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Effects of Light Exposure and Nitrogen Source on the Production of Oil from Freshwater and Marine Water Microalgae

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Abstract: The biomass yield and oil content of *Chlorella saccharophila* (freshwater) and *Tetraselmis suecica* (marine) microalgae were investigated using various nitrogen source (ammonium nitrate, ammonium phosphate, ammonium sulfate and combination of nutrients) at various light durations (9, 16 and 24 h). $NaHCO_3$ was used as the carbon source. The nitrogen concentration, temperature and pH were maintained at 70 mg/L, 22°C and 8.5, respectively. The results indicated that *T. suecica* produced higher cell yield compared to the *C. saccharophila* under all levels of parameters tested. Light exposure of 24 h produced the highest biomass yield. However, the difference in cell yields between light duration of 16 and 24 h was not significant. The combination of nutrients resulted in the highest growth for both species of microalgae. However, high growth did not necessarily result in high oil yield. The oil content was much higher for *C. saccharophila* than *T. suecica.* Varying light duration had no direct effect on oil yield. The nutrient type significantly influenced the production of oil. *C. saccharophila* produced the highest oil yield using ammonium phosphate while *T. suecica* achieved the highest oil yield using ammonium nitrate. The results indicated that high algal growth does not necessarily result in high oil yield. Both, the generation of new cells and storage of oil require energy. When the cells used energy for generation of new cells they store less oil. Thus, growing *C. saccharophila* using the combination of nutrients at 16 h light exposure would be the optimal growth conditions for producing oil for biodiesel production.

Keywords: Microalgae, Freshwater, Marine, $NaHCO_3$, CO_2, Nitrogen Source, Light, Biomass, Oil, Extraction

Introduction

The increase in the annual global energy consumption over the past century has relied heavily on fossil fuels (oil, coal and natural gas) for powering up cars, farms, factories and for production of electricity (Areva, 2011). The world consumption of crude oil, coal and natural gas in 2011 was 87.4 million barrels/day, 8 144 million short tons/day (4.64 billion barrels of oil/day) and 3 368 billion m^3 (2 118 barrels of oil/day), respectively (Barrientos and Soria, 2011). Fossil fuel burning has accelerated Carbon Dioxide (CO_2) emissions on a global scale from 1.1% per year in 1990 to more than 2.6% per year in 2010 (Adams, 2013). This has contributed to global warming which impacted all living organisms (Root *et al*., 2003). An increase in the earth's temperature has been attributed to a decline in the Adelilie penguins species, melting of glaciers, increased sea level and increased precipitation resulting in floods (Forcada *et al*., 2006). In Canada, forest fires, floods, insect infestations and drought have all been attributed to global warming (Epstein, 2000).

The environmental concerns associated with greenhouse gas emissions emphasise the need for alternative energy sources that are more environmentally friendly. Various types of biomass can be used as renewable energy sources that offer immediate prospects of producing liquid fuels such as biodiesel and bioethanol which can be used as substitutes for petroleum products (Singh and Gu,

2010). Using biofuels, offer the benefits of greater energy security, foreign exchange savings and reduced environmental effects (Balat, 2009; Kan, 2009; Yenikaya *et al*., 2009). Biomass feedstocks for energy production include food waste, municipal waste, agricultural waste, edible and nonedible oilseeds, aquatic plants and algae.

Microalgae, which are abundant in nature, can be used as an alternate fuel source because of their high growth rate and their ability to produce lipids that can be used for the production of biodiesel (Chisti, 2007; Hu *et al*., 2008; Song *et al*., 2008). The majority of lipids produced by microalgae have a low degree of unsaturation, making them a good energy source for replacement of fossil fuels (Singh and Gu, 2010). Microalgae are photosynthetic microorganisms capable of surviving in marine and fresh water environments, tend to have a much higher oil content than vegetable plants, can produce and store large amounts of oil without the production and release of harmful wastes into the environment, are extremely resilient and often unaffected by fluctuations in the environment and utilize the carbon dioxide for their growth, thus help reduce greenhouse gas emissions (Demirbas, 2010; Singh *et al*., 2010; Wahlen *et al*., 2011; Pokoo-Aikins *et al*., 2010; Demirbas *et al*., 2011).

Biodiesel, as a liquid fuel, can be produced by the transesterification of oil (triacylglycerols) extracted from microalgae by the addition of methanol and the use of a catalyst such as acid, alkali or enzyme (Leung *et al*., 2010; Demirbas and Demirbas, 2011; Wahlen *et al*., 2011; Demirbas, 2005; Chen *et al*., 2009). The waste generated from the microalgal biomass can be further utilized to produce other biofuels such as methane and ethanol via fermentation or used as animal feed and organic fertilizer (Chen *et al*., 2009; Demirbas, 2010; Demirbas, 2011). Biodiesel from microalgae generates the same amount of energy as that generated from petroleum diesel without the release of harsh compounds (NOx, SOx and hydrocarbons) into the atmosphere, it is biodegradable and nontoxic and it can be utilized in existing diesel engines without any modifications (Ulusoy *et al*., 2004; Demirbas, 2005; Kalam and Masjuki, 2005; Singh and Gu, 2010).

The main objectives of this study were: (a) to select two strains of microalgae, one freshwater and one marine microalgae based on growth and oil production, (b) to evaluate the effect of light duration (9,16 and 24 h) and nutrients type (ammonium nitrate, ammonium phosphate, ammonium sulfate and a mix of all three), while maintaining the nitrogen concentration at 70 mg/L and using $NaHCO_3$ as a carbon source, on the microalga biomass yield and oil content of these species.

Materials and Methods

Experimental Apparatus

A fully automated multiple open pond system (Fig. 1) consisted of a frame, 18 open pond units, a cooling unit, a lighting unit, a supernatant collection unit and control unit was used in this study.

The frame (244 cm in width ×41 cm in depth ×283 cm in height) consisted of three shelves (76 cm apart) and housed the open pond, light, cooling, water collection and control units. Each shelf was divided vertically into two sides by a 1.2 cm thick plywood sheet to provide a better control of light and feed.

The open pond unit consisted of six ponds, each was made of galvanized steel and was divided into three compartments (each was 38 cm in length ×38 cm in width ×12.5 cm in depth and can holds up to 18 L).

The lighting unit provided 430 hectolux of illumination per shelf (480 μmol m^{-2} s^{-1}) using a mixture of fluorescent and incandescent lamps (six 40 W cool white fluorescent lamps 122 cm in length and four 100 W incandescent bulbs) mounted on each shelf, that sit 100 cm away from the ponds.

A cooling unit was designed to continuously remove the heat produced by the lamps to avoid heating of the algae media on the upper and middle shelves. A 5 cm diameter PVC pipe (having 6 mm diameter holes spaced 6 cm apart and facing out) was placed under the backside of the ponds. Two metal blocks placed under each pond provided a 5 cm space between the pond and the lighting system of the shelf below it. A 5 cm diameter PVC pipe was attached vertically to the left side of the frame and acted as a manifold through which air was blown by means of a motor driven fan (Model AK4L143A Type 821, Franklin Electric, Bluffton, Indiana).

The supernatant from each tray was collected in a separate container (2.7 L each) located at the bottom of the system. The outlets were connected to plastic tubes of 1 cm outside diameter, which were passed through a specially designed solenoid valve.

A computer was used to operate and control the various components of the open pond system and record the various measurements. The light intensity was measured using a Quantum Sensor (SQ-316 Series, Apogee, Logan, Utah). The pH was measured using pH electrodes (EW-59001-65, Cole Parmer, Montreal, Quebec). The temperature was measured using thermocouples (WD-08541-12, Nova-Tech International, Houston, Texas). A basic computer program (BASIC Stamp Editor v 2.5) allowed the configuration of the operating frequency and duration

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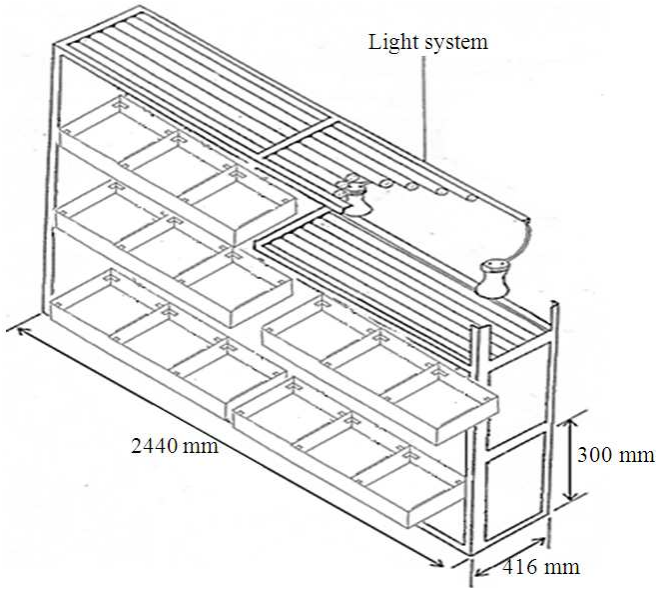
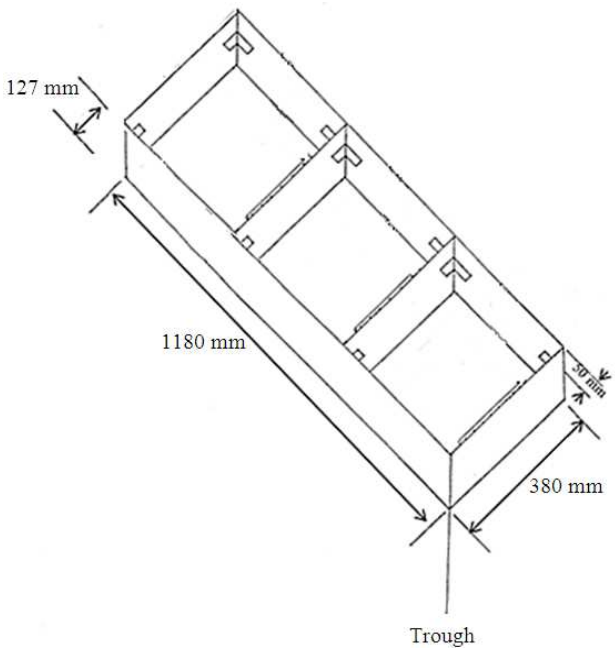
of the light, aeration unit and collection system. The computer was connected to a data coordinator (cDAQ-9178, National Instruments, Vaudreuil-Dorion, Quebec) which had 24 digital output ports and 24 digital input ports. The digital output ports were connected to electronic circuits which were responsible for the lighting, cooling and collection systems.

