

**SUSTAINABLE CULTIVATION OF MICROALGAE BIOMASS FOR
BIOENERGY PRODUCTION**

**THESIS SUBMITTED TO THE UNIVERSITY OF NOTTINGHAM
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY**

BY

ANGELA PAUL A/P PETER



**University of
Nottingham**
UK | CHINA | MALAYSIA

**DEPARTMENT OF CHEMICAL AND ENVIRONMENTAL
ENGINEERING, FACULTY OF SCIENCE AND ENGINEERING,
UNIVERSITY OF NOTTINGHAM MALAYSIA, JALAN BROGA,
43500 SEMENYIH, SELANGOR DARUL EHSAN, MALAYSIA**

21st MARCH 2022

Declaration

I declare that this written submission represents my ideas in my own words, and where others' ideas or words have been included, I have adequately cited and referenced the original sources. I also declare that I have adhered to all principles of academic honesty and integrity and have not misrepresented or fabricated or falsified any idea/data/fact/source in my submission. I understand that any violation of the above will be a cause for disciplinary action by the Institute and can also evoke penal action from the sources that have thus not been properly cited, or from whom proper permission has not been taken when needed. I also declare that that work presented in this thesis has been reviewed by a few Journal editors and experts in the field that leads for acceptance for publication.



(signature)

Angela Paul Peter

(ID: 18812919)

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- 2) Shazia Ali, **Angela Paul Peter**, Kit Wayne Chew, Heli Siti Halimatul Munawaroh, Pau Loke Show (2021). Resource recovery from industrial effluents through the cultivation of microalgae: A review. Bioresource technology [IF: 9.642 (2020)].
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- 5) Kreena Gada, **Angela Paul Peter**, Wai Siong Chai, Pau Loke Show (2022). Industry Perspectives of Microalgae 4.0. [Book: Microalgae for Environmental Biotechnology; Page 363 – 395; CRC Press]

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Nomenclature

AAS	: Atomic Absorption Spectrometry
APIs	: Application Programming Interface
AP	: Average Precision
BECCS	: Bioenergy with carbon capture and storage
BG-11	: Inorganic medium
BOD	: Biological oxygen demand
BSA	: Bovine serum albumin reagent
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	: Calcium chloride
C_c	: Costs for consumables
CCTV	: Closed circuit television
C_e	: Energy costs
CFD	: Computational fluid dynamics
CIP	: Clean in place
C_I	: Total investment cost
C_l	: Costs for labour
C_{loss}	: Optimal term which represents the loss of algae
CMYK	: Cyan, Magenta, Yellow, Black
CO_2	: Carbon dioxide
$\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$	: Cobaltous nitrate hexahydrate
$\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$	: Copper (II) sulfate pentahydrate
DB & B	: Industrial dairy powder waste: biscuit waste
DCW	: Dry cell weight
DM	: Industrial dairy powder waste: milk waste
DSLR	: Digital single lens reflex
EDTA	: Ethylenediaminetetraacetic acid
EDX	: Energy-dispersive X-ray
EPS	: Element of polysaccharide
EU	: European Union
FAME	: Fatty acid Methyl Esters
FESEM	: Field emission scanning electron microscopy
FSP-E	: <i>Chlorella vulgaris</i>
FTIR	: Fourier-transform infrared spectroscopy
GGE	: Gasoline gallon equivalent
HPLC	: High-performance liquid chromatography
HSL	: Hue, Saturation, and lightness
H_2SO_4	: Sulfuric acid
H_3BO_3	: Boric acid
I	: Rate of interest
IBG	: BG-11 (Inorganic medium)
IDEs	: Integrated Development Environments
IoT	: Internet of Things
IRENA	: International Renewable Energy Agency
ISO	: International Organization for Standardization
ISWM	: Integrated solid waste management
KOH	: Potassium hydroxide
K_2HPO_4	: Dipotassium phosphate
LCA	: Life cycle analysis

LCI	: Life cycle Inventory
LED	: Light-emitting diode
M	: Maintenance costs
MPBR	: Microfiltration-based membrane photo-bioreactor
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	: Hydrated magnesium sulfate
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	: Manganese (II) Chloride
NASA	: National Aeronautics and space Administration
NaNO_3	: Sodium nitrate
Na_2CO_3	: Sodium carbonate
$\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$	: Sodium molybdate dihydrate
NH_3	: Ammonia
NH_4	: Ammonium
$(\text{NH}_4)_2\text{HPO}_4$	: Diammonium phosphate
NO^2	: Nitrite
NO^3	: Nitrate
N_2O	: Nitrous oxide
OMPBR	: Osmosis membrane photo-bioreactor
PBR	: Photo-bioreactor
POME	: Palm oil mill effluent
PUFAs	: Polyunsaturated fatty acids
RB	: Recycled medium (Industrial dairy powder waste: biscuit waste)
RGB	: Red, green, blue
RSM	: Response surface methodology
SDK	: Software development kit
TAC	: Total annual cost
TFF	: Tangential flow filtration
TSS	: Total suspended solid
URL	: Uniform Resource Locator
XML	: Extensible Markup Language
Y_{loss}	: Total produced biomass in a year
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	: Zinc sulphate

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CHAPTER 1

INTRODUCTION

1.1 Background study

In recent years, it is known that energy consumption has been increasing drastically with an estimated growth of global energy demand to more than 85% by 2040. The limitation of fossil fuels has driven many research to explore into renewable energy sources such as geothermal energy, bioenergy, hydropower energy, solar energy many more. However, it was then discovered that the biofuel produced by microalgae is a promising substitution of fossil fuels. In general, microalgae are a versatile species as they can sustain and continue growing even in a harsh surrounding with low nutrients concentration. Hence, microalgae are preferred over other biomass source are due to its high rates of photosynthesis by mitigating carbon dioxide, CO₂ emission effectively for the removal of nitrogen and phosphorus, no competitions with feed, food or arable land of agriculture, and cultivation using wastewater. Although microalgae are likely to be a feasible source for bioenergy, there are still challenges and limitations to be overcome to scale up for commercialization.

As the demand for microalgae-based products increases in the market, there is also an urge to further investigate the cultivation process into large-scale mode in a more sustainable method. Although open pond cultivation is cost saving in terms of energy and materials, closed scale photo bioreactor, PBR has been developed as it has control over the growth conditions and contamination. Moving forward, the challenge in implementing PBR system in large scale is its operating cost that requires large volume of culture medium as it is associated with modified chemical medium. Hence, in this study the discovered the microalgae grown in BG-11 alone (IBG), the combination of biscuit waste produced higher biomass concentration (44%), with increased lipid (11%), protein (20%), and carbohydrate (57%) contents. *Chlorella vulgaris* was able to absorb nutrients from the culture medium with combination of food processing waste

and IBG thus enhancing its growth. Moreover, it was also proven that 40% of the replacement ratio yielded highest biomass concentration (4.11 g/L), lipid (260.44 mg/g), protein (263.93 mg/g), and carbohydrate (418.99 mg/g) content compared with all the other culture ratio combination. In addition, a novel photo-to-biomass concentration estimation was conducted via image processing for three different colour model pixels and based on linear regression analysis, it was found that the red, green, blue (RGB) colour model can interpret its colour variance precisely. Digitalizing the microalgae monitoring will be useful for engineers, researchers, and industrialist in moving forward the implementation of continuous PBR system. However, the problem statement for scaling up a bioprocess was identified using life cycle analysis. The study emphasizes on the techniques that can be used in large-scale algal operations, for photo-bioreactor cleaning and maintenance. LCA indicated that recycling of remaining culture water obtained from dried biomass back into the photo-bioreactor was identified to be the most sustainable *Chlorella vulgaris* production process that can be considered for a commercial facility for bio-fuel production. Large scale production of microalgae biomass for biofuel production is quite a challenge due to several factors such as costly photo-bioreactor, sustainable medium for optimal growth, and time-consuming algae growth monitoring procedures. Thus, a mobile application (FindAlgae) was developed with a smart algae growth monitoring which analyses the colour differences in a single click. The use and development of mobile applications is a relatively new growing industry. Overall, this thesis focuses on the implementation of sustainable culture medium for the cultivation of *Chlorella vulgaris* and the implementation of machine learning and Internet of Things, IoT in microalgae growth monitoring to ease large scale development facilities.

1.2 Aims and Objectives:

The research topic mainly focuses on solving the challenges faced on the production of sustainable growth medium of microalgae and exploiting the potential techniques on

digitalization of microalgae growth monitoring. Hence five technical problem statement have been examined throughout the process which is essential in upscaling the growth of microalgae production plant. The problem statement rectified in this research are:

1. Recognizing the best organic medium to replace chemical based culture medium: Evaluate the nutrient content of dairy food processing waste remediation to be integrated with microalgae culture system for the growth of biomass.
2. Identifying the optimized, organic medium culture with chemical based nutrient based on vision imaging systems for biomass growth monitoring.
3. Analyzing the feasibility of the bioprocess recommended using life cycle analysis assessment of *Chlorella vulgaris* cultivation process using industrial waste powder as nutrient source in large scale.
4. Minimizing the tedious lab growth monitoring system using UV-Vis: Developed a mobile application (FindAlgae) for microalgae growth monetarization based on vision imaging system.
5. Test run the functionality of the FindAlgae app by evaluating and optimizing the recycling of culture medium in semi-continuous cultivation; biomass growth monitoring based on the developed mobile application (FindAlgae).

1.3 Research scopes:

Chapter 1: Literature Review

This chapter reviews about the benefits of microalgae for biofuel, wastewater treatment and as bioindicators. It also discusses the impact of processes based on microalgae using life cycle assessment. The importance of microalgae cultivation was presented.

Chapter 2: Integrated Microalgae Culture System with Dairy Food Processing Waste Remediation and Biomass Productivity Enhancement

This chapter mainly focusses the ability of integrating dairy food processing waste remediation with BG-11 for *Chlorella vulgaris* and its nutrient absorption. The waste powders investigated in this chapter were obtained from industrial milk, and biscuit manufacturing facilities. The culture medium was modified by combining both BG-11 and food processing waste powders to identify the optimal algal growth and biochemical content. This chapter mainly focusses the ability of integrating dairy food processing waste remediation with BG-11 for *Chlorella vulgaris* and its nutrient absorption.

Chapter 3: Microalgae Cultivation with Industrial Biscuit Waste Remediation Based on Vision Imaging Systems for Biomass Growth Monitoring

This chapters investigates on optimizing the proportion of industrial biscuit processing waste powder, substituted into BG-11 as a source of cultivation medium for the growth of *Chlorella vulgaris*. Various percentages of industrial biscuit processing waste powder were substituted in the inorganic medium to analyses the algal growth and biochemical composition. Secondly, the exploitation of colour acquisition was performed onto *Chlorella vulgaris* growth phases, and a novel photo-to-biomass concentration estimation was conducted via image processing for three different colour model pixels.

Chapter 4: Life Cycle Analysis: A Comparison of *Chlorella Vulgaris*, FSP-E Cultivation Pathway for Industrial Scale

This chapters investigate the scaling up of *Chlorella vulgaris*, FSP-E cultivation by discovering the best culture pathway using life cycle assessment, LCA. The techniques that can be used in large-scale algal operations, for photo-bioreactor cleaning and maintenance. Culture mediums plays a significant role in overall costing for microalgae growth processes, and their supply were addressed during the scaling up cultivation systems in terms of flow mixing, pipeline costing.

Chapter 5: A Digital Platform for Microalgae Growth Monetarization Via Mobile Application

This chapter made use of modern digital technologies such as sensors, automation applications, and the Internet of Things (IoT). The goal of this study was to develop a mobile application (FindAlgae) with a smart algae growth monitoring by analyzing colour differences in a single click. This section covers, the Java programming language that was used to code the application framework.

Chapter 6: Semi-Batch *Chlorella Vulgaris* Cultivation: Custom Vision Model for Biomass Growth Monitoring.

This chapter aims at improving the strategy of recycling culture media for semi-batch cultivation of *Chlorella vulgaris*. In two cycles, different percentages of recycled media were replaced to examine algal development and biochemical content. Next, custom vision and (FindAlgae) was applied to in monitoring the *Chlorella vulgaris* maturing stages, and a unique digital architecture framework was developed.

