Nigerian Research Journal of Engineering and Environmental Sciences 9(1) 2024 pp. 21-48
p ISSN: 2635-3342; e ISSN: 2635-3350

Nigerian Research Journal of Engineering and Environmental Sciences
Journal homepage: www.rjees.com

Review Article

Isolation and Identification of *Dunaliella* Species for the Production of Biocrude using Hydrothermal Liquefaction Technique: A Mini Review

***[1,2]Ikhazuangbe, P.M.O., [2,4]Eboibi B.E, [4]Amabogha, B., [2]Orugba, H. and [2,3,4]Agarri, S.E.**

[1]Department of Chemical Engineering, Faculty of Engineering, Edo State University Uzairue, Nigeria
[2]Department of Chemical Engineering, Faculty of Engineering, Delta State University Oleh Campus, Nigeria.
[3]Department of Chemical Engineering, Faculty of Engineering, Ladoke Akintola University, Ogbomosho, Nigeria.
[4]Department of Chemical Engineering, Faculty of Engineering, Federal University Otuoke, PMB 126 Yenagoa, Nigeria.
*ikhazuangbe.prosper@edouniversity.edu.ng

http://doi.org/10.5281/zenodo.12599317

ARTICLE INFORMATION

Article history:
Received 10 Jan. 2024
Revised 11 May 2024
Accepted 21 May 2024
Available online 30 Jun. 2024

Keywords:
Dunaliella
Biocrude
Elemental composition
Identification
Isolation

ABSTRACT

Screening, isolation, identification and cultivation of microalgae for production of biocrude have been explored by many researchers. The different techniques for isolation (micropipette washing and streak-plating techniques) and culturing (convectional and novel techniques) used by these researchers were reviewed in this work. The elemental and biochemical composition of microalgae were reviewed, and Van Kreven chart was generated and compared for Dunaliella, Chlorella, Nannochloropsis and Spirullina species. The effects of light, oxygen content, temperature, carbon dioxide, nutrient concentration, salinity, mixing, and dilution rate, which affects the rate of growth of microalgae, composition of the Dunaliella microalgae biomass, molecular identification of the morphological characteristics was also reviewed. The various challenges and prospect encountered in the isolation and screening of pure microalgae species were evaluated. Despite numerous research investigations in this field, several challenges still exist including isolation and cultivation of pure species of Dunaliella for the commercialization of biocrude production from microalgae.

1. INTRODUCTION

Microalgae are members of the heterogeneous group of photosynthetic autotrophs that grows vigorously in different water bodies such as rivers, pools, lakes, seas (Show *et al*., 2019). They can be applied as sources of food for man's consumption (Picardo *et al*., 2013), wastewater purification (Randrianarison and Ashrate, 2017), production of

biofertilizer (Selman *et al*., 2013), they provide oxygen and food for different species in the aquatic environment (Hameed, 2016), CO_2 sequestration (Barros *et al*., 2015) and as viable source of biofuels Tang *et al*., 2011). Microalgae are diverse, and they include different phyla with unique properties and characteristics such as eukaryotic or prokaryotic, unicellular structure or simple multicellular structure (Selman *et al*., 2013).

In 1942, it was first reported by Harder and Witsch that microalgae have what it takes to be a good source of lipids which can be converted to food or used for the production of biocrude (Harder and von Witsch, 1942). This has led to active research on microalgae and the various (Tan *et al*., 2015; Chowdury *et al*., 2020). In the last few decades, microalgae biofuels have become an attractive research field in renewable, green, and sustainable source of energy (Maga, 2017). Microalgae do not have complex structure and organs in comparison with other plants, yet, microalgae perform photosynthesis because of the presence of chlorophyll in their cells (Show *et al*., 2019). They are photosynthetic organisms and also aquatic organisms that do not have vascular tissues, roots, leaves, stems but have single reproductive system (Randrianarison and Ashraf, 2017).

The range of renewable fuels generated from microalgae algae include: Biomethane (Barro *et al.,* 2015; Phwan *et al*., 2018; Bose *et al*., 2020), bioethanol (Goyal *et al.,* 2008; Maga, 2017; da Maia *et al*., 2020), Syngas (Liu *et al*., 2012; Hong *et al*., 2017; Lee *et al*., 2020), biohydrogen (Jiang and Savage, 2017; Show *et al*., 2020; Antoniana *et al*., 2021) and bio-crude (Zhang *et al*., 2018; Eboibi, 2019; Hu *et al.,* 2019). Among all these fuels produced from the biomass of microalgae, bio-crude is commonly known because of its energy, density and cleaner fuel (Maga, 2017; Panahi *et al*., 2019; Liu *et al*., 2019).

Momentarily, there are over 50,000 species of microalgae known globally (Silva *et al*., 2021), while only about 30,000 of them have been studied (Hammed, 2016). Among these species is the *Dunaliella* genus. These microalgae are distinct from other green microalgae morphologically, because they don't have rigid cell wall and they have outer cell membrane which is made up of glycoproteins (Tang *et al.,* 2011). Among 20 species that belong to the *Dunaliella* genus are *Unaliella purva*, *Dunaliella primolecta*, *Dunaliella bioculata, Dunaliella salina, Dunaliella virdies, Dunaliella bardawil, Dunaliella* media, *Dunaliella tertiolecta* and *Dunaliella acidophila*. These species can be found in every continent and they have been studied over decades (Tang *et al*., 2011).

Microalgae, which is the source of the third-generation fuel is produced from the derived lipids (Taghipour *et al*., 2021). The amount of lipid present in microalgae is much higher than its equivalent present in seeds currently used for biodiesel production (Randrianarison and Ashraf., 2017; Mondal, *et al*., 2017; Mofijur *et al*., 2019).

Shuping *et al*, (2010) obtained biocrude yield of about 25.8% from *Dunaliella tertiolecta* by hydrothermal liquefaction operation at a reaction temperature of 360℃ and 50min, using a catalyst of 5% Na_2CO_3. Their report showed that the *Dunaliella tertiolecta* biomass they used for their work was purchased, they did not screen, culture or harvest the microalgae they used for their work. Also, there was no report on kinetic and thermodynamic behavior of the production of the biocrude. The characterization of the *Dunaliella tertiolecta* shows protein as 39.8%; fat 2.87% carbohydrate 21.69% carbon 39.80%; hydrogen 5.37%; oxygen 53.02% and heating value 20.08 MJ/kg. Xue *et al*, (2017) obtained strain of *Dunaliella tertiolecta* from Chinese Academy of science, institute of hydrobiology, freshwater algae culture collection. They cultivated the strain in a medium sterilized for 30 min at 121℃ before the inoculation. The *Dunaliella tertiolecta* cells were cultivated 13/11 hr light/dark cycle. They reported that at the expiration of the cultivation period of *Dunaliella tertiolecta*, the group treated with triethylamine had much lower optical density. The cells under the treatment of triethylamine maintained moderate level for the 72 hr, during which the total lipid yield was high. The microalgae were not screened before pure strain of *Dunaliella tertiolecta* was obtained. Also, the mode of harvest was not reported in their work. Shang *et al*, (2016) reported that they obtained the strain of *Dunaliella parva* from Chinese Academy of science, institute of hydrobiology, freshwater Algae culture collection. They reported that two samples were cultured, one with limited nitrogen and the other with nitrogen sufficient sample. They monitored the cell growth by measuring its optical density at $680nm$ using UV spectrophotometer, and the *Dunaliella perva* was harvested by centrifugation technique while the pellets were transferred to a storage section and frozen with liquid nitrogen at -86℃. The optical density $OD_{(260/280)}$, of the nitrogen

limited and sufficient were obtained as 2.14 and 2.17 respectively. Lima *et al*, (2020) isolated the microalgae species from samples using both filtration method (5 to 20µm) pore size sieves and serial dilutions in microplates. They then carried out microalgae isolation by characterizing the microscopic morphological analysis before molecular analysis, in order to access the isolated microalgae. To correctly identify the *Dunaliella* strain, a polymerase chain reaction (PCR) was used so that the ITS region could be amplified. They used four strains, *Nanochloropsis gaditana* and *Chlorella sorokiniana* were purchased from Scotland association of material science and universita di Napoli Federico II respectively. *Chlorella pozzillo* and *Dunaliella tertiolecta* were obtained along the coast of Sicilan, isolated and identified by rDNA 185 sequencing. But the characteristics that identify each of the strains were not reported in their work. The amount of lipids removed from the biomass was not also reported in the work. Liang, Xue & Jiang (2018) cultivated *Dunaliella tertiolecta* using a two stage with triethylamine and glycerol for high lipid production. They obtained the *Dunaliella* strain from freshwater algae culture, China. The microalgae were cultivated on a medium that contains 0.42 g/l $NaNO_3$, 0.015 g/l $NaH_2.2H_2O$, 0.840 g/l $NaHCO_3$, 1.230 g/l $MgSO_4.7H_2O$, 0.074 g/l KCl, 0.044 g/l $CaCl_2.H_2O$, 0.5 ml/l Fe-EDTA solution and A5 trace elements solution 1ml/l with solution of 2.0 M NaCl at a temperature of 26 ℃ under a dark/light cycle 14/10 hr for about 24 days. They obtained the value of the optical density (OD) of the *Dunaliella* microalgae during culture at about 630 nm. Wang *et al*, (2020) cultured *Dunaliella* salina in a 500 ml sterilizer f/2 medium, maintained at a pH of 7.3±0.1. They maintained the culture in a growth chamber of about 25±2 ℃, a light/dark cycle of 12/12 hours and the initial optical density was 630 nm. Wang *et al*, (2017) investigated the kinetics of arsenite and arsenate bioaccumulation in *Dunaliella* species at different regimes of phosphate. They obtained the *Dunaliella* strain from institute of oceanology, China. The microalgae cells were maintained in an f/2 culture medium that has been sterilized. The pH of the culture medium was kept at about 7.3±0.2 for 25 ±2 ℃ under a 12/12 hours light/dark cycles. The initial OD of the medium was adjusted to 630 nm = 0.05 (approximately 4 x 10^5 cell ml^{-1}). The yield of the *Dunaliella salina* microalgae was not reported. Liu and Yildiz, (2018) obtained the *Dunaliella salina* cells from Dalhouse aquaculture, Canada. They used Bold basal medium in a controlled environment of about 20 ℃ with a light/dark cycle of 16/8 hr. The salinity was maintained at 30ppt and the initial optical density was regulated to about 10^5 cell ml^{-1}. They obtained a maximum biomass yield of 169.5 mg/l at about 383.5%. Zhu *et al*, (2018) reported the cultivation of *Dunaliella* specie in a floating photobioreactor using concentration of seawater desalination. They obtained the strain of *Dunaliella salina* from the culture collection center of microalgae and protozoa, Scotland. They used a modified seawater medium containing 87.75 g/l NaCl, 8.4 g/l $NaHCO_3$, 0.5 g/l KNO_3, 0.08 g/l $K_2HPO_4.3H_2O$, 0.111 g/l $CaCl_2$, 0.507 g/l $MgCl_2.6H_2O$, 0.123 g/l $MgSO_4.7H_2O$, $FeCl_2.6H_2O$, 0.0006 g/l and 1ml/l A5 was used. Their report showed 300g of *Dunaliella salina* can be produced in 12 days culture. Kim *et al*, (2017) cultivated *Dunaliella* on bicarbonate for the utilization of carbon efficiency. They isolated the cell from salt pond in Korea and were cultured in a modified Johnson's medium containing 2 M NaCl. Then cultivated the cell with several concentrations of (0 - 30 g/l) $NaHCO_3$ to determine the cells' ability of the microalgae to grow with carbonate as a source of carbon. It was cultivated for 2 – 3 days and obtained optical density at 680 nm. The microalgae cells were maintained at 25 ℃ and light intensity of 340 μEm^{-2}. They reported in their work that the specific gravity of the *Dunaliella* species increased from 0.34/day to optimum of 0.97/day using 5 g/l $NaHCO_3$ but the percentage yield of the microalgae biomass was not reported. Mirzale *et al*, (2021) study was on shifting strategy in flash light on the growth curve of *Dunaliella salina* cell to enhance β-carotene biosynthesis. The *Dunaliella salina* (CCAP 19/18) cells were obtained from culture collection of microalgae and protozoa, Scotland. The medium contains 300 mg/l KNO_3, 20 mg/l KH_2PO_4, 80 mg/l K_2HPO_4, 47 mg/l $CaCl_2$, 10 mg/l, $MgSO_4.7H_2O$, and trace element. The medium was supplemented the medium with 33 g/l NaCl and adjusted its pH and temperature to 7.0 and 25℃ respectively. The result showed the dry weight biomass reached an optimum value of 1.3 g/l after 25 days.

In 2021, Keramati *et al*, studied audible sound frequency effect on *Dunaliella salina* rate of growth and β-carotene production (Keramati *et al*., 2021). The *Dunaliella salina* strain was collected from the Islamic Azad University, Iran. The *Dunaliella salina* was cultured in a Johnson's media containing1.5 g/l $MgCl_2.6H_2O$, 0.5 g/l $MgSO_4.7H_2O$, 0.2 g/l KCl, 0.2 g/l $CaCl_2$, 1.0 g/l KNO_3, 0.043 g/l $NaHCO_3$, 0.035 g/l KH_2PO_4, 10 ml/l of stock Fe-solution, 10 ml/l trace

element solution. The pH was adjusted to 7.5, the optimum salinity was 1 M/l and the temperature of the system was maintained at 23±2 ℃ but the biomass yield was not reported. Bredda *et al*, (2019) research investigation was on the effect of wavelength of light on *Dunaliella salina* cultivation for lipid production. The *Dunaliella salina* BMAK 110 strain was obtained from seawood culture collection Sao Paulo, Brazil. The strain was kept in a 5l Elemeyer with Guillard f/2 medium. They kept the flasks under a 30 μmol/m^2.S LED white light and aerated with sterile air. An optimum biomass yield of 136.67 mg/l.day was obtained when cultivated with blue, red and green LED equal proportions.

Almutairi (2020), investigated the effects of phosphorous and nitrogen on methyl ester and the fuel properties of *Dunaliella* specie. They obtained the *Dunaliella salina* strain CCAP 19/12 from culture center of microalgae and protozoa, UK. The liquid *Dunaliella salina* was subcultured in a f/2 growth medium and a temperature of 25±2℃ with continuous illumination with 70 μmol/m^2 S LED light. They measured the optical density (OD) at 650nm and reported an optimum growth of 0.911 ± 0.020 g/l after 10 days.

Despite several investigations on *Dunaliella* algae, there are challenges in the commercialization of biocrude from this microalgae specie. Some of the challenges include screening of species, molecular identification, cultivation, harvesting, and cost of large-scale production (Tan *et al*., 2011; Muvlaet , 2017; Tripathi and Kumar, 2017; Zabed *et al*., 2020; Wang *et al*., 2021), hence extensive research investigation in this field is necessary.