Microalgae

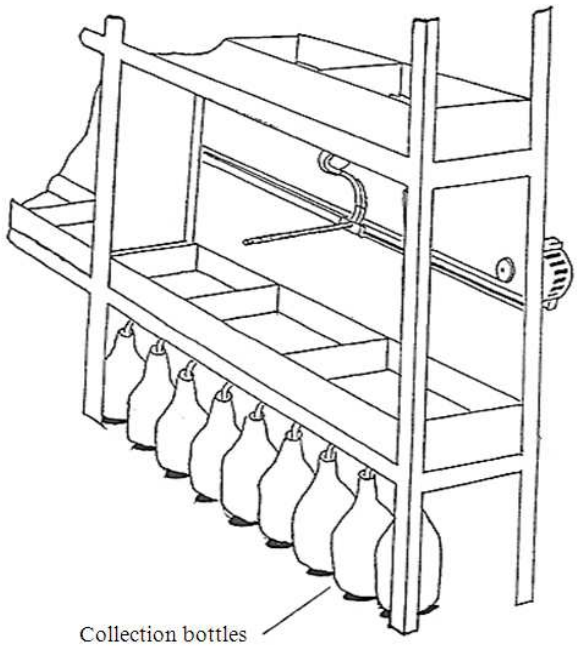
Eight freshwater microalgae (*Botrycoccus brauni, Chlorella protothecides, Chlorella pyrenoidsa, Chlorella saccharophila, Chlorella sorokiniana, Chlorella vulgaris, Chlorococcum sp.* and *Scenedesmus obliqus*) and six marine (*Chaetoceros muelleri, Isochrysis sp., Nannochloropsis sp., Pavlova salina, Phaeodactylum tricornutum, Skeletonema costatum* and *Tetraselmis suecica*) microalgae species were selected based on their ability to yield high biomass and store lipids (Table 1). The relationship between lipid content (%) and biomass yield (g/L) is



(a) Schematic view of the open pond system



(b) Open pond unit



(c) Collection unit

Fig. 1. Experimental apparatus

Table 1. Lipid contents, temperature and pH of freshwater and marine water microalgae species

Species	Biomass (g/L)	Lipid Content (%)	Lipid Yield (g/L)	Lipid Productivity (mg/L/d)	Temp. (°C)	pH	Reference
Freshwater							
Botryococcus brauni	1.84	25.20	0.46	5.51	20	7.6	Velichkova *et al*. (2012)
Chlorella protothecides	1.32	31.23	0.41	39.60	25	6.0	Liu *et al*. (2011); Shi *et al*. (2006)
Chlorella pyrenoidosa	2.84	38.95	1.11	107.90	25-30	7.4	Liu *et al*. (2011)
Chlorella saccharophila	3.88	45.46	1.76	153.38	20-24	7.5-9	Liu *et al*. (2011)
Chlorella sorokiniana	3.22	19.30	0.62	44.70	30	7-8	Rodolfi *et al*. (2009); Moronta *et al*. (2006)
Chlorella vulgaris	1.01	27.66	0.28	27.61	25-30	7.0	Liu *et al*. (2011)
Chlorococcum sp.	3.92	19.30	0.76	53.70	25-30	8-8.5	Rodolfi *et al*. (2009)
Scenedesmus obliqus	4.36	38.98	1.70	117.00	20-30	8.0	Liu *et al*. (2011)
Marine							
Chaetoceros muelleri	0.98	33.60	0.33	21.80	20-30	8.0	Rodolfi *et al*. (2009)
Isochrysis sp.	2.38	22.40	0.53	37.70	25	8.0	Rodolfi *et al*. (2009); Liu and Lin (2001)
Nannochloropsis sp.	2.80	24.40	0.68	48.20	20-25	8.4	Rodolfi *et al*. (2009); Spolaore *et al*. (2006)
Phaeodactylum tricornutum	3.36	18.70	0.63	44.80	25-30	8.0	Okauchi and Tokuda (2003)
Skeletonema costatum	1.12	21.10	0.24	17.40	25	7.4	Rodolfi *et al*. (2009); Yan *et al*. (2002)
Tetraselmis suecica	4.48	23.00	1.03	36.40	18-24	7-9	Rodolfi *et al*. (2009)

illustrated in Fig. 2 and 3 for the freshwater and marine microalgae species, respectively. The selection of the microalgae was based on the oil yield (g/L). The oil yield was calculated by determining the portion of the weighted biomass that corresponds to the lipid content, by multiplying the percentage that is made up of the lipids.

The freshwater strain *Chlorella saccharophila* was selected for the study because of its high lipid content (45%). This strain is capable of achieving a biomass yield of 3.88 g/L, which is not the highest among the freshwater species, but can however be offset by the fact that it achieves the highest lipid content. This results in a lipid yield of 1.75 g/L. The highest biomass yielding algae *Scenedesmus obliqus* of 4.34 g/L only achieves a lipid content of 38%, which intern results in a lipid yield of 1.69 g/L. In addition this species has the highest lipid productivity when compared to the other freshwater species. However, this was not the basis for selection since productivity can change depending on the length of growth and the phase of growth in which the productivity was measured. *Chlorella saccharophila* is a green unicellular microalga belonging to the Chlorella genus (Lewis, 1997). The cells have an average size of 7.3 μm (Bock *et al*., 2011). The cells contain a single chloroplast enclosed in a spherical or subspherical form (Fig. 4a). These cells reproduce asexually through production of non-motile autospores (John *et al*., 2002). This species is able to use glucose (Singh *et al*., 2013), bicarbonate and carbon dioxide as the carbon source for growth (Matsuda and Colmen, 1996). The optimal temperature and pH for growth are 20-24°C and 7.5-8, respectively.

The marine microalgae strain *Tetraselmis suecica* was selected for the study because of its high biomass yield of 4.48 g/L and comparatively high lipid content (23%). This species did not achieve the highest lipid content among the other species but can, however, be offset by the fact that it achieves the highest biomass yield. This results in a lipid yield of 1.03 g/L, while the *Chaetoceros muelleri* which has the highest lipid content of 34% and a biomass yield of 0.98 g/L results in a lipid yield of 0.33 g/L. The productivity value for this species is not the highest when comparing it to the other marine microalgae species. The productivity can change depending on the length of growth and the growth phase in which the productivity was measured. It is for this reason that the selection of the species was based on the lipid yield (g/L). *Tetraselmis suecica* grows as single cells. They are motile and can be compressed or curved (Fig. 4b), but they are never twisted (Acuna and Kiefer, 2000). The cells are spherical or elliptic with a length of 35 μm and a width of 14 μm. This species is able to use both sodium bicarbonate (White *et al*., 2012) and carbon dioxide (de Castro Araujo and Garcia, 2005) as the carbon source for growth. The optimal temperature and pH for growth are 18-24°C and 7-9, respectively (Lavens and Sorgeloos, 1996).

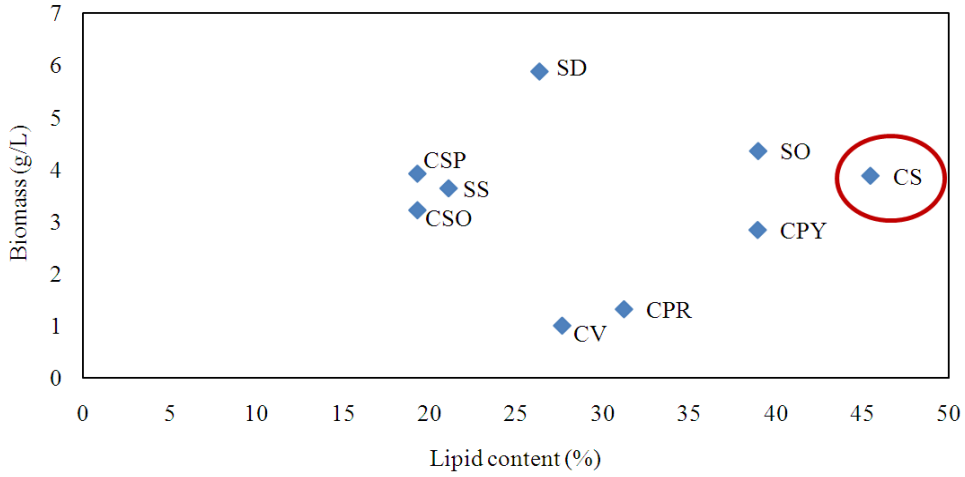


Fig. 2. Biomass Vs. lipid content of freshwater microalgae species (BB: *Botryococcus brauni*, CPR: *Chlorella protothecides*, CS: *Chlorella saccharophila*, CSO: *Chlorella sorokiniana*, CV: *Chlorella vulgaris*, CSP: *Chlorococcum* sp., SD: *Scenedesmus dimorphus*, SO: *Scenedesmus obliqus*, SS: *Scenedesmus* sp)

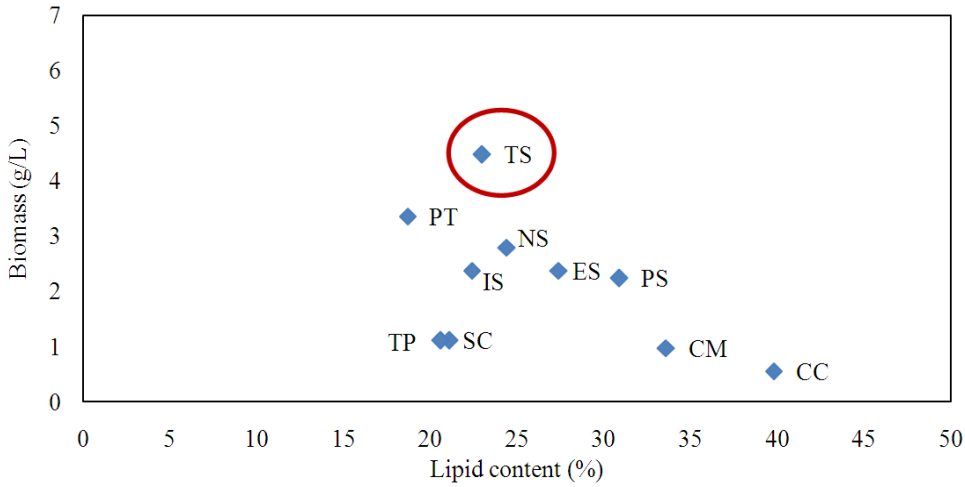


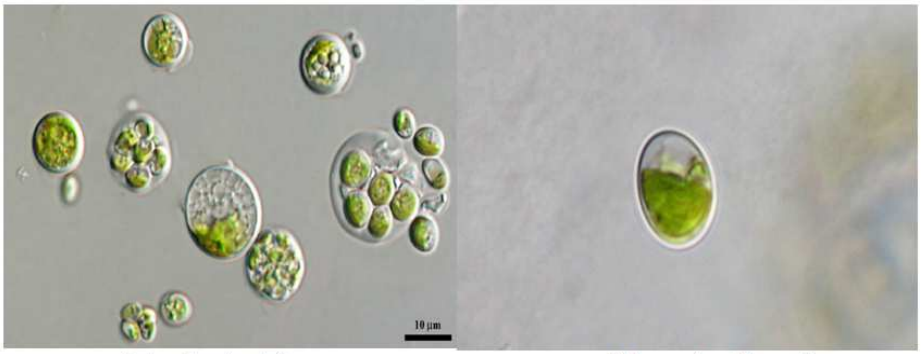
Fig. 3. Biomass Vs. lipid content of marine microalgae species (CC: *Chaetoceros calcitrans*, CM: *Chaetoceros muelleri*, SC: *Skeletonema costatum*, TP: *Thalassiosira pseudonana*, PS: *Pavlova salina*, ES: *Ellipsoidion sp*., IS: *Isochrysis sp*., NS: *Nannochloropsis sp*., PT: *Phaeodactylum tricornutum*, TS: *Tetraselmis suecica*, TP: *Thalassiosira pseudonana*)

Experimental Design

The selected freshwater (*Chlorella saccharophila*) and marine (*Tetraselmis suecica*) microalgae species were grown under various environmental conditions in the open pond system. The effects of light duration and nutrient on the algae biomass and oil content were evaluated using $NaHCO_3$ as a carbon source while maintaining the nitrogen content, pH and temperature constant at 70 mg/L, 8.3-8.9 and 22°C, respectively. Sodium bicarbonate ($NaHCO_3$) was administered at a concentration of 1300 mg/L. The light intensity was kept at 480 µmol m^{-2} s^{-1} and the algae was exposed to three light periods throughout the cultivation process: (a) the shortest day light in the winter of ~9 h, (b) the longest day light in the summer of ~16 h and (c) full light exposure (24 h) using the automated lighting and control units in the open pond system. Ammonium nitrate, ammonium phosphate, ammonium sulfate and a mix of all three were evaluated as sources of nitrogen (Table 2). Each experiment was carried out for 10 days with three replicates, giving a grand total of 72 runs for both species.