1.4 Research Connectivity and Relationship:

Figure 1.1 illustrates the connectivity of each research scopes tackled in this thesis. It begins by integrating microalgae culture system with dairy food processing waste remediation (Chapter 3), followed by implementation of vision imaging to monitor large scale algae growth (Chapter 4). Then the feasibility of upscaling the lab study was analyzed using LCA (Chapter 5). On the other hand, a digital platform was built to monitor the growth of microalgae via Mobile Application which enable to reduce the challenges faced for upscaling the bioprocess (Chapter 5). Lastly, a semi-batch cultivation was conducted to test out the custom vision model developed by the mobile application for biomass growth monitoring.

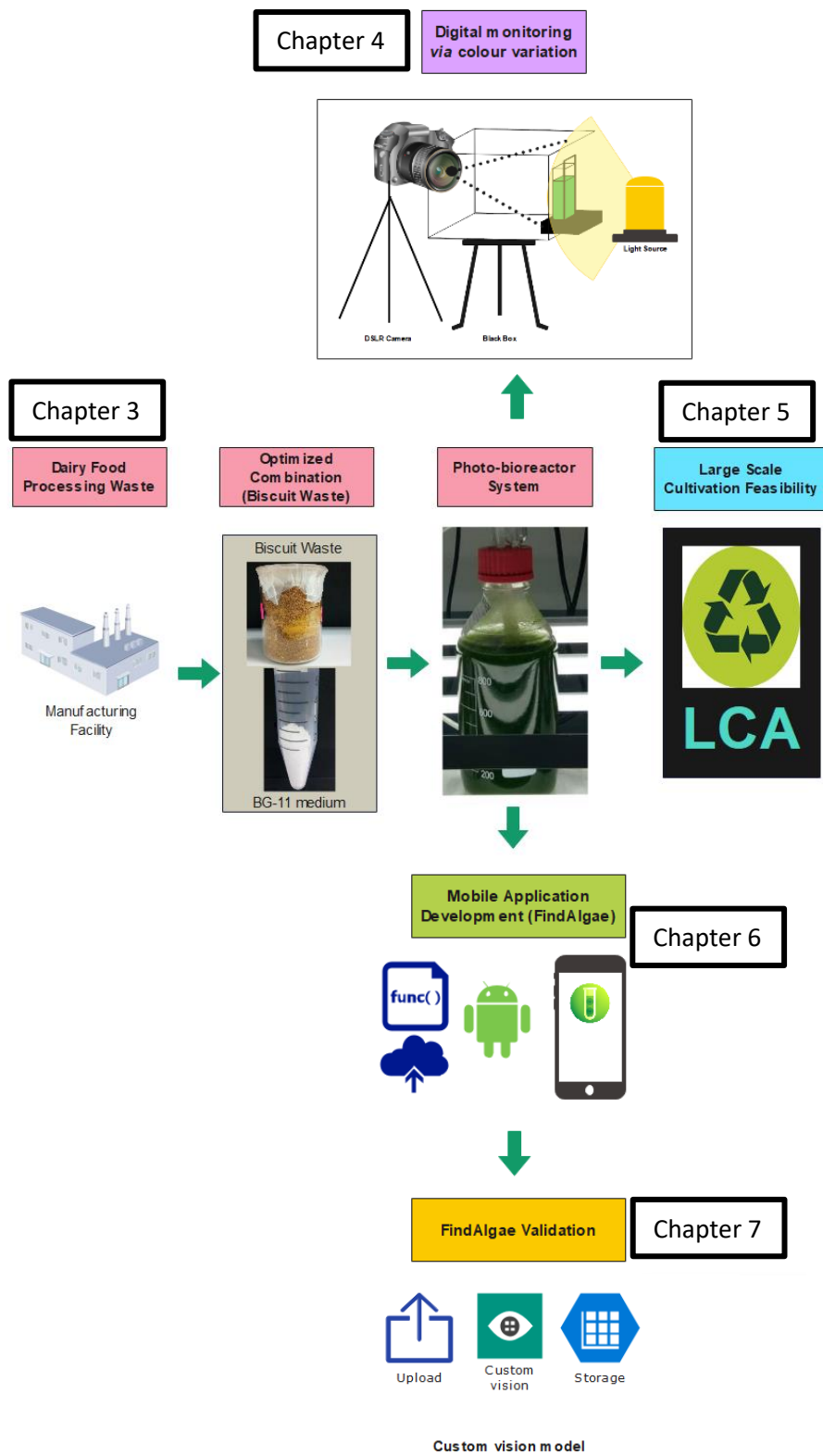


Figure 1.0.1: Graphical layout of the research scopes.

CHAPTER 2

LITERATURE REVIEW

In the context of climate change and the increase of the energy demand, there is a need for carbon sequestration methods and sustainable fuels. This can be done by cultivation of microalgae, a unicellular microscopic alga that converts carbon dioxide into high value bioproducts and energy. Moreover, microalgae can be used to assess the health of ecosystems such as lakes. This chapter reviews microalgae for biofuel, for wastewater treatment and as bioindicators. This chapter also discuss the impact of processes based on microalgae using life cycle assessment. The co-cultivation of microalgae with other microbes were presented, and comparison of conventional processes with processes integrating auto-flocculation, in situ transesterification and excretion.

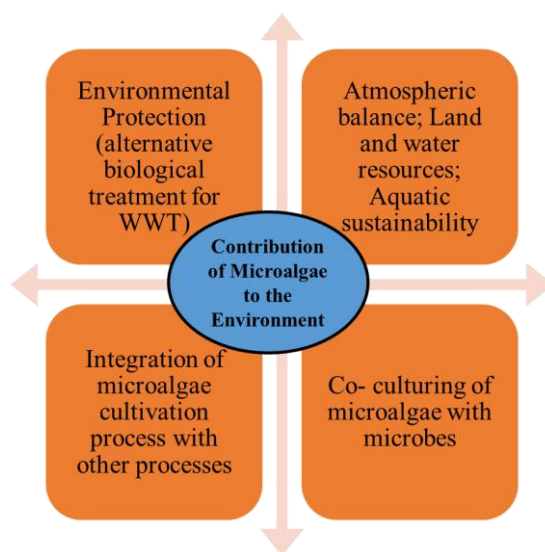


Figure 2.1: Graphical Abstract of Literature Review

Keywords: Atmospheric; Aquatic; Biotechnology; Sustainability; Wastewater.

This chapter are published in Environmental Chemistry Letters. [IF: 9.027 (2020)]:

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2.1 Microalgae for environmental protection

2.1.1 Biological assessment of water pollution

Water pollution is a global threat that is faced by most countries globally. Apart from industrial and domestic waste dump into the rivers, it is also known that loads of pesticides enter into the aquatic ecosystem from agricultural runoff or leaching [1]. Some of these organic pollutants could be found rapidly in the aquatic ecosystems as well. Government agencies have invested their concern about maintaining the quality of aquatic resources by implementing physical and chemical measurements that provide quantitative data on the levels of aquatic pollution and degradation. This leads to the need for an effective and efficient method to assess the toxicity of different pollutants and microorganisms which are embedded in the ecosystem. Microalgae can be the efficient tool here, as it has the potential to be used as biological indicators of pollution in eco-toxicity due to its predominant role in the first level of the food chain [2].

Microalgae are considered as a useful indicator of environmental quality due to its characteristics that consumes toxic contaminants and heavy metals in the wastewater [3]. Besides, microalgae also act as representative members of phytoplankton in the water flow that could be utilized for the indication of trophic level and water quality status [4]. The increase in nitrogen and phosphorus in the water systems causes biological productivity to be high which leads to the generation of phytoplankton. It was observed that high nutrients concentration (i.e., nitrogen and phosphorus) causes larger phytoplankton biomass which indicates the poor quality of the waste system. Moreover, microalgae species are highly sensitive when exposed to groups of harmful pollutants as compared to other microorganisms [5]. Table 2.1 lists down the dominant algal indicator species for a trophic status of various lake types [6]. The selection of algal indicators is usually done based on the mean phosphorus and chlorophyll concentration of the lake water. Thus, the selection of algal indicators is based on the water quality.

Table 2.1: Various algae species utilized as an indicator of environmental assessment

Lake types	Algal indicators	Mean phosphorus concentration (µg/L)	Mean chlorophyll concentration (µg/L)
Oligotrophic	^{a*} <i>Cyclotella comensis</i> ; ^{b*} <i>Staurodesmus</i> sp;	4 – 10	1 – 2.5
Mesotrophic	^{a*} <i>Tabellaria Flocculosa</i> ; ^{b*} <i>Sphaerocystis schroeteri</i> ; ^{c*} <i>Gomphosphaeria</i> sp.	10 – 35	2.5 – 8
Eutrophic	^{a*} <i>Aulacoseira</i> sp; ^{b*} <i>Eudorina</i> sp; ^{c*} <i>Anabaena</i> sp.	35 – 100	8 – 25
Hypertrophic	^{a*} <i>Stephanodiscus hanteschii</i> ; ^{b*} <i>Scenedesmus</i> sp; ^{c*} <i>Aphanocapsa</i> sp.	>100	>25

Note: a*= Diatoms; b*= Green algae; c*= Cyanobacteria

A single microalgae species has been used for the toxicity evaluation and its testing procedure are standardized by the European Community Commission and the Environmental Protection Agency for U.S [7]. The respective unialgal testing can perform in a laboratory under batch cultures, where the toxicant will be added to the assay. There will be no replacement of culture medium or pollutants into the assays [8]. Researchers have also used more batteries of bio-assays while independently tested on different microalgae species along with the same toxicant [9]. This is because, not all species of algae can be used in assessing the effects of environmental pollutants due to its different sensitivities [10]. Correlatively, it has been proven that batteries have their ability to compare the sensitivity of different microalgae species to a specific pollutant [9].

Research was conducted on 5 different toxicities using 5 types of green algae species (i.e., *Selenastrum capricornutum*, *Scenedesmus quadricauda*, *Scenedesmus obliquus*, *Chlorella vulgaris* and *Chlorella pyrenoidosa*), and 3 types of cyanobacteria (i.e., *Anabaena flos-aquae*, *Microcystis flos-aquae* and *Mirocystis aeruginosa*). Since the pollutant properties are not

constant, the sensitivity test will be measured in decreasing order of the aquatic ecological risk: beta-cyfluthrin > fentin hydroxide > cyhexatin > azocyclotin > fenbutatin oxide [11]. Besides, Schipper et al. (2010) discovered the ability of bioassays in providing valuable information and being the source of biological data for risk assessment on various pollutants [12]. The most frequently used microalgae in bioassays for a wide range of contaminants are *genera Chlamydomonas, Chlorella, Scenedesmus, and Selenastrum* [13]. In short, the usage of microalgae to assess the toxicity of different pollutants and microorganisms which are embedded in the ecosystem is definitely a cost and energy efficient. This is because, while accessing the toxicity microalgae could also treat the waterbed by up taking mainly the nitrite and phosphorus.

2.1.2 Microalgae for wastewater treatment

Water is a natural resource that is widely available on the globe, and recently clean water supply has been an issue in many industrial based developing countries. Water pollution is mostly due to the industries, municipal, agricultural wastewater with a huge number of organic and inorganic contamination. The pollutants, presences in most wastewater sources are heavy metals [14], xenobiotics [15], high concentration of nitrates, phosphates [16] and carbon compounds [17]. The main objectives of wastewater treatment will be focused on eliminating contaminants that are embedded in the water. Department of Environment, in every country has its standard wastewater quality regulation that must be achieved before releasing it out. The idea of aquatic microalgae culture could fulfil the criteria which includes reduction of biological oxygen demand (BOD), total suspended solids (TSS), pathogens organisms, and lastly concentration of nitrogen and phosphorus in the wastewater [18]. Table 2.2 describes specifically on the properties of microalgae that tackles the main objectives of a wastewater treatment.

Table 2.2: Impact of microalgae application on the biochemical oxygen demand (BOD), nutrient, heavy metal, and pathogen removal of wastewater treatment.

