Cultivation Medium Design Via Elemental Balancing for *Tetraselmis suecica*

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The primary requirements for high-biomass-concentration microalgal cultivation include a photon source and distribution, efficient gas exchange and suitable growth medium composition. However, for mass outdoor production of microalgae, growth medium composition is a major controlling factor as most of the other factors such as light source and distribution are virtually uncontrollable. This work utilises an elemental balance approach between growth medium and biomass compositions to obtain high-density microalgal cultures in an open system. F medium, commonly used for the cultivation of marine microalgae such as *Tetraselmis suecica* was redesigned on the basis of increasing the biomass capacity of its major deficient components to support high biomass concentrations ($\varphi \sim 5.0$ % for N, S and $\varphi \sim 10$ % P), and the entire formulation was dissolved in 0.2 μm sterile filtered natural seawater. Results show that the new medium (F′) displayed a maximum biomass concentration and total lipid concentration of 1.29 g L^{-1} and 108.7 mg L^{-1} respectively, which represents over 2-fold increase compared to that of the F medium. Keeping all variables constant except growth medium, and using F medium as the base case of 1 medium cost (MC) unit mg^{-1} lipid, the F′ medium yielded lipid at a cost of only 0.35 MC unit mg^{-1} lipids. These results show that greater amounts of biomass and lipids can be obtained more economically with minimal extra effort simply by using an optimised growth medium.

Key words:
Microalgae, growth medium, elemental balancing, flue gas, biomass, economics

Introduction

The past few years have witnessed considerable activities relating to the production of microalgae for commercial purposes. From a modest beginning in the late fifties, new endeavours have emerged as specialised industries are aiming to produce animal feed, food additives, biofertilizers and different natural products using microalgae.[1,2] Production of biodiesel from lipids extracted from microalgae has been of recent interest due to increasing petrol prices resulting from fossil fuels depletion, and/or the alarming rate of global warming issues.[3–5] Biodiesel is mainly produced via *trans*-esterification reaction of triglyceride molecules present in microalgal lipids with alcohol, such as methanol. In this process, methyl/ethyl alcohol is deprotonated with a base which turns it into a stronger nucleophile to convert lipid triglycerides into methyl/ethyl-esters.[4,5] Microalgal biomass is obtained from a renewable source, does not require a large area of land for cultivation, possesses a high growth rate and accumulates a satisfactory amount of lipid for biodiesel production. Stationary phase microalgal lipids are mostly neutral lipids due to their lower degree of unsaturation and their accumulation in the microalgal cells at the early or late end of growth stage depending on the strain.[6,7] A variety of photo-bioreactors have been utilised for microalgae production.[8–10] Large open outdoor pond cultivation is one of the earliest industrial systems for microalgal cultivation. Closed systems have been described for the production of microalgal biomass and some fine chemicals.[11,12] Enhancements in the performance of different photo-bioreactors have been obtained by optimising illuminating techniques, efficient gas and liquid exchange units and controlled nutrient supply.[13,14] However, few studies have focused on detail medium optimisation for the production of high-density microalgal biomass. Growth medium formulation dramatically affects the performance of microalgal cultivation processes, especially for an open photo-bioreactor system where most of the major cultivation factors are virtually uncontrollable.[15] The type, source, concentration and the proportion of elemental ingredients present in the cultivation medium affect the amount of biomass produced, as well as lipid volumetric titres. It is also probable that nutrient concentration directly bears on the physiology of the microalgal cells by influencing their intricate regulatory systems and therefore controls their mitosis or proliferation mechanism as well as lipid production.

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In a typical aerated closed transparent photo-bioreactor exposed to the atmosphere, growth inhibition is likely to occur through the depletion of specific major nutrients in the growth medium or the secretion of inhibitory compounds by the microalgal strain.[15] Secretion of growth inhibitors is usually not typical of most marine microalgae species including *Tetraselmis suecica*.[16] Hence, growth medium optimisation is essential to achieve high microalgal biomass levels. A number of growth media for the cultivation of microalgae have been formulated. Many of these are derived from chemical analysis of the natural habitats of the microalgae.[17] There exists few growth media designs based on detailed study of nutrient requirements and uptake.[15,18] The major elements required for the growth of microalgae are N, P, K, Mg, Ca, S, Fe, Cu, Mn and Zn and these elements are added in the form of inorganic or organic salts.[15,19,20] It has also been observed that the nutrient uptake is a function of various factors including biomass density, light intensity and distribution, temperature, and pH.[15,21] Generally, reports on the experimental investigations of the effects of optimising biomass and lipid production from a growth medium perspective is limited. This limitation makes growth medium selection more of a trivial process and this contributes partly to the routine attainment of low microalgae biomass and lipid yields. This work is geared toward the optimisation of growth medium composition based on the elemental composition of the microalgal biomass. The optimisation of growth medium composition based on the cellular biomass content renders the physiological nature of the cells intact, improves lipid storage capacity and enhances the proliferation rate of the microalgae cells. This will potentially have a positive influence on biomass and lipid concentrations. An economic evaluation of the performance of the growth medium (as in cost of medium per mg of lipid produced) which has generally not been considered in the literature to date is outlined here. Also, analysis of CO_2 fixation rate of microalgae cultivation systems is presented in this paper.