Preparation of Liquid Medium for Inoculum Growth

The freshwater microalgae medium was prepared on algal proteose medium (ATCC Catalog Medium No. 847, American Type Culture Collection, Manassas, VA, USA) and was made up by adding 1 g of proteose Peptone (Difco 0120) to 1 L of Bristols solution (Table 3). Bristols solution was prepared by adding the



(a) *Chlorella saccharophila* (Skaloud, 2007)

(b) *Tetraselmis suecica* (Reefsnow, 2012)

Fig. 4. Microscopic illustrations of *Chlorella saccharophila* and *Tetraselmis suecica*

Table 2. Concentration of micronutrient components

Compound	Molecular Weight (g/mol)	Amount (mg/L)	Concentration (mg/L)		
			Nitrogen	Phosphorus	Sulfur
Ammonium nitrate (NH_4NO_3)	80	200.0	70		
Diammonium phosphate $(NH_4)_2HPO_4$	132	330.0	70	78	
Ammonium sulfate (NH_4SO_4)	132	330.0	70		80
Combination					
Ammonium nitrate (NH_4NO_3)	80	85.7	30		
Diammonium phosphate $(NH_4)_2HPO_4$	132	194.3	20	22	
Ammonium sulfate (NH_4SO_4)	132	94.3	20		23

following amounts from the prepared stock solutions: 10 mL $NaNO_3$, 10 mL $CaCl_2$, 10 mL $MgSO_4$ $7H_2O$, 10 mL K_2HPO_4, 10 mL KH_2PO_4, 10 mL NaCl, 0.05 mL $FeCl_3$ and 940 mL distilled water. The stock solutions were prepared as follows: 10 g $NaNO_3$ in 400 mL distilled water, 1 g $CaCl_2$ in 400 mL distilled water, 3 g $MgSO_4$ $7H_2O$ in 400 mL distilled water, 3 g K_2HPO_4 in 400 mL of distilled water, 7 g KH_2PO_4 in 400 mL of distilled water and 1 g NaCl in 400 mL distilled water.

The marine microalgae medium was prepared in F/2 medium (Guillard and Ryther, 1962). The trace element liquid medium stock solution (Table 4) was prepared by the addition of 4.16 g Na_2 EDTA, 3.15 g $FeCl_3$•$6H_2O$, 0.01 g $CuSO_4$•$5H_2O$, 0.022 g $ZnSO_4$•$7H_2O$, 0.01 g $CoCl_2$•$6H_2O$, 0.18 g $MnCl_2$•$4H_2O$ and 0.006 g Na_2MoO_4•$2H_2O$ into 1 L autoclaved seawater (Halifax Waterfront, Halifax, Nova Scotia). The vitamin mix stock solution was prepared by the addition of 0.1 g Thiamine HCl and 0.0005 g biotin into 1 L autoclaved seawater. The liquid medium was prepared by the addition of 0.075 g $NaNO_3$, 0.00565 g NaH_2PO_4•$2H_2O$, 1.0 mL trace element stock solution and 1 ml of vitamin mix stock solution.

Preparation of Solid Medium for Inoculum Growth

The marine microalgae grow only on a marine liquid medium. The freshwater microalgae solid medium was prepared on algal proteose agar medium (ATCC Catalog Medium No. 847). The solid medium (Table 3) was

Table 3. Components of the freshwater liquid and solid media

Component	Quantity	
	Proteose agar medium	Proteose broth medium
Agar (g)	15.00	
Proteose peptone (g)	1.00	1.00
Bristols solution (L)	1.00	1.00
$NaNO_3$ Solution (mL)	10.00	10.00
$CaCl_2$ Solution (mL)	10.00	10.00
$MgSO_4 \cdot 7H_2O$ Solution (mL)	10.00	10.00
K_2HPO_4 Solution (mL)	10.00	10.00
KH_2PO_4 Solution (mL)	10.00	10.00
NaCl Solution (mL)	10.00	10.00
$FeCl_3$ Solution (mL)	0.05	0.05
Distilled Water (mL)	940.00	940.00

Table 4. Components of the F/2 marine liquid media (Guillard and Ryther, 1962)

Component	Quantity (g/L*)
Trace element stock solution	
Na_2 EDTA	4.1600
$FeCl_3 \cdot 6H_2O$	3.1500
$CuSO_4 \cdot 5H_2O$	0.0100
$ZnSO_4 \cdot 7H_2O$	0.0220
$CoCl_2 \cdot 6H_2O$	0.0100
$MnCl_2 \cdot 4H_2O$	0.1800
$Na_2MoO_4 \cdot 2H_2O$	0.0060
Vitamin mix stock solution	
Thiamine HCl	0.1000
Biotin	0.0005

*per liter of autoclaved seawater

made up by the addition of 1 g proteose peptone (Difco 0120) and 15 g agar to 1 L Bristols solution.

Preparation of Inoculum

Sufficient amounts of inoculum were prepared for all the experimental runs for both freshwater and marine microalgae in order to maintain consistency. The procedures for preparing the inocula are depicted in Fig. 5.

The freeze dried *Chlorella saccharophila* sample (ATCC® 30408™, Catalog Medium No. 847, American Type Culture Collection, Manassas, VA, USA) was revived in 5 mL of Bristols liquid media. Using an inoculating loop, cells were transferred from the liquid media onto 3 petri dishes containing proteose agar media. The plates were incubated for 3 days at room temperature and a photocycle of 14 h light and 10 h dark. The cells were then scraped off the solid media using an inoculating loop and submerged them into a 125 mL Erlenmeyer flask containing 25 mL of Bristols liquid medium. These cells were then left to grow for 2 weeks at a photocycle of 14 h light and 10 h dark. The mixture was then transferred to a 500 mL Erlenmeyer flask containing 250 mL of Bristols liquid media which was left to grow for 2 weeks at a photocycle of 14 h light and 10 h dark. Finally, the medium was transferred from the 500 mL flask into a 30 L bioreactor containing 25 L of Bristols liquid media and left to grow for 2 more weeks at a photocycle of 14 h light and 10 h dark.

The inoculum for *Tetraselmis suecica* microalga was prepared by taking 5 mL of the liquid sample (UTEX LB 2286, Cedarlane, Burlington, Ontario)and adding it to 125 mL Erlenmeyer flask containing 25 mL of F/2 liquid medium and then left to grow at room temperature for 2 weeks at a photocycle of 14 h light and 10 h dark. The mixture was then transferred to a 500 mL Erlenmeyer flask containing 250 mL of F/2 liquid media and was left to grow for 2 weeks at a photocycle of 14 h light and 10 h dark. Finally, the medium was transferred from the 500 mL flask into a 30 L bioreactor containing 25 L of F/2 liquid media and left to grow for 2 additional weeks at a cycle of 14 h light and 10 h dark.

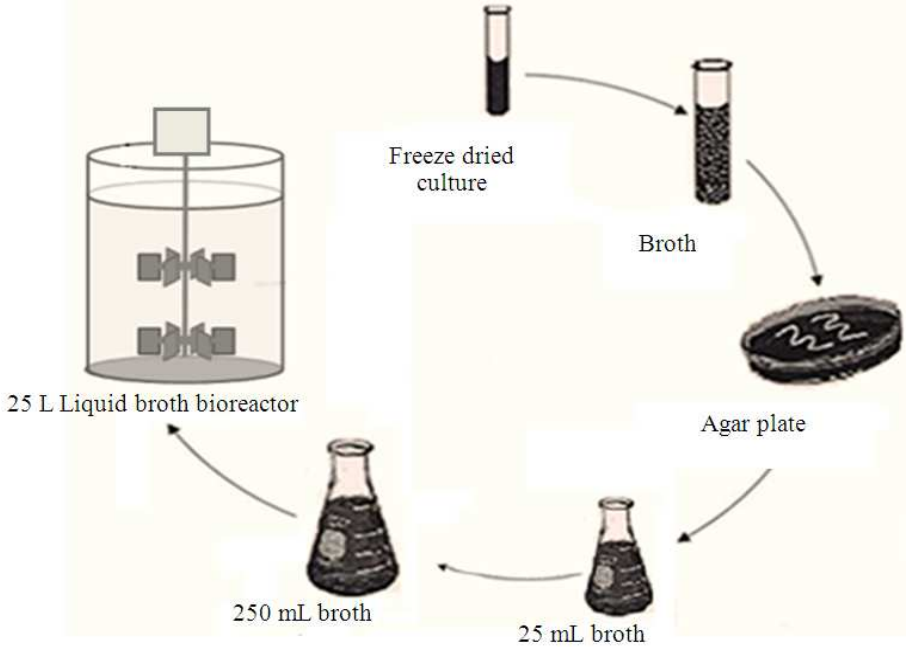
Preparation of Algae Production Media

The freshwater production medium is a modification of the Fitzgerlad medium (Hughes *et al*., 1959). The preparation of the stock solutions for this media is shown in Table 5. The medium was made up by the addition of 1 mL of each of the stock solutions A, B, C and D into 1 L distilled water (Table 6).