In this present review work, elemental and biochemical compositions, microalgae screening, molecular identification, culture and biomass harvest methodology of *Dunaliella* species microalgae were reviewed. The various techniques applied for the isolation of microalgae are not well available and known to many researchers. Presently, most researchers purchased microalgae concentration from hatcheries for a particular strain of microalgae. Consequently, this review is undertaken to explore possibility of modifying the biochemical compositions of *Dunaliella* species that favors the production of biocrude and ways of properly identifying *Dunaliella* species microalgae.

2. ELEMENTAL COMPOSITION OF MICROALGAE BIOMASS

The composition of biomass determines the characteristics, composition and percentage yield of the biocrude. Elemental composition of microalgae may vary quantitatively, depending on the factors affecting its chemical composition such as microalgae strain, the environmental conditions, the amount and quality of light, type of cultivating technique, the composition and temperature of the culture medium (Zanella and Vianello, 2020). Biomass of microalgae consist of five major elements which are Carbon, Hydrogen, Nitrogen, Sulphur and Oxygen, they are presented with their composition in Table 1. These elements majorly formulate the amount of energy of the biomass of microalgae. The ratio of oxygen to carbon and that of carbon and oxygen plays important role in the determination of heating value, the combustibility, the amount of energy and the amount of moisture of the biomass. The higher the oxygen to carbon ratio the lower the energy density. Elemental analysis is one of the most effective characterization tools of energy generated from the biomass of microalgae (Hossain *et al*., 2019).

Generally, microalgae have about 35.2 – 57% carbon content, except for *Botryococcus braunii* which contain about 77.04%. It contains about 24 – 35% of oxygen, 6 – 9% and 2 – 12% hydrogen and nitrogen respectively, while the sulphur content is ≈1.5% sulphur (Mathimaniani and Mallick, 2019). Among all these elements, the bio-crude higher heating value (HHV) is highly dependent on the oxygen content, as higher oxygen lowers its HHV (Gollakota *et al.,* 2018).

Table 1: Elemental composition of *Dunaliella Chlorella*, *Spirulina* and *Naochloropsis* microalgae

Strain	Carbon (%)	Hydrogen (%)	Nitrogen (%)	Oxygen (%)	Sulphur (%)	H/C	O/C	HHV (MJ/kg)	Citation
D. Salina	27.13	nr	15.94	51.19	2.70	nr	22.64	nr	Mishra *et al*., 2011
D. Tertiolecta	39.00	5.37	1.99	59.02	nr	1.65	16.31	20.08	Shuping *et al*., 2010
D. Salina	27.00	7.10	8.20	32.20	0.50	3.16	14.31	14.09	Mathimani and Mallick, 2019
D. Salina	48.10	7.10	9.40	23.30	0.90	1.77	5.81	21.20	Gong *et al*., 2014
D. Tertiolecta	38.63	6.41	2.54	nr	nr	1.99	nr	nr	Goo *et al*., 2013
D. Tertiolecta	53.30	5.20	9.80	31.70	0.50	1.46	7.14	19.78	Biller and Ross, 2011
C. vulgaris	58.23	7.10	8.52	25.80	0.35	1.46	5.32	25.22	Mahima *et al*., 2021
Chlorella sp.	56.20	6.90	7.70	28.70	0.60	1.47	6.13	19.90	Biller *et al*., 2015
C. vulgaris	50.00	7.10	5.80	33.80	0.53	1.70	8.11	20.29	Peng *et al*., 2017
C. vulgaris	48.30	7.30	3.00	32.90	nr	1.81	8.17	nr	Figueira *et al*., 2015
C. pyrenoidosa	51.20	6.80	11.30	30.70	0.70	1.59	7.20	22.61	Gai *et al*., 2015
C. vulgaris ESP-31	53.01	8.67	3.26	35.05	nr	1.96	7.93	nr	Bach *et al*., 2017
Spirulina sp.	74.80	8.20	6.90	9.10	1.00	1.32	1.46	34.83	Eboibi, 2021
S. platensis	49.60	6.20	10.80	33.40	0.80	1.50	8.08	21.08	Gai *et al*, 2015
S. almeriensis	41.90	6.70	5.90	44.70	0.80	1.92	12.80	88.94	Earreiro *et al*., 2014
S. obliquus CNW-N	37.37	5.80	6.80	50.02	nr	1.86	16.06	nr	Chen *et al*., 2014
S. platensis	42.26	5.86	3.47	47.26	1.15	1.66	13.42	16.83	Toor *et al*., 2013
S. specie	55.70	6.80	11.20	26.40	0.80	1.46	5.69	24.64	Biller and Ross, 2011]
Nannochloropsis sp.	75.40	9.80	5.40	8.90	0.50	1.56	1.42	36.92	Barreiro *et al*., 2015
Nannochloropsis gaditana	74.80	9.90	5.30	9.50	0.50	1.59	1.52	36.77	Barreiro *et al*., 2015
N. gaditana	49.40	7.70	7.00	34.70	1.10	1.87	8.43	22.74	Barreiro *et al*., 2014
N. gaditana	47.60	7.50	6.90	25.10	0.50	1.89	6.33	22.81	Barreiro *et al*., 2015
N. oculata	53.90	8.44	2.65	34.35	0.66	1.88	7.65	36.90	Kumar *et al*., 2018
N. oculata	57.80	8.00	8.60	25.70	nr	1.66	5.34	36.70	Biller and Ross, 2011

2.1. Carbon Element

This is one of the most significant constituents of *Dunaliella* species biomass. Carbon element obtained from CO_2 in the atmosphere is taken-up by the plant and become part of its composition during photosynthesis (Gollakota *et al*., 2018). Total content of carbon element present in the biomass of *Dunaliella* species has direct impact on the yields and quality of biocrude. The higher the content of carbon element in the microalgae biomass, the higher its calorific value and that of the corresponding bio-crude produced (Chouhan and Sarma, 2013). Carbon element is the major contributor to the heating value of the biomass, and it is converted to -CO2 during combustion, which is eventually released into the atmosphere from where it is used to become part of the photosynthetic process. Majorly, the content of carbon element in the biomass is estimated through the compositions of hemiscellulose, lignin and cellulose. Biomass rich in lignin has carbon content that is higher than 50wt% (Gollakota *et al*., 2018). The design of the reactor for the hydrothermal liquefaction operation also relays on the fixed carbon content in the *Dunliella* species sample, as it determines the extent of oxidation, reduction reactions in the reactor and the gas composition (Chouhan and Sarma, 2013).

2.2. Hydrogen Element

Hydrogen is another significant constituent of the *Dunaliella* species biomass, and as an organic substance, it can be observed in carbohydrates, phenolic polymers. The combustion process of biomass involves the conversion of hydrogen component to water (H_2O), and this contributes greatly to the higher heating value (HHV) of the *Dunaliella* species biomass. Total content of hydrogen element present in *Dunaliella* species biomass has significant impact on the quality of biocrude. Increasing the weight percentage of hydrogen element in the *Dunaliella* microalgae biomass will increase the production yield of biocrude, and the higher the value of hydrogen element in the *Dunaliella* species biomass, the more the calorific value of the biomass and biocrude respectively (Gollakota *et al*., 2018). The high production rates of hydrogen element by *Dunaliella* microalgae are stated in parts, on the efficient growth of biomass. The setups for biomass hydrogen element production normally make use of single-celled microalgae, specifically the ones with good possession of metabolic and enzymatic qualities required for hydrogen element production (Deboroski *et al*., 2018). The amount of hydrogen element is normally lower in herbaceous biomass, that is, 5.5 – 6%, than in woody biomass which is about 6 – 8% (Gollakota *et al*., 2018).

2.3. Nitrogen Element

Nitrogen constitutes a vital nutrient that is obtained from the biomass of *Dunaliella* species. It is applied to the soil as fertilizer to enhance plant growth and its overall yield (Gollakota *et al*., 2018). *Dunaliella* species biomass contains necessary nutrients such as proteins, carbohydrates, and vitamins. This type of microalgae biomass can be used in green fertilizer production. *Dunaliella* species have been observed to grow faster, grow healthier and also, more tolerant to stress. The application of microalgae biomass for the production of fertilizer has not received extensive attention (Slinksiene *et al*., 2022). One of the characteristics of pure microalgae biomass is the slow release of nitrogen, which actually satisfies the needs of the microalgae. The most frequently used bio-fertilizer is the dry biomass of microalgae, although, drying requires energy consumption. The wet microalgae biomass (suspension) can be applied as raw material for the production of fertilizer, thereby eliminating cost of energy for drying while the content of water in the biomass is served in the fertilizer granulation process (Suanbul *et al*., 2020). The amount of nitrogen element contributes greatly to the process of degradation in fermentation or digestion, but during combustion of the biomass, nitrogen does not oxidize, therefore has no effect on the overall heating values of *Dunaliella* species biomass or the bio-crude obtained from it (Gollokota *et al*., 2018). At low concentrations (about 100 ppm), ammonium nitrogen (NH_4–N), nitrogen containing compound has no effect on the rate of growth of *Dunaliella* species, but as the concentrations of nitrogen element becomes high (>200ppm) in form of ammonium nitrogen, it reduces the biomass yield by about 30wt% (Slinksiene *et al*., 2020).

2.4. Sulphur Element

Sulphur is another significant element similar to nitrogen in the structure of amino acids, enzymes and proteins for improved growth of the plant. The sulphur content of herbaceous crops is higher than that of woody biomass, thereby, their high growth rate. The amount of sulphur in the biomass of wood can be below detection limit (0 wt%) and can be up to 1% on exceptional cases. While the amount can be up to 0.2wt% or higher in herbaceous biomass but is most significant in gaseous emissions, corrosion and syngas cleaning in gasification processes (Gollakota *et al*., 2018). The sulphur element is present in the structure of proteins, enzymes, peptides, some amino acids, vitamins and coenzymes found in the microalgae cell and is also a component of many others organic cell compounds. Physiological role of sulphur in *Dunaliella* species is linked with cell division process metabolism of protein and the synthesis of fatty acid (Fernandes *et al*., 2020). Cell sulphur demand can be provided by introducing inorganic sulphur into the microalgae culture medium. The movement of sulphur by active transportation into the microalgae; which is a process that requires light and it is also temperature-sensitive. Therefore, injections of SO_2 with bubbling air, which is used as a nutrient by the microalga cell, enhances microalgae biomass production yield. Sulphur deficiency in the microalgae culture medium is a stress factor for the cells and this usually led to lipid accumulation and to cell division disorder. At the same time, when the SO_2 concentration in the bubbling air decreases, the microalgae pH of the culture medium will tend to stop the growth of the microalgae (Zhang *et al*., 2020).

2.5. Oxygen Element

When considering the biomass chemical composition, oxygen constitutes an important element. The oxygen content in the microalgae biomass influences the heating value. The oxygen content in phenolic compounds is difficult to break so as to enhance the heating values. The oxygen content measurement is not done directly, it is estimated by subtracting the concentrations of carbon (C), hydrogen (H), nitrogen (N), sulphur (S) and ash obtained at superficial condition from 100, whether wet or dry biomass (Gollakota *et al*., 2018; Zhang *et al*., 2019; Khoo *et al*., 2020). *Dunaliella* species consumes carbon dioxide (CO_2) during phototrophic growth and produces oxygen (O_2). When a closed cultivation system such as photobioreactor is used, the oxygen produced can easily build up to as high as 8 – 10 mg/l.min and can even reach 100 mg/l.min even when frequent gas exchange is carried out. This process can cause adverse effect on yield of microalgae biomass production reducing microalgal cell growth rate (Gao *et al*., 2022). The major reactions that occur at high concentration of dissolved oxygen element are photorespiration (the consumption of oxygen which depends on light for the production of CO_2, Mehler process (water-water cycle) and photoinhibition (occurs as a result of prolonged exposure of microalgae to high intensity of light, which results to the formation of reactive oxygen species, and can cause damage of cellular components). When these conditions arise, high oxygen concentration can play an active role through Mehler reactions, by giving out excess energy (Kazbar *et al*., 2022). Gas-liquid transfer operation is significant to avoid the accumulation of oxygen (O_2) in the culture medium and to ensure provision of the required carbon dioxide (CO_2) to run photosynthesis (Abiusi *et al*., 2020).

The presence of oxygen in biofuel reduces the energy density, although other heteroatoms like nitrogen may also be present in the biocrude, but oxygen-containing functional groups attract water, which can also cause corrosion of the storage tanks and pipelines (van Dyke *et al*., 2019). The H/C ratio (Equation 1) indicate the extent to which upgrading will be required to produce deoxygenated biocrude, while the biocrude produced from biomass with high H/C ratio has the tendency of high energy, dense biocrude when compared with low H/C ratio biomass. Figure 1 shows the plot of this relationship with O/C (Equation 2) of selected microalgae as Van Kreven chart. Table 1 shows that the H/C of previous works carried out by different researchers on *Dunaliella* species are relatively lower than those of *Chlorella* specie, *Nannochloropsis* specie and *Spirullina* specie, but the O/C ratio of *Dunaliella* specie is relatively higher than the other microalgae considered.

$$\frac{H}{C} = \frac{weight\ percent\ of hydrogen\ \ x\ molecular\ weight\ of\ carbon}{weight\ percnet\ of carbon\ x\ molecular\ weight\ of hydrogen} \quad (1)$$

$$\frac{O}{C} = \frac{weight\ percent\ oxygen\ of\ x\ molecular\ weight\ of\ carbon}{weight\ percnet\ of carbon\ \ x\ molecular\ weight\ of\ hydrogen} \quad (2)$$

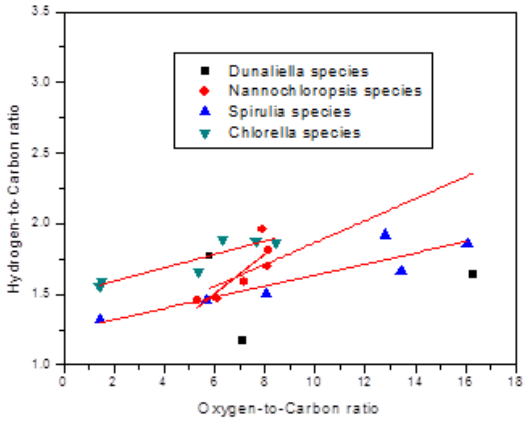

Figure 1: Van Kreven chart of hydrogen-to-carbon and oxygen-to-carbon of selected microalgae

3. BIOCHEMICAL COMPOSITION OF THE BIOMASS FEEDSTOCK

The biochemical analysis of microalgae biomass includes the carbohydrates, lipids, and protein, presented in Table 2. In addition to the elemental or ultimate composition of microalgae, microalgae biochemical composition is of utmost significance in the selection of strain to be used for the production of biocrude. The percentage yield of biocrude from different microalgae is dependent on the algae compositions. Aside lipid-rich microalgae which majorly favour the biocrude production, low lipid producing microalgae biomass can also be converted to biocrude. Protein-rich biomass of microalgae is preferable for hydrothermal liquefaction operation because when compared to carbohydrate-rich biomass, it can be readily converted to biocrude (Mathimani and Mallick, 2019). Carbohydrates-rich biomass of microalgae mostly decomposes into polar materials that are water-soluble, rather than nonpolar hydrocarbons, this in turn dissolves in the aqueous phase of the hydrothermal liquefaction operation of biomass, making carbohydrate contribute only slightly to the production of biocrude (Hao *et al*., 2021). Carbohydrates-rich biomass can actually be converted into different compounds at various operating temperatures, while the conversion efficiency of organic matter actually increases with temperature (Kumar *et al.*, 2018). At higher temperature, repolymerization of small-molecule substances of carbohydrates can be promoted, while solid depolymerization and gas aggregation to generate biocrude can be improved upon (Wadrzyk *et al*., 2018). Protein-rich biomass can be hydrolyzed, the peptide bonds broken into amino acids when subjected to hydrothermal liquefaction operation. They are subsequently decarboxylated, deaminated, repolymerized and dehydrated, in order to form organic and carbonic acids, nitrogen compounds, ammonia, carbon dioxide, and other gases. Crude proteins or crude polysaccharides do not really have linear relationship with biocrude yield. At reaction temperatures of 220 – 260 ^{o}C, polysaccharides actually have negative effect on the conversion of protein-rich biomass to biocrude. However, at about 300 ^{o}C polysaccharides can promote the conversion of protein-rich biomass into biocrude (Yang *et al*., 2015).