Criteria	Microalgae Application	Reference
BOD removal	Microalgae are utilizing for the treatment of brewery effluent and at the same time, its biomass production has been evaluated. It was reported that the maximum dry biomass weight reach is at 0.917g/L with 91.43% of BOD reduction.	[19]
Nutrient removal	Studies have involved the phytoremediation technology using microalgae which indicate an excellent nutrient removal efficiency with low implementation and maintenance cost compared to other conventional physicochemical methods such as ion-exchange, membrane separation, reverse osmosis, etc.	[20]
Heavy metal removal	Microalgae can possess a large number of heavy metals as the maximum capacity depends on its biomass growth condition. It is reported that call grown in photoautotrophic conditions have the ideal biosorption characteristics.	[21]
Pathogen removal	The photosynthesis process of microalgae enhances the dissolved oxygen produced which leads to an increase in the pH value. This mechanism contributes to the reduction of bacteria (pathogen) present in the effluent.	[22]

In the conventional method of wastewater treatment, there will be a combination of both chemical reactions (i.e., chlorination, chemical precipitation, ion exchange, and biological organisms (i.e., anaerobic and aerobic mechanism). However, microalgae can be used as an alternative to biological treatment for wastewater [23]. The application of microalgae in wastewater treatment is mainly due to its ability that could direct uptake or consume the contaminant for growth. It is known that microalgae require nitrogen, phosphorus for the cultivation process to occur, which can be obtained directly during wastewater treatment [24]. Thus, the main targeted pollutants will be nitrogen and phosphorus. A group of researchers investigated the efficiency of microalgae on the removal of pollutants from wastewater that consists of a mixture of the river, industrial, and domestic water discharge using two different microalgae species. It was reported that the COD, BOD, and TSS removal were similar however, in terms of nitrate and phosphorus removal *Chlorella vulgaris* species is more efficient than *Chlamydomonas* species for treating wastewater [25].

Furthermore, microalgae could also improvise the purification performance of bacterial systems by providing additional oxygen from photosynthesis [26]. This also reduces directly the total energy cost (e.g., gassing process) and indirectly on oxygen supply (e.g., stirring performance) [27]. Microalgae can perform chemoheterotrophic metabolism, where the inorganic carbon from carbon dioxide can be fixed into organic carbon in the presence of water [28]. This mechanism will be appropriate to be applied specifically on industrial wastewater containing the high organic load.

Recently there were multiple studies carried out focusing on algae-based wastewater treatment using conventional microalgae and cyanobacteria such as *Chlorella* sp. [23], *Scenedesmus* sp. [29] and *Nannochloropsis* sp. [30]. The common characteristic of the mentioned species is their potential to accumulate high levels of lipids and starch from wastewater and concludes that microalgae can be utilized as a biological treatment method of wastewater. Microalgae-based wastewater treatment represents a low operating cost alongside with environmentally friendly alternative compared to the conventional wastewater treatment process [31]. In summary, the usage of microalgae for wastewater treatment, would reduce the treating cost and also the microalgae biomass obtain from the treating process can be utilize as a use feedstock for the production of lipid and biofuels.

2.1.3 Microalgae as a renewable biomass feedstock

Microalgae are known as a sustainable source of renewable energy due to its characteristics that can grow vigorously and be cultivated in a wide range of mediums such as wastewater, wasteland, lakes, and pond [32]. It was discovered that the growth of algae is the fastest compared to edible crops (i.e., rice, wheat, corn potato, sugarcane, and barley) and have the capacity to produce a large amount of biomass per acre [33]. Environmental regulator simply means an environmental standard set by the government for power generation, transportation, and consumer goods industries [34]. These regulations indirectly subsidize the economy of

each country by preventing negative effects associated with any pollution. The transportation industry has been growing rapidly and this increases the burning of fossil fuel for transportation fuel which would significantly contribute its effect on the release of greenhouse gases [35]. This was supported by Josefina and Meza (2014) who reported that the transportation sector alone contributes to about 28% of greenhouse gas emission worldwide, thus it is important to identify an alternative feedstock for fuel production [36].

The implementation of microalgae-based biofuel production has been proposed as early as the 1950s and lately, it has been widely explored by researchers [37]. This is because, algae consist of lipids, carbohydrates, protein, and phytonutrients. It is also known that these compounds can be utilized as feedstock for multiple added-value products [38]. Lipids extracted from algae biomass can be refined into fatty acids, and further processed to produce biodiesel by transesterification process [39]. Transesterification reactions can also be performed with the use of a heterogeneous catalyst such as zinc and aluminium that can be obtained from wastewater effluent. Moreover, the gasification of microalgae biomass via anaerobic digestion or thermal cracking could produce biogas [40]. Gasification is a process that produces syngas by converting carbon-rich raw material via partial oxidation within a limited range of oxygen and air steam supply. In case the supply of air steam is costly, the microalgae biomass can also undergo direct combustion to generate power and syngas [41].

The carbohydrate fraction from algae biomass could be fed as feedstock for the production of bioethanol under anaerobic fermentation conditions [42]. Although bioethanol is known as the carbon-neutral fuel that is produced from plant waste materials, the challenge relies on its requirement for pre-treatment of algae (e.g. ultrasound-assisted, microwave-assisted and electricity-assisted) before fermentation process [43]. Microalgae biomass could also produce solid, liquid, and gaseous products via the pyrolysis process [44]. A study was carried out using *Chlorella* sp. in a microwave oven and it was recorded that the produced bio-oil was

approximately 28.6% w/w of the biomass [45]. Algae residual biomass could also initiate the production of biomethane through the anaerobic fermentation process [46]. It is proven that microalgae have the potential to produce a wide range of sustainable biofuels and promote the sequencing of CO₂ which complies with carbon capture and storage, BECCS strategy [47]. In brief, microalgae-based biomass produces biofuel in an eco-friendlier, sustainable manner which justifies the classification of microalgae as a renewable source of biomass feedstock for an environmental regulator.

2.2 Environmental impacts from the microalgae-based production processes

2.2.1 Atmospheric balance

Carbon dioxide becomes a poisonous gas when there is too much (>0.4%) of it in the atmosphere which leads to greenhouse gasses and constitutes a major challenge to global sustainability [47]. Lately, there is various technology being developed to enhance CO₂ mitigation such as the implementation of an environmentally friendly bio-reactor that achieves maximum productivity, energy, and operational costs efficiency [48]. Conjunctionally, microalgae cultivation process on a large scale has a wider impact on the atmosphere [49]. This is because microalgae can enhance the biological fixation of CO₂ presence in the atmosphere via the photosynthesis process. Many studies have been conducted to evaluate the quantified theory where microalgae as the source to absorb carbon from the atmosphere [50]. Recently, a group of researchers has conducted a comparison study between microalgae species and diatom and observed that *P. tricornutus* (diatom) adsorbs carbon at 1.5 mg.L/min which are lower compared to cyanobacteria *A. micropia nageli* which able to uptake 28 mg.L/min [51].

Furthermore, response surface methodology (RSM) was also developed to identify the ability of microalgae to uptake carbon in optimize growth conditions [52]. The results indicated that it is important to cultivate the microalgae in an optimized condition as it also enhances the uptake of carbon. This is because the high photosynthetic efficiencies of microalgae in utilizing

solar energy allows the fixation of CO₂. Figure 2.2 demonstrates an example of carbon capture and storage mechanism using microalgae.

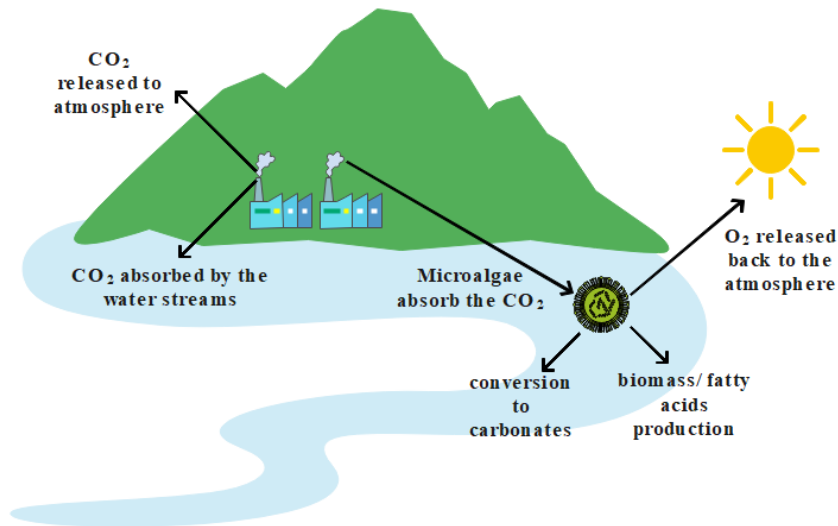


Figure 2.2: Carbon capture storage mechanism.

In addition to these, investigation on the emission of nitrous oxide, N₂O gas from microalgae cultivation has also been conducted. This is to ensure that the natural circulation of nitrogen among the atmosphere does not merge along with the oxygen gas released by microalgae through photosynthesis [27]. It is believed that N₂O gas is approximately 264 times more harmful than CO₂ to the greenhouse effect [53]. Traditionally, there are two main possibilities that the production of nitrous oxide could take place during microalgae cultivation under non-axenic condition either: (1) the autotrophic bacteria which uses either hydrogen or sulphur compounds as the electron donor that releases N₂O gas, (2) the heterotrophic denitrifiers which use organic compounds [54]. Bacterial denitrification occurs through a series of reduction reactions that trigger the generation of N₂O gas.

A study was carried out in an open pond cultivation system of *N. salina* sp. under oxic condition (free oxygen molecular conditions), and the results indicate the N₂O levels were too low

(negligible); whereby nitrous oxide gas was increasing under anoxic conditions (dissolved oxygen conditions) [55]. Another study was conducted in a raceway pond and discovered that the N_2O gas was depleted during that cultivating process of *Staurosira* species, where the water body acts as a sink of N_2O instead of the source [56]. Thus, it was concluded that the atmospheric mass transfer of nitrous oxide represents an insignificant fraction compared to the overall CO_2 adsorption mechanism by microalgae. It is also discovered that dried microalgae biomass produced from anaerobically digested dairy manure treatment be utilized as fertilizers for nutrient recycling replacing commercial chemical fertilizers [57]. In short, microalgae cultivation process on a large scale can be used for carbon storage and also uptakes the nitrogen source surrounding the culture which also reduce the impurities on water streams.

2.2.2 Land and water resources

Microalgae is a third-generation biofuel feedstock that does not interrupt the food chain or feed crops. Microalgae do not require a large space of land for its cultivation but still can equally produce the amount of biofuel as other crops due to its high biomass productivity per unit area [58]. The essential criteria of site selection for large operation microalgae cultivation are to have sufficient access to water supply with appropriate salinity, suitable landscape, climate atmospheric conditions, and easy source to nutrients and carbon dioxide [59]. Desert locations are not suitable areas due to their high ambient temperature which leads to a lack of fresh water supply and nutrient availability.

An advantage to microalgae species is that they can still able to grow on marginal land, pastures, and forested area [60]. The benefit of land space for the cultivation process is its measures the greenhouse gas emission from building on large scale raceway ponds that lead to changes in gas fluxes [61]. Reuse of land takes place, where food farmlands are utilizing for fuel crops after the harvesting season. By doing so, there will be a lack of nutrient exposure to the food crops which causes defects in the quality of the human food chain [62]. Hence, the

proposal of offshore-based cultivation is highly recommended to avoid the utilization of food farmland. In the beginning, the cultivation of microalgae will be on open pond system which demands land space, however with the help of technology regards to the development of photo-bioreactors helps to reduce the land space [63]. Yet, there are many concerns that the amount of biofuels production is dependent on the land space area allocated for cultivation. With the implementation of photo-bioreactors, microalgae could generate 200×10^9 L/year of oil in 2011 [64]. Recently, the US transport fuel supply has been estimated to be replaced by 50% with a land requirement of 1540 M ha of corn, 594 M ha of soybeans, and 42 M ha of microalgae [65].

On top of these, microalgae could also grow on a floating wastewater semipermeable which is believed to have an advantage of no land requirement. The preliminary study was conducted at the National Aeronautics and Space Administration, NASA Ames Research Centre under project OMEGA to enhance the growth of microalgae [66]. An enclosed semipermeable membranes filled with industrial/domestic wastewater which consist of sufficient nutrients can also support the growth of freshwater algae as the waves of the ocean will provide the mixing operation naturally [67]. In brief, the proposed microalgae application for biofuel production not only maintains balanced atmospheric surroundings but also contributes to the environment by reducing the usage of agricultural land.

2.2.3 Aquatic environmental sustainability

Water consumption and quality play an essential role in an environmental sustainability evaluation. As for microalgae, a wide range of wastewater source (i.e. domestic or industrial) can be utilized for the cultivation process and researchers have proven that in both the selection of wastewater differs according to the alga strain and regardless the type of medium, algae can grow. The application of domestic and industrial wastewater is believed to provide economic and environmental benefits, especially for large scale microalgae cultivation. This because,

microalgae can consume the nutrients present in the wastewater during photosynthesis and simultaneously acts as a wastewater cleaning agent which reduces wastewater treatment cost [68]. However, the rate of nutrient consumption relies on the algae strain and environmental conditions such as light intensity, the surrounding temperature, and surface volume ratio expose to algae. Nutrients such as carbon, nitrogen, and phosphorus are the several specific key nutrients that wastewater needs to be provided for the microalgae cultivation system [69].