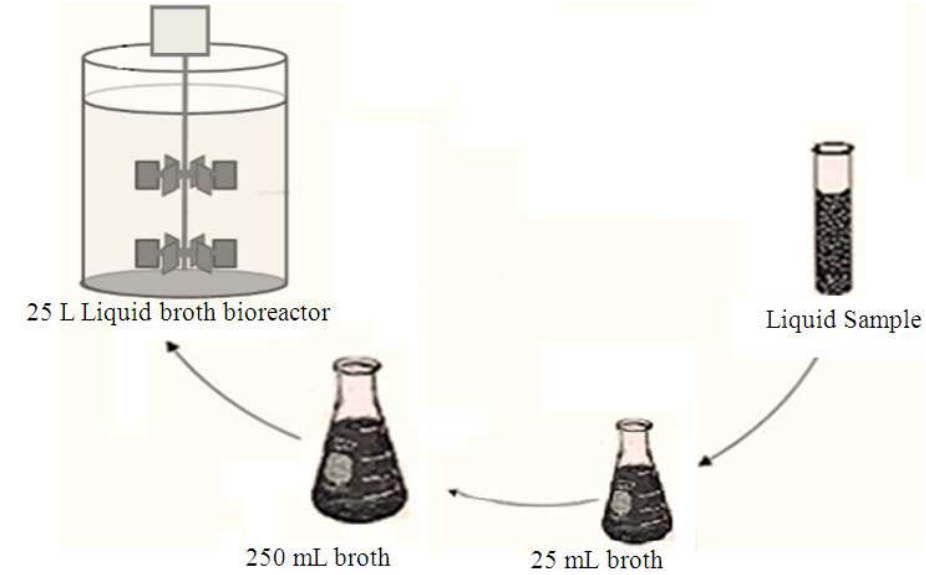
A modified F/2 medium (Guillard and Ryther, 1962) was used as the production medium for the marine microalga. The medium was modified by eliminating the addition of sodium nitrate. The medium consists primarily of autoclaved ocean water (Halifax Waterfront, Halifax, NS, Canada). Table 7 shows the elemental analysis of the components present in the marine water which was performed at the Mineral Engineering Center of Dalhousie University.

Experimental Protocol

To each open pond a total of 4.75 L of freshwater production medium were added. The desired nutrient (ammonium nitrate, ammonium phosphate, ammonium sulfate or combination of all three) was added to the production medium. This solution was enriched with 1.3 g/L of sodium bicarbonate (total of 6.5 g) and 250 mL of *Chlorella saccharophila* inoculum was added to each pond. The cells were exposed to either 9, 16 and 24 h or light and left to grow for 10 days. Every other day, 100 mL sample was taken for analyses. The biomass was harvested from the liquid media using a Sorvall T1 Centrifuge (Thermo Scientific, Ohio, USA). The supernatant from the centrifuge tubes was decanted and the cells were collected for biomass yield and oil content analyses. The marine medium was used with marine algae and the same procedure was followed.



(a) Freshwater microalgae



(b) Marine microalgae

Fig. 5. Preparation of inocula

Microalgae Biomass Determination

The freshwater mciroagla biomass yield was determined by measuring the optical density at 484 nm from a standard curve between the cell count and optical density. The number of Colony Forming Units (CFU) for *Chlorella saccharophila* was determined using a series of dilutions. A 1 mL aliquot sample was added to a test tube containing 9 mL autoclaved distilled water. The contents of the tube were vortexed (Thermolyne Maxi Mix, Thermolyne Corporation, Hampton, New Hampshire, USA) to distribute the cells. A 1 mL aliquot of this solution was added to another test tube containing 9 mL autoclaved distilled water. This tube was again vortexed to distribute the cells. This was repeated 7 times to obtain dilutions of 1:1, 1:10, 1:100, 1:1000, 1:10 000, 1:100 000, 1: 1 000 000. For each of the dilutions made, 0.1 mL of the solution was added to a petri dish containing solid freshwater or marine water medium. The plates were sealed with parafilm, inverted and

Table 5. Formulation of stock solutions for *Chlorella Saccharophila* production medium

Stock solutions (per 200 mL)	Composition
A	24.648 g $MgSO_4 \cdot 7H_2O$
B	1.360 g KH_2PO_4
	8.700 g K_2HPO_4
C	1.392 g $FeSO_4 \cdot 7H_2O$
	1.864 g EDTA tri Na
D	0.620 g H_3BO_3
	0.340 g $MnSO_4 \cdot H_2O$
	0.057 g $ZnSO_4 \cdot 7H_2O$
	0.018 g $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$
	0.027 g $CoCl_2 \cdot 6H_2O$
	0.024 g KBr
	0.017 g KI
	0.023 g $CdCl_2 \cdot 5/2H_2O$
	0.091 g $Al_2(SO_4)_3(NH_4)_2SO_4 \cdot 24H_2O$
	0.040 mg $CuSO_4 \cdot 5H_2O$
	0.560 mL H_2SO_4 (97%)

Table 6. Components of freshwater production medium

Component	Amount (mL)
A	1
B	1
C	1
D	1
Distilled water	996

Table 7. Elemental analysis of autoclaved ocean water used as a marine production medium

Element	Amount (mg/L)
Na	10254.00
Mg	1078.00
S	1010.00
K	395.00
Ca	386.00
Sr	6.79
Si	2.80
P	0.10
Ba	0.05
Al	0.05
Ni	0.04
Zn	0.02
Mo	0.01
Cd	0.01
Co	0.01
Cu	0.01

incubated at room temperature (~24°C) at a photo cycle of 14 h light and 10 h dark for 3 days. The plates were then removed and the colonies were counted using a colony counter (Model No. 7-910, Fisher Scientific, Ottawa, Ontario). The plates consisting of 30-300 CFU were used for calculating the CFU of the sample and the standard curve was prepared by plotting the optical density against the CFU (Fig. 6a). The following equation was used to calculate *Chlorella saccharophila* cell yield:

$$Cell\ Yield = \left(\frac{Optical\ Density}{5 \times 10^{-7}} \right) \times 10^3 \quad (1)$$

The marine microalgae yield was also determined by optical density measurements. A standard curve between the cell count and optical density (measured at 750 nm) was developed (Fig. 6b) and the following equation was used to calculate *Tetraselmis suecica* cell yield:

$$Cell\ Yield = \left(\frac{Optical\ Density}{23 \times 10^{-4}} \right) \times 10^4 \quad (2)$$

Oil Content Determination

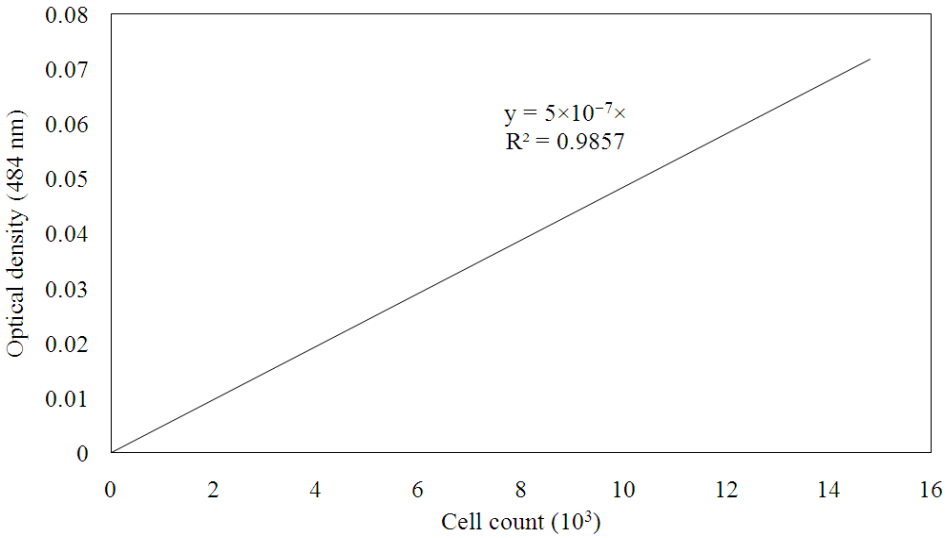
The oil content in the algae was determined using ultrasound assisted solvent extraction according to Bligh and Dyer method described by Araujo *et al*. (2013). Firstly, the algae biomass was homogenized and mixed with 25 mL methanol, 12.5 mL chloroform and 5 mL distilled deionized water. This mixture was exposed to ultrasonic energy (Branson 2510R-DTH, Branson Ultrasonics Corporation, Danbury, USA) for 40 min. Then, an additional 12.5 mL chloroform and 12.5 mL sodium sulfate solution (1.5% w/v) were added and sonicated for another 20 min. The solid biomass particles were filtered out of the solution and the liquid fraction was transferred to a separatory funnel with the addition of 75 mL KCl (0.88% w/v). The mixture was vigorously shaken and left to separate for 24 h. The solubility of oils in the chloroform solvent and the insolubility of solvents in water allowed for separation to occur into two phases (organic and aqueous). The oil containing phase (on the bottom) was drained out of the separatory funnel and collected into a pre-weighed distill flask. The flask was distilled using a rotary evaporator (HiTEC RE-51, Yamato Scientific America, California, USA) set at 45°C. The oil left behind was weighed in the flask and the yield was determined as follows:

$$Oil\ Yield\ (\%) = \frac{weight\ of\ Oil(g)}{weight\ of\ Algae\ Biomass(g)} \times 100 \quad (3)$$

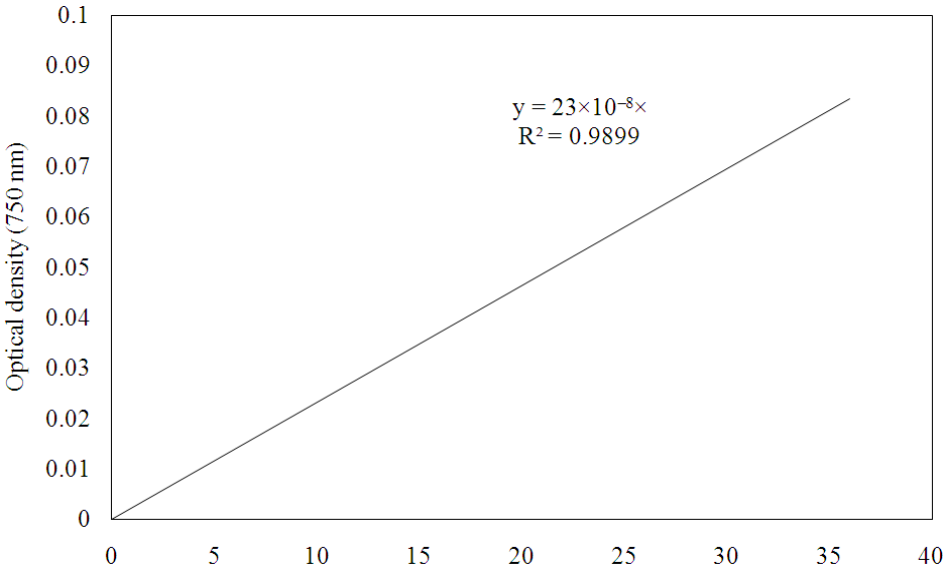
Results and Discussion

Microalgae Biomass

The cell number results are shown in Table 8. Analysis of the Variance (ANOVA) was performed on the cell yield data as shown in Table 9 using Minitab statistics software (Minitab® 16.2.2., Minitab Inc., Canada). The effects of microalgae type, light duration and nitrogen source on the cell yield were significant at the 0.001 level. There were also significant interactions



(a) *Chlorella saccharophila* at 484 nm



(b) *Tetraselmis suecica* at 750 nm

Fig. 6. Standard curves

between these parameters. Tukey's grouping was used to test the differences among the levels of each parameter as shown in Table 10. The two algae were significantly different from one another at the 0.05 level. The marine microalgae *Tetraselmis suecica* had the highest mean cell yield (2.89×10^6 cells/mL). The nutrients ammonium nitrate, ammonium phosphate and ammonium sulfate were not significantly different from one another, but were significantly different from the nutrient combination at the 0.05 level. The highest mean cell yield of 4.01×10^6 cells/mL was achieved using the combination of nutrients. The light exposures 16 and 24 h were not significantly different from one another, but were significantly different from the 9 h light exposure at the 0.05 level. However, the highest mean cell yield of 2.28×10^6 cells/mL was achieved at 24 h light exposure.