Microalgae populate aquatic ecosystem, therefore lack combined support structure like complex matrix of lignin, lignocellulosic biomass, cellulose and hemicellulose, which negatively affects the system. The biomass composition and yield of biomass, selectively depend on the type of microalgae species, this is in turn greatly affected by the nutritional and growth conditions of the microalgae. Unicellular microalgae are effective source of lipids and proteins, while macroalgae or seaweeds are effective source of carbohydrates (Suparmaniam *et al*., 2019).

The biochemical constituents of most microalgae is generally known to consist of high proteins of about 40 – 70%, medium carbohydrates of about 12 – 30%, considerable amount of lipids of about 4 – 20%, carotenoids of about 8 – 14%, but variable concentrations of vitamins and minerals (Vaquero, 2021). However, there is limited information on how the biochemical constituents of the microalgae biomass can be modified genetically or with the use of biotechnology, using any of these techniques, genome editing with

site-directed nucleases, genome editing directed by synthetic oligonucleotides, RNA directed DNA methylation, cisgenesis and intragenesis, transgrafting, agro-infiltration, haploid induction and accelerated breeding techniques (Harvey and Ben-Amotz, 2020), thereby improving its protein and carbohydrate contents for higher biocrude yield.

Table 2: Biochemical analysis of *Dunaliella* specie

Species	Proteins (wt%)	Lipids (wt%)	Carbohydrate (wt%)	Citation
Dunaliella	20-30	10-20	50.00 - 80.00	Harvey and Ben-Amotz, 2020
Dunaliella tertiolecta	61.32	2.87	21.69	Shuping *et al*., 2010
Dunaliella bioculata	49.30	8.00	4.00	Pourkarimi *et al*., 2020
Dunaliella salina	57.00	6.00	32.00	Pourkarimi *et al*., 2020
Dunaliella teriolecta	29.10	11.00	14.00	Pourkarimi *et al*., 2020
Dunaliella species	49-57	6-80	4-32	Sarkar *et al*., 2022
Dunaliella Salina	69.70	9.10	5.40	Debinath *et al*., 2021
Dunaliella tertiolecta	46.40 - 50.60	18.00 - 23.50	8.30 - 31.30	Debnath *et al*., 2021
Dunaliella salina	32.00	6.00	57.00	Hossain *et al*., 2019
Dunaliella tertiolecta	24.00	22.00	46.00	Reddy *et al*., 2014
Dunaliella salina	26.00 – 29.00	18.0 – 25.00	16.00	Amorim *et al*., 2020
Dunaliella	13.17	7.30	36.17	Najeeb *et al*., 2024
Dunaliella	20.47	11.04	36.83	Najeeb *et al*., 2024
Dunaliella sp. ABRIINW-B1	19.00	42.00	8.00	Gbarajeh *et al*., 2020
Dunaliella sp. ABRIINW-G2/1	41.00	36.00	4.00	Gbarajeh *et al*., 2020
Dunaliella sp. ABRIINW-I1	39.00	47.00	4.00	Gbarajeh *et al*., 2020
Dunaliella bioculata	49.00	8.00	4.00	Krimpen *et al*., 2013

3.1. Lipids

Generally, lipids produced by *Dunaliella* microalgae include neutral lipids, wax esters, polar lipids, sterols, hydrocarbons and prenyl derivatives. These lipids can be categorized into two groups, storage lipids also known as non-polar lipids, and structural lipids also known as polar lipids, and their yield from a particular microalgae species highly affects its efficiency of energy production (Chowdury *et al*., 2020). Lipids produced from microalgae usually take the form of triacylglycerols (TAGs), which mostly have aliphatic character and they are majorly non-polar and they are made up of glycerol backbone which is bonded to three fatty acids. At room temperature, the fats are generally not soluble in solvents and as temperature changes; they tend to be polar (Sajjadia *et al*., 2018). When TAGs are hydrolyzed, glycerol is one of the products obtained and this glycerol can be processed to acetaldehyde, methanol, propionaldehyde, acrolein, ethanol, allyl alcohol and formaldehyde, and gas mixture (CO, CO_2 and H_2), when subjected to hydrothermal liquefaction (Gollakota *et al*., 2018). Examples of triacylglycerols and their specific heat are presented in Table 3. Fatty acids (FA) present in triacylglycerols are targeted for production of biocrude (Morales *et al*., 2021). Microalgae generally make fatty acids with carbon chain lengths of 12, 16 and 18, but some microalgae species can produce fatty acids with chain length up to 24 carbons. Triacylglycerols (TAGs) majorly contain saturated fatty acids (SFAs) and mono-unsaturated fatty acids (MUFAs), such as C14:0 (Myristic acid, MA), C16:0 (Palmitic acid, PA), C16:1 (Palmitoleic acid, POA), C18:0 (Stearic acid, SA) and C18:1 (Oleic acid, OA), while polyunsaturated fatty acids (PUFAs) can also be present (Sharma *et al*., 2012). The amount and ratio of saturated and unsaturated fatty acid in microalgae specie determines its suitability for biofuel feedstock. Some microalgae such as *Botryococcus* specie*, Chlorella vulgaris*, and *Scenedesmus* species can also produce a large quantity of polyunsaturated fatty acids such as C22:6 (42%) in *Aurantiochytrium* specie, C22:5 + C22:6 (39.4%) in *Schizochytrium limacinum*, C20:5 (25%) in *Porphyridium cruentum* (Zarrinmehr *et al*., 2019). The properties of biocrude produced from microalgae largely depend on the constituent of the fatty acids. For example, the biocrude produced from microalgae with low saturated fatty acid, is bond to have better cold temperature properties because the pour and cloud point of the biocrude are increased by long-chain saturated fatty esters. However, when the biocrude contains

high number of unsaturated compounds, it gets oxidized faster than conventional crude. The percentage weight of lipid produced from different species of *Dunaliella* microalgae by various researchers are presented in Table 2. Therefore, while seeking efficient microalgae strains for biocrude production, the ability of microalgae to generate high quantity of lipid and high quality of the fatty acid constituents should be considered (Sajiadia *et al*., 2018). Nevertheless, these researches did not consider how the *Dunaliella* microalgae can be modified in order to increase the lipid productivity, which will eventually boost the biocrude yield.

Table 3: Specific heat capacity of triacylglycerols

Triacylglcerols	Specific heat capacity (kJ/kg)
Tristearin	1.938
Triolein	1.886
Trilinolein	1.813
Trilinolenin	1.765

3.2. Proteins

This is one of the significant compositions of microalgae biomass and it is made up of many peptide chains which reduce to the polymers of amino acids. Protein contents of microalgae specie and their strain can be vastly different, and this is substantially so because of the environments in which they are cultivated (Wang *et al*., 2021). Several species of microalgae may contain high levels of protein, typically 40–60wt%, while some of the species have relatively low protein levels, especially those selected for the production of oil and biocrude. It has been reported that crude protein content in the biomass of microalgae ranges from 6 – 63w%, while most species have over 40w% (Barka and Blecker, 2016). This variability of protein content reported by different researchers is remarkable for some microalgae species such as *Dunaliella* specie, as can be seen in Table 2. The yield of amino acid when subjected to HTL is low because they undergo decarboxylation, which produces carbonic acids and amines; also, they undergo deamination which produces organic acids and ammonia (Hao *et al*., 2021). These products from the reactions may then repolymerized to form aromatic ring structure such as nitrogen heterocyclics (pyrole or indole) or phenols and long chain hydrocarbons. The main sources of nitrogen in the derived biocrude are proteins (Sun *et al*., 2017; Yu *et al*., 2018; Obeid *et al*., 2021). Microalgae, just like other sources of food such as soy, meat and eggs, are rich sources of protein. The quality of the protein from microalgae is determined by the amount of the essential amino acids which serves various functions. The efficiency of protein extraction from microalgae biomass is influenced by the chemical composition, the characteristics of its structure and the morphology of the microalgae, while higher cellular content of protein is peculiar with increase in light irradiation over a long photoperiod (Morales-Jimenez *et al*., 2020; Orejuela-Escobar, 2021; Bumbac *et al*., 2023). Many species of microalgae that are high-protein producers have been identified, these include *Dunaliella* species (Brasil *et al*., 2018; Vingiani *et al*., 2019), and the corresponding percentage weight of the various strains are presented in Table 2.

3.3. Carbohydrates

Carbohydrate is an intracellular component, in which increase in the theoretical biomass, increases its productivity. It makes up the structural component of microalgal cell wall which acts as an intracellular energy storage compound. This stored carbohydrate helps to generate maintenance energy when the photosynthetic energy yield remains zero during the dark cycle (Debinath *et al*., 2021). On the contrary, cell structural wall of carbohydrates acts as blockage for molecules that are toxic, which may tend to breach the cell (de Carvalbo *et al*., 2020). Microalgal has complicated carbohydrates which comprise mixture of neutral sugar, amino sugar and uronic acid, while it has different compositions among the several species and different conditions of cultivations (Deninath *et al*., 2021). The glycogen molecules are the primary intracellular storage carbohydrates which are accumulated by prokaryotic blue-green algae, whereas beta-glucans also known as starch are preferably accumulated by eukaryotic microalgae. For example, the genus from Euglenophyta and Rhodophyta absorbs floridean starch and paramylon as the primary storage carbohydrates respectively, while Chlorophyta, Dinophyta and Cryptophyta absorbs starch (Abdallah *et al*., 2016). The oleaginous microalgae under Heterokontophyta and Bacillariophyceae do not absorb

carbohydrate but are known to absorb lipids as storage energy molecules. When nutrient depletion occurs under adverse environmental conditions, rather than accumulating high molecular weight polysaccharides, microalgae accumulate high levels, low molecular weight sugar alcohols and sugars (Debinath *et al.*, 2021). The sugar alcohols and sugars, act as scavengers of oxygen together with the regular function under stress condition, as energy storage compound. But the structural carbohydrates are essentially different among the species and different conditions of growth; therefore, it is applied as a marker for the identification of different species (Alhattab *et al.*, 2019). Consequently, microalgal strains having inherent higher growth rates must be selected.

3.4. Carotenoids

Carotenoids are among the largest classes of natural pigments having more than 750 structures present in several organisms including microalgae and higher plants. They are juxtaposed isoprenoids which are produced by all organisms of photosynthesis used for light-harvesting and for photoprotection of microalgae. Carotenoids are located on chloroplast, in green tissues of plants and they function as secondary molecules of photosynthetic pigments. Also, it prevents photooxidative damage and under stress, act as antioxidants (Zhu *et al.*, 2020). Carotenoids containing one β ring not substituted are significant for the pathway of vitamin A and are also needed to produce retinoids in the human diet. These are very significant in differentiating cell, growth, and apoptosis. They are also very important for the control of vision defects. Molecules of carotenoid that are intact and the products of carotenoid cleavage other than retinoids, may also act as scavengers of lipid radical and also as singlet oxygen quenchers which protect against oxidant stress, and also act as filters of blue light for photoprotection of skin and eyes (Xu *et al.*, 2018). The market of carotenoids is projected to be up to about USD 2.0 billion by 2026, largely because of increase in the use of natural carotenoids especially β-carotene as food colorants, and of the innovations of technology of extraction (Harvey and Ben-Amotz, 2020). β-carotene is well known among carotenoids as a precursor of vitamin A and a potential treatment for cancer and cardiovascular disease (Rammuni *et al.*, 2019). However, this β-carotene cannot be directly synthesized and must be obtained by humans through diet. Several enzymes are involved in biosynthesis of β-carotene in plants (Sun *et al.*, 2018). Research into biosynthesis β-carotene is still limited in microalgae (Sui and Vlaeminck, 2019). Among these microalgae, *Dunaliella* species has been the focused. The cell from *Dunaliella* microalgae can absorb β-carotene under stress (Pick *et al.*, 2019). Harey and Ben-Arotz, (2020) reported that the total carotenoid of *Dunaliella* specie is between 60 – 90g/kg. Zhu *et al*, (2020) reported in their work that pigments they used were extracted from *Dunaliella salina* cells using 90% (v/v) acetone, before it was incubated at 55℃ for 15 min and vortex mixing was carried out at maximum speed for about 1 min. The extracts were centrifuged, and the filtrate obtained from the filtration operation (0.22-um nylon filter) were analyzed using high performance liquid chromatography (HPLC). β-carotene was separated in the HPLC using acetonitrile, methanol and isopropanol (45:10:45) as solvent after 20 min. The temperature of the HPLC column was set at 25℃ and a constant flow rate of 1.0ml/min. They obtained the concentration of β-carotene at 450nm, from the HPLC calibrated profiles with β-carotene standard.

4. SCREENING AND ISOLATION OF *DUNALIELLA* STRAIN

4.1. Screening of Microalgae

Screening of microalgae is a preprocessing operation applied to microalgae cultures. The percentage efficiency of microalgae screening depends on the cell size and screen openings (Christina, 2017). The devices used primarily for the screening operation are microstrainers and vibrating screens. Microstrainers are made up of rotary drum that is covered with a straining fabric, polyester or stainless steel, and which is frequently subjected to backwash. The overall production cost is largely determined by the factor of the flow-through rate. When larger microalgae are screened with openings that are larger, it results in faster flow rates and lower cost of operations. This is a simple technique of screening microalgae and low investment is involved, but its percentage efficiency in the recovering of bacterial sized microalgae is very low that the mesh screen that can be used has wide character, therefore, this method requires further processing.