Experimental

Materials

Potassium nitrate (KNO_3) (BDH Chemicals Pty Ltd, MW 101.10, 99.0 %), sodium phosphate monobasic (NaH_2PO_4) (Merck Pty Ltd, MW 119.98, 99.5 %), manganese chloride ($MnCl_2$) (BDH Chemicals Pty Ltd, MW 125.84, 98.0 %), zinc sulphate ($ZnSO_4$) (BDH Chemicals Pty Ltd, MW 161.47, 99.5 %), cobalt nitrate ($Co(NO_3)_2$) (BDH Chemicals Pty Ltd, MW 182.94, 97.5 %), copper sulphate ($CuSO_4$) (Prolabo Pty Ltd, MW 159.61, 98.5 %), sodium molybdate ($Na_2MoO_4 \cdot 2H_2O$) (AJAX Chemicals Pty Ltd, MW 241.95, 98.0 %) and glycerol ($C_3H_5(OH)_3$) (Merck Pty Ltd, MW 92.10, 99.5 %) were used for growth medium preparation for microalgal cultivation, hexane ($CH_3(CH_2)_4CH_3$) (Sigma-Aldrich, MW 86.18, 99.0 %) was used for lipid extraction from microalgae and methanol (CH_3OH) (Sigma-Aldrich, MW 32.04, 99.0 %) was used for chlorophyll extraction from microalgae.

Microalgae strain

The microalgal strain used throughout this body of work is *Tetraselmis suecica* cultivated in bag photo-bioreactor located at Monash University, Clayton (Victoria, Australia). The culture was obtained from CSIRO Microalgae Research Centre (Hobart, Australia). *Tetraselmis suecica* is a marine green flagellate with a satisfactory amount of lipid, thus making it a potential source for biodiesel production.[22]

Growth medium preparation

F medium commonly used for the cultivation of most marine microalgae is used as a base medium to develop an optimised medium. This work seeks to develop an optimum growth medium to boost the biomass capacities of the vital elemental ingredients present in F medium by balancing the elemental composition of the microalgae with that of the growth medium. This technique has previously been reported by Mandalam and Palsson, 1998.[15] The elemental composition of *Tetraselmis* species (in mole per mole of phosphorus) as reported by Ho *et al.* 2003[23] is shown in Table 1. The source of carbon for microalgae growth is CO_2 which will be supplied by flue gas from Monash University Engineering boiler house, and compressed air mixture sparged into the bag photobioreactor. The remaining elements N, P, S, K, Mg, Ca, Sr, Fe, Mn, Zn, Cu, Co, Cd and Mo must be provided by the growth medium. The composition of F medium is as shown in Table 2. The elemental constituents of F medium were evaluated for biomass capacities based on the average values of the dry cell mass composition. The concentration of each element in the F medium (as in gram of element per litre of F medium) was calculated from the elemental compositions given in Table 2. The maximum biomass capacity of each element was determined by considering the concentration of each element, g L^{-1} of F medium and the fraction of the element in the dry cell mass as given in Table 1. The biomass capacity (%) of element i is calculated as in eq. (1).

$$\delta_i = \frac{\gamma_{i,E}}{w_f \rho_f w_E} \cdot 100 \quad (1)$$

Table 1 – *Elemental composition of Tetraselmis sp. as reported by Ho et al., 2003*[23]

Elemental constituents	Compositions/ mol mol^{-1} P	Average mass fraction, $\overline{w}$/%
C	199 ± 43	81.626
N	26 ± 5.3	12.442
P	1	1.059
S	1.33 ± 0.01	1.455
K	1.47 ± 0.04	1.959
Mg	0.23 ± 0.01	0.188
Ca	0.86 ± 0.019	1.176
Sr	$(25 \pm 1.01) \cdot 10^{-3}$	0.075
Fe	$(3.9 \pm 0.42) \cdot 10^{-3}$	$7.451 \cdot 10^{-3}$
Mn	$(4.0 \pm 0.25) \cdot 10^{-3}$	$7.520 \cdot 10^{-3}$
Zn	$(0.44 \pm 0.075) \cdot 10^{-3}$	$9.844 \cdot 10^{-4}$
Cu	$(0.51 \pm 0.009) \cdot 10^{-3}$	$1.107 \cdot 10^{-3}$
Co	$(0.112 \pm 0.018) \cdot 10^{-3}$	$2.256 \cdot 10^{-4}$
Cd	$(0.15 \pm 0.007) \cdot 10^{-3}$	$5.777 \cdot 10^{-4}$
Mo	$(0.028 \pm 0.006) \cdot 10^{-3}$	$9.229 \cdot 10^{-5}$