Effect of Microalgae Type

The effects of microalgae type on the cell yield are shown in Fig. 7. *Tetraselmis suecica* achieved higher cell yields than the *Chlorella saccharophila* at all nutrient types and light durations. *Tetraselmis suecica* achieved cell yields of 0.266×10^6, 1.664×10^6,

Table 8. Average cell number and oil content of *Chlorella saccharophila* (freshwater) and *Tetraselmis suecica* (marine) microalgae using various nitrogen sources at different light exposures

Species	Nutrient type	Light (h)	Cell number (cells/mL)	Oil content (%)
Freshwater	Ammonium nitrate	9	$0.279\times10^6\pm0.07\times10^6$	14.162±4.85
		16	$0.336\times10^6\pm0.05\times10^6$	13.176±2.58
		24	$0.460\times10^6\pm0.04\times10^6$	7.714±3.44
	Ammonium phosphate	9	$0.138\times10^6\pm0.03\times10^6$	29.075±13.40
		16	$0.186\times10^6\pm0.05\times10^6$	27.353±1.43
		24	$0.489\times10^6\pm0.08\times10^6$	7.861±2.24
	Ammonium sulfate	9	$0.139\times10^6\pm0.02\times10^6$	13.247±0.58
		16	$0.157\times10^6\pm0.03\times10^6$	7.168±1.00
		24	$0.507\times10^6\pm0.09\times10^6$	3.782±3.56
	Combination	9	$0.295\times10^6\pm0.02\times10^6$	22.443±17.6
		16	$0.630\times10^6\pm0.03\times10^6$	18.781±0.89
		24	$0.689\times10^6\pm0.01\times10^6$	12.907±7.85
Marine	Ammonium nitrate	9	$0.266\times10^6\pm0.08\times10^6$	2.742±0.76
		16	$0.619\times10^6\pm0.02\times10^6$	1.482±1.03
		24	$0.750\times10^6\pm0.02\times10^6$	1.102±0.67
	Ammonium phosphate	9	$1.664\times10^6\pm0.05\times10^6$	1.876±0.78
		16	$2.122\times10^6\pm0.58\times10^6$	1.213±1.38
		24	$2.354\times10^6\pm0.10\times10^6$	0.640±0.55
	Ammonium sulfate	9	$0.664\times10^6\pm0.02\times10^6$	2.158±0.43
		16	$0.978\times10^6\pm0.01\times10^6$	1.589±1.70
		24	$2.793\times10^6\pm0.02\times10^6$	1.175±0.31
	Combination	9	$2.689\times10^6\pm0.02\times10^6$	0.450±0.21
		16	$9.415\times10^6\pm0.06\times10^6$	0.435±0.16
		24	$10.342\times10^6\pm0.13\times10^6$	0.270±0.06

Values are the average of three replicates

Table 9. Analysis of the variance for cell yield

Source	DF	SS	MS	F	P
Total	71	5.854×10^{14}			
Model					
Species (S)	1	1.152×10^{14}	1.151×10^{14}	60.29	0.001
Nutrient (N)	3	1.413×10^{13}	4.709×10^{13}	24.65	0.001
Light (L)	2	2.896×10^{13}	1.448×10^{13}	7.58	0.001
S*N	3	1.228×10^{14}	4.092×10^{13}	21.42	0.001
S*L	2	1.894×10^{13}	9.469×10^{12}	4.96	0.010
N*L	6	3.546×10^{13}	5.909×10^{12}	3.09	0.010
S*N*L	6	3.116×10^{13}	5.193×10^{12}	2.72	0.020
Error	48	9.169×10^{13}	1.910×10^{12}		

DF: Degrees of Freedom; SS: Sum of Squares; MS: Mean Sum of Squares; F: F Distribution; P: Probability-Value; $R^2 = 84.34\%$; CV = 17.69%

Table 10. Tukey's grouping for cell yield

Factors	Level	N	Mean yield	Tukey's grouping
Species	Freshwater	36	358921	B
	Marine water	36	2888406	A
Nutrient	Ammonium nitrate	18	451837	A
	Ammonium sulfate	18	873226	A
	Ammonium phosphate	18	1159062	A
	Combination	18	4010527	B
Light (h)	9	24	766963	A
	16	24	1821984	B
	24	24	2282043	B

Groups with the same letter are not significantly different from each other at the 0.05 level

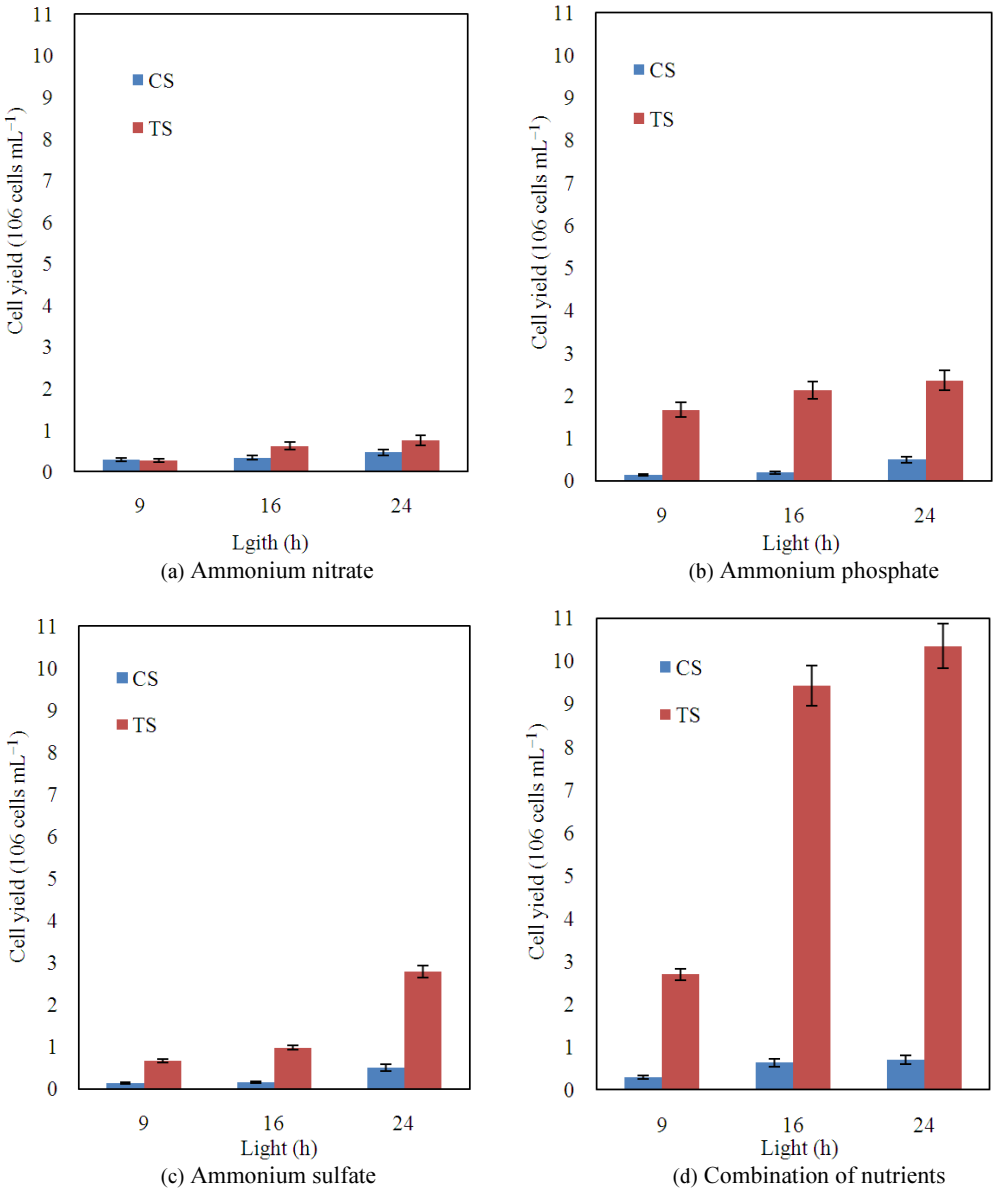


Fig. 7. Effect of microalgae type on the cell yield (cells/mL) at different nutrients light exposures (CS: *Chlorella saccharophila*, TS: *Tetraselmis suecica*)

0.664×10^6 and 2.689×10^6 cells/mL while *Chlorella saccharophila* achieved cell yields of 0.28×10^6, 0.14×10^6, 0.14×10^6 and 0.30×10^6 cells/mL at 9 h light exposure using ammonium nitrate, ammonium phosphate, ammonium sulfate and combination of nutrients, respectively. Similar trends were observed with other light exposures (16 and 24 h).

Singh *et al*. (2013) reported a dry cell yield of 378 mg/L for *Chlorella saccharophila*. Isleten-Hosoglu *et al*. (2012) reported a dry cell weight yield of 138 mg/L for *Chlorella saccharophila*. Herrera-Valencia *et al*. (2011) reported a biomass productivity of 154.3 mg/L/d for *Chlorella saccharophila*. Chinnasamy *et al*. (2010) reported a biomass productivity of 23 mg/L/d for *Chlorella saccharophila*. Danquah *et al*. (2010) reported a biomass yield 1.29 g/L for the *Tetraselmis suecica*. Michels *et al*. (2013) achieved a biomass productivity of 350 mg/L/d for *Tetraselmis suecica*. Thomas *et al*. (1984) reported a biomass productivity of 192 mg/L/d for *Tetraselmis suecica*. Moheimani (2013) achieved a biomass productivity of 320 mg/L/d for *Tetraselmis suecica*. Bondioli *et al*. (2012) noted a biomass productivity of 237 mg/L/d for *Tetraselmis suecica*. These reports clearly indicate that both the cell yield and productivity were much higher for the marine microalga *Tetraselmis suecica* (1290-2480 mg/L)

compared to the freshwater microalga *Chlorella saccharophila* (138-378 mg/L).

In this study the highest cell yield obtained for *Chlorella saccharophila* was 175 mg/L with a biomass productivity of 17.5 mg/L/d while the highest cell yield obtained for *Tetraselmis suecica* was 2.48 g/L with a biomass productivity of 247 mg/L/d. These results are within the range reported in the literature. The differences in the results can be attributed to the different growth conditions used and the varying harvesting techniques.