Moreover, there is formation of bacterial and microalgal biofilm on the mesh or fabric, which makes it susceptible to regular maintenance. Microstraining operation is greatly influenced by the initial microalgal concentration and this can result to incomplete solids removal. For example, *Coelastrum proboscideum* that is harvested by microstraining technique resulted in 1.5% total suspended solids (TSS), consuming about 0.2 kW/m^3, while the use of vibrating screens in both continuous and batch mode allows the recovery efficiency of about of 5-6% total suspended solids (TSS) and 7-8% TSS, respectively. Therefore, it is pertinent to conclude that greater microalgal solid content is achievable by the use of vibrating screens method. This technique is currently in use for large-scale production of multicellular filamentous blue-green microalgae (Barros *et al*., 2015).

4.2. Isolation of Microalgae

Isolation of a microalgal single cell is a method whereby a particular cell is picked from the sample mixture, using a treated glass capillary or micropipette under a microscope observation. The single cell is transferred to a sterile droplet of water or suitable selected media (Singh *et al*., 2015). This is the first step to obtain pure cultures of microalgae strain selection (Duong *et al*., 2012). There are several methods used for microalgae isolation, such as, micropipette technique (Cobos et al., 2020), cytometry technique, bioinformatics approach (Duong *et al*., 2012) and streak-plating technique, but two are of cosmopolitan importance; that is, micropipette washing method and streak-plating technique (Toyub *et al*., 2008; Hassi and Alouani, 2020).

4.2.1. Micropipette washing technique

The micropipette technique is operated in two stages: the preparation of the micropipette followed by the washing technique. This technique can be applied for different range of microalgae samples and it is less expensive (Doung *et al*., 2012). This technique involves the preparation of the micropipette followed by the washing step. In this technique, 10 – 120 μm of microalgae sample is collected with plankton net and stored on a 50ml transparent bottle. 50 ml of bold basal medium or Conway medium (BBM/CD) would be mixed with the sample and taken to the laboratory. Fresh BBM/CD would be prepared in 8 different sterile bottles (10 ml). 10 drops of sterile water would be added to the groove of glass slide and a drop of the stored microalgae would be added to the groove. Cells of the microalgae would be picked up by the micropipette and placed on the groove of the slide, then mixed. A single cell will be picked from it and placed in a 10 ml bottle and mixed thoroughly. The entire operation would be repeated to confirm the picked single cell and the cell will be allowed to grow in a BBM/CD for future use (Hassi and Alouani, 2020). The composition of the Bold Basal Medium (BBM) is presented in Table 4 (Toyub *et al*., 2008; Eckerstorfer *et al*., 2019).

4.2.2. Streak plate technique

This technique is carried out in three phases: the preparation of agar plate phase, centrifuge washing phase and streak-plating phase. It involves the collection of 10 - 120μm microalgae sample using plankton net and the sample kept in a 50ml plastic bottle. The sample would be mixed with 20ml fresh BBM/CD and further mixed with distilled water and loaded into 4 tubes in a centrifuge at 5000rpm for minutes (Haoujar *et al*., 2020). The supernatant would be removed and the residue mixed with equal volume sterile water. The centrifugation would be repeated for 5 minutes at 5000rpm and the entire operation would be repeated at least 3 times to be sure it's free of bacterial. The microalgae sample is streaked by loop on the agar plate and the microalgae cells would be allowed to grow. Again, the microalgae cell is streaked in a new plate and the entire operation repeated if free of contamination. The pure cell would be placed on a BBM/CD and the microalgae allowed to grow, for further use (Hassi and Alouani, 2020).

4.3. Molecular Identification

The complexity of the arrangement of carbon pattern and the variation among microalgae species, makes the identification of pure microalgae for each specific application not to be straight forward (Radha *et al*., 2013). The standard procedure involved in the identification of microalgae include, single cell insolation of the strain, the DNA extraction, purification, amplification, sequencing and taxonomic identification (Jahn *et al*., 2014). Several novel taxonomic methods have been encouraged to identify *Dunaliella* species, the first is

based on the morphological and physiological characteristics of the microalgae, however, this is not an effective tool to unambiguously identify all *Dunaliella* species because different *Dunaliella* species vary greatly under different culture conditions (Borowitzka and Silva, 2007). Another taxonomic method for the identification of *Dunaliella* species is based on morphological and biochemical criteria. This method involves the reversal of the *Dunaliella* species taxonomy based on stigma condition, cell length, optimum salinity, flagella length, type of refractile granules, the type of symmetry, cell color, the maximum total carotenoid content, the type of carotenoid and the formation of aplanospores (Borowitzka and Silva, 2007). But these characteristics vary greatly, depending on the culture conditions such as temperature, nutrient availability and light intensity.

Table 4: Culture medium nutrient concentrations

Nutrient	Concentration in stock solution	Amount in culture medium (mll^{-1})
$NaNO_3$	5.0g/200ml	10ml
$MgSO_4.7H_2O$	1.5g/200ml	10ml
NaCl	0.5g/200ml	10ml
K_2HPO_4	1.5g/200ml	10ml
KH_2PO_4	3.5g/200ml	10ml
$CaCl_2.2H_2O$	0.5g/200ml	10ml
Trace elements solution		1.0ml
i. ZnSO4.H2O	8.82g/l	-
ii. $MnCl_2$.4H2O	1.44g/l	-
iii. MoO_3	0.71g/l	-
iv. $CuSO_4.5H_2O$	1.57g/l	-
v. $Co(NO_3)_2.6H_2O$	0.49g/l	-
H_2BO_3	1.14g/100ml	1.0ml
EDTA Stock		1.0ml
i. KOH	31g/l	-
ii. $EDTA.2H_2O$	50g/l	-
iii. Distil. H_2O	1.0L	-
Iron solution		1.0ml
i. $FeSO_4.7H_2O$	4.98g/l	-
ii. Conc. H_2SO_4	1.0ml	-
iii. Distil. H_2O	1.0L	-

Therefore, this technique cannot help to convincingly discriminate *Dunaliella* species (Dehghani *et al*., 2020). Consequently, new approaches using molecular biological method are being used to classify algae, including *Dunaliella* species. Cytochrome C-oxidase subunit I (COXI) gene is widely applied for molecular identification of animal. Similarly, this method has been tested with microalgae, focusing on the 18S rRNA, rigulose-1-5-bisphosphate carboxylase/oxygenase (rbcl), 23S rRNA nuclear internal transcribed spacer, 2 (ITS-2), tufA (plastid factor of elongation) (Vieira *et al*., 2016) and COXI gene (Lee *et al*., 2012). Consequently, several number of 18S rDNA primers have been applied for identification of the cell of microalgae (Khaw *et al*., 2019). The basic process of microalgae identification is to obtain 18S rDNA sequence in order to determine the nearest relative of the particular microalgae cell. Therefore, 18S rDNA is a "frontier gene" used in microalgae cell identification (Haoujar *et al*., 2020). This involves extraction of the DNA of the microalgae followed by desalting or without desalting operation and the extract accessed in line with DNA quantity and quality (Khaw *et al.*, 2019). The 18S rDNA primer performance of polymerase chain reaction (PCR) is carried out to obtain the microalgae molecular identity which would be compared with the morphology of known microalgae characteristics (position of stigma and size of microalgae) (Dehghani *et al*., 2020). The base pairs (Gene size bp), accession numbers and sequence which identify and confirm a particular microalgae specie using the DNA are presented in Tables 5 – 7. PCR analysis carried out on any microalgae specie using a specified primer will produce base pair image with gene size while sequencing produces accession number of the microalgae used as confirmation of the specie by matching

the accession number of the microalgae with the once on the National Center for Biotechnology Information (NCBI) database. The gene size (base pair) and accession number are the characteristics of the microalgae determined by molecular identification as shown in Tables 5 – 7. El-Sheekh *et al*, (2020) worked on molecular identification of chlorophyte *Chlorella* species. They reported that the cells were harvested by centrifugation at the exponential growth phase and the pellet was washed several times with a polysaccharide elimination buffer. The extraction of the DNA was carried out to amplify the sequences of 18S ribosomal RNA gene, using the procedure of GeneJet Plant genomic DNA purification Kit (Thermo) # K0791.

5. FACTORS THAT INFLUENCES THE GROWTH OF MICROALGAE

The growth of algal has a salient component such as medium having a source of light energy that enable photosynthesis to take place, air flow or $CO_2$and appropriate nutrients (Chowdury *et al*., 2020). The mechanics of microalgae growth can be determined on the basis of constant environment, nutrient supply and internal nutrient content, using the Droop model while the rate of growth based on the concentration of external supplement to the culture media can be simulated using Monod model (Hossain *et al*., 2019). Equations (3) and (4) represent the Droop and Monod models respectively.

$$\mu_D = \mu_{Dmin} \text{ x } \left(1 - \frac{q_{min}}{q}\right) \quad (3)$$

$$\mu_D = \mu_{Dmin} \text{ x } \frac{[N]}{K_N + \{N\}} \quad (4)$$

Where μ_D is the Droop model specific growth rate (S^{-1}), μ_{Dmax} is the Droop model maximum rate of growth q_{min} is the minimum cell growth, q = cell quota (S^{-1}). K_N is the nutrients half-saturation constant (mg/l), N is the nutrient concentration (Eze *et al*., 2018).

Under suitable parameters and culture conditions, microalgae biomass cell number doubled within 24 hours (Hossain *et al*., 2019). The growth rate of the cell life cycle occurs in five stages: the lag, exponential or log, the linear growth, stationary and the decline or death phases. Among these stages, the exponential phase is considered to be the stage that is most productive where the population of the microalgae numbers within about 3.5 hr incubation, and this time required for the cell population to double, is called the doubling time.

Table 5: The 18S rDNA sequences of *Dunaliella* species (NCBI database as in Dehghani *et al*, 2020)

Dunaliella species	Gene size (bp)	Accession number	Geographic origin
D. bardawil strain KMMCC 1346	2054	JQ315779.1	Republic of Korea
D. bardawil UTEX LB 2538	2088	DQ009777.1	USA
D. bioculata UTEX LB 199	1687	DQ009761.1	USA
D. parva	2585	M62998.1	Unknown
D. peircei strain UTEX LB 2192	2065	DQ009778.1	USA
D. primolecta UTEX 1000	1620	KJ018734.1	USA
D. salina strain KMMCC 1428	1647	JQ315781.1	Korea
Dunaliella sp. ABRIINW M1/2	2120	EU678868.1	Iran
D. salina UTEX LB 200	2065	DQ009779.1	USA
D. salina strain KU07	2069	KF825551.1	Thailand
D. salina strain KU11	2067	KF825550.1	Thailand
D. salina strain KU13	2068	KF825552.1	Thailand
D. salina isolate BAK	1784	KU641617	Iran
Dunaliella sp. SAS11133	1722	KF054056.1	China
D. pseudosalina isolate MAH	1735	U641615	Iran
D. salina isolate BAK	1784	KU641617.1	Iran
Asteromonas gracilis isolate BA	1687	KU351659.1	Iran
D. viridis strain CONC002	2494	DQ009776.1	USA
D. tertiolecta CCMP 364	1620	KJ018735.1	USA
D. tertiolecta UTEX 999	1620	KJ018733.1	USA
Chlamydomonas reinhardtii	1641	AB701555	Japan
D. lateralis strain Nepal	1692	DQ009762.1	USA
D. polymorpha	2991	KJ756825.1	UK

Table 6: List of some primers sequences and the sequencing success rate for four strains

Primer	Molecular marker	Sequence	Annealing T°C	Citation
18SF	18S rDNA	AACCTGGTTGATYCTGCCAG	56, 60	Haoujar *et al*., 2020
Euk516r	18S rDNA	ACCAGACTTGCCCTCC	56, 60	Haoujar *et al*., 2020
Tetra_rbcl_F	Rbcl	GKACTTGGACAACTGTATGGACKGATGGT	56	Haoujar *et al*., 2020 Haoujar *et al*., 2020 Beatrice-Linder *et al*., 2018
Tetra_rbcl_R		GRTCTTTTTCWACRTAAGCATCACGCATTA	56	Beatrice-Linder *et al*., 2018
RbcL_13F		AATGGCTCCACAAACAGAAAC	50-55	Beatrice-Linder *et al*., 2018
RbcL_8R		TCACAAGCAGCAGCTAATTC	50-55	
RbcL_14R		ATCAAGACCACCACGTAAACA	50	Beatrice-Linder *et al*., 2018
ITS1		TCCGTAGGTGAACCTGCGG	50	
ITS4	rbcl	TCCTCCGCTTATTGATATGC	50	Beatrice-Linder *et al*., 2018
SalmF		ACCCTCACCCCTCTTTCCTTT	63	
SalmR		TTGGGAAAGCCAGATCCACC	63	Beatrice-Linder *et al*., 2018
SalmProbe		6FAM-GTTAGCTTCTCAGCTGG	63	
UnivF		TTGGAGGGCAAGTCTGGT	63	Beatrice-Linder *et al*., 2018
UnivR		CGAGCTTTTTAACTGCAACAA	63	
UnivProbe		VIC-CGGTAATTCCAGCTCC	63	Beatrice-Linder *et al*., 2018
				Haydoen *et al*., 2006
				Hayden *et al*., 2006
				Beatrice-Linder *et al*., 2018

Table 7: The rbcl sequences of *Dunaliella* species (NCBI database, as in Dehghani *et al*., 2020)

Dunaliella species	Gene size (bp)	Accession number	Geographic origin
D. bardawil strain KMMCC 1346	798	JQ315489.1	Republic of Korea
D. bardawil UTEX LB 2538	1038	DQ313194.1	USA
D. bioculata UTEX LB 199	1038	DQ313195.1	USA
D. parva	1040	DQ173091.1	Chile
D. peircei strain UTEX LB 2192	869	DQ313196.1	USA
D. primolecta UTEX 1000	1038	DQ313198.1	USA
D. acidophila strain CCAP 19/35	667	HQ142901.1	Spain
D. salina strain KMMCC 1428	894	JQ315491.1	Korea
Dunaliella sp. ABRIINW M1/2	1320	KC149893.1	Iran
D. salina UTEX LB 200	869	DQ313197.1	USA
D. salina strain KU07	427	KF825555.2	Thailand
D. salina strain KU11	613	KF825554.1	Thailand
D. salina strain KU13	632	KF825553.1	Thailand
D. salina isolate BAK	789	KU682279	Iran
Dunaliella sp. SAS11133	717	KF054057.1	China
D. pseudosalina isolate MAH	799	KU641616	Iran
D. viridis strain CONC002	1038	DQ313206.1	USA
D. tertiolecta CCMP 364	1038	DQ313204.1	USA
D. tertiolecta UTEX 999	1038	DQ313203.1	USA
Chlamydomonas reinhardtii	1128	AB511846	Japan

The cell growth rate and the doubling time can be evaluated with Equations (5) and (6).

$$\text{Growth rate (K')} = \ln \frac{n_2/n_1}{t_2 - t_1} \quad (5)$$

$$\text{Doubling time (t')} = \frac{1}{K'/\ln(2)} \quad (6)$$

Where t_1 is the initial stage culture period, t_2 is the final stage of the culture period (h), n_1 and n_2 are the microalgae biomass total population at t_1 and t_2 respectively (Singh *et al*., 2018).