In most scenarios, microalgae obtained its CO_2 via carbonic anhydrase by photosynthesis however, the limiting factor for cultivation to occur will be its inefficient mass transfer of CO_2 from the atmosphere and low solubility in water. Utilization of flue gas can be considered as an alternative source, but it has a high potential to cause dissolution to other nutrients present in the aquatic environments. Researchers have reported that microalgae have their capability to uptake carbon supply from the bacterial degradation of organic matter present in the wastewater [70]. Nitrate, nitrite and ammonium ions and many dissolved organic nitrogenous compounds (urea, free amino-acids and peptides) are the main sources of nitrogen for the algae during cultivation, which used mainly for the synthesis of amino-acids and proteins [43]. Usually, in aquatic environment nitrogen can be attained from the inorganic species found in the wastewater stream such as nitrate, NO_3^- or nitrite, NO_2^- [16]. This would provide a proper balance of nitrogen, as there are possibilities of excess nitrogen amount embed in the wastewater stream which leads to the development of toxics. Therefore, the uptake of nitrogen for algae biomass production may reduce the negative impacts on the aquatic ecosystem.

Phosphorus is also required for the growth of microalgae as a source of energy. The presence of phosphorus is very limited in most freshwater streams and nitrogen will be the limiting nutrient in marine environments [71]. Excess concentration of phosphorus could also lead to eutrophication. However, it has been discovered that phosphorus obtained from waste sources usually exists in an inorganic form [72]. It is discovered that microalgae can be used for the

phosphorus recovery with an environmental advantage compared to other phosphorus recovery methods such as leaching and thermal treatment [73]. Besides, there have been several studies conducted on identifying the average water footprint of various transport fuel as it a tool of environmental sustainability measurement [74]. It was reported that a closed production of biofuel using photo-bioreactor has a lower water footprint than the other biofuel feedstock from first-generation biomass such as soy, palm, and sugarcane [75]. In summary, the characteristics of microalgae that specifically uptakes carbon, nitrogen, phosphorus able to generate well concentrated biomass and simultaneously act as a wastewater cleaning agent.

2.3. Ecotechnologies based on microalgae

2.3.1 Co-culturing of microalgae with microbes

In natural habitats, the presence of microalgae exists in a wide range of dynamic environments that are beneficial for its survival. However, environmental aspects and nutrient feeds are essential when it comes to the industrial production of high value-added products using microalgae. Co-culturing plays an important role in providing an efficient amount of nutrients to microalgae without harming the wastewater ponds [76]. Co-culturing simply means that two different types of cells will be cultured in the same medium in which both cells will be in contact between them. The application of co-culturing has begun between two types of microalgae strains (i.e., *Scenedesmus bicellularis* and *Chlorella* sp.) which promotes an outstanding increase in the growth rate of algal strains [77]. In recent years, scientists have developed, co-culturing of yeast and microalgae for the production of bioenergy and biofuel. Moreover, it is also discovered that the co-culturing of anaerobic bacteria (*Clostridia* sp.) with aerobic (*Bacillus* sp.) could enhance the quality of bio-hydrogen [78].

Scientists have also invented a technology to bio-flocculate microalgae (*Chlorella vulgaris*) species by co-culturing it with a filamentous fungus (*Mucor circinelloides*) which contributes to the reduction of algae biofuel cost [79]. On top of these, the co-culturing of microalgae and

fungi could also enhance the element of polysaccharide, EPS in biomass which are considered to be the emerging source of bioactive value-added compounds [80]. Bio-flocculation is performed by biological agents that can separate the biomass from the culture medium which is considered to be a cost-effective technique especially for industrial-scale operations. As for interactions between algae species, bio flocculation usually non-flocculating microalgae are inserted with other auto flocculants which are considered as an ideal alternative method for the microalgae harvesting process [81].

This has been proven in a research that was cultured in a marine medium and it was reported that the culture with *Tetraselmis suecica* algae species managed to harvest only 46% of biomass from *Neochloris oleoabundans* microalgae [81], while *Chlorella vulgaris* obtained a maximum efficiency of 68% [82]. In addition to these, bio-flocculation contributes to the environment by avoiding the use of inorganic and chemically synthetic flocculants in microalgae harvesting due to their biodegradation and non-toxicity properties [83]. It is also an economical and sustainable method as it does not demand various cultivation conditions which allow reusing of wastewater and also reduces unwanted contamination. In short, the entire co-culturing and bio flocculation studies emphasize more on the contribution of symbiotic relationship upon the participating of co-culturing via microorganisms-microalgae species which enhances the bio-production of biomolecules.

2.3.2 Integrating microalgae with other biotechnological applications

Microalgae can adapt in a wide range of environment; hence its growth can be performed in an integrated system of several biotechnological applications. The concept of process integration has been exploited to recover multiple compounds from microalgae-based biomass. Integration of processes can be done for either energy, nutrients, or carbon dioxide supplies during the microalgae cultivation [84]. A study has been carried out on microalgae on flue gas released by manufacturing facility for CO₂ treatment [51]. Figure 2.3 demonstrates the process flow that

of microalgae pond which can be a cost-saving implementation for CO₂ separation out of the gas stream. The respective process is considered as an environmentally friendly integration with economically attractive as well. Energy integration could occur in closed photo-bioreactors especially for light supply and it is discovered that the greenhouse gases emissions reduction is around 2 – 7% [52].

An integrated algal bio refinery process combined algal processing was studied and it was proven that the combination of algae processing reduces the bio-fuel cost by 9% with an improved energy yield of 126 GGE/ton biomass [85]. The microalgae process integration for nutrients is mostly done using the wastewater streams. This is because microalgae have the capability to uptake the nutrients present in the wastewater flow which also simultaneously acts as a treating agent [86].

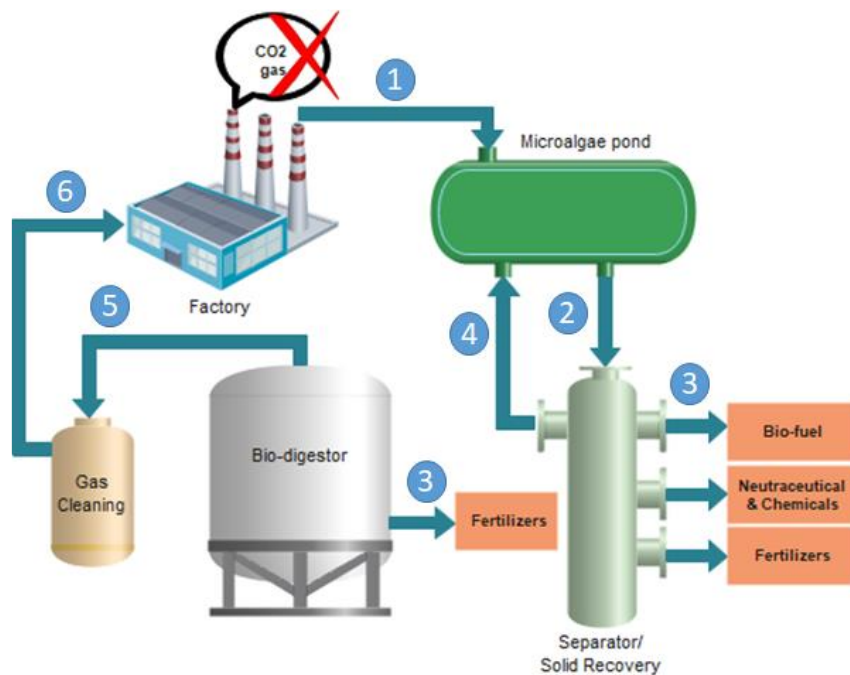


Figure 2.3: The microalgae pond can be merged with the wastewater tank collection.

Apart from these external integrations, microalgae production flow could also undergo internal interactions of the process which contributes a reduction in technical steps to obtain a value-added product. The interactions of product recovery and cultivation can save the cell-harvesting

process and lead to a continuous product stream [87]. There have been multiple discoveries on the reduction process such as auto flocculation, in situ transesterification, excretion, etc. [88]. Table 2.3 explains the reduction of technical steps in each of the processes. Nevertheless, the challenge in implementing these techniques for large scale is its overall process efficiencies in terms of biomass concentration and quality although it is cost savings in terms of energy supplies. In a study, it was reported that the in situ transesterification process promotes its biodiesel yield by stirring process although the moisture content of biomass is high [89]. This significantly leads to the reductions of the equilibrium of Fatty Acid Methyl Esters (FAME) conversion yield.

As for the auto flocculation process, even though it is proven that interrupting CO₂ supply to algae promotes its flocculation, the challenge is faced in the harvesting process [90]. This significantly leads to the reductions of the equilibrium of FAME conversion yield. As for the auto flocculation process, even though it is proven that interrupting CO₂ supply to algae promotes its flocculation, the challenge is faced in the harvesting process [91]. In short, the integrating of microalgae-based process with the wastewater treatment and production flow, could lower the operating cost and save the overall energy resources for the production plant.

Table 2.3: Comparison of technical stages between non- integrated and integrated microalgae to bio-fuel pathway processes.

Pathways for microalgae	Non-integrated	Integrated process		
Type of Process Flow	Conventional process flow [92]	Auto flocculation [90]	In situ transesterification [89]	Excretion [93]
Stages of process				
Cultivation	✓	✓	✓	✓
Flocculation	✓	×	✓	×
Centrifugation	✓	✓	✓	×

Disruption	✓	✓	✓	×
Recovery	✓	✓	×	✓
Further Processing	✓	✓	×	✓
Final Product (Further refinery processes are performed based on the required specific products)				

*Note: Cross symbol represents the skipped conventional steps due to the integration of microalgae processes with auto-flocculation, in-situ transesterification, excretion processes.

2.3.3 Environmental life cycle analysis

Life cycle assessment, LCA is an important methodology that will be carried out to analyse the environmental impacts which associate with all the stages of a process. This is because LCA reports provide an early baseline aspect to be considered especially in terms of ecological balance for scaling up. By doing so, it enables scientists to enhance the application of technology to innovative development. There are four standard stages of an LCA structure which start from goal definition and scoping, inventory analysis, impact evaluation, and final interpretation [91]. Thermal algae dewatering demands a high amount of fossil fuel-derived energy which was quantified as an obstacle for the development of algae technology [94]. In conjunction with this, there have been many studies that have been conducted to discover process improvements. This leads to various discoveries lately, such as the development of two-stage microalgae dewatering, implementation of tray drier, solar power drying, and many more [95].

It is known that microalgae have the characteristic to adsorb CO₂ that presence in the atmosphere during the cultivation process, and relatively a comparative life cycle assessment has been carried out on the production of microalgae biomass between synthetic commercial CO₂ and upgrading waste gas (biogas to bio-methane) [96]. According to the results obtained, it was reported that the CO₂ from upgrading biogas is more feasible and sustainable than the synthesis CO₂ supply as it leads to an improved overall environmental profile of the process

without affecting its productivity. Since the development of photo-bioreactors has been proven to be a progressed technology from open raceway cultivation, thus it is essential to also conduct a life cycle assessment before proceeding further for industrial scale.

A LCA study was performed on each stage of the biomass production from the manufacturing aspects by involving in the infrastructure materials, construction of photo-bioreactors and the cultivation, flocculation, filtration, biomass drying and cleaning processes [97]. The overall results indicate that the application of photo-bioreactors for the cultivation of microalgae has the potential to reduce environmental impacts however there will still be limitations in terms of cost, continuous supply of nutrients, and CO₂. Moreover, LCA studies have also been undertaken to investigate the energetic balance of the entire process chain from the production of biomass production to the combustion of biodiesel. In this particular study, two different culture conditions (nitrogen & fertilizers) and extraction options (dry & wet extraction) were tested [98]. The outcome of the analyses confirms that microalgae have their capability to be an energy source by controlling the nitrogen stress during the cultivation process and optimization of wet extraction. In summary, LCA is an environmental management tool that provides information such as cost, performance, potential impacts to the environment to the decision-makers of any selective microalgae-based process.