Effect of Light

The effect of light duration on the cell yield of *Chlorella saccharophila* and *Tetraselmis suecica* is shown in Fig. 8. The results indicate a slight increase in cell yields as the light duration was increased for both microalgae species. For the ammonium sulfate nutrient, an increase in light duration from 9 to 24 h, increased the cell yields from 0.138×10^6 cells/mL to 0.368×10^6 cells/mL and from 0.664×10^6 cells/mL to 2.79×10^6 cells/mL for the *Chlorella Saccharophila* and *Tetraselmis suecica*, respectively. Similar trends were observed with the other nutrients.

Al-Qasmi *et al*. (2012) stated that the biomass yield is directly linked to light duration. Larsdotter (2006) noted large fluctuations in algal biomass and removal of nutrients efficiencies due to seasonal variations in light and temperature. Khoeyi *et al*.

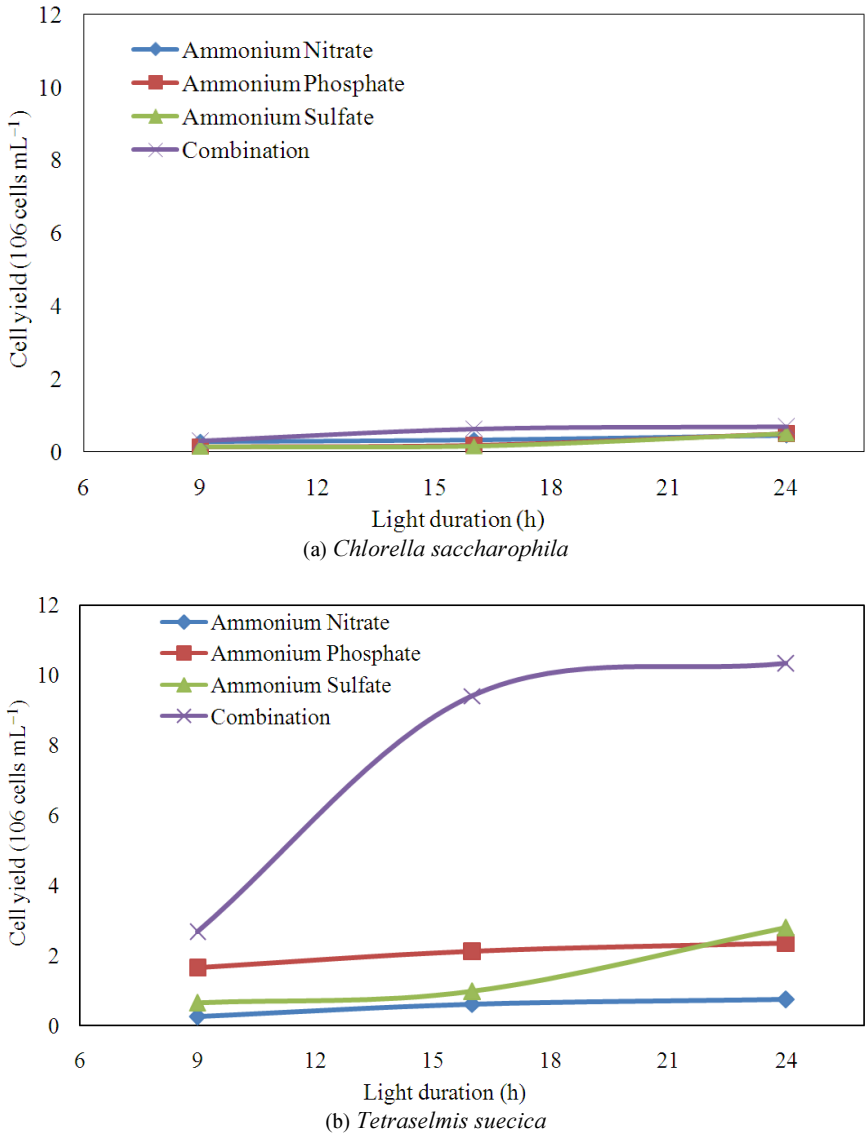


Fig. 8. Effect of light duration on the cell yield (cells/mL) of freshwater (*Chlorella saccharophila*) and marine (*Tetraselmis suecica*) microalgae using different nutrients

(2012) reported *Chlorella vulgaris* cell numbers of 39×10^6 cells/mL, 60×10^6 cells/mL and 75×10^6 cells/mL at the light exposures of 8, 12 and 16 h, respectively. Wahidin *et al*. (2013) noted that *Nannochlorpsissp*. achieved an increase in cell concentration from 1.3×10^7 cells/mL to 2.1×10^7 cells/mL as the light duration was increased from 12 to 18 h. Mata *et al*. (2012) reported cell yields for *Scenedesmus obliquus* of 0.30 and 0.45 g/L at light exposures periods of 12 and 24 h, respectively.

The highest duration had exceptionally higher effect on the cell yield when the combination of nutrient was used. Increasing the light duration from 9 h to 24 h for this treatment increased the cell yield from 0.295×10^6 to 0.689×10^6 cells/mL and 2.689×106 to 10.342×106 cells/mL for *Chlorella Saccharophila* and *Tetraselmis suecica*, respectively. Increasing the light duration resulted in higher biomass production as a result of increased photosynthetic activity. Kaplan *et al*. (1986), Martinez *et al*. (1999), Hessen *et al*. (2002) and Sato and Murata (1980) reported that longer light exposures results in higher phosphorous uptake by the cells. Larsdotter (2006) reported that additional illumination in the winter for algal culture increased the uptake of phosphorus and nitrate uptake.

Effect of Nutrient Type

The effect of nutrient type on the cell yields of *Chlorella saccharophila* and *Tetraselmis suecica* microalgae is shown in Fig. 9. The combination of nutrients achieved the highest cell concentration for the two microalgae. The highest cell concentration of 0.295×10^6, 0.630×10^6 and 0.689×10^6 cells/mL for *Chlorella Saccharophila* and the highest cell concentrations of 2.689×10^6, 9.41×10^6 and 10.34×10^6 cells/mL for *Tetraselmis suecica* were achieved using the combination of nutrient system at 9, 16 and 24 h, respectively. The ammonium nitrate, ammonium phosphate and ammonium sulfate nutrients had similar effect on the cell yield cut were significantly less compared to the combined nutrients.

Becker (1994) stated that ammonia assimilation in microalgae is easier due to the simplicity of the molecule and its presence in a solution inhibits the nitrogenase activity. Odum (1983) noted that microalgae are capable of using nitrate because of its presence in nature.

Makareviciene *et al*. (2011) achieved the best biomass productivity of *Chlorella* sp. using sodium nitrate. Li *et al*. (2008) showed that *N. oleoabundans* increased in cell yield from 1.2 to 2.4 g/L in day 2 using nitrate. Costa *et al*. (2001) reported that *Spirulina platensis* cell yields of 1.559, 0.993 and 0.081 g/L when using sodium nitrate, ammonium nitrate and ammonium sulfate, respectively.

Abe *et al*. (2002) noted that *Trentepohlia aurea* biomass was 1.5 times higher in culture grown with sufficient nitrogen and phosphorus. Fried *et al*. (2003) stated a significant positive effect on algae growth with both nitrogen and phosphorus as nutrient sources. Li *et al*. (2011) noted that the species *Scenedesmus sp*. produced a higher cell productivity of 2.21×10^6 cells/mL/d with nitrogen and phosphorous contents of 12.1 and 0.27 mg/L, respectively. Totsche *et al*. (2006) noted that the growth of *Chlamydomonas sp*. and *Ochromonas sp*. was stimulated with the addition of phosphorus into the media.

In this study, the ammonium sulfate did not prove to be suitable for yielding high biomass. Guzman-Murillo *et al*. (2007) noted that using ammonium sulfate as a nitrogen source for *Phaeodactylum tricornutum* species resulted in lower cell yields than ammonium nitrate. Rocha *et al*. (2003) also showed that an increase in ammonium sulphate concentration from 5 mM to 10 mM decreased the cell concentration from 4×10^7 to 2.9×10^7 cells/mL. Costa *et al*. (2001) reported that freshwater algae *Spirulina platensis* achieving biomass yields of 0.993 and 0.081 g/L using ammonium nitrate and ammonium sulfate, respectively. All these reported indicate that sulfate is not effective nutrient for promoting microalgae cell growth.

Different nutrients are responsible for the operation of different cell parts and lack of one nutrient affects the overall efficiency of the cells (Juan, 2006; Chen *et al*., 2009; Ji *et al*., 2013). In this study, the combination of nutrients (ammonium nitrate, ammonium phosphate, ammonium sulfate) provided the algae with a full spectrum of nutrients (nitrate, phosphate and ammonium) as opposed to the individual nutrients containing only nitrogen with one other element.

Microalgae Oil Content

The oil yield results are shown in Table 8. Analysis of the Variance (ANOVA) was performed on the oil yield data as shown in Table 11. The effects of microalgae type and nitrogen source on oil yield were significant at the 0.001 and 0.003 levels, respectively. However, the light duration did not have any significant effect. The interaction between the algae and nitrogen source also has a significant effect. However, the interactions between light duration, algae type and nitrogen source were not significant. Tukey's grouping was used to test the differences among the levels of each parameter as shown in Table 12. The two microalgae *Chlorella saccharophila* and *Tetraselmis suecica* were significantly different from one another at the 0.05 level. The highest mean oil yield (15.9%) was obtained from