Where δ_i is the biomass capacity of element *i*, $\gamma_{i,E}$ is the mass of element *i* per litre of F medium, w_f is the dry mass fraction of fresh cells, w_E is the mass fraction of element *i* in dry mass of cell and ρ_f is the density of fresh microalgal cells. Dry mass fraction and density of the microalgal cell were taken to be ~0.25 and ~1.01 g mL^{-1} respectively.[24] The biomass capacity of each element in F medium is as shown in Table 3. The results show that the medium is deficient of N, P and S and zero amounts of Mg and Ca, and the lack of these vital nutrients presents various effects on the growth of the microalgal cells. For example, the absence or limitation of Ca and Mg results in growth retardation and reduction of photosynthetic activity and chlorophyll content. Also, cell mitosis is hindered in sulfur-deficient microalgae cultures.[25–27] The results also show that F medium is rather rich in micronutrients and trace metals such as Mn, Zn, Cu, Co, Mo and Fe. F medium was redesigned by increasing the biomass capacities of each deficient macronutrient (φ ~ 5.0 % for N, S and φ ~ 10.0 % P) to support high biomass concentration. The concentrations of the micronutrients (Mn, Co, Mo and Fe) were slashed down to a third as their concentrations exceeded the maximum biomass capacity; thus impacting positively on the cost of medium preparation. The composition of the new medium F′ is as shown in Table 2. The entire formulation

Table 2 – *Compositions of F and F′ growth media in mg compound per L of solution*

Nutrient compounds	F medium compositions/ mg L^{-1}	F′ medium composition/ mg L^{-1}
KNO_3	150	1155
NaH_2PO_4	11.3	103
$MnCl_2$	7.2	2.4
$ZnSO_4$	0.4	4.5
$Co(NO_3)_2$	0.2	0.07 ~ 0.1
$CuSO_4$	0.196	0.71
Na_2MoO_3	0.126	0.04 ~ 0.1
$FeC_6H_5O_7$	9	3

Table 3 – *Calculated biomass capacities of the elemental constituents of F and F′ growth media*

Elemental constituents	F medium biomass capacity, φ/%	F′ medium biomass capacity, φ/%
N	0.67	5.12
P	1.01	9.95
S	0.03	4.98
K	11.72	90.12
Mn	165.81	55.27
Zn	65.20	65.2
Cu	27.84	27.84
Co	113.36	37.79
Mo	272.89	90.96
Fe	109.98	36.66

was dissolved in 0.2 μm sterile filtered natural seawater with an ionic fraction of ~3.4 % obtained from Chelsea Beach (Victoria, Australia). The availability of Ca^{2+} and Mg^{2+} ions in seawater supplemented the F′ growth medium as a source of calcium and magnesium for microalgae growth.

Simple outdoor cultivation of microalgae in bag photo-bioreactor

Two φ = 20 % inoculum cultures were established in two 100 L outdoor bag photo-bioreactors containing F and F′ growth media with compositions dissolved in 0.2 μm sterile filtered natural seawater as shown in Fig. 1. The temperatures of the cultures were recorded to be between 17–24 °C, corresponding to ambient temperatures between 20–28 °C. The cultures were aerated with flue gas (sourced from Monash University Engineering boiler house) and compressed air mixture (20 : 80)