To obtain optimal growth of microalgae and its biomass production, several other environmental factors or their combination which may impact the growth must be considered. These factors include: light, temperature, salinity, mixing and dilution rate (Radha *et al*., 2013). Since culture conditions vary with species, growth factors must also be specified for the success of cultivation of the microalgae for specific purpose (Radha *et al*., 2013; Chowdury *et al*., 2020). Research shows that addition of supplements such as salts and vitamins to the culture of microalgae also helps to boost the production of biocrude (Hossain *et al*., 2019).

5.1. Effect of Temperature

Temperature is a significant variable in the growth rate of *Dunaliella* microalgae, so it is important to control this factor playing a critical role in the *Dunaliella* microalgae growth rate. The strain of *Dunaliella* microalgae is selected on the basis of the prevailing temperature condition (Slocombe and Benemann, 2016). Also, the uptake of nutrients and strain selection of *Dunaliella* microalgae are based on the effect of changes in temperature (Daneshvar *et al*., 2021). In most cases, the growth rate of *Dunaliella* microalgae increases with increase in temperature until it reaches an optimal temperature after which further increase in temperature will result to a decrease in the rate of growth of the *Dunaliella* microalgae (Figure 2). The growth of majority of the microalgae is negatively affected by temperature below 16℃ and temperature above 35℃ (Pachiappan *et al*., 2016). Guedes *et al*, (2011) reported that *Dunaliella salina* when cultivated in a semi continuous system at 25 ℃, biomass yield of about 80 g/(m^3.day) was obtained. Kumar *et al*, (2018) reported in their work that *Dunaliella tertiolecta* was cultivated using white LED's at 23±0.25 ℃ and an optimum biomass of 1.7972g/l was obtained. Xue and Jiang, (2017) reported that *Dunaliella tertiolecta* was cultivated at 26℃ on a day and night cycle of 14/10 hour, but the percentage yield of the *Dunaliella salina* biomass was not reported, rather 20% and 80% respectively were reported as the total lipid production and the content of lipid per cell. Shang *et al*, 2016 reported that the cells of *Dunaliella parva* were cultivated in a batch mode at 24 ℃ and the optimum lipid content per cell was reported as 40%.

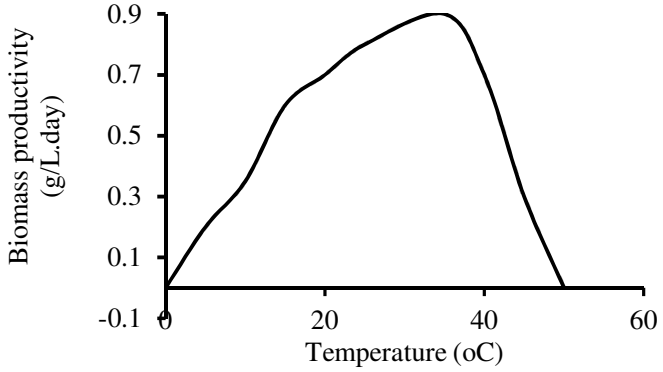

Figure 2: Effect of temperature on the biomass productivity of *Dunaliella* microalgae

5.2. Effect of pH

The pH of *Dunaliella* microalgae has been observed to be one of the major factors which control the cell formation and metabolism of its biomass (Lutzu *et al*., 2020). Water pH is tightly related to the pH of microalgae. When *Dunaliella* microalgae grow in an alkaline environment, it is easier for it to capture CO_2 and this will result to high biomass productivity (Ren, 2014). Most species of *Dunaliella* microalgae tolerate

wide range of pH (5.5 – 10.5) represented in Figure 3, but beyond this interval, the productivity of the biomass will greatly reduce (Chowdury *et al*., 2020). The *Dunaliella* microalgae medium pH is affiliated to the concentration of CO_2 it produces. This means that, the pH of the medium increases regularly as the CO_2 is consumed in the system. The pH of the medium also influences the amount of nutrients like iron and organic acid in the medium (Lutzu *et al*., 2020). Therefore, pH is an environmental factor that has to be controlled by carbonate equilibrium in the medium. *Dunaliella* microalgae survives in both acidic and alkaline pH (Yin *et al*., 2014), but pH for the growth of most species of microalgae is 7.0 – 9.0 (Blinova *et al*., 2016). Kumar *et al*., (2018) reported that *Dunaliella tertiolecta* growth rate was monitored in a culture medium which was allowed to vary between *pH* of 7.5 to 8.5 and the maximum biomass yield was obtained as 1.7972 g/l.

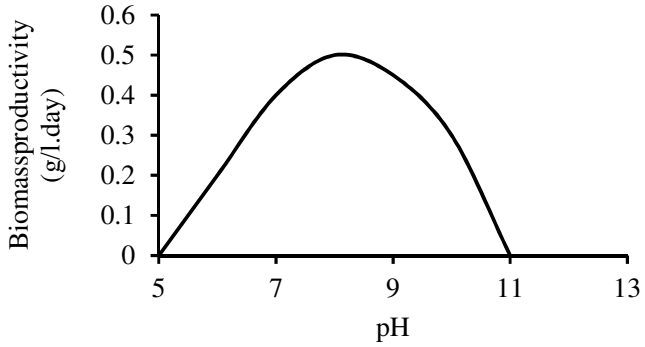

Figure 3: Effect of pH on the *Duanliella* microalgae biomass productivity

5.3. Effect of Salinity

Dunaliella microalgae are highly tolerant of salinity changes Ren, 2014). Most *Dunaliella* species grow better with the salinity reached by diluting sea water with tap water, which is slightly less than that of their original habitat (Blinova *et al*., 2016). The optimal salinity for the *Dunaliella* microalgae growth rate is about 20 – 24g/l (Pachiappan *et al*., 2021). The salinity of microalgae tends to increase in the open culture because of excessive evaporation. Base on the salinity tolerance of *Duanliella* microalgae, its salinity can be divided into three categories such as, when the *Duanliella* microalgae is formed in water with low salinity (Oligohaline), when the *Duanliella* microalgae develop in a moderate saline water environment of 5 to 18g/kg (Mesohaline) and when the *Duanliella* microalgae develop in a extremely saline water environment between 18 to 30g/kg (Polyhaline) (Chowdury *et al*., 2020).

5.4. Effect of Light

In the rate of growth of *Dunaliella* microalgae, light is one of the very essential factors (Blinova *et al*., 2016). *Dunaliella* microalgae assimilate inorganic carbons and convert them to organic matter by photosynthesis. This source of energy that drives this conversion is light, and as a result, photoperiod, spectral quality and intensity of the light which aid this reaction are considered. Light intensity requirement varies with the depth of the *Duanliella* microalgae culture medium and the algae density. When the culture depth and cell concentration are high, increased light intensity is required to penetrate the culture (Pachiappan *et al*., 2021). When *Duanliella* microalgae cell is cultivated under limited source of light, it assimilates carbon and synthesizes it to amino acid and other important constituents. When the light is saturated, sugar and starch are formed through the pathway of pentose phosphate-reducing, but continuous light in the culture are often used in the industry and in research because it achieves maximum growth rate. When light/dark cycles are used instead of having continuous light, it either increase light concentration or lower the cost of production of the *Duanliella* microalgae because cell division occurs in the dark for many unicellular cell cultures, while division for other cells occurs in both dark and in the illuminated condition. When the illuminated phase is

interrupted, cell division is more frequent while CO_2 fixation and some enzymes of photosynthesis are inactive during illumination (Blinova *et al.*, 2016). Light wavelength for most *Duanliella* microalgae is generally from 400 – 700nm for photosynthesis and artificial illumination should not be less than 18 hr duration per day, while white light fluorescence lamps may be used in controlled room (Schulze *et al.*, 2014). Figure 4 shows how rate of photosynthesis varies with light intensity. As the intesnity of light increases, photosynthesis of the *Duanliella* microalgae also increases and reaches maximum point at saturation. Further increase above the saturatiion point leads to photoinhibition (Chowdury *et al.*, 2020).

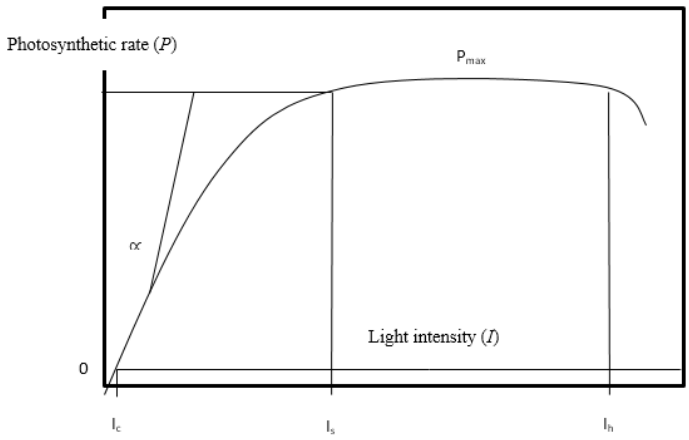

Figure 4: Photosynthetic rate variation with light intensity on the *Duanliella* microalgae

5.5. Effect of Carbon Dioxide

Carbon dioxide is another significant factor that affect the rate of growth of *Duanliella* microalgae (Chowdury *et al.*, 2020). Considering the production of known mass (1 kg) of *Duanliella* microalgae biomass, 1.8 – 2.0 kg carbon dioxide is required (Acien-Fermandez *et al.*, 2013). The amount of carbon dioxide (CO_2) in the air has increased from 260 ppm to 380 ppm as a result of the combustion of fossil fuels, and this is the mainly responsible for global warming. Different suggestions have been made to minimize this level of CO_2 in the air (Minillo *et al.*, 2013), among them, biological fixing of carbon dioxide can be applied to eliminate CO_2 from air. The *Duanliella* microalgae used for sequestering CO_2 will depend on the proper selection of the specie that is affected by the amount CO_2 in the air. This is very significant to the rate of growth of the *Duanliella* microalgae. The higher the CO_2 concentration in the air, the better the rate of the *Duanliella* microalgae growth (Khairy *et al.*, 2014), but the growth rate of *Dunaliella salina* is not affected by CO_2 concentration from 230 – 5100 ppm (Business bliss Consultants, 2018).

Duanliella microalgae cultivation can be grouped as photoautotrophic, heterotrophic, mixotrophic, and photoheterotrophic, depending on the sources of carbon that is used for the cultivation (Hu *et al.*, 2017). In photoautotrophic mode, *Duanliella* microalgae utilize light and inorganic materials such as carbon dioxide (CO_2), water, and inorganic salts for the synthesis of organic materials and photosynthesis (Ruiz *et al.*, 2022). In photoheterotrophic mode, the *Duanliella* microalgae utilize carbon sources outside the culture medium to grow in a dark environment (Zhang *et al.*, 2022). While in heterotrophic mode, *Duanliella* microalgae provides both the carbon dioxide (CO_2) and the sources of organic carbon. Heterotrophic and mixotrophic mode of *Duanliella* microalgae also have the behavior of photoautotrophic metabolism. In mixotrophic mode, rapid cell division of *Duanliella* microalgae also takes place (Cheng *et al.*, 2022). Consequently, heterotrophic and mixotrophic modes might be observed in some photoautotrophic *Duanliella* microalgae species and photoheterotrophic modes as secondary metabolic pathways. Mixotrophic modes have significantly higher yield of biomass and lipids, when compared with those in autotrophic mode. Increase in carbon dioxide (CO_2), which is one of the factors that influences the photosynthetic process of the *Duanliella*

microalgae; is beneficial to the photosynthetic efficiency of the *Duanliella* microalgae, thereby increasing its yield of biomass (Zhang *et al*., 2022).

5.6. Effect of Nutrients

It is very important to know the environmental requirement of the natural habitat of the *Duanliella* microalgae in order to know if it is nutrient-rich or nutrient-poor environment ((Pachiappan *et al*., 2021)]. A culture medium that is ideal for *Duanliella* microalgae should contain elements such as iron, nitrogen and phosphorus, and these may vary according to cultivated species of *Duanliella* microalgae. The minimum nutritional requirement for the *Duanliella* microalgae growth rate can be obtained by the expression $C\ O_{0.48}\ H_{1.83}\ N_{0.11}\ P_{0.01}$. Trace metals such as manganese, copper, iron, cobalt, nickel and zinc are present in the *Duanliella* microalgae cells in extremely small quantity (Acien-Fermandez *et al*., 2013). Table 8 shows how addition of nutrients increases the production of *Duanliella* microalgae biomass, thereby reducing the cultivation time (Chowdury *et al*., 2020). Base on the molecular formula, about 50% biomass *Duanliella* microalgae is made up of carbon (C), which is a vital component of all organic substances that is synthesized by the cells like vitamin, carbohydrates, proteins, nuclei acids and lipids (Sacristan *et al*., 2018).

5.7. Effect of Mixing

Mixing of the culture of *Duanliella* microalgae is of paramount importance in the balance of pH and gases of the system. This helps to evenly distribute light to all the cells of the *Dunaliella* specie and eliminate the challenges of shading (prohibition of the cells of microalgae from absorbing light) which inhibit the microalgae growth (Ren, 2014). Mixing reduces the presence of gradients that will limit the cell performance and helps to avoid the sedimentation of the cells in the system, so that they can move between light and dark zones. Mixing of *Dunaliella* specie cells can be carried out by the use of pump in the column for recirculation, use of magnetic stirrer in the column and by bubbling air into the column (Ravelonandro *et al*., 2018). Although some of the microalgae species do not tolerate serious agitation; but *Dunliella* specie is not one of them. Whatever mixing method that is applied, the energy supplied imposes cost which has to be optimized (Wang *et al*., 2012; Chowdury *et al*., 2020).