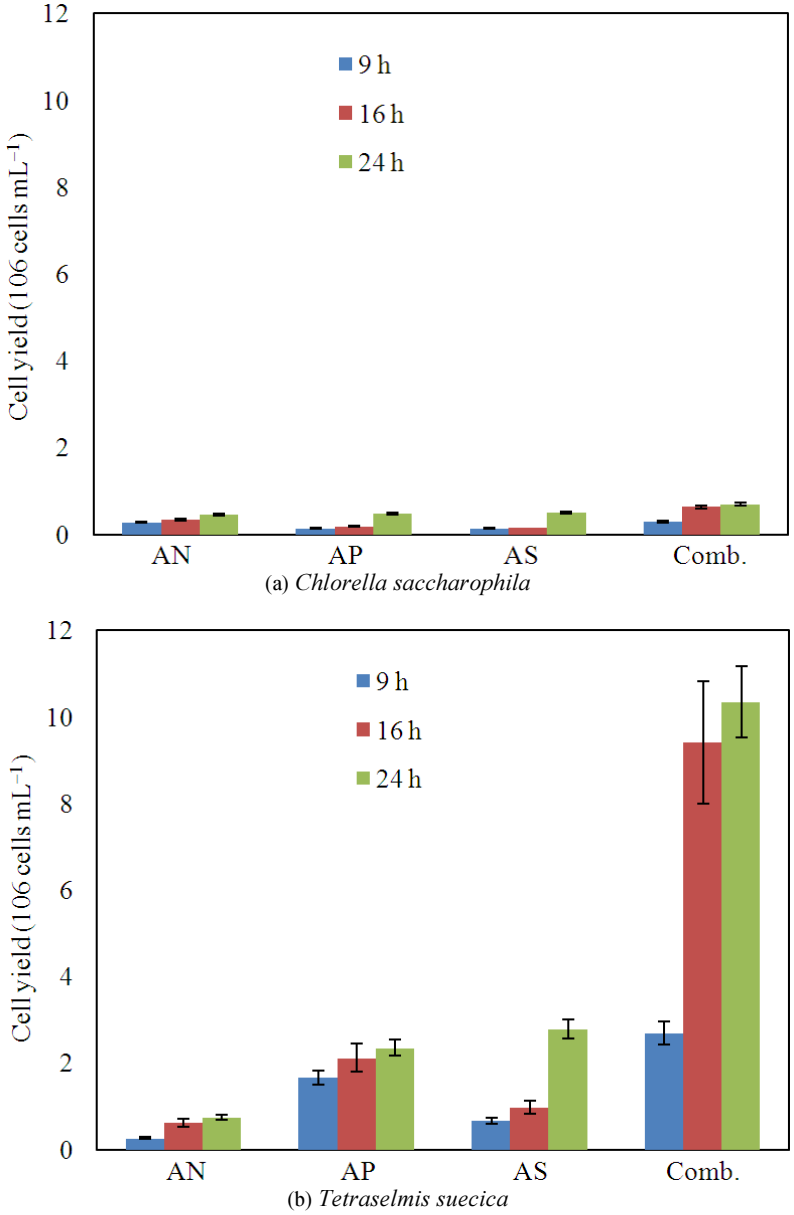


Fig. 9. Effect of nutrient type on the cell yield (cells/mL) of freshwater (*Chlorella saccharophila*) and marine (*Tetraselmis suecica*) microalgae at varying light durations (AN: Ammonium Nitrate, AP: Ammonium Phosphate, AS: Ammonium Sulfate, Comb: Combination of nutrients)

Table 11. Analysis of the variance for oil content

Source	DF	SS	MS	F	P
Total	71	8296.85			
Model					
Species (S)	1	3866.82	3866.82	80.37	0.001
Nutrient (N)	3	765.33	255.11	5.30	0.003
Light (L)	2	31.62	15.81	0.33	0.722
S*N	3	895.03	298.34	6.20	0.001
S*L	2	50.24	25.12	0.52	0.597
N*L	6	210.31	35.05	0.73	0.629
S*N*L	6	168.01	28.00	0.58	0.743
Error	48	2309.49	48.11		

DF: Degree of Freedom; SS: Sum of Square; MS: Mean of Square; F: F Distribution; P: Probability-Value; ^{2} = 0.72; CV = 12.59%

Table 12. Tukey's grouping for oil content

Factors	Level	N	Mean oil content (%)	Tukey's grouping
Species	Freshwater	36	15.918	B
	Marine water	36	1.261	A
Nutrient	Ammonium phosphate	18	13.560	A
	Combination	18	9.214	A B
	Ammonium nitrate	18	6.730	B
	Ammonium sulfate	18	4.853	B
Light (h)	9	24	9.523	A
	16	24	8.194	A
	24	24	8.051	A

Groups with the same letter are not significantly different from each other at the alpha significance level of 0.05

Chlorella saccharophila. The nutrient types ammonium nitrate, ammonium sulfate and the combination of nutrients were not significantly different from one another, but were significantly different from the ammonium phosphate at the 0.05 level. However, the ammonium phosphate system was not significantly different from the combination of nutrient system. The highest mean oil yield of 13.56% was achieved using the ammonium phosphate system. The levels of light exposure were not significantly different from one another. The highest mean oil yield of 29.1% was achieved with the 9 h light duration.

Effect of Microalgae Type

The effect of microalgae type on the microalgae oil content is shown in Fig. 10. *Chlorella saccharophila* achieved the highest oil yields using all nutrient types at all light durations. It achieved in oil yields of 13.17, 29.08, 7.17 and 22.44% while *Tetraselmis suecica* achieved oil yields of 1.48, 1.21, 1.17 and 0.45% at the 9 h light exposure using ammonium nitrate, ammonium phosphate, ammonium sulfate and combination of nutrients, respectively. Similar trends were observed at the other light exposures (16 and 24 h).

The results showed that the oil yields obtained from *Chlorella saccharophila* were 10 fold higher than those obtained from *Tetraselmis suecica,* despite the higher biomass yields obtained from the *Tetraselmis suecica* species. This can be attributed to the use of energy as different species use their energy for different metabolic pathways (Pittman *et al*., 2011). Sharma *et al*. (2012) stated that the occurrence and extent to which lipids are produced by microalgae is species/strain specific. *Chlorella saccharophila* cells are better at accumulating lipids at the expense of cell division while the *Tetraselmis suecica* cells are better at cell division at the expense of storing lipids. Rodolfi *et al*. (2009) reported that higher biomass yields correspond to lower cellular lipid content and noted that *Prophyridum cruentum, Scenedesmus, Chlorella* and *Chaetoceros calcitrans* resulted in biomass productivities of 0.37, 0.26, 0.23 and 0.04 g/L/d and lipid contents of 9.5, 21.1, 18.7 and 39.8%, respectively. Pai and Lai (2011) achieved a 9 fold increase in cell yield of the oleaginous algae (from 28.3 mg/L to 254 mg/L), but the oil content only increased by 1.6 fold (from 20.4% to 33.6%).

Several researchers (Herrera-Valencia *et al*., 2011; Isleten-Hosoglu *et al*., 2012; Chinnasamy *et al*., 2010; Tan and Johns, 1991) indicated that higher oil productivities were achieved using the *Chlorella saccharophila* (63.3-153 mg/L/d) microalgae as opposed to *Tetraselmis suecica* (14.8-32 mg/L/d). Isleten-Hosoglu *et al*. (2012) reported a lipid content of 29.5% from *Chlorella saccharophila*. Liu *et al*. (2011) reported a lipid content of 45% and a lipid productivity of 153 mg/L/d for *Chlorella saccharophila.* Chinnasamy *et al*. (2010) reported a lipid content of 12.90% for *Chlorella saccharophila*. Tan and Johns (1991) reported a lipid content of 47% for *Chlorella saccharophila*. Danquah *et al*. (2010) reported a lipid yield of 108.7 mg/L for *Tetraselmis suecica.* Griffiths and Harrison (2009) reported a lipid productivity of 32 mg/L/d for *Tetraselmis suecica*. Montero *et al*. (2011) reported a lipid productivity of 27 mg/L/d for microalga *Tetraselmis suecica.* Moheimani (2013) reported a lipid productivity of 14.8 mg/L/d for *Tetraselmis suecica.* These values are higher than those obtained in this study. The differences can be attributed to the varying cultivation periods, variation in nutrient systems and the effectiveness in the oil extraction methods used.

Effect of Light

The effect of light duration on the oil content for *Chlorella saccharophila* and *Tetraselmis suecica* is shown in Fig. 11. Increases in the light exposure from 9 h to 24 h resulted in decreased oil yields for all nutrient systems for both algae species. Increasing the light duration increased the photosynthetic activity and this decreased the oil content, as the cells use energy for generation of new cells at the expense of lipid storage (Sharma *et al*., 2012). Khotimchenko and Yakovleva (2005) stated that increasing light periods stimulates the growth, fatty acid synthesis and the formation of membrane components (chloroplast). Bandarra *et al*. (2003) noted that a shorter light exposure period increased the oil content in *Isochrysis galbana*.

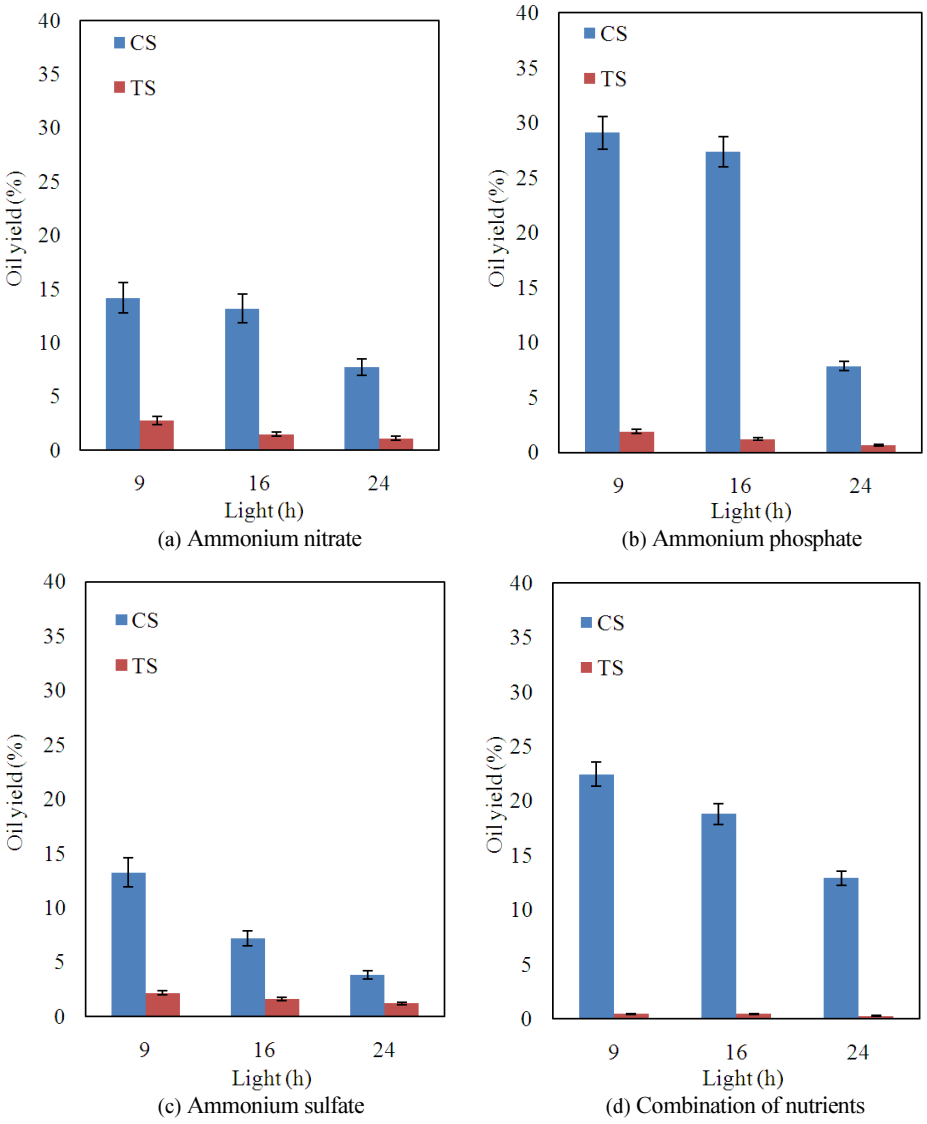


Fig. 10. Effect of microalgae type on the oil content (%) using different nutrients and light exposures (CS: *Chlorella saccharophila*, TS: *Tetraselmis suecica*)

Wahidin *et al*. (2013) cultured marine microalgae *Nannochloropsis sp*. using a light intensity of 100 µmol/m^2/s at photoperiods of 18 and 24 h and found that the highest lipid content of 31.3% was achieved at the 18 h light exposure and an increase in light exposure to 24 h resulted in a lipid content of 27.9%. Lim and Zaleha (2013) reported that the marine species *Chaetoceros calcitrans*, *Chlorella sp*. and *Nannochlorosis* had a higher lipid content at 12 h light exposure as opposed to 24 h light exposure. Herrera-Valencia *et al*. (2011) noted a lipid content of 40% for *Chlorella saccharophila* grown at 16 h light exposure. Perez-Pazos and Fernandez-Izquierdo (2011) achieved lipid yields *Chlorella sp*. of 0.25 and 0.17 g/L at 6 and 18 h light exposures. These results are similar to those obtained in this study.