2.4 Future perspectives

Microalgae are a third-generation feedstock that can potentially replace the application of fossil fuel with an added advantage of providing environmental benefits alongside such as clean water supply from wastewater treatment, balance atmospheric air, and aquatic sustainability. However, certain gaps need to be taken into consideration in terms of environmental aspects near the future for further progress into large/industrial-scale production. A summary of the main potential impacts is discussed with further research requirements. Firstly, the cultivation

of microalgae must be exploited on a large scale in photo-bioreactors where its atmospheric emission will be recorded.

A case study was conducted using natural seawater as a culture medium on 75 liters' glass container photo-bioreactor [99]. Seawater was chosen specifically, as it provides a promising way to produce massive biomass without a demand for large land space and can avoid the consumption of fresh water for cultivation. However, it is also equally important to monitor the large emission of gasses that will occur during the cultivation process. This is because theoretically on large scale the gaseous emission tends to bind among each other in the atmospheric which may not be noticeable in lab-scale tryouts. Therefore, both the uptake CO_2 by algae and the emission of gasses during the algae growth phase must be studied. Besides, it is also important to include in actual industries to enhance the environmental advantages that microalgae-based processes provide. Some of the listed advantages are, extraction of nutrients from waste streams in terms of flue gases, sewage (domestic and manufacturer), and CO_2 gas effluent from industrial processing. Only then, the actual economic feasibility, environmental impacts, and limitations can be evaluated.

Moreover, there has also been plenty of technical investigation on microalgae to be utilized for wastewater treatment. The discussion mostly focusses on its comparisons on whether microalgae-based treatments are environmentally friendly and more sustainable than existing methods. This makes it easy to justify that microalga are the best alternative as most conventional existing wastewater treatment requires high levels of energy and chemical which also results in an expensive process. However, there is also a requirement for a depth investigation in case of excessive nutrients and minerals in the water bodies that leads to eutrophication. Whereas, the development of a microfiltration-based membrane photo-bioreactor, MPBR and forward osmosis based osmotic membrane photo-bioreactor, OMPBR can be utilized for continuous tertiary wastewater treatment. The integration of these

technologies benefits the treating process with several challenges such as bio-fouling, salt accumulation, concentration polarization to be tackled [100].

Moving on, much focus is required to evaluate the accumulation of toxins in biomass when wastewater medium is used especially on a large scale. This could limit its use both in pharmaceutical and food as a fertilizer and as a feedstock biofuel due to its high concentration of heavy metals. The presence of toxins can be negligible during the lab-scale case study; thus, it is essential to venture into pilot-scale of microalgae cultivation on terrestrial biodiversity.

Moreover, an extension to the structure of LCA could also be improved for the environmental effects of microalgae measurement with more boundaries structure. This is because, boundaries such as policymakers, industries, and public stakeholders, the national department of environment should also be taken into consideration for future the development of genetic microalgae-based technology. The introduction of Life Cycle Inventory evaluation quantifies all of the process inputs (energy, raw materials) and output emissions such as air, water, co-products and solid waste. Recently a LCI evaluation, was conducted according to the actual pilot-scale process data and scaled up to industrial microalgae plant for the production of biofuel [101]. It was reported that the inventory values obtain contribute to a complete LCA modeling which can be used to identify a sustainable large-scale algae-based plant. In short, LCA studies proves that the application of microalgae provides a positive contribution to the environment.

2.5 Conclusion

In summary, environmental effects are one of the side effects of fossil fuel-based processes, and the commercialization of new technologies based on microalgae is proven to be an alternative source. The recovery of lipids and other beneficial bio-compounds which can be further refined to biofuel, biodiesel, or health pharmaceutical supplements. Apart from being a feedstock to produce biofuel and pharmaceutical products, microalgae can also act as environmental protection. The application of microalgae diverse must be commercialize in large scale especially as biological assessment, alternative biological treatment for wastewater treatment, and its biomass can be utilized for an environmental regulator as well. Moreover, microalgae also contribute back to the environment by taking into consideration atmospheric balance, aquatic environmental sustainability, land, and water resources. Research enhancement on the implementation of eco-technology in process integration using microalgae plays an important in producing a sustainable product which could tackle the energy demand without harming global environment. This goal can be achieved, with continuous research and development on commercializing microalgae upstream-downstream based technologies.

CHAPTER 3

INTEGRATED MICROALGAE CULTURE SYSTEM WITH INDUSTRIAL DAIRY WASTE REMEDIATION AND BIOMASS PRODUCTIVITY ENHANCEMENT

Overview

Waste produced from manufacturing plants poses a severe risk such as environmental degradation, water pollution, and soil contamination, due to its high elemental composition. Specifically, powder waste disposal requires additional energy sources in terms of scientific treatment, structured collection, and disposal packaging according to the safety regulation. Thus, this research discusses the viewpoint of integrating food processing waste as an organic carbon source with BG-11 medium for *Chlorella vulgaris* (FSP-E) growth. The waste powders investigated in this study were obtained from milk, and biscuit manufacturing facilities. The culture medium was modified by combining both BG-11 and food processing waste powders to identify the optimal algal growth and biochemical content. Compared to the microalgae grown in BG-11 alone (IBG), the combination of biscuit waste produced higher biomass concentration (44%), with increased lipid (11%), protein (20%), and carbohydrate (57%) contents. *Chlorella vulgaris* was able to absorb nutrients from the culture medium with combination of food processing waste and IBG thus enhancing its growth. The results obtained also indicate that an integrated culture system using food processing waste and synthetic sources can generate energy out of waste by improving the bio-composition of the microalgae biomass.

Keywords: Medium contamination; Food processing; Waste powder; Organic medium; Waste remediation

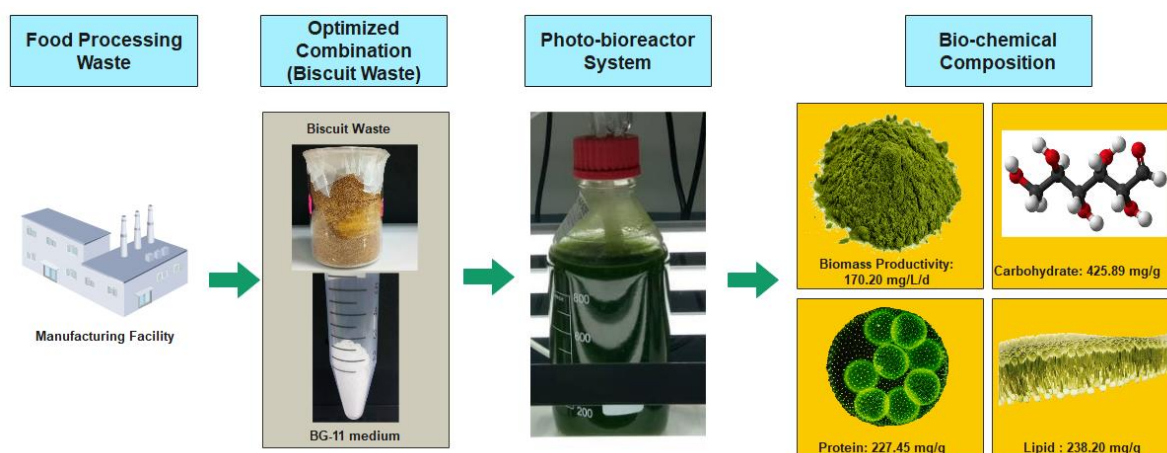


Figure 3.1: Graphical Abstract Integrated Microalgae Culture System with Industrial Dairy Waste Remediation and Biomass Productivity Enhancement.

3.1 Introduction

The global demand for dairy food products is increasing, and as a result, dairy industries produce variety of products while generating both solid and liquid waste [102]. A standard dairy factory produces approximately 4–11 million tons of waste annually, with a high concentration of organic matter (fat, protein, and carbohydrates) and nutrients (primarily nitrate and phosphorous) derived from milk based items [103]. All liquid waste sources are usually combined to create a drainage reservoir, where wastewater will be treated before being discharged into the surrounding water system. Kolev et al.(2017) discovered that industrial dairy wastewater consists of three major sources such as processing cooling waters, pipeline and tankers cleaning waters and sanitary waste (by-products of process) [104]. Although this type of generated effluent is highly unstable in nature, there are various advanced technologies for industrial dairy wastewater treatment such as physio-chemical process (electrocoagulation, adsorption, membrane treatment), aerobic process (sequencing batch reactor), anaerobic process (up flow anaerobic sludge blanket, hybrid anaerobic digesters, anaerobic sequencing batch reactors) [105].

Apart from wastewater, dairy food processes also generate solid waste such as leftover dust powders after packaging, waste powder recovery from dust collector following the spray-drying process and damage stocks [106]. Mostly, dust powders produced by dairy industry will be further processed to livestock feed or disposed by mixing it with the wastewater pond [102]. The disposal of dairy food processing waste is more challenging than managing dairy wastewater treatment. This is because powdered dairy waste tends to clog water streams and has the ability to clump together to form a hard rock texture when exposed to air. Moreover, dairy dust powders are a significant threat to the environment since milk-based dust suspensions with density of 75–1000 g/m³ in air will combust or self-ignite upon contact with hot surfaces [107]. Hence, most industrial corporations prefer disposing out their powder waste to livestock farmers. On the other hand, researchers have ventured in utilizing the milk-based dust powders for fermentation process which enhances acetone-butanol-ethanol production by *Clostridium acetobutylicum* and *Clostridium beijerinckii* [108].

Microalgae can contribute to various sectors such as biofuels, biopharmaceuticals, and food supplementation industry. High value-added products in microalgae extracts are used for health supplements, biofuels, biomass (fertilizers and animal feed), and also in cosmetics [109]. In depth, microalgae consist of high health promoting properties such as anticoagulant, antioxidants, antitumor, anticancer, and antihypertensive which widen the research scope in microalgae based processes [110]. Therefore, studies related to enhancing the cultivation of microalgae are important in order to achieve a promising biomass feedstock. For scaling up and commercialization of microalgae production large quantity of cost- saving culture medium is required without compromising the nutrients content. There have been many interventions in using solid waste (Palm Oil Mill Effluent, fertilizers) as organic culture medium for the production of microalga biomass [111]. This strategy can be used to repurpose industrial dairy food processing waste for the cultivation of *Chlorella vulgaris*, a microalgae species that

absorbs nitrate, phosphorus, carbon, and hydrogen from water [112]. Hence, this research investigates various sources of industrial dairy waste as a potential source of microalgae growth medium.

Integrated solid waste management, (ISWM) is an advance and productive approach that implies various technology to reduce, reuse, recycle, and manage waste which can benefits human and natural environment. ISWM strategic varies based on the solid waste properties and mixing conditions. Hence, the integration of industrial dairy food processing waste and BG-11 medium (IBG) was proposed to determine its efficiency in enhancing microalgae productivity than IBG culture alone. The integration of mediums has high probability of increasing the carbon source which enhances the cultivation process and its biochemical properties. This would result in a more efficient microalgae growth of bio-refineries in near future. Thus, the focus of this study was to examine the prospective of integrating BG-11 medium (IBG), with industrial dairy powder waste: milk waste (DM), and biscuit waste (DB) as nutrient medium to produce *Chlorella vulgaris* microalgae species. The possibility of integrating industrial dairy food processing waste for microalgae production was investigated by assessing the accumulation of algae biomass and biochemical content using four different types of organic mediums. The productivity of biomass was examined by identifying whether organic industrial waste produces the greatest biomass concentration and biochemical composition of all the growth medium combinations.

3.2 Methodology

3.2.1 Microalgae species and culture medium preparation

The *Chlorella vulgaris* species was collected from a reservoir field in southern Taiwan. The modified BG-11 medium contained of 1.5 g NaNO₃, 0.03 g K₂HPO₄, 0.075 g MgSO₄·7H₂O, 0.006 g anhydrous citric acid combined with four stock solutions. The first stock solution made of 2 g Na₂CO₃; The second stock solution made of 3.6 g CaCl₂·2H₂O; The third stock solution

made of 0.6 g ferric ammonium citrate and 0.1 g EDTA; The fourth stock solution made of 2.86 g H_3BO_3 , 1.81 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.222 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.07 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.04 g $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$. All these four-stock solutions are mixed with 1 L of deionized water. In an indoor photo-bioreactor (PBR), 200 ml of the IBG medium was used for the pre-culturing. The PBR consisted of a 250 ml glass vessel, and the top vessel is covered with a layer of rubber that holds connections for compressed air and CO_2 supply, culture removal stream, and oxygen releasing stream. The PBR was set up at room temperature with constant stirring source from the magnetic stirrer with hotplate (FAVORIT) at 200 rpm with 2.5 cm length magnetic stirrer bar for a 250 ml glass vessel. Then the framework is place in a rack that holds external horizontal LED light sourced on the back and front of the vessel. LED, 3000K warm white light was used (50/60 Hz) on the PBR wall. Air was purified using (0.45 μm filter) and blended with 1.5% of compressed CO_2 at the rate of 200 ml/min. The culture broth was continuously aerated (0.2 vvm, volume gas per volume medium per minute). After 7 days of pre-culture, the subsequent batch cultures, were performed using similar PBR operating parameters in 1L glass vessel for each combination with 4.5 cm length magnetic stirrer bar in agitation. The initial growth of algae cells occurs in a petri dish; thus, it is advisable to upscale the growth from 250ml then gradually into a 1 litre bottle. This allows the cells to adapt to a glass vessel growing surroundings. A total of 5 glass vessels were used for one complete set cultivation cycle and the experiment were repeated three times. A total of 10 days was required to complete each cultivation cycle. The average readings were recorded.