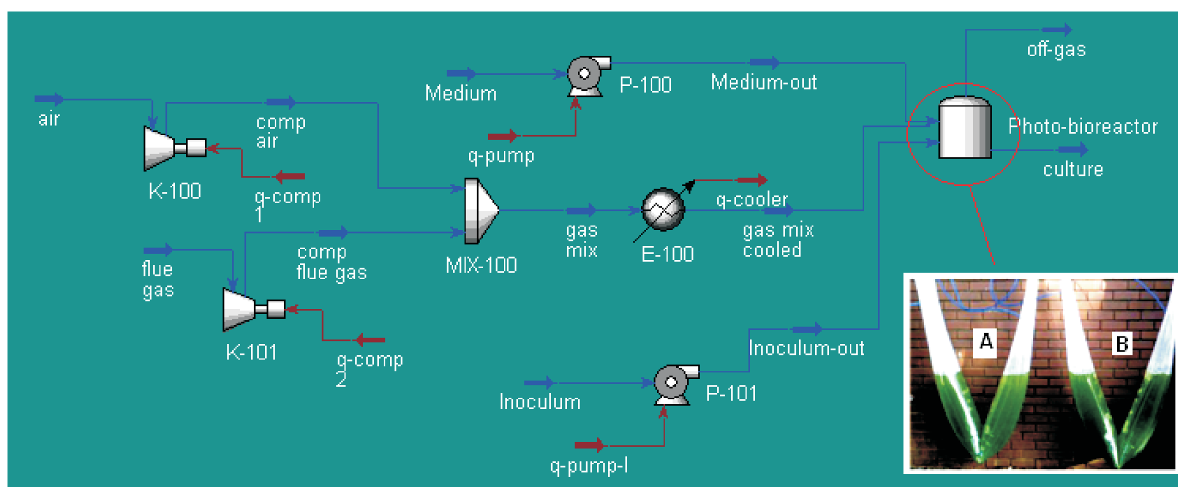


Fig. 1 – *Schematic diagram showing the flow of flue gas/compressed air system, inoculum and growth medium into the bag photo-bioreactors. The flue gas/compressed air mixture is cooled first before feeding to the photo-bioreactor. Insert: Two bag photo-bioreactors for microalgae cultivation. Bag photo-bioreactor A contains F medium and 20 % microalgae inoculum, and bag photo-bioreactor B contains F' medium and 20 % microalgae inoculum. The picture was taken during the accelerating phase of the growth profile.*

at 34.5 kPa (gauge) mainly as a source of CO_2. A schematic view of the gas systems, growth medium and inoculum additions is shown in Fig. 1. The sparging rate of the gas also creates uniform culture mixing. The composition of the original flue gas stream is N_2 (78–80 %), CO_2 (11.7–12.5 %), O_2 (4.8–5.9 %), CO ($70–110 \cdot 10^{-6}$), SO_2 ($418–430 \cdot 10^{-6}$), NO_x ($120–130 \cdot 10^{-6}$) and ash (48.5–53.4 mg L^{-1}). Dry cell mass concentration, lipid concentration, and culture pH were monitored offline by taking daily samples in the early mornings before sunrise and/or afternoons before sunset. The evaporation rate of water from the cultures was estimated to be less than 60 mL d^{-1}. The cultivation was terminated after the stationary growth phase was obtained in the two media.

Determination of dry cell mass concentration of microalgae

The microalgal cultures were mixed thoroughly before each sample was collected. 50 mL aliquot of the culture was pipetted and transferred into a 50 mL centrifuge tube. Centrifugation (Heraeus, multifuge 3S-R, Germany) was performed at 4500 × g rpm for 20 min. The supernatant was carefully poured off from the tube in order not to disturb the pellet or pour off any unsettled material. The pellet from the centrifugation was rinsed with 10 mmol L^{-1} HCl solution, transferred to a pre-weighed heat-resistant crucible and dried at 105 °C in an oven overnight.[28] The sample was removed from the oven, kept in a desiccator and weighed. This weight minus the weight of the empty crucible gives the dry cell mass which was converted to dry cell mass concentration by dividing it by the sample culture volume. Samples were generally run in triplicate for each data point.

Solvent extraction of chlorophyll *a* and *b*

Chlorophyll *a* and *b* were extracted from the microalgal culture samples using 100 % methanol. The samples were diluted 10 times by methanol, wrapped with aluminum foil and kept at 4 °C for 40 min. The samples were then centrifuged at 10 000 × g rpm for 10 min. The absorbance of the green supernatant was measured at two wavelengths: λ = 650 and 665 nm. The chlorophyll *a*, chlorophyll *b* and total chlorophyll concentrations in mg L^{-1} were then calculated using the equations described by Hitkins and Baker, 1986.[29]

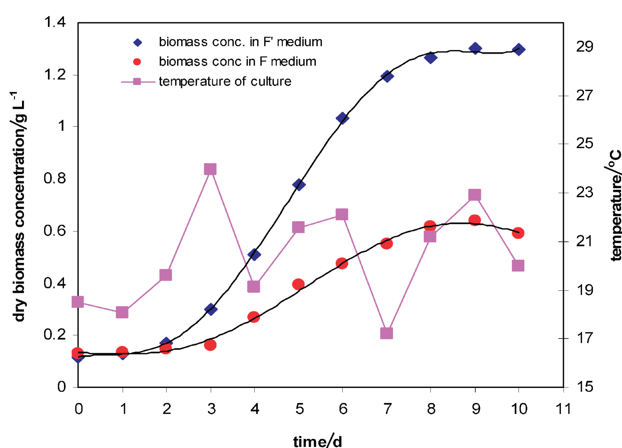
Solvent extraction of lipid from microalgae biomass

Oven dried microalgae biomass samples were pulverized and interacted with 20–40 mL of 99 % pure hexane. The mixtures were tightly covered, vortexed for 30 min and incubated overnight at 30 °C. The resulting mixture was filtered and the biomass residue was washed twice with the same volume of hexane and added to the extracted fraction. The hexane fraction was evaporated to dryness and the residual extract, mainly lipids, was weighed and the total lipid content determined gravimetrically. Each of the lipidic extracts obtained was analyzed by gas chromatography to determine their fatty acid composition.[30] All experiments were carried out in triplicate.

