (2021) 2689, https://doi.org/10.3389/FMICB.2021.718933/BIBTEX.
[37] K. Kumar, D. Banerjee, D. Das, Carbon dioxide sequestration from industrial flue gas by Chlorella sorokiniana, Bioresour. Technol. 152 (2014) 225–233, https://doi.org/10.1016/j.biortech.2013.10.098.
[38] H. Wu, X. Miao, Biodiesel quality and biochemical changes of microalgae Chlorella pyrenoidosa and Scenedesmus obliquus in response to nitrate levels, Bioresour. Technol. 170 (2014) 421–427, https://doi.org/10.1016/j.biortech.2014.08.017.
[39] Q.V. Bach, W.H. Chen, A comprehensive study on pyrolysis kinetics of microalgal biomass, Energy Convers. Manag. 131 (2017) 109–116, https://doi.org/10.1016/j.enconman.2016.10.077.
[40] S. Paniagua, L.F. Calvo, C. Escapa, R.N. Coimbra, M. Otero, A.I. García, Chlorella sorokiniana thermogravimetric analysis and combustion characteristic indexes estimation, J. Therm. Anal. Calorim. 131 (2018) 3139–3149, https://doi.org/10.1007/s10973-017-6734-1.
[41] A. Raheem, V. Dupont, A.Q. Channa, X. Zhao, A.K. Vuppaladadiyam, Y.H. Taufiq-Yap, M. Zhao, R. Harun, Parametric characterization of air gasification of Chlorella vulgaris biomass, Energy Fuels (2017), https://doi.org/10.1021/acs.energyfuels.6b03468.
[42] W.-H. Chen, Y.-S. Chu, J.-L. Liu, J.-S. Chang, Thermal degradation of carbohydrates, proteins and lipids in microalgae analyzed by evolutionary computation, Energy Convers. Manag. 160 (2018) 209–219, https://doi.org/10.1016/j.enconman.2018.01.036.
[43] A.M. Rizzo, M. Prussi, L. Bettucci, I.M. Libelli, D. Chiaramonti, Characterization of microalga Chlorella as a fuel and its thermogravimetric behavior, Appl. Energy 102 (2013) 24–31, https://doi.org/10.1016/j.apenergy.2012.08.039.
[44] M.A.P. Muniz, M.N.F. Dos Santos, C.E.F. Da Costa, L. Morais, M.L.N. Lamarão, R. M. Ribeiro-Costa, J.O.C. Silva-Júnior, Physicochemical characterization, fatty acid composition, and thermal analysis of Bertholletia excelsa HBK oil, Pharmacogn. Mag. 11 (2015) 147–151, https://doi.org/10.4103/0973-1296.149730.