3.2.2 Preparation of industrial dairy waste as culture medium

The industrial dairy waste was provided by local dairy manufacturers located in Malaysia. In a 250 ml conical flask, 2 g of each type of dairy waste was combined with 200 ml deionized water. The flasks were fitted in a water bath and rotated for 30 min at 150 rpm at room temperature. Specifically, DM, and DB were evaluated in this study with IBG. The culture

medium was comprised of 200 ml dairy waste solution and the remaining 800 ml with IBG medium in a 1L photobioreactor set up. Throughout the study, all mixture combinations have a pH range of 6-7.5 which was measured using Mettler Toledo SevenCompact™ pH Meter S210, Brand (Merck).

3.2.3 Evaluation of microalgae cell growth

UV-Vis's spectrophotometer (UV-1800, Shimadzu) was used at wavelength of 680 nm to measure the microalgae biomass growth concentration. The dry cell weight (DCW) of the microalgae biomass was calculated by filtering 5 ml of aliquots of culture via a cellulose acetate membrane filter (25 mm diameter, 0.45µm pore size). All filters were dried for 8 hours at 90 °C. The microalgae DCW was determined by subtracting the weight of the empty filter from that of loaded filter. Then, by calibrating the absorbance, OD₆₈₀ the absorbance value obtained from the UV-Vis measurement which were translated to biomass concentration. A blank sample of each culture combination was used to reduce the transmission of medium colour in the absorbance readings. Next, the transparent supernatant was extracted from the microalgae culture stock for each mixture variation after centrifugation at 6000 rpm for 15 min. The remaining pellet was freeze dried for 8 hours and used for detailed bio-composition analysis and characterization studies.

Eq. (1) represent the biomass growth productivity, P_b (mg/L/d) and Eq. (2) represent the specific growth rate, μ (d⁻¹):

$$P_b = \frac{W_2 - W_1}{t_2 - t_1} \quad (1)$$

$$\mu = \frac{\ln \frac{W_2}{W_1}}{t_2 - t_1} \quad (2)$$

Where W_1 and W_2 are DCW (g/L) at time t_1 (start) and t_2 (end), respectively.

3.2.4 Evaluation of nitrate and phosphorus concentration of culture combinations

The spectrophotometric approach was used to determine the nitrate content [113]. Every 24 hours, the microalgae specimens were collected out of the PBR and are diluted with deionized water to measure the absorbance value using UV-Vis spectrophotometer at 220 nm optical density. The standard curve was constructed using various concentration of pure sodium nitrate (NaNO_3). As for the phosphorus content measurement, the atomic absorption spectroscopy (AAS, PerkinElmer A Analyst 400) was used [114]. At day 0, day 6 and day 20 of the cultivation cycle, 20 ml of each medium variety was removed from the PBR and heated with regular mixing to promote phosphorus removal from the mediums. The standard association between absorbance and phosphorus concentration was developed using dibasic ammonium phosphate, $(\text{NH}_4)_2\text{HPO}_4$ with known concentration. The AAS spectrophotometer was set to detect light at 213.6 nm, which is the phosphorus resonance axis. The difference in content over the original was used to measure the overall phosphorus concentration removal (%).

3.2.5 Microalgae-based lipid production

Bligh and dryer approach was used to remove the lipid content through microalgae biomass [115]. Chloroform, methanol, and deionized water were mixed with ratio of 1:2:0.8 (w/v) and 50 mg of harvested biomass was submerged in the solution with total volume of 7.6 ml. Then, an ultrasonic water bath (Elma, Germany) was used to sonicate the mixture at 35 kHz frequency for 5 min. Next, the mixture was then sonicated for another 5 min after applying 2 ml of chloroform and filtered water. After sonication, the mixture was stirred vigorously for 30 seconds before centrifuging at 3000 rpm for 5 min. The bottom layer was poured into a pre-weighted glass vial and was heated in an oven at 80 °C for 30 min. After heating, the vials were placed in a room temperature desiccator and allowed to cool for 5 hours before being weighed. The lipid content was measured as a percentage difference (wt %) of DCW as per Eq. (3).

$$\text{Lipid content (wt\%)} = \frac{\text{Final vial weight} - \text{Initial vial weight}}{50 \text{ mg}} \times 100\% \quad (3)$$

3.2.6 Protein extractions

The microalgae biomass was exposed to cell disruption using ultra sonication to retrieve its protein [116]. Firstly, 0.1g of dried microalgae biomass was combined with 10 ml of deionized water containing 0.5N KOH. The mixture was then vortexed for 60 seconds before being ultra-sonicated for 20 min at a frequency of 35 kHz and centrifuged for 5 min at 7000 rpm. The supernatant layer was used for protein quantification and its remaining pellet are drained. Bradford method was implemented to test the protein content in the extracted microalgae biomass [117]. In a dark setting, 2.5 ml Bradford reagent was mixed with 0.25 ml biomass sample (supernatant layer). The mixture then was analysed at 595 nm using UV-Vis's spectrophotometer. The standard interaction among absorbance (OD_{595}) and protein concentrations was established using bovine serum albumin reagent (BSA).

3.2.7 Carbohydrate extractions

A modified technique was used to remove carbohydrate compounds from microalgae biomass. [118]. The mixture of 50 mg powdered biomass and 0.5 ml 18M H_2SO_4 was kept in a biosafety chamber for 30 min. Then, 4.5 ml of deionized water was included to the mixture and autoclaved the solution at 120 °C for 30 min, followed by 10 min of centrifugation at 6000 rpm. The carbohydrate content of the transparent supernatant was determined using the phenol-sulphuric method [119]. The mixture of 1 ml 18M H_2SO_4 and 0.2 ml of 4% (w/v) phenol with 10 μ l of supernatant was measured using the UV-Vis spectrophotometer using 490 nm. The standard relationship between OD_{490} and carbohydrate content was identified using starch extracts with various concentration.

3.2.8 Elemental analysis - characteristics of industrial dairy waste powder

3.2.8.1 Functional group characterisation (ftir)

The active compound present in DM, and DB were identified using Fourier transform infrared (FTIR) spectroscopy where the types of functional groups and its concentrations were analysed and evaluated. Two different waste samples were tested from both milk, and biscuit processing facility as DM1, DM2, DB1, and DB2 respectively. The industrial dairy waste powder was scanned using an FTIR model (Perkin Elmer Frontier, USA) with a default range of 400 cm^{-1} to 4000 cm^{-1} that allows the discovery of chemical bonds (functional groups).

3.2.8.2 Average elemental weight percentage of industrial dairy waste (edx).

The elemental composition of industrial dairy food processing waste (DM1, DM2, DB1, and DB2) were determined using energy dispersive X-Ray spectroscopy, EDX (FEI Quanta 400F FESEM). To detect a more precise weight percentage, all the samples were scanned at four different locations with magnification times of $200\times$, $1200\times$, $2500\times$, $5000\times$, and $6000\times$. The average weight percentage of each scanning are reported. The results were used to evaluate the comparison of microalgae biomass as a potential energy source. The study was carried out using an Oxford Instrument INCA 400, which offers material characterization that collects precise data at the micro-nano scales. EDX processing was used to obtain results that consists of spectra with peaks that corresponded to the actual substance properties.

3.2.8.3 Reversed-phase high performance liquid chromatography.

Chromatograms were recorded using a liquid chromatograph (Shimadzu, Kyoto), a UV–Vis's spectrophotometer (SPD 6A), and a C-R6A Chromatopac processor. The injection valve was a Rheodyne 7725i with a 100 or 20-ml loop. A reversed-phase Nova-Pack C ($150 \times 3.9\text{ mm} \times 4\text{ }\mu\text{m}$) from Waters 18 (Milford, MA, USA) type of HPLC column was used in this study. The industrial dairy food processing waste (DM1, DM2, DB1, and DB2) were diluted with water at 1:10 ratio and injected with 1 mg/l of B_{12} stock solution for solid phase extraction [120]. The

specimen was then added to the column in a 200-ml aliquot. Using a reference sample provided by Fort Dodge, this technique was verified in terms of repeatability, linearity, and accuracy.

3.2.9 Statistical analysis

IBM SPSS Statistics version 26, one way ANOVA with two-tailed- test was used in data analyses of the total protein, lipid, and carbohydrate, biomass growth concentration, nitrate and phosphorus content. In this study, descriptive statistical analysis is provided as mean $\pm$ standard deviation using Sigma Plot V.10. Values reported in this study are the average of three replicates. The statistical analysis of the data was conducted to assess the sum of differences with significance $p \leq 0.05$.

3.3 Results and discussion

3.3.1 Fourier transform infrared (FTIR) spectroscopy

FTIR can classify chemical bonds in a molecule by generating an infrared adsorption spectrum. The peaks observed in the spectrum Figure 3.2, represents the existence of specific functional groups of each industrial dairy waste powder. Not all peaks are considered, as there will be some external disturbance such as frequency of the vibration and interference in light absorption through the sample which leads to the tiny peaks. The binding sites of the powders reported are based on the sample peaks detected within the absorption range [121].

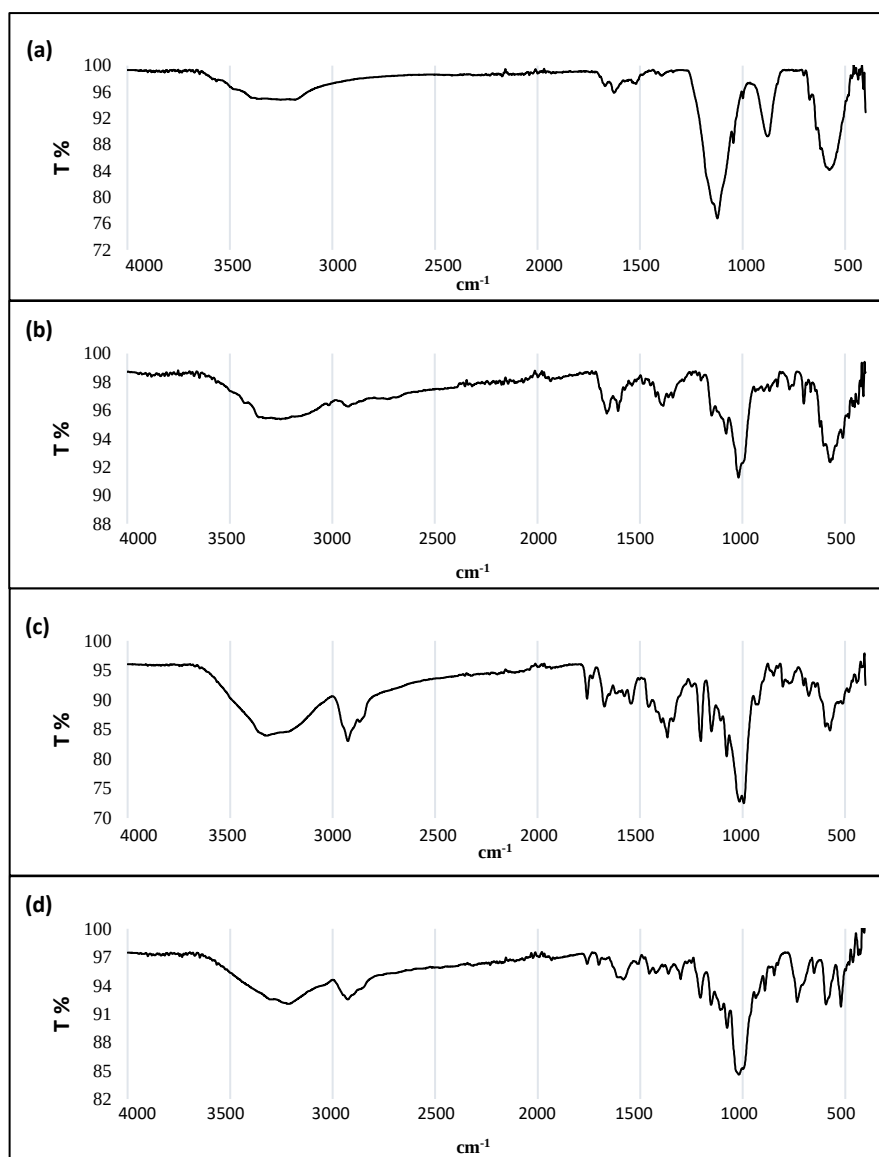


Figure 3.2: FTIR Spectrum of the industrial dairy waste (a) DM1 (b) DM2 (c) DB1 (d) DB2.