Results and discussion

Effect of growth medium composition on biomass concentration

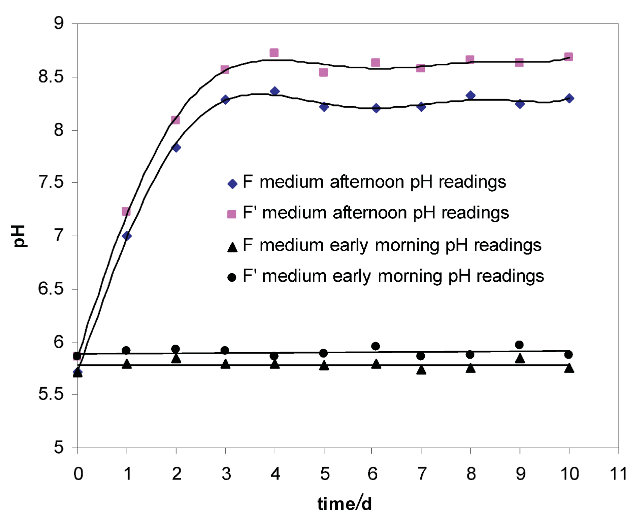
The biomass growth rate of microalgal cells in F and F′ growth media and daily temperature readings of the culture suspensions during cultivation are shown in Fig. 2. Different biomass growth profiles of the microalgae cells were portrayed by the different media. The results show a general increase in the biomass concentration up to 0.58 g L^{-1} and 1.29 g L^{-1} in the stationary phase for F and F′ growth media respectively. The exponential phase of the microalgal cultivation process exists from day 3 to day 6, where the biomass concentration increased from 0.16 g L^{-1} to 0.48 g L^{-1} for F medium and 0.3 g L^{-1} to 1.04 g L^{-1} for F′ medium. This is the region with the highest differential biomass growth per unit time and F′ medium displays a better biomass concentration increment in this phase than F medium. The growth rate of the cells in the different media was about the same in the first 2 days of lag period. The microalgae growth in F medium started stabilizing after 6 days to reach a maximum concentration of 0.64 g L^{-1} and declined slightly to 0.58 g L^{-1}, whilst F′ medium increased through the exponential phase and stabilised at 1.29 g L^{-1}. The significant variation existing in the cell density profiles of the two media is attributed to differences in the amount of active nutrients present in the different media. The declination of microalgae concentration after day 9 is due to the exhaustion of active nutrients present in the medium. Microalgal cells in F and F′ media attained a maximum growth rate of 0.17 and 0.33 respectively. The cultivation was halted in day 10.



F i g . 2 – *Dry biomass concentration and temperature profiles for 2 x 100 L outdoor cultivation of microalgae in bag photobioreactors containing F and F′ growth media. F′ medium displays a higher final biomass concentration than F medium. Both cultures displayed a temperature range of 17–24 °C. The results represent the average values of 3 replicates (n = 3, σ ≤ 0.03).*

Effect of growth medium composition on pH and dissolved CO_2 profiles

F and F′ media cultures presented different pH profiles during the cultivation process. The results show that pH values recorded for F′ medium are higher than that of F medium, and this is due to the different consumption rates of CO_2 by microalgal cells present in the different media. Microalgal cells in F′ medium are fed with stoichiometrically optimised nutrients in the right proportion; hence have a higher metabolic rate than those of F medium. Also, nitrate uptake generates alkalinity. Higher nitrate concentrations are generally accompanied by higher absorption rate and an increase in alkalinity. This results in a higher CO_2 consumption rate by the cells in F′ medium, thus, increasing its pH. According to Fig. 3, the daily (afternoon) pH of the cultures increased rapidly from 5.7 to a maximum value of 8.4 for F medium, and 5.8 to a maximum of 8.7 for F′ medium after the introduction of the inoculum. This is due to the initial uptake of CO_2 by the microalgal cells during the day. The daily pH values of the cultures decreased slightly and stabilised at 8.2 and 8.4 respectively for F and F′ media after the fifth day – resulting from the buffering capacity of the seawater and the complete adaptation of the microalgal cells to the culture environment. During the night, the pH values of the cultures decrease massively as the microalgal cells do not photosynthesise (do not make use of CO_2) but undergo respiration to release more CO_2 which turns the culture suspension acidic with pH ~5.7 for both F and F′ media. This result is confirmed by early morning and afternoon quantifications of dissolved CO_2 levels during the cultivation process. As shown in Fig. 4, the afternoon dissolved CO_2 level de-



F i g . 3 – *Early morning and afternoon pH profiles during the cultivation of microalgae in outdoor bag photobioreactors using F and F′ media. The results represent the average values of 3 replicates (σ ≤ 0.05).*