6. PROSPECT AND CHALLENGES IN ISOLATION AND IDENTIFICATION *DUNALIELLA* MICROALGAE

As a promising source of renewable biomass for biocrude production, *Dunaliella* species of microalgae can be cultivated on land and also utilizes any wastewater for its cultivation. Although, *Dunaliella* microalgae have numerous advantages, but many technical difficulties are encountered in its screening, isolation and identification, which are as follows:

(1) Ease of screening of a pure specie of *Dunaliella* microalgae
(2) Preservation of a pure specie at a very low temperature after it has been screened before it is been cultured
(3) Preservation of a pure specie from contamination with another microorganism, after it has been screened
(4) Ease if rapid identification of the species

7. CONCLUSION

The significance of *Dunaliella* species as a good source of food, effectiveness in wastewater treatment and an alternative source of energy cannot be overemphasized. *Dunaliella* species with high lipid content, which is the main source of biocrude, can only be identified when correctly isolated in order to cultivate this specific microalgal specie for a particular purpose with the most effective and efficient technique, to avoid contamination and low biomass productivity. In this article, the works of several researchers on the isolation, identification and conditions affecting the growth rate of *Dunaliella* species for the production of high yield biocrude were reviewed. The areas where challenges are still being encountered have been identified, together with areas where more research is needed, to effectively explore the production of biomass from *Dunaliella* species.

8. CONFLICT OF INTEREST

There is no conflict of interest associated with this work.

REFERENCES

Abdallah, Q.A., Nixon, B.T. and Fortwendel, J.R. (2016). The Enzymatic Conversion of Major Algal and Cyanobacterial Carbohydrates to Bioethanol. *Frontiers in Energy Research,* 4 (36)*,* pp. 1 – 15.

Abiusi, F., Rene. H., Wijffels, R.H. and Janssen, M. (2020). Doubling of microalgae productivity by oxygen balanced mixotrophy. *ACS Sustainable Chemistry & Engineering,* pp. 1 – 33.

Acien-Fernandez, F.G., Fernandez-Sevilla, J.M. Molina, G.E. (2013). Photobioreactors for the Production of Microalgae. *Reviews in Environmental Science and Bio-Technology*, 12, pp. 131 – 151.

Alhattab, M., Kermanshahi-Pour, A. and Brooks, M.S.L. (2019). Microalgae disruption techniques for product recovery: influence of cell wall composition. *Journal of applied phycology*, 31, pp. 61–88.

Almutairi, A.W. (2020). Effects of nitrogen and phosphorus limitations on fatty acid methyl esters and fuel properties of *Dunaliella salina. Environmental Science and Pollution Research*, pp. 1 – 8.

Amaro, H.M., Guedes, A.C. and Malcata, F.X. (2011). Advances and Perspectives in Using Microalgae to Produce Biodiesel. *Applied Energy*, 88, pp. 3402 – 3410.

Amorim, M.L., Soares, J.J., Coimbra, J.S.R., Leite, M.O., Albino, L.F.R. and Martins, M.A. (2020). Microalgae proteins: production, separation, isolation, quantification, and application in food and feed. *Critical Reviews in Food Science and Nutrition*, pp. 1 – 28.

Antonina, R.L., Emanuele, V., Maria De, P.R., Rosa B., Giuliana, M. and Giuseppe, (2021). Biohydrogen from Microalgae: Production and Applications. *Applied Sciences,* pp. 1 – 14.

Aratboni, A.H., Rafiei, N., Garcia-Granados, R., Alemzadeh, A. and Morones-Ramírez, J.R. (2019). Biomass and lipid induction strategies in microalgae for biofuel production and other applications. *Microbial Cell Factories,* 18 (1), pp. 1 - 17.

Bach, Q.V., Chen, W.H., Lin, S.C., Sheen, H.K. and Chang, J.S. (2017). Wet torrefaction of microalga *Chlorella vulgaris ESP-31* with microwave-assisted heating. *Energy Conversion Management,* 141, pp. 163 - 170.

Barka, A. and Blecker, C. (2016). Microalgae as a potential source of single-cell proteins. A review. *Biotechnology Agronomy Society and Environment*, 20 (3), pp. 427 – 436.

Barreiro, D.L., Riede, S., Hornung, U., Kruse, A. and Prins, W. (2015). Hydrothermal liquefaction of microalgae: effect on the product yields of the addition of an organic solvent to separate the aqueous phase and the biocrude oil. *Algal Research*, 12, pp. 206 - 212.

Barreiro, D.L., Samori, C., Terranella, G., Horiung, U., Kruse, A. and Prins, W. (2015). Assessing microalgae biorefinery routes for the production of biofuels via hydrothermal liquefaction. *Bioresources Technology*, 174, pp. 256 – 265.

Barreiro, D.L., Samorì, C., Terranella, G., Hornung, U., Kruse, A., Prins, W. (2014). Assessing microalgae biorefinery routes for the production of biofuels via hydrothermal liquefaction. *Bioresource Technology*, 174, pp. 256 - 265.

Barros, A.I., Gonçalves, A.I., Simões, M. and Pires, J.C.M. (2015). Harvesting techniques applied to microalgae: A review. *Renewable and Sustainable Energy Reviews,* 41, pp. 1489–1500.

Barros, A.L., Gonçalves, A.L., Simões, M. and Pires, J.C.M. (2015). Harvesting techniques applied to microalgae: A review. *Renewable and Sustainable Energy Reviews,* 41, pp. 1489–1500.

Beatrice-Lindner, P., Garrido-Cardenas, J.A., Sepulveda, C., Acien-Fernandez, F.G. (2018). A new approach for detection and quantification of microalgae in industrial-scale microalgal cultures. *Applied Microbiology and Biotechnology*, pp. 1 – 8.

Biller, P. and Ross, A.B. (2011). Potential yields and properties of oil from the hydrothermal liquefaction of microalgae with different biochemical content. *Bioresource Technology*, l02, pp. 215 – 225.

Biller, P. Brajendra, K., Sharma, B.K., Kunwar, B. and Ross, A.B. (2015). Hydroprocessing of bio-crude from continuous hydrothermal liquefaction of microalgae. *Fuel,* 159*,* pp. 197 – 205.

Blinová, L., Bartošová, A., Gerulová, K.S. (2106). Cultivation of microalgae (*Chlorella vulgaris*) for biodiesel production. Research papers Faculty of materials science and Technology in Trnava Slovak University of technology, Bratislava, 23 (36), pp. 87 – 95.

Borowitzka, M.A. and Silva, C.J. (2007). The taxonomy of the genus *Dunaliella* (Chlorophyta, Dunaliellales) with emphasis on the marine and halophilic species. *Journal of Applied Phycology*, 19, pp. 567–590.

Bose, A., O'Shea, R., Lin, R. and Murphy, J.D. (2020). A perspective on novel cascading algal biomethane biorefinery systems. *Bioresource Technology*, pp. 1 – 54.

Brasil, B.D.S.A.F., de Siqueira, F.G., Salum, T.F.C., Zanette, C.M. and Spier, M.R. (2018). Microalgae and cyanobacteria as enzyme biofactories, *Algal Resources*, 25, pp. 76–89.

Bredda, E.H., Da Silva, A.F., Silva, M.B., Da Rós and P.C.M. (2019). Mixture design as a potential tool in modeling the effect of light wavelength on *Dunaliella salina* cultivation: an alternative solution to increase microalgae lipid productivity for biodiesel production. *Preparative Biochemistry & Biotechnology*, pp. 1 – 12.

Bumbac, M., Nicolescu, C.M., Olteanu, R.L., Gherghinoiu, S.C., Bumbac, C., Tiron, O., Manea, E.E., Radulescu, C., L., Gorghiu, L.M., Stanescu, S.G., Serban, B.C., Buiu, O. (2023). Preparation and Characterization of Microalgae Styrene-Butadiene Composites Using *Chlorella vulgaris* and *Arthrospira platensis* Biomass. *Polymers*, 15 (1357), pp. 1 – 18.

Business Bliss Consultants FZE. Factors Affecting the Growth of Various Microalgal Species, (November 2018), Retrieved from https://ukdiss.com/examples/factors-affecting-growth-microalgal-species.php?vref=1

Chen, W-H., Huang, M-Y., Chang, J-S., Chen, C-Y. (2014). Thermal decomposition dynamics and severity of microalgae residues in torrefaction. *Bioresource Technology*, 169, pp. 258 – 264.

Cheng, P., Huang, J., Song, X., Yao, T., Jiang, J., Zhou, C. (2022). Heterotrophic and mixotrophic cultivation of microalgae to simultaneously achieve furfural wastewater treatment and lipid production. *Bioresource Technology*, 349 (126888).

Chouhan, A.P.S. and Sarma, A.K. (2013). Critical Analysis of Process Parameters for Bio-oil Production via Pyrolysis of Biomass: A Review. *Recent Patents on Engineering,* 7.

Chowdury, K.H., Nahar, N. and Deb, U.K. (2020). The Growth Factors Involved in Microalgae Cultivation for Biofuel Production: A Review. *Computational Water, Energy, and Environmental Engineering*, 9, pp. 185 – 215.

Christina, E. (2017). Algal Biofuels: production, cultivation and lipid extraction for biofuel production. *Think Indian Journal*, 22 (17), pp. 615 – 632.

Cobos, M., Castro, J.C., Paredes, J.D., Pérez, S., Maddox, J.D., Estela, S.L., Rodríguez, H.N., Tresierra, A.B., Saldaña, J.E., Marapara, J.L., Adrianzén, P.M. and Gonzales, R. (2020). Isolation, Characterization, and Biotechnological Potential of Native Microalgae From the Peruvian Amazon. *Microalgae - From Physiology to Application.*

da Maia, J.L., Cardoso, J.S., da Silveira Mastrantonio, D.J., Bierhals, C.K., Moreira, J.B., Costa, J.A.V., de Morais, M.G. (2020). Microalgae starch: A promising raw material for the bioethanol production. *International Journal of Biological Macromolecules,* pp. 1 – 49.

Daneshvar, E., Ok, Y.S., Tavakoli, S., Sarkar, B., Shaheen, S.M., Hong, H., Luo, Y., Rinklebe, J., Song, H. and Bhatnagar, A. (2021). Insights into upstream processing of microalgae: A review. *Bioresource technology*, 329.

de Carvalho, J.C., Magalhaes, A.I., de MeloG.V., Pereira, A.B.P., Medeiros, E.B., Sydney, C. and Rodrigues (2020). Microalgal biomass pretreatment for integrated processing into biofuels, food, and feed. *Bioresources Technology*, 300 (122719).

Debnath, C., Bandyopadhyay, T.K., Bhunia, B., Mishra, U., Narayanasamy, S., Muthuraj, M. (2021). Microalgae: Sustainable resource of carbohydrates in third-generation biofuel production. *Renewable and Sustainable Energy Reviews*, 150 (111464), pp 1 – 21.

Dębowski, M., Rusanowska, P., Zieliński, M. Dudek, M. and Romanowska-Duda, Z. (2018). Biomass Production and Nutrient Removal by *Chlorella vulgaris* from Anaerobic Digestion Effluents. *Energies*; 11 (7), pp. 1 - 11.

Dehghani, J., Atazadeh, E., Omidi, Y. and Movafeghi, A. (2020). The use of 18S ribosomal DNA, ITS and rbcL molecular markers to study the genus Dunaliella (Dunaliellaceae) in Iranian samples: A phylogenetic approach. *International Journal of Oceanography and Hydrobiology*, 49 (1), pp. 88 – 98.

Dineshkumar, R., Narendran, R., Jayasingam, P., Sampathkumar, P. (2017). Cultivation and Chemical Composition of Microalgae *Chlorella Vulgaris* and its Antibacterial Activity against Human Pathogens. *Journal of Aquaculture & Marine Biology*, 5 (3), pp. 2 – 8.

Duong, V.T., Li, Y., Nowak, E., Schenk, P.M. (2012). Microalgae Isolation and Selection for Prospective Biodiesel Production. *Energies*, 5, pp. 1835-1849.

Eboibi, B.E. (2019). Impact of time on yield and properties of biocrude during downstream processing of product mixture derived from hydrothermal liquefaction of microalga. *Biomass Conversion Biorefinery,* 9, pp. 379 – 387

Eboibi, B.E. (2021). Analysis of Processing Hydrothermal Liquefaction of Microalgae Reaction Product with and without Dichloromethane Solvent for Biocrude Recovery. *FUOYE Journal of Engineering and Technology,* 6 (1), pp. 96 – 102.

Eckerstorfer, M.F., Engelhard, M., Heissenberger, A., Simon, S., Teichmann, H. (2019). Plants Developed by New Genetic Modification Techniques—Comparison of Existing Regulatory Frameworks in the EU and Non-EU Countries. *Frontiers in Bioengineering & Biotechnology*, 7 (26), pp. 1 – 16.

El-Sheekh, M., Abu-Faddan, M., Abo-Shady, A., Nassar, M.Z.A., Labib, W. (2020). Molecular identification, biomass, and biochemical composition of the marine chlorophyte Chlorella sp. MF1 isolated from Suez Bay. *Journal of Genetic Engineering and Biotechnology*, 18 (27), pp. 1 – 10.

Eze, V.C., Velasquez-Orta, S.B. and Hernandez-Garcıa, A. (2018). Kinetic modelling of microalgae cultivation for wastewater treatment and carbon dioxide sequestration. *Algal Research*, 32, pp. 131–141.

Fernandes, T., Fernandes, F., Andrade, C.A.P. and Cordeiro, N. (2020). Assessing the impact of sulfur concentrations on growth and biochemical composition of three marine microalgae. *Journal of Applied Phycology*.

Figueira, C.E. Moreira, P.F. and Giudici, R. (2015). Thermogravimetric analysis of the gasification of microalgae *Chlorella vulgaris. Bioresource Technology*, 198, pp. 717 -724.

Gai, C., Zhang, Y., Chen, W.T., Zhang, P. and Dong, Y. (2015). An investigation of reaction pathways of hydrothermal liquefaction using *Chlorella pyrenoidosa* and *Spirulina platensis. Energy Conversion Management*, 96, pp. 330 - 339.

Gao, S., Edmundson, S. and Huesemann, M. (2022). Oxygen stress mitigation for microalgal biomass productivity improvement in outdoor raceway ponds. *Algal Research*, 68 (102901), pp. 1 – 8.

Gharajeh, N.H., Valizadeh, M., Dorani, E. and Hejazi, M.A. (2020). Biochemical profiling of three indigenous *Dunaliella* isolates with main focus on fatty acid composition towards potential biotechnological application. *Biotechnology Reports*, 26, pp. 1 – 9.

Gollakota, A.R.K., Kishore, N. and Gua, S. (2018). A review on hydrothermal liquefaction of biomass. *Renewable and Sustainable Energy Reviews,* 5

Gong, X., Zhang, B., Zhang, Y., Huang, Y. and Xu, M. (2014). Investigation on Pyrolysis of Low Lipid Microalgae *Chlorella vulgaris* and *Dunaliella salina. Energy Fuels*, 28, pp. 95−103.

Goo, B.G., Baek, G., Choi, D.J., Park, Y., Synytsya, A., Bleha, R., Seong, D.H., Lee, C.G. and Park, J.K. (2013). Characterization of a renewable extracellular polysaccharide from defatted microalgae *Dunaliella tertiolecta. Bioresource Technology*, 129, pp. 343 – 350.