All the waste powders tested for this study observed C-O and C=O stretch vibrations (carbonyl group), suggesting the presence of carbon and oxygen in the culture. However, nitrate and phosphate are the most essential nutrients needed in order to enhance the growth of microalgae [122]. From Figure 3.2, it was observed that DB1 and DB2 both had double bond nitro groups (N=O) bend as the spectrum detects peaks between at $1400 - 1300\text{cm}^{-1}$. This gives an early indication that these two wastes will be appropriate to be replaced with IBG medium as the double bond nitro groups shares more nitrate electrons among them which act as nutrients as

compared to other powders waste for the growth of microalgae. On the other hand, the spectrum detected a peak at 3252.39 cm^{-1} in DM2 where it can be amines – primary and secondary. As nitrate breaks loose from amines, hydrogen atom will be released. These hydrogen atoms have a high risk of forming bonds with oxygen, that further dilutes the culture medium [123]. Lack of nitro bonds peaks also predicts that DM2 may not be the best option to enhance microalgae growth. As for DM1, it is observed that there is a mixture of both double layer nitro groups (N=O) at 1386.31 cm^{-1} and single layer amines bonds (N-H) at 1661.02 cm^{-1} [124]. Furthermore, detailed elemental analysis was carried to identify the weight percentage of each element present in the industrial dairy waste powders individually which could be able to predict the best option for replacing IBG medium for microalgae growth medium.

3.3.2 Energy dispersive x-ray spectroscopy (EDX)

The industrial dairy food processing waste was used in this study are well-recognised as supply source of protein, carbohydrate, dietary fibre and minerals for human/animal diet [125]. In general, dairy-based minerals are a combination of chemically complex compounds with wide range of stability and resistant to physical and chemical assault. The mineral consistency of each waste powder varies depending on variety of factors. Physical and chemical factors such as temperature, water content, pH, preservative additives, flavours, inhibitors influence the durability and properties of dairy waste materials [126]. Therefore, elemental analysis was recommended to analyse the specific element (nutrient) present in each of the industrial powder waste sample which plays an important factor for microalgae biomass productivity.

Table 3.1 lists the weight percentages of carbon, oxygen, and nitrate are substantially higher than the weight percentage of other elements. The EDX spectrum peaks are shown in Appendix Figure 1. Nitrate is a fundamental component of amino acids, which is the molecular building blocks of protein [127]. Nitrate, phosphorus, and carbon forms the backbone of microalgae ($\text{CH}_{1.7}, \text{O}_{0.4}, \text{N}_{0.15}, \text{P}_{0.0094}$) [128]. Nitrate and phosphorus limitation in microalgae culture, can

reduce the growth and biomass productivity. Based on the EDX analysis conducted, DB1 carries the highest nitrate content (25.56 wt %) while DM2 detects the highest phosphorus content (0.27 wt %). Although the elemental distributions in the powder dairy waste vary due to the sources of their origins, they still consist of both nitrate and phosphorus, which is needed for microalgae cultivation. The presence of other micronutrients are only required in small trace amounts as they influence many enzymatic activities in algal cell [129]. All four types of dairy waste contain nitrate and phosphorus with less toxic heavy materials that can replace the IBG medium's nutrient source. Thus, further study on the contribution of each dairy waste on the biomass productivity and production of carbohydrates, lipid and protein was carried out.

3.3.3 Cobalamin (vitamin - B₁₂) detection

Dairy products usually have high bioavailability of vitamin B₁₂ (cobalamin) as it is more easily absorbed *via* gastrointestinal tract than any other synthetic vitamins [130]. Thus, presence of vitamin B₁₂ from industrial dairy waste powders used for this study was also tested. Vitamin B₁₂ refers to a group of compounds known as cobalamins, which all contain a cobalt complex with a corrin system [131]. Table 3.1, represent the concentration of vitamin B₁₂ that was found in all the industrial dairy food processing waste used in combination with BG-11 combinations.

Table 3.1: Average weight percentage of elements and concentration of vitamin B₁₂ (cobalamin) in industrial dairy food processing waste (a) DM1 (b) DM2 (c) DB1 (d) DB2

Element	Average Weight, %			
	DM1	DM2	DB1	DB2
C	56.20 ± 4.88	28.60 ± 1.18	52.35 ± 4.15	52.76 ± 4.45
O	25.65 ± 5.97	56.90 ± 6.92	32.08 ± 3.13	29.71 ± 5.41
N	14.46 ± 0.39	2.53 ± 1.18	25.56 ± 0.11	13.66 ± 2.84
P	0.15 ± 0.01	0.27 ± 0.06	0.07 ± 0.01	0.01 ± 0.01
Zn	1.31 ± 0.67	-	-	-
S	1.04 ± 0.10	11.41 ± 5.81	0.22 ± 0.10	1.28 ± 0.66
Al	0.42 ± 0.13	-	-	1.50 ± 0.39
Si	-	-	-	1.08 ± 0.02
Cl	0.07 ± 0.21	0.29 ± 0.02	0.25 ± 0.05	-
Vitamin	Concentration of vitamin B ₁₂ , µg/100g			
B ₁₂	46.9	47.5	46.0	41.8

*Note: DM1 & DM2: Dairy Milk Waste (sample 1 & 2); DB1 & DB2: Dairy Milk Waste (sample 1 & 2)

Considering the results shown in Table 3.1, DM2 consists of the highest concentration of vitamin B₁₂ at 47.5µg/100g followed by DM1, DB1, and DB2 at 46.90 µg/100 g, 46.00 µg/100 g, and 41.80µg/100g. According to Dalto et al., (2018), vitamin B₁₂ concentrations fluctuate depending on the temperature at which dairy goods are stored, and external contamination exposure that could range from 25 to 250 µg/100g [130]. However, the industrial dairy food processing waste used in this study was collected from leftover dust powders, and waste powder recovery from dust collector which is also extremely vulnerable to external contamination and storage temperature. On the other hand, microalgae can also absorb vitamin B₁₂ as growth factor [132]. Micronutrient absorption by microalgal groups is tough since they are available in extremely low quantities in sea and fresh water. The concentration of vitamin B₁₂ in seawater ranges from 0 – 70 ng/L which is still quite low to sustain vitamin B₁₂ dependent microalgae development [133]. Grant et al., (2014) discovered that microalgae require a minimum concentration of cobalamin of 20 – 50 ng/L to thrive [134]. This suggests that, although river water and sea waters have their potential to enhance the algae development, these water bodies cannot serve as the only nutrient supply for algal growth [67].

3.3.4 Comparisons of industrial dairy waste on microalgae biomass yield.

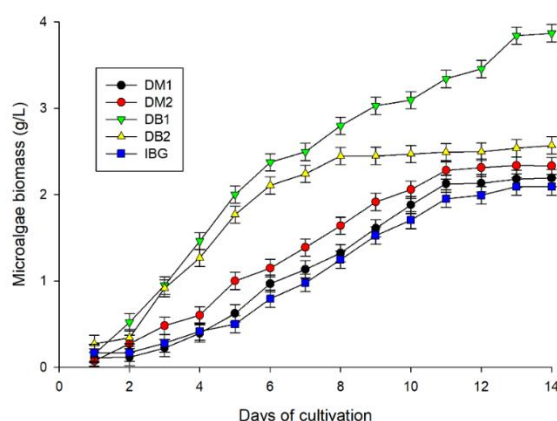


Figure 3.3: Cultivation curve of *Chlorella vulgaris* grown in IBG and industrial dairy food processing waste culture mixtures.

Nutrients' availability, carbon dioxide and light are the main contributors for the growth of microalgae. Nitrate is the most essential source of nutrient for the enhancement of bulk microalgae biomass quantities [109]. Microalgae has the ability to utilize wide range of nitrate sources including ammonium, organic nitrogen, and nitrate. The productivity of cell growth and nitrate intake are proportional to each other, as the cells of microalgae aggressively consume nitrate to facilitate growth. Although the DB1, indicates the highest average weight percentage of nitrate, it is also important to observe the growth efficiency of *Chlorella vulgaris* FSP-E with all other medium combinations.

Figure 3.3 demonstrates the cultivation growth of *Chlorella vulgaris* using industrial dairy food processing waste as its source of nutrients. The DB1 medium mix achieved the highest microalgae biomass concentration at 3.61 g/L, followed by 3.22 g/L, 3.13 g/L, and 2.78 g/L was obtained for DB2, DM2, and DM1 mixtures respectively, while IBG medium source alone achieved a biomass production of 2.67 g/L after 14 days. Usually, the cultivation of microalgae can take up to 16 to 21 days to approach the stationary growth phase [135]. However, with industrial dairy food processing waste as nutrient source, the cultivation days could be minimized to 12 - 14 days. This enhances the overall microalgae biomass productivity. Within 14 days, the microalgae biomass productivity growth in all types of industrial dairy food processing waste medium was observed to be higher than the inorganic medium, IBG. The growth pattern of microalgae consists of four-phases: lag, exponential, stationary, and death phase [110]. Main function of the growth media is to trigger the initial phase till the microalgae adapt to the surrounding environment and to sustain its stationary phase [136]. The presence of lipid and carbohydrate will be initiated and developed during the stationary phase [137]. Thus, the behaviour of microalgae during the stationary phase decides on quality of biomass and its capability for bioenergy production. Based on

Figure 3.3, it can be said that DB2 mixtures, grew rapidly within the first 4 days and was in stationary phase for the remaining 10 days, however DB1 took approximately 13 days to reach its stationary phase. The growth of microalgae in DB2 is noticed to be higher than the control inorganic IBG medium till 8th day of the culture period, and eventually started to be stationary from 9th day onwards. This is due to the very low amount of nitrate content in DB2 medium mixture. On the other hand, the growth of DM2, DM1, and IBG grew rapidly for the first 11 days, and continued its stationary phase till the 14th day. This shows that the microalgae have reached its maximum productivity and may starts to degrade if harvesting process does not take place. It is also not advisable to prolong the stationary growth as apoptosis of the algae may occur due to insufficient carbon and nitrate source [138]. The IBG medium source, was found to be the lowest compared with all the other medium mixture combination. Apart from increasing the biomass productivity of microalgae, using the industrial dairy food processing waste would save cost and energy for the cultivation of microalgae. This is because, there is no additional pre-treatment and filtration technology is required for the medium preparation.

Table 3.2: Characteristics of *Chlorella vulgaris* grown in IBG and industrial dairy waste culture mixtures.

Culture mixtures	DCW (g/L)	P _b (mg/L/d)	μ (d ⁻¹)
DM1	3.13 ± 0.05	86.68 ± 4.23	0.25 ± 0.01
DM2	2.78 ± 0.08	101.09 ± 2.89	0.29 ± 0.03
DB1	3.61 ± 0.05	170.20 ± 5.41	0.37 ± 0.06
DB2	3.22 ± 0.04	134.44 ± 2.15	0.19 ± 0.01
IBG	2.67 ± 0.05	81.08 ± 2.33	0.13 ± 0.01

The average specific growth rates (μ) measured throughout the 14 days culture is shown in Table 3.2. The daily average growth rate was also reported to be highest for DB1 followed by DM2, DM1, and DB2 as compared to the control IBG medium. Addition of industrial dairy food processing waste is likely to increase the micronutrient percentage in the culture medium which enhance the amount of biomass produced. Hence, the combination of industrial dairy waste with BG-11 intensifies the culture mediums' nutrient content. Microalgae biomass yield

(P_b) can be increased by adding a significant volume of micronutrients resulting in a higher biomass yield. Arefin et al., (2020) revealed that neither a concentration of industrial dairy waste that is too high nor too low will not be suitable for processing microalgae biomass since the medium may include residues that prevent the growth and production of biochemical material in the microalgae [106]. Hence, a pre-trial study was carried out and it was identified that 2 g of each industrial dairy waste provides the best growth of microalgae biomass for 1 L capacity PBR. Moreover, the industrial dairy food processing waste have no significant influence on the colour intensity of its culture medium. Unlike commercial industry wastewater, leachate, food waste compost which may contribute to darken the culture medium [122]. Dark mediums prevent light from reaching microalgae, inhibiting their growth as light is an essential factor in microalgae growth quality [139]. Table 3.3 compares the biomass growth of *Chlorella vulgaris* using other organic sources combinations with inorganic nutrients alongside with discoveries of this study. According to the culture condition comparison in Table 3.3, it can be concluded that the supply of CO₂ gas (50 PPM) and pH range (7- 8) generates greater microalgae biomass productivity. Besides, the integration of culture medium is reported to be the most influential factor that influences the biomass growth and cell composition. Thus, further analysis into the biochemical content was conducted to investigate whether the integration of expired dairy premixes can be combined with IBG to produce high quality bio-composition microalgae biomass.

Table 3.3: Comparisons of *Chlorella vulgaris*, biomass productivity cultivated using various organic nutrient integrated sources.

Organic nutrition	Integrated sources	Culture Conditions	Biomass Productivity (mg/L/d)	Reference
Organic Fertilizer	Bold's Basal medium	A: without CO ₂ pH: 6.8 B: 80 %	30.84 ± 8.15	[140]
Palm Oil Mill Effluent, POME	Urea	A: without CO ₂ pH: 6.8 – 7.2 B: 50 %	58.40 ± 5.26	[141]

Industrial Waste	Bold's Basal medium	A: with CO ₂ pH: 6.8 B: 1 %	120.01 ± 0.01	[142]
Food Waste Compost	BG-11	A: with CO ₂ pH: 7.2 B: 25 %	120.31 ± 7.84	[122]
Biscuit processing waste (DB1)	BG-11	A: with CO ₂ pH: 7.4 B: 20 %	170.20 ± 5.41	Optimized findings from this study

*Note: A: Carbon dioxide, CO₂ supply; B: Integration percentage

3.3.5 Nutrient removal efficiencies

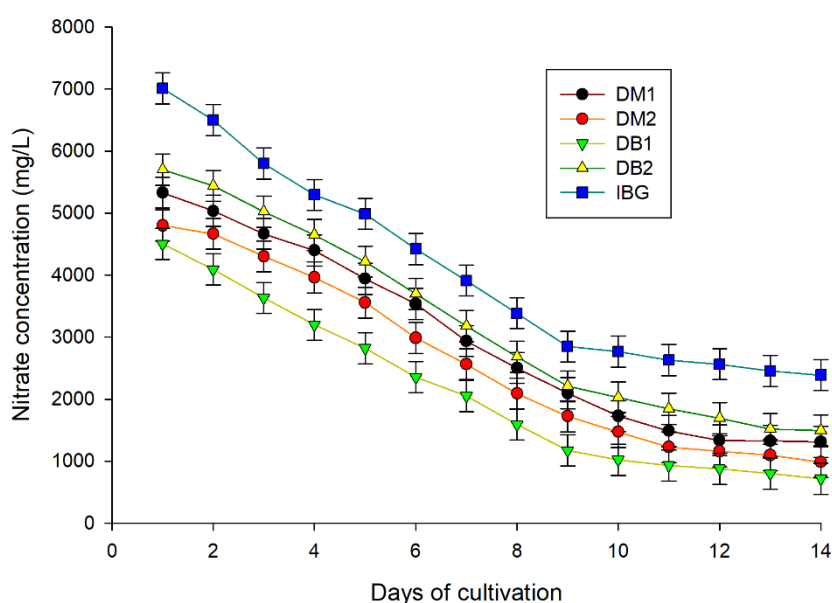


Figure 3.4: Nitrate content throughout the growth of *Chlorella vulgaris* grown in IBG and industrial dairy food processing waste medium.

During the cultivation phase, microalgae can accumulate nourishment while still removing organic matter from the medium. Nitrate and phosphorus are one of the most important sources for microalgae development and these nutrients will be absorbed from its culture medium to enhance the cell formation which leads to greater biomass productivity [142]. As per the FTIR analysis (Figure 3.2), the composition of industrial dairy food processing waste mainly consists of carboxylic acid, nitrate and amides functional group which will be providing the adequate amount of nutrients to enhance the growth microalgae biomass greater than the chemically modified nutrients source [143]. Despite having excellent nutritional qualities based on

elemental analysis, industrial dairy food processing waste is not suitable for recycling back into dairy food production operations since these powders are exposed to open air where the atmospheric dust and external heavy material will contaminate and cause deficiency onto the product quality. The potential of microalgae to effectively consume nitrate, phosphorus and B₁₂ vitamin provides an advantage for dairy product manufacturers to recycle their powder waste which can save them disposal cost and venture into a more sustainable production. Thus, the integration of industrial dairy food processing waste with BG-11 can improve the growth rate of microalgae and recovery of bio-products becomes a worthwhile interest.

Throughout the 14 days of cultivation process of this analysis, the nitrate content decreased. It is observed that the nitrate reduction rate at the end was more stable compared to the initial stage. This is because, microalgae tend to consume large amount of nitrate in its initial growth phase, where the ability of phosphorus is also active [144]. The highest nitrate removal percentage within the first 8 days occurred for DB1 of 74% followed by DM2, DB2, and DM1 of 64%, 61%, and 60% respectively. However, for IBG medium, the nitrate reduction rate was at 59% which is the lowest compared to all the other premix combination. Although the initial nitrate content was high for inorganic medium, the growth of microalgae is slightly slower in IBG as compared with the mixture of industrial dairy food processing waste with BG-11 medium. This is because, the integration of BG-11 accelerates the growth process resulting in higher nitrate reduction percentage. Besides, the shades of mediums have no influence on the light penetration for all culture combinations, and an equivalent volume of CO₂ is supplied to all mediums throughout the 14-day cycle. DB1 are observed to uptake 84% of nitrate from its medium which is the highest compared to DM2 (79%), DB2 (75%), DM1 (74%) and IBG (67%) combinations in Figure 3.4. Previously, a study of microalgae cultivation using paper industry waste as an organic medium was conducted and it was reported that a maximum of 57% removal of nitrate was achieved at the end of a 10 days' cultivation period [145].

Additionally, the use of food waste compost as organic cultivation medium achieves a maximum of 12% of nitrate reduction [122]. This signifies that industrial dairy food processing waste for a segment of a chemical medium could generate a comparable and perhaps greater amount of biomass as the availability of nitrate is adequate.

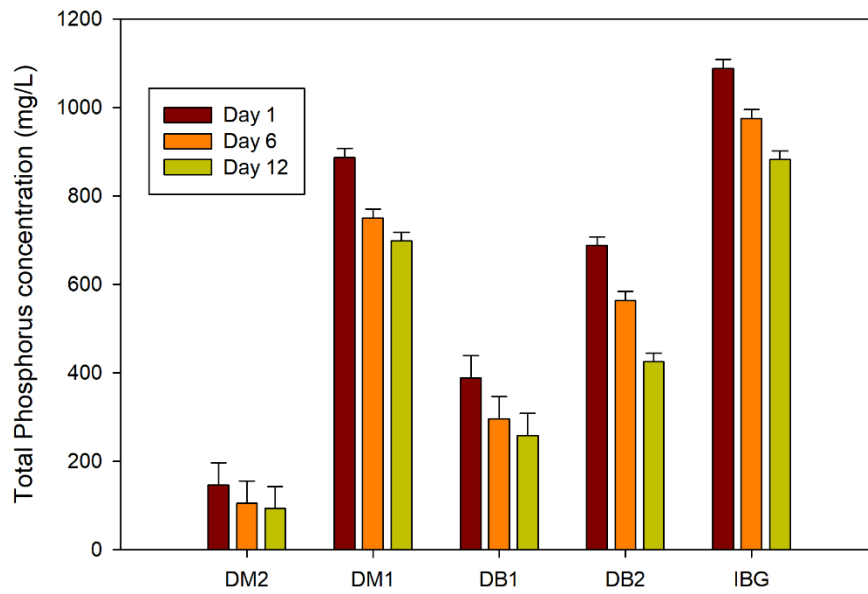


Figure 3.5: Reduction trend of phosphorus content in each combination of industrial dairy food processing waste with BG-11 medium.

Inorganic culture media such as BG-11, Basal, and Zarrouk have high phosphorus content, which is necessary for microalgae production [146]. Hence, it is important that the organic medium would be able to provide sufficient phosphorus source for the growth of microalgae. According to the elemental analysis, DM2 had the highest phosphorus content, whereas all the other industrial dairy food processing waste had about 30% lower phosphorus content. Phosphorus is the element that provides flavouring and moistness particularly for frozen food, hence DM2 had the most phosphorus content [147]. It was observed that, the mixing of industrial dairy food processing waste with BG-11 did not extremely increase the mediums' initial phosphorus content but instead the combination modified the overall culture medium to have slightly lower phosphorus concentration as compared to IBG alone. Liang Fu et al., (2019) concluded that excess amount of phosphorus could result in producing low quality

microalgae biomass as it has the possibility to effects the genetic materials and energy for cell division [148]. Thus, neither too little nor too much of phosphorus content would inhibit the microalgae growth phase. In this study, the industrial dairy food processing waste were combined with IBG (ratio of 1:4) of 1 L culture, and DM1 measures the highest initial phosphorus concentration in comparison with DM2, DB1, and DB2. Although DM1 has highest phosphorus content, the biomass productivity of DB1 is 50% more than DM1. Although the culture medium consists of high initial phosphorus content as per Figure 3.5, microalgae only consume adequate amount of nutrients that is needed for its growth as the requirement of phosphorus differs according to the microalgae species [149]. IBG consists highest phosphorus concentration with lowest phosphorus removal percentage of 18.93%. However, DM2 indicates the highest removal at 36.30%, followed by DB1, DB2, and DM1 at 33.68%, 31.23% and 21.31% phosphorus removal, respectively. DM2 uptakes more phosphorus than other industrial dairy food processing waste due to its lowest initial phosphorus content. This is because, under phosphorus limited conditions, microalgae species tend to uptake all the available phosphorus in the medium for its cell survival as well as enhance its lipid accumulation [148]. Hence, it is predicted that DM2 and DB1 have possibilities to be the best combination of industrial dairy waste and IBG for microalgae cultivation. Detailed analysis on lipid, protein and carbohydrate are conducted to identify the best industrial dairy waste culture combination.

3.3.6 Biochemical compositions

3.3.6.1 Lipid content

Lipid content produced from microalgae biomass varies according to the types of culture medium used for growth as listed in Table 3.4. The lipid concentration for DM2 was the highest at 239.69 mg/g, followed by DB1, DB2, and DM1, at 238.20 mg/g, 220.95 mg/g, and 220.15 mg/g, respectively. In comparison with the IBG medium, the biomass had the lowest lipid

production all the medium combinations at 211.99 mg/g. The proportion of phosphorus in the culture medium affects lipid accumulation, and it is well known that lipid production is optimum if phosphorus is lacking [149]. The high initial phosphorus content in IBG does not provide the cells a stress condition during its growth phase which also leads to a lower lipid accumulation and DM2 based biomass generates the highest lipid content due to the phosphorus starvation condition. Similar previous studies have been conducted on *Chlorella* species algae, and it was discovered that culture mediums with temperature, light intensity and nitrates stress conditions can also activate the lipid synthesis [150]. In this study, the temperature, agitation, duration, and light intensity were kept constants for all medium combinations to avoid any additional energy input to the overall cultivation process.

Table 3.4: Bio-chemical concentration of *Chlorella vulgaris* in IBG and industrial dairy waste mediums.

Culture mixtures	Lipid (mg/g)	Protein (mg/g)	Carbohydrate (mg/g)
DM1	220.15 ± 5.50	192.99 ± 4.10	293.09 ± 8.00
DM2	239.69 ± 2.10	189.99 ± 3.90	314.01 ± 1.90
DB1	238.20 ± 5.50	227.45 ± 0.90	425.89 ± 0.40
DB2	220.95 ± 4.10	225.69 ± 3.00	235.05 ± 4.10
IBG	211.99 ± 5.60	182.77 ± 0.20	134.58 ± 4.40

IBG had the highest initial nitrate content followed