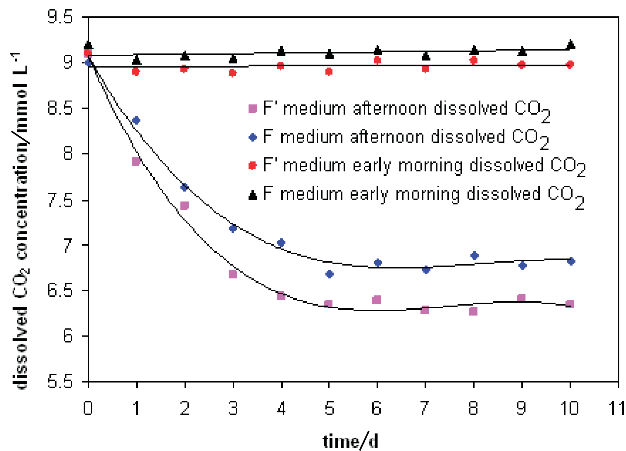


Fig. 4 – *Early morning and afternoon dissolved CO_2 concentration profiles during the cultivation of microalgae in outdoor bag photobioreactors using F and F′ media. The results represent the average values of 3 replicates ($\sigma \leq 0.04$).*

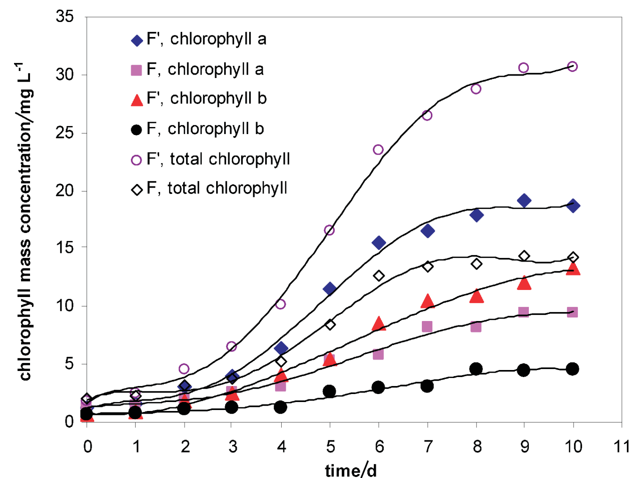


Fig. 5 – *Concentration profiles for chlorophyll a, chlorophyll b and total chlorophyll content during the cultivation of microalgae in outdoor bag photobioreactors using F and F′ growth media. The results represent the average values of 3 replicates ($\sigma \leq 0.11$).*

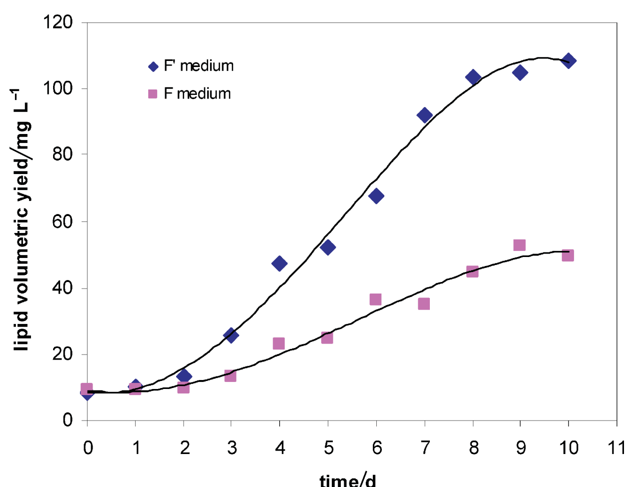
creased from 9.05 mmol L^{-1} to 6.83 mmol L^{-1} for F medium, and from 9.10 mmol L^{-1} to 6.34 mmol L^{-1} for F′ medium during the first 4 days after inoculation and remained stationary afterwards, whilst the early morning dissolved CO_2 level maintained a constant and the similar value of ~9.12 mmol L^{-1} for both F and F′ media after inoculation. Dissolved CO_2 quantification was performed by titrating the culture samples with standard HCl to determine the stoichiometric amount of HCO_3^- converted into CO_2 gas. In brief, there is a maximum accumulation of CO_2 during the dark phases of the cultivation cycle when the microalgal cells do not photosynthesise but rather respire. Hence, low pH levels are encountered during the night.