Effect of Nutrient Type

The effect of nutrient type on the oil content is shown in Fig. 12. Nutrient type plays an important role in oil yield. Ammonium nitrate, ammonium phosphate, ammonium sulfate and combination of nutrients resulted in oil content of 13.18, 7.71 and 14.16%, 29.08, 27.35 and 7.86%, 7.17, 13.24 and 3.78% and 22.44, 12.91 and 18.78% for *Chlorella saccharophila* and 1.48, 1.10 and 2.74%, 1.21, 0.64 and 1.88%, 1.18, 2.16 and 1.59% and 0.45, 0.44 and 0.27%, for *Tetraselmis suecica* at the 9, 16 and 24 h, respectively.

In this study, the results indicate the highest oil content for the freshwater (*Chlorella saccharophila)* microalgae were achieved using ammonium phosphate nutrient as the addition of phosphorus stimulates the production and storage of lipids. This can also be seen

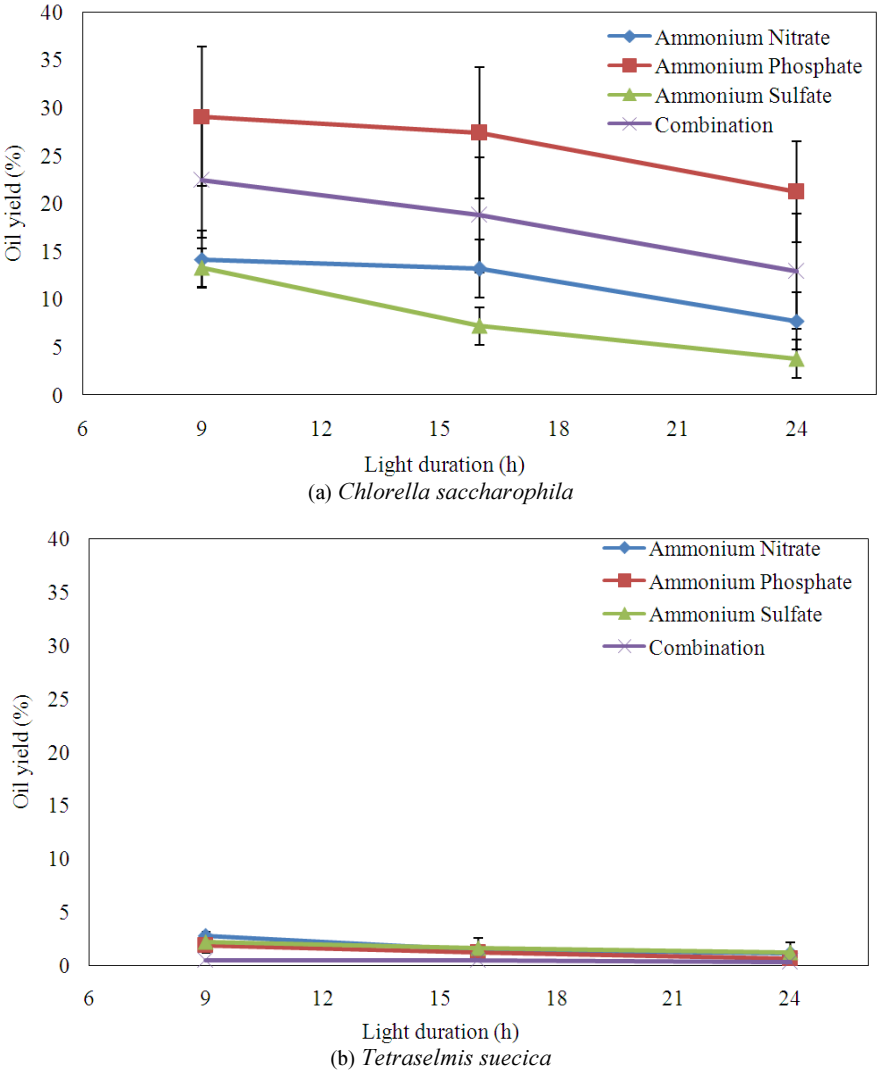


Fig. 11. Effect of light duration on the oil content of *Chlorella saccharophila* and *Tetraselmis suecica* at different nutrients and light durations

in the combination of nutrients system which resulted in the second highest oil content for this species, due to the presence of phosphorus. However, the difference between the two treatments was not significant. Similarly, the results for the marine microalgae *Tetraselmis suecica* indicate that the highest lipid content were achieved with the ammonium nitrate system and the lowest oil yields were achieved with the combination of nutrients (the opposite is true for biomass yields) which indicates that the medium with ammonium nitrate enhanced cell growth and the lack of sulfur and phosphorous caused the cells to store lipids instead of growth.

Kumar *et al*. (2012) noted increases in biomass (0.9-2.9 g L^{-1}) and decreased lipid content (48-32%) with increased concentrations of nitrogen. Converti *et al*. (2009) achieved an increase in lipid production upon nitrogen depletion for *Nannochloropsis oculata* and *Chlorella vulgaris*. Mutlu *et al*. (2011) noted that *Chlorella vulgaris* increased lipid content from 12.29 to 35.6% when the culture was deprived of nitrogen but was phosphorus sufficient. Illman *et al*. (2000) reported a 40% increase in lipids in a low nitrogen containing medium. Reitan *et al*. (1994) reported that the microalgae *Nannochloris atomus* and *Tetraselmis sp*. had decreased lipid content due to phosphorous starvation. Sato *et al*. (2000) achieved an increase in lipid content with limitation of sulphur in *Chlamydomonas reinhardtii*. Hu *et al*. (2008) stated the sulfate limitation in microalgae promotes lipid accumulation.

Conclusion

The biomass and oil yields of *Chlorella saccharophila* (freshwater) and *Tetraselmis suecica* (marine) microalga were investigated using different

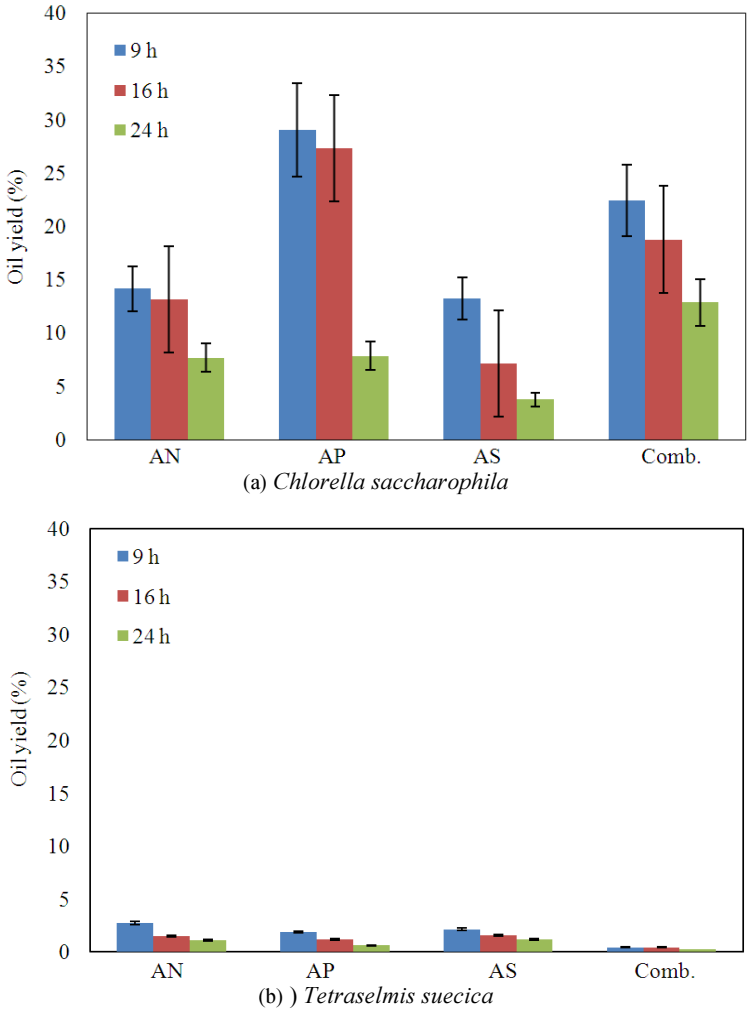


Fig. 12. Effect of nutrient type on the oil content of freshwater (*Chlorella saccharophila*) and marine (*Tetraselmis suecica*) microalgae at varying light durations. (AN: Ammonium nitrate, AP: Ammonium Phosphate, AS: Ammonium Sulfate, Comb: Combination of nutrients)

nitrogen sources (ammonium nitrate, ammonium phosphate, ammonium sulfate and combination of nutrients) at various light durations (9 h, 16 h and 24 h). $NaHCO_3$ was used as the carbon source and the nitrogen, temperature and pH were maintained at 70 mg/L, 22°C and 8.5, respectively. The results indicated that *Tetraselmis suecica* produced higher cell yields compared to the *Chlorella saccharophila* under all operating parameters. The biomass yields slightly increased with increasing light duration for both the *Tetraselmis suecica* and *Chlorella saccharophila*. However, there were no statistically significant differences between the light duration of 16 and 24 h. The combination of nutrients resulted in the highest growth for both species of microalgae, but high growth did not necessarily result in high lipid yields. Both cell generation and lipid production require energy; when the cells use energy for production of new cells they produce less oil for storage. Higher oil yields were achieved with the freshwater (*Chlorella saccharophila*) microalgae compared to the marine (*Tetraselmis suecica*) microalgae. No significant differences between light durations on oil yield were noted. *Chlorella saccharophila* produced the highest lipid content (and lowest biomass yield) using ammonium phosphate nutrient while *Tetraselmis suecica* achieved the highest oil yields (and lowest cell yields) using ammonium nitrate. The combination of nutrients at 24 h light exposure resulted in the highest biomass yields for *Chlorella saccharophila* which resulted in the highest total lipid yield. However, the economics of increasing the light exposure period from 16 to 24 h (50%) is not offset by the slight increase in lipid yield (5%).

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Author's Contributions

The experimental work and data analysis were carried out by first author under the supervision of second author. The first draft was prepared by the first author and the review, correction and organization of the paper were made by second author.

Ethics

There are no ethical issues.

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