Goyal, H.B., Seal, D. and Saxena, R.C. (2008). Bio-fuels from thermochemical conversion of renewable resources: a review. *Renewable and Sustainable Energy Reviews*, 12 pp. 504 – 517.

Harder, R. and von Witsch, H. (1942). Bericht uber versuche zur fettsynthese mittels autotropher microorganismen, *forschugsdient sonderheft,* 16, pp. 270 -275.

Hamed, I (2016). The evolution and versatility of microalgal biotechnology: A Review. *Compressive review in food science and food safety*, pp. 1 – 20.

Hao, B., Xu, D., Jiang, G., Ahmed, T., Jing, S.X. and Guo, Y. (2021). Chemical reactions in the hydrothermal liquefaction of biomass and in the catalytic hydrogenation upgrading of biocrude. *The Royal Society of Chemistry,* 23, pp. 1562–1583

Haoujar, I., Cacciola, F., Manchado, M., Abrini, J., Haoujar, M., Chebbaki, K., Oteri, M., Rigano, F., Mangraviti, D., Mondello, L., Essafi, A., Chairi, H. and Senhaji, N.S. (2020). Isolation of Microalgae from Mediterranean Seawater and Production of Lipids in the Cultivated Species. *Foods*, 9 (1601), pp. 1 – 13.

Harvey, P.J. and Ben-Amotz, A. (2020). Towards a sustainable *Dunaliella salina* microalgal biorefinery for 9-cis βcarotene production. *Algae research*, 50, pp. 1 – 9.

Hassi, M. and Alouani, M. (2020). Isolation and identification of marine microalgae from the atlantic ocean in the south of Morocco. *American Journal of Innovative Research and Applied Sciences*, pp. 1 – 6.

Hayden, K., Ivors, K., Wilkinson, C., Garbelotto, M., (2006). TaqMan chemistry for phytophthora ramorum detection and quantification, with a comparison of diagnostic methods. *Phytopathology*, 96, pp. 846 – 854.

Hong, Y., Chen, W., Luo, X., Pang, C., Lester, E. and Wu, T. (2017). Microwave-enhanced pyrolysis of macroalgae and microalgae for syngas production. *Bioresource Technology,* pp. 1 – 33.

Hossain, N., J. Zainia, J., Mahliab, T.M.I. and Azad, A.K. (2019). Elemental, Morphological and Thermal Analysis of Mixed Microalgae Species from Drain Water. *Renewable Energy,* 131, pp. 617– 624.

Hossain, N., Meurah, T. and Mahlia, I. (2019). Progress in physicochemical parameters of microalgae cultivation for biofuel production. *Critical Review Biotechnology*, 39, pp 835–59.

Hu, J., Nagarajan, D., Zhang, Q., Chang, J. D. and Lee, D. (2017). Heterotrophic cultivation of microalgae for pigment production: A review. *Biotechnology Advances*, 36, 54–67.

Hu, Y., Gong, M., Feng, S., Xu, C.C. and A. Bassi, A. (2019). A review of recent developments of pre-treatment technologies and hydrothermal liquefaction of microalgae for bio-crude oil production. *Renewable and Sustainable Energy Reviews,* 101*,* pp. *476–492.*

Jahn, M.T., Schmidt, K. andMock, T. (2014). A novel cost effective and high-throughput isolation and identification method for marine microalgae. *Plant methods*, 10(26), pp. 1 – 10.

Jiang, J. and Savage, P.E. (2017). Influence of process conditions and interventions on metals content in biocrude from hydrothermal liquefaction of microalgae. *Algal Research,* 26*,* pp. 131–134.

Karatzos, S., van Dyk, J.S., McMillan, J.D., and Saddler, J. (2017). Drop-in biofuel production via conventional (lipid/fatty acid) and advanced (biomass) routes: Part I. *Biofuels, Bioproducts and Biorefining*, 11 pp. 344–362.

Kazbar, A., Cogne, G., Urbain, B., Marec, H., Le-Gouic, B., Tallec, J., Takach, H. and Ismail, A. and Pruvost, J. (2019). Effect of dissolved oxygen concentration on microalgal culture in photobioreactors. *Algal Research,* 39 (101432), pp. 1 – 11.

Keramati, A., Shariati, F.P., Tavakoli, O., Akbari, Z. and Rezaei, M. (2021). The effect of audible sound frequency on the growth and beta-carotene production of *Dunaliella* salina. *South African Journal of Botany*, 141, pp. 373 – 382.

Khairy, H.M., Shaltout, N.A., El-Naggar, M.F. and El-Naggar, N.A. (2014). Impact of elevated CO_2 concentrations on the growth and ultrastructure of non-calcifying marine diatom (Chaetoceros gracilis F.Schutt). *Egyptian Journal of Aquatic Research*, 40, pp. 243–250.

Khaw, Y.S., Khong, N.M.H., Shaharuddin, N.A. & Yusoff, F.M.D. (2019). A simple 18S rDNA approach for the identification of cultured eukaryotic microalgae with an emphasis on primers. *Journal of Microbiological Methods*, pp. 1 – 56.

Khoo, C.G., Lamb, M.K., Mohamed, A.R. and Lee, K.T. (2020). Hydrochar production from high-ash low-lipid microalgal biomass via hydrothermal carbonization: Effects of operational parameters and products characterization. *Environmental Research*, 188 (109828), pp. 1 – 12.

Kim, G.Y., Heo, J., Kim, H.S. and Han, J.I. (2017). Bicarbonate-based Cultivation of *Dunaliella salina* for Enhancing Carbon Utilization Efficiency. *Bioresource Technology*, pp. 1 – 25.

Krimpen, M.V., Bikker, P., Meer, I.V., Peet-Schwering, C.V. and Vereijken, J. (2013). *Cultivation, Processing and Nutritional Aspects for Pigs and Poultry of European Protein Sources As Alternatives for Imported Soybean Products,* Wageningen UR Livestock Research, pp. 1 – 63

Kumar, A., Guria, C. and Pathak, A.K. (2018). Optimal cultivation towards enhanced algaebiomass and lipid production using *Dunaliella tertiolecta* for biofuel application and potential CO2 biofixation: Effect of nitrogen deficient fertilizer, light intensity, salinity and carbon supply strategy. *Energy*.

Kumar, P.K., Krishna, S.V., Verma, K., Pooja, K., Bhagawan, D., Srilatha, K. and Himabindu, V. (2018). Bio oil production from microalgae via hydrothermal liquefaction technology under subcritical water conditions. *Journal of Microbiological Methods,* 153, pp. 108–117.

Lee, G., Park, S.Y., Yum, S., Woo, S., Lee, Y.H. and Hwang, S.Y., Park, S.H., Moh, S.H., Lee, S. and Lee, T.K. (2012). Development of DNA chip for verification of 25 microalgae collected from southern coastal region in Korea. *BioChip Journal*, 6, pp. 325–334.

Lee, X.J., Ong, H.C., Gan, Y.Y., Chen, W-H. and Mahlia, T.M.I. (2020). State of art review on conventional and advanced pyrolysis of macroalgae and microalgae for biochar, bio-oil and bio-syngas production. *Energy Conversion and Management,* 210 (112707), pp. 1 – 34.

Liang, M.H. Xue, L.L. and Jiang, J.G. (2018). Two-stage cultivation of *Dunaliella tertiolecta* with glycerol and triethylamine for lipid accumulation: A viable way to alleviate the inhibitory effect of triethylamine on biomass. *Applied and environmental microbiology*, pp. 1 – 18.

Lima, S., Villanova, V., Grisafi, F., Caputo, G., Brucato, A. and Scargiali, F. (2020). Autochthonous microalgae grown in municipal wastewaters as a tool for effectively removing nitrogen and phosphorous. *Journal of Water Process Engineering*, 38, pp. 1 – 12.

Liu, G., Jiang**,** R., You, J., Muir, D.C.G. and Zeng, E.Y. (2019). Microplastic Impacts on Microalgae Growth: Effects of Size and Humic Acid. *Environmental Science and Technology,* 54 (3), pp. 1782 – 1789.

Liu, Y. and Yildiz, I. (2018). The effect of salinity concentration on algal biomass production and nutrient removal from municipal wastewater by *Dunaliella* salina. *International Journal of energy research,* pp. 1–10.

Liu, Y.Q., Lim, L.R., Wang, J., Yan, R. and Mahakhant, A. (2012). Investigation on Pyrolysis of Microalgae Botryococcus braunii and Hapalosiphon specie. *Industrial & Engineering Chemistry Research*, 51, pp. 10320 – 10326.

Lutzu, G.A., Ciurli, A., C. Chiellini, C., Di Caprio, F. and Concas, N.T. (2020). Dunford, Latest developments in wastewater treatment and biopolymer production by microalgae. *Journal of Environmental Chemical Engineering*, pp 1 – 80.

Maga, D (2017). Life cycle assessment of biomethane produced from microalgae grown in municipal waste water. *Biomass Conversion and Biorefinery,* 7 (1), pp. 1–10.

Mahima, J., Sundaresh, R.K., Gopinath, K.P., Rajan, P.S.S., Arun, J., Kim, S., Pugazhendhi, A. (2021). Effect of algae (*Scenedesmus obliquus*) biomass pre-treatment on bio-oil production in hydrothermal liquefaction (HTL): Biochar and aqueous phase utilization studies. *Science of the Total Environment,* 778, pp. 1 – 9.

Mathimani, T. and Mallick, T.N. (2019). A review on the hydrothermal processing of microalgal biomass to bio-oil - Knowledge gaps and recent advances. *Journal of Cleaner Production,* 217, pp. 69 – 84.

Minillo, A. Godoy, G.G., and Fonseca, G.G (2013). Growth performance of microalgae exposed to CO_2. *Journal of Clean Energy Technology*, 1 (110).

Mirzaie, M.A.M., Lak, Z.J. and Meybodi, F.H. (2021). A New Shifting Strategy in Flashing Light Based on the Cell Growth Curve to Enhance β-carotene Biosynthesis in *Dunaliella salina. Current Microbiology*, 78, pp. 2733–2740.

Mishra, A., Kavita, K. and Jha, B. (2011). Characterization of extracellular polymeric substances produced by micro-algae *Dunaliella salina. Carbohydrate polymers*, 83 (2), pp. 852–857.

Mofijur, M., Rasul, M.G., Hassan, N.M.S. and Nabi, M.N. (2019). Recent Development in the Production of Third Generation Biodiesel from Microalgae. *Energy Procedia,* 156, pp. 53 – 58.

Mondal, M., Goswami, S., Ghosh, A., Oinam, G., Tiwari, O.N., Das, P., Gayen, K., Mandal, M.K. and Halder, G.N. (2017). Production of biodiesel from microalgae through biological carbon capture: a review. *Biotechnology,* 7 (2), pp. 1 – 21.

Morales, M., Aflalo, C., Bernard, O. (2021). Microalgal lipids: A review of lipids potential and quantification for 95 phytoplankton species. *Biomass and Bioenergy*, 150 (106108), pp. 1 – 25.

Morales-Jiménez, M., Gouveia, L., Yáñez-Fernández, Y., Castro-Muñoz, R., Barragán-Huerta, B.E. (2020). Production, Preparation and Characterization of Microalgae-Based Biopolymer as a Potential Bioactive Film. *Coatings*, 10 (120), pp. 1 – 15.

Muylaert, K. (2017). Harvesting of microalgae: Overview of process options and their strengths and drawbacks. *Microalgae-Based Biofuels and Bioproducts,* (2017) 113 – 132.

Najeeb, M.L., Ahmad, M.D., Anjum, A.A., Maqbool, A., Ali, M.A., Nawaz, M., Ali, T. and Manzoor, R. (2024). Distribution, screening and biochemical characterization of indigenous microalgae for bio-mass and bio-energy production potential from three districts of Pakistan. *Brazilian Journal of Biology*, 84 (261698), pp. 1 – 9.

Obeid, F., Van, T.C., Guo, B., Surawski, N.C., Hornung, U., Brown, R.J., Ramirez, J.A., Thomas-Hall, S.R., Stephens, E., Hankamer, B. and T. Rainey, T. (2021). The fate of nitrogen and sulphur during co-liquefaction of algae and bagasse: Experimental and multi-criterion decision analysis. *Biomass and Bioenergy,* 151 (106119), pp. 1 -13.

Orejuela-Escobar, L., Gualle, A., Ochoa-Herrera, V., Philippidis, G.P. (2021). Prospects of Microalgae for Biomaterial Production and Environmental Applications at Biorefineries. *Sustainability,* 13 (3063), pp. 1 – 19.

Pachiappan, P., Prasath, B.B., Perumal, S., Ananth, S, Devi, S.A., Kumar, S.D. and Jeyanthi, S. (2016). Isolation and Culture of Microalgae. *Advances in Marine and Brackishwater Aquaculture*, pp. 1- 16.

Panahi, H.K.S., Tabatabaei, M., Aghbashlo, M., Dehhaghi, M., Rehan, M., Nizami, A-S. (2019). Recent updates on the production and upgrading of bio-crude oil from microalgae. *Bioresource Technology Reports,* pp. 1 – 53.

Peng, G., Vogel, F., Refardt, D. and Ludwig, C. (2017). Catalytic supercritical water gasification: continuous methanization of *Chlorella vulgaris. Industrial Engineering Chemistry Research*, 56, pp. 6256 - 6265.

Phwan, C.K. Ong, H.C., Chen, W-H., Ling, T.C., Ng, E.P. and Show, P.L. (2018). Overview: Comparison of pretreatment technologies and fermentation processes of bioethanol from microalgae. *Energy Conversion and Management,* 173 pp. 81–94.

Picardo, M.C., Medeiros, J.L., Monteiro, J.G.M., Chaloub, R.M., Giordano, M. and Arau´jo, O.Q.F. (2013). *A methodology for screening of microalgae as a decision making tool for energy and green chemical process applications.* Clean Technology Environment Policy 15, pp. 275–291.

Pick, U., Zarka, A., Boussiba, S., Davidi, L. (2019). A hypothesis about the origin of carotenoid lipid droplets in the green algae *Dunaliella* and *Haematococcus*. *Planta,* 249, pp. 31–47.

Pourkarimi, S., Hallajisani, A., Alizadehdakhel, A., Nouralishahi, A., Golzary, A. (2020). Factors affecting production of beta-carotene from Dunaliella salina microalgae. *Biocatalysis and Agricultural Biotechnology*, 29 (101771), pp, 1 - 14.

Radha, S., Fathima, A.A., Iyappan, S. and Ramya, M. (2013). Direct colony PCR for rapid identification of varied microalgae from freshwater environment. *Journal of Applied Phycology,* 25, pp. 609 – 613.