Dependency of chlorophyll concentrations on growth medium composition

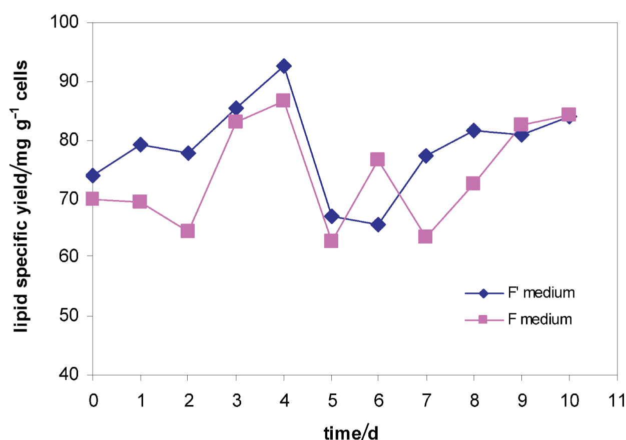
A significant difference was observed in the concentration profiles of chlorophyll *a* and *b* and total chlorophyll content for the two media, F and F′, during the cultivation process. As shown in Fig. 5, chlorophyll *a* and *b* contents of F′ medium culture cells increased continuously to a maximum of ~19.1 mg L^{-1} and 13.4 mg L^{-1} respectively, and this is 2-fold higher than that of F medium in terms of chlorophyll *a,* and ~3-fold higher than that of F medium in terms of chlorophyll *b*. The higher chlorophyll content of cells in F′ medium reflects the enhanced capacity of the medium to maintain photoautotrophic cultures. The pattern of increase in total chlorophyll content was similar to that of biomass growth in the case of the F′ medium, but the scenario was different for F medium. The chlorophyll content in F medium cultures did not increase after the 6th day although its biomass increased up to the 8th day. The maximum total chlorophyll content achieved in F and F′ media cultures are 14.2 mg L^{-1} and 30.7 mg L^{-1} respectively. This study has used the simple cellular elemental composition as the basis for redesigning microalgal growth media stoichiometrically. It is often assumed that the limiting nutrients are nitrogen and phosphorus, and the other elements are always in excess in the medium so it is always suggested that addition of the major elements can increase the overall capacity of the medium in terms of biomass growth. However, a stoichiometric balance in the medium composition needs to be established in order to continuously increase the chlorophyll content as well as the biomass concentration.[15]

Analysis of lipid extracts from microalgae

Extraction of microalgal lipids from dry biomass with hexane is a very promising method as only non-polar intracellular molecules are extracted. This is because of the highly non-polar nature of hexane; hence there are no issues with co-extraction of chlorophyll. The concentrations of the extracted lipids were analyzed gravimetrically and their fatty acid compositions were determined using gas chromatography. According to Fig. 6, analysis of lipid samples extracted from F and F′ media biomass showed different lipid yield kinetics. After 2 days of near negligible lipid production, the volumetric yield of the lipid extract increased over the next 8 days reaching 47.9 mg L^{-1} and 108.7 mg L^{-1} for F and F′ media respectively. There is a clear distinction between the lipid extract volumetric yield characteristics of F and F′ media; this is attributed to the compositional balance and stoichiometrically designed F′ growth medium con-



F i g . 6 – *Lipid volumetric yield profiles during the cultivation of microalgae in outdoor bag photobioreactors using F and F′ growth media. The results represent the average values of 3 replicates (σ ≤ 0.15).*



F i g . 7 – *Lipid specific yield profiles during the cultivation of microalgae in outdoor bag photobioreactors using F and F′ growth media. The results represent the average values of 3 replicates.*

taining optimized concentrations of major and minor nutrients necessary for lipid production, hence presents a higher lipid yield profile. Fig. 7 shows the lipid specific yield trends for the different media, F and F′. The specific yield of lipid from the microalgae was determined between 62.5–92.6 mg g^{-1} cells, with a maximum of 92.6 mg g^{-1} cells for F′ medium and 86.5 mg g^{-1} cells for F, both occurring in day 4. The higher maximum specific yield of lipid obtained from the F′ medium has an advantageous effect on lipid purity since the quantity of unwanted intracellular molecules existing with the lipid extract molecules per unit mass of cell is minimal. Lipid extracted from F and F′ media biomass displayed an identical fatty acid profile and this is shown in Table 4.

T a b l e 4 – *Fatty acid profile of lipids extracted from microalgae obtained from F and F′ media cultures*

Abbreviation	Fatty acid	% FAME
16:00	hexadecanoic acid	23
16:1 w5	11-hexadecenoic acid	2.9
16:1 w7	9-hexadecenoic acid	2.5
16:02	7,10-hexadecadienoic acid	1.6
16:03	7,10,13-hexadecatrienoic acid	5.8
20:05	5,8,11,14,17-eicosapentaenoic acid	11.7
18:00	octadecanoic acid	–
18:1 w9	9-octadecenoic acid	24.6
18:1 w7	11-octadecenoic acid	–
18:2 w6	9,12-octadecadienoic acid	5.1
18:3 w3	9,12,15-octadecatrienoic acid	22.7

Economic analysis and CO_2 fixation

The cost inventory for typical batch microalgal cultivation for high-density microalgae biomass production and lipid extraction encompasses all materials and equipment for cultivation and extraction. The cost of lipid extraction is disregarded as it is identical for the two media, hence it will not affect the comparative cost analysis. For an open system of production where most of the parameters are environmentally dependent, the medium is a key critical input as it has direct impact on the biomass yield, lipid yield (as shown earlier) and the cost. Growth medium selection for microalgal growth and lipid production is often based on following previously developed protocols or, at best, trial and error with no prior cost per yield analysis. This is one of the main reasons for the low lipid yields attained even for expensive medium formulations. A medium cost (MC) per mg lipid analysis was performed for F and F′ medium using F medium as a base scenario of 1.0 MC mg^{-1} lipid. The cost of medium per mg lipid was calculated based on the following equation.

$$C_k = \frac{1}{m_{l,k}} \left(\sum_{i=1} \alpha_{i,k} m_{i,k} + \sum_{j=1} \beta_{j,k} V_{j,k} \right) \quad (2)$$

Where C_k is the total cost of medium k per unit mass of lipid, $m_{l,k}$ is the mass of lipid produced from biomass from medium k, $\alpha_{i,k}$ is the cost per unit mass of components i existing in medium k, $m_{i,k}$ is the mass of component i present in medium k, $\beta_{j,k}$ is the cost per unit volume of components j existing in medium k, and $V_{j,k}$ is the volume of component j present in medium k. From the results obtained from the economic study, the cost of F′ medium per mg lipid was 0.35 MC. F′ medium in-

creased its lipid yield to an extent that reduced its cost of medium per mg lipid drastically. However, costs of compressed air and utilities cannot be omitted in a complete cost evaluation of the batch run. Considering this medium cost evaluation, there is a great cost per yield advantage of using F′ medium for small-scale batch production as well as large-scale production of lipid from microalgae.

In determining the CO_2-capture attributed to microalgae, Benemann and Oswald, 1996[31] determined that the ash-free dry mass of microalgae may be multiplied by 2.193 to obtain the mass of CO_2 fixation. The elemental compositions of the microalgae ash-free dry mass, microalgae cell culture supernatants and photosynthetic rates are useful to determine CO_2 fixation levels. A mathematical model of the growth of microalgae on CO_2 as a substrate is useful to understand the mass transfer kinetics of the system in order to quantify and maximize CO_2 consumption and the microalgae growth rate. Important parameters such as the mass transfer co-efficient, volumetric, specific and time yields are needed as the key parameters of the system. Fig. 8 shows the CO_2 fixation profiles obtained for F and F′ growth media cultures. Generally and as expected, the CO_2 fixation rate increased rapidly during the exponential growth phase and stabilised in the stationary growth phase. F′ medium displayed a higher CO_2 capturing rate than F medium, thus presents a better carbon audit than F medium.

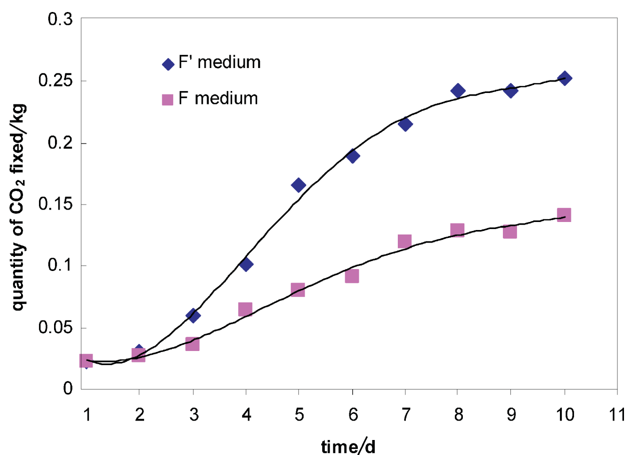


Fig. 8 – *CO_2 fixation profiles during the cultivation of microalgae in outdoor bag photobioreactors using F and F′ growth media*

Conclusion

The vast range of industrial uses of microalgae requires the need for high-density viable cultures. The main objective of this body of work was to display the achievement of higher biomass concentration and hence lipid yield economically simply by using an optimized and elementally balanced growth medium. The pivotal factor here is the medium cost (MC) per unit mass of lipid produced. Since most outdoor microalgae cultivation practices have a high degree of uncontrollability and are valued identical in terms of compressed air usage, sunlight, utilities, pH and temperature, the medium composition is the main factor that differentiates cultivation yields and cost in a comparative study. It is therefore essential to know how much is consumed and how much is being produced. This enables increased productivity using cheaper growth medium materials. The increase in biomass concentration, chlorophyll content and hence lipid yield in F′ medium cultures has demonstrated the enhanced capacity of the medium to support high-density cultures for the development of vital consumer products. This study has underlined the significance of medium development in achieving high-density cultures for lipid and chlorophyll production.

List of symbols

C – total cost, mg^{-1}
m – mass; mg, g
n – number of replicates
V – volume, L
w – mass fraction, %, 10^{-6}
β – cost per unit volume, L^{-1}
α – cost per unit mass, g^{-1}
γ – mass concentration, mg L^{-1}
δ – biomass capacity, %
ρ – density, mg L^{-1}
φ – volume fraction, %
λ – wavelength, nm

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