Rammuni, M., Ariyadasa, T.U., Nimarshana, P. and Attalage, R. (2019). Comparative assessment on the extraction of carotenoids from microalgal sources: astaxanthin from H.
pluvialis and $\beta - carotene$ from *Dunaliella salina. Food Chemistry*, 277, pp. 128–134.

Randrianarison, G. and Ashraf, M.A. (2017). Microalgae: a potential plant for energy production. *Geology, Ecology, and Landscapes,* 1 (2), pp. 104-120.

Ravelonandro, P.H., Ratianarivo, D.H., Joannis-Cassan, C., Isambert, A., M. and Raherimandimby, M. (2011). Improvement of the growth of Arthrospira (Spirulina) platensis from Toliara (Madagascar): Effect of agitation, salinity and CO_2 addition, *Food and Bioproducts Processing,* 8 (9), pp. 209 – 216.

Reddy, H.K., Muppaneni, T., Rastegary, J., Shirazi, S.A., Ghassemi, A. and Deng, S. (2014). Hydrothermal Extraction and Characterization of Bio-Crude Oils from Wet *Chlorella Sorokiniana* and *Dunaliella Tertiolecta. Environmental Progress & Sustainable Energy*, 32 (4), pp. 910 – 915.

Ren, T. (2014). Primary Factors Affecting Growth of Microalgae Optimal Light Exposure Duration and Frequency. Graduate Theses and Dissertations, Iowa State University, Ames, Iowa 13793, pp 1 – 80.

Ruiz, J., Wijffels, R.H., Dominguez, M., and Barbosa, M.J. (2022). Heterotrophic vs autotrophic production of microalgae: bringing some light into the everlasting cost controversy. *Algal Research*, 64 (102698).

Sacristán, M., de Alva, V.M. Luna, P., Orta Ledesma, M.T., Cruz Gómez, M.J. (2018). Carbon, Nitrogen, and Phosphorus Removal, and Lipid Production by Three Saline Microalgae Grown in Synthetic Wastewater Irradiated with Different Photon Fluxes. *Algal Research*, 34, (103).

Sajjadia, B., Chena, W-Y., Aziz, A., Raman, A. and Ibrahim, S. (2018). Microalgae lipid and biomass for biofuel production: A comprehensive review on lipid enhancement strategies and their effects on fatty acid composition. *Renewable and Sustainable Energy Reviews*, 97, pp. 200 – 232.

Sarkar, S., Sarkar, S., Manna, M.S., Gayen, K. and Bhowmick, T.K. (2022). Chapter 12 - Extraction of carbohydrates and proteins from algal resources using supercritical and subcritical fluids for high-quality products. *Innovative and Emerging Technologies in the Bio-marine Food Sector*, pp, 249 – 275.

Schulze, P.S.C., Barreira, L.A., Pereira, H.G.CJ.A. Perales, J.C.S. Varela (2014). Light emitting diodes (LEDs) applied to microalgal production. *Trends in Biotechnology*, 32 (8), pp. 422 – 30.

Selmani, N., Mirghani, M.E.S. and Alam, M.Z. (2013). Study the Growth of Microalgae in Palm Oil Mill Effluent Waste Water. 4th International Conference on Energy and Environment, pp. 1 – 5.

Shang, C., Bi, G., Yuan, Z., Wang, Z., Alam, M.A. and Xie, J. (2016). Discovery of genes for production of biofuels through transcriptome sequencing of *Dunaliella* parva. *Algae research*, 13, pp 318 – 326.

Sharma, K.K., Schuhmann, H., and Schenk, P.M. (2012). High Lipid Induction in Microalgae for Biodiesel Production. *Energies*, 5, pp. 1532 – 1553.

Show, K-Y., Yan, Y., Zong, C., Guo, N., Chang, J-S. and Lee, D-J. (2019). State of the Art and Challenges of Biohydrogen from Microalgae. *Bioresource Technology,* pp. 1 – 53.

Show, P.L., Tan, J.S., Lee, S.Y., Chew, K.W., Lam, M.K., Lim, J.W. and Ho, S. (2020). A Review on Microalgae Cultivation and Harvesting, and Their Biomass Extraction Processing Using Ionic Liquids. *Bioengineered*, pp. 1 – 38.

Shuping, Z., Yulong, W., Mingde, Y., Kaleem, I., Chun, L. and Tong, J. (2010). Production and characterization of bio-oil from hydrothermal liquefaction of microalgae Dunaliella tertiolecta cake. *Energy,* 35, pp. 5406 – 5411.

Silva, M.R.O.B., Moura, Y.A.S., Converti, A., Porto, A.L.F., Marques, D.A.V, Bezerra, R.P. (2021). Assessment of the potential of *Dunaliella* microalgae for different biotechnological applications: A systematic review. *Algal Research*, 58 (102396), pp. 1 – 14.

Singh, K.S., Anis, A. and Ghosh, S. (2018). Mathematical modeling of light energy flux balance in flat panel photobioreactor for *Botryococcus braunii* growth, CO2 biofixation and lipid production under varying light regimes. *Biochemical Engineering Journal*, 134, pp. 44–56.

Singh, P., Gupta, S.K., Guldhe, A., Rawat, I. and Bux, F. (2015). Microalgae Isolation and Basic. *Culturing Techniques*, chapter 4, pp. 43 – 54.

Slinksienė, R., Sendzikiene, E., Mikolaitiene, A., Makareviciene, V., Paleckiene, R., Ragauskaite, D. Use of microalgae biomass for production of granular nitrogen biofertilizers. *Green chemistry letters and reviews*, 15 (2), pp. 416–426.

Slocombe, S.P. and Benemann, J.R. (2016). *Production for Biomass and High-Value Product.* Proceedings of Microalgal*,* pp. *1 – 16.*

Sui, Y. and Vlaeminck, S.E. (2019). *Dunaliella* microalgae for nutritional protein: an undervalued asset. *Trends in Biotechnology*, pp. 1 – 3.

Sumbul, A., Ansari, R.A., Rizvi, R. and Mahmood, I. (2020). Azotobacter: A potential bio-fertilizer for soil and plant health management. *Saudi Journal of Biological Sciences*, pp. 1 – 25.

Sun, J., Yang, J. and Shi, M. (2017). Review of Denitrogenation of Algae Biocrude Produced by Hydrothermal Liquefaction. *Transactions of Tianjin University,* 23, pp. 301–314.

Sun, X.M., Ren, L.J, Zhao, Q.Y., Ji, X.J. and Huang, H. (2018). Microalgae for the production of lipid and carotenoids: a review with focus on stress regulation and adaptation. *Biotechnology and Biofuels,* 11 (272), pp. 1 – 16.

Suparmaniam, U., Lam, M.K., Uemura, Y., Lim, J.K., Lee, K.T., Shuit, H.S. (2019). Insights into the microalgae cultivation technology and harvesting process for biofuel production: A review. *Renewable and Sustainable Energy Reviews*, 115 (109361), pp. 1 – 23.

Taghipour, A., Ramirez, J.A., Brown, R.J., Rainey, T.J, (2021). Fractional distillation of algae based hydrothermal liquefaction biocrude for co-processing: changes in the properties, storage stability, and miscibility with diesel. *Energy Conversion and Management,* 236, 114005.

Tan, C.H., Show, P.L., Chang, J.S., Ling, T.C., and Lan, J.C.W. (2015). Novel Approaches of Producing Bioenergies from Microalgae: A Recent Review. *Biotechnology Advances*, pp. 1 -30.

Tang, H. Abunasser, N., Garcia, M.E.D., Chen, M., Ng, K.Y.S. and Salley S.O. (2011). Potential of microalgae oil from *Dunaliella tertiolecta* as a feedstock for biodiesel. *Applied Energy,* 88, pp. 3324 – 3330.

Toor, S.S., Reddy, H., Deng, S., Hoffmann, J., Spangsmark, D., Madsen, L.B., Holm-Nielsen, J.B. and Rosendahl, L.A. (2013). Hydrothermal liquefaction of *Spirulina* and *Nannochloropsis salina* under subcritical and supercritical water conditions. *Bioresource Technology*, 131, pp. 413 – 419.

Toyub, M.A., Miah, M.I., Habib, M.A.B and Rahman, M.M. (2008). Growth performance and nutritional value of *Scenedesmus obliquus* cultured in different concentrations of sweetmeat factory waste media. *Bangladesh Journal Animal Science*, 37 (1), pp. 86 – 93.

Tripathi, B.N. and Kumar, D. (2017). *Generation and Harvesting of Microalgae Biomass for Biofuel Production.* Prospects and Challenges in Algal Biotechnology, Chapter 3, pp. 89–111.

van Dyk, S., Su, J., Mcmillan, J.D., Saddler, J.J. (2019). Potential synergies of drop-in biofuel production with further co-processing at oil refineries. *Biofuels, Bioproducts and Biorefining,* pp. 1 – 16.

Vaquero, M.G. (2021). Chapter 8 - Food applications, Microalgae, pp. 207-238,

Vieira, H.H., Bagatini, I.L. Guinart, C.M. and Vieira, A.A.H. (2016). tufA gene as molecular marker for freshwater Chlorophyceae. *Algae* 31, pp. 155–165.

Vingiani, G.M., De P., Luca, P., Ianora, A., Dobson, D.W., Lauritano, C.L. (2019). Microalgal enzymes with biotechnological applications. *Marine Drugs*, 17 (459), pp. 76 – 89.

Wądrzyk, M., Janus, R., Vos, M.P. and Brilman, D.W.F. (2018). Effect of process conditions on bio-oil obtained through continuous hydrothermal liquefaction of Scenedesmus sp. Microalgae. *Journal of Analytical and Applied Pyrolysis,* pp. 1 – 12.

Wang, B., Lan, C.Q. and Horsman, M. (2012). Closed photobioreactors for production of microalgal biomasses. *Biotechnology Advances*, 30 (4), pp 904–912.

Wang, Q., Oshita, K. and Takaoka, M. (2021). Flocculation properties of eight microalgae induced by aluminum chloride, chitosan, amphoteric polyacrylamide, and alkaline: Life-cycle assessment for screening species and harvesting methods. *Algal Research,* pp. 1 - 11.

Wang, Y., Tibbetts, S.M. and McGinn, P.J. (2021). Microalgae as sources of high-quality protein for human food and protein supplements. *Foods*, 10 (3002), pp. 1 – 18.

Wang, Y., Zhang, C., Yu, X., Ge, Y. (2020). Arsenite Oxidation by *Dunaliella* salina is affected by External Phosphate Concentration. *Bulletin of Environmental Contamination and Toxicology*, pp. 1 - 6.

Wang, Y., Zhang, C., Zheng, Y. and Ge, Y. (2017). Bioaccumulation kinetics of arsenite and arsenate in Dunaliella salina under different phosphate regimes. *Environmental science pollution research,* pp. 1 – 9.

White, T.J., Bruns, T., Lee, S. and Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: PCR Protocols: A guide to methods and applications. Academic press, pp. 315 - 322.

Xu, Y., Ibrahim, I., Wosu, C., Ben-Amotz, A. and Harvey, P. (2018). Potential of new isolates of *Dunaliella salina* for natural β-carotene production. *Biology*, 7 (14), pp. 1 – 15.

Xu, Y., Lu, A., Chen, X., Su, D., Wang, W., Zhang, Y. (2018). Isolation, Identification and Growth Optimisation of Freshwater Microalgae. *Turkish Journal of Fisheries and Aquatic Sciences*, 18, pp. 1293-1302.

Xue, L.L., and Jiang, J.G. (2017). Cultivation of *Dunaliella tertiolecta* intervened by triethylamine enhances the lipid content. *Algal Research,* 25, pp. 136–141.

Yang, W., Li, Z., Li, Z., Tong, C. and Feng, L. (2015). Understanding low-lipid algae hydrothermal liquefaction characteristics and pathways through hydrothermal liquefaction of algal major components: Crude polysaccharides, crude proteins and their binary mixtures. *Bioresource Technology,* 196, pp 99–108.

Ying, K., Gilmour, D.J. and Zimmerman, W.B. (2014). Effects of CO2 and pH on Growth of the Microalga *Dunaliella salina. Journal of Microbial & Biochemical Technology*, 6 (3), pp. 167 – 173.

Yu, J., Maliutina, K. and Tahmasebi, A. (2018). A review on the production of nitrogen-containing compounds from microalgal biomass via pyrolysis. *Bioresource Technology*, pp. 1 – 40.

Zabed, H.M., Akter, S., Yun, J., Zhang, G., Zhang, Y, and Qi, X. (2020). Biogas from microalgae: Technologies, challenges and opportunities. *Renewable and Sustainable Energy Reviews,* 117 (109503), pp 1 - 21.

Zanella, L. and Vianello, F. (2020). Microalgae of the genus Nannochloropsis: Chemical composition and functional implications for human nutrition. *Journal of Functional Foods*, 68, 103919.

Zarrinmehr, M.J., Farhadian, O., Heyrati, F.P., Keramat, J., Koutra, E., Kornaros, M. and Daneshvar, E. (2019). Effect of nitrogen concentration on the growth rate and biochemical composition of the microalga, Isochrysis galbana. *Egyptian Journal of Aquatic Research.*

Zhang, B., He, Z., Chen, H., Kandasamy, S., Xu, Z., Hu, X. and Guo, H. (2018). Effect of Acidic, Neutral and Alkaline Conditions on Product Distribution and Biocrude Oil Chemistry from Hydrothermal Liquefaction of Microalgae. *Bioresource Technology,* pp. 1 – 32.

Zhang, S., Zhang, L., Xu, G., Li, F., Li, X. (2022). A review on biodiesel production from microalgae: Influencing parameters and recent advanced technologies. *Frontiers in microbiology*, 13 (970028), pp. 1 – 20.

Zhang, Y., Huang, Z., Zheng, H., Wang, Q. and Li, A. (2020). Growth, biochemical composition and photosynthetic performance of Scenedesmus acuminatus under different initial sulfur supplies. *Algal Research,* 45 (101728), pp. 1 – 9.

Zhang, Z., Wang, C., Cao, G., Chen, W-H. and Ho, S.H. (2019). Comparison and characterization of property variation of microalgal biomass with non-oxidative and oxidative torrefaction. *Fuel*, 246, pp. 375–385.

Zhu, C., Zhai, X., Jia, J., Wang, J., Han, D., Li, Y., Tang, Y. and Chi, Z. (2018). Seawater desalination concentrate for cultivation of *Dunaliella salina* with floating photobioreactor to produce $\beta - carotene$. *Algae research*, 35, pp. 319 – 324.

Zhu, Q.L., Zheng, J.L., Liu, J. (2020). Transcription activation of β-carotene biosynthetic genes at the initial stage of stresses as an indicator of the increased $\beta - carotene$ accumulation in isolated *dunaliella salina* strain GY-H13. *Aquatic Toxicology*, 222 (105472), pp. 1 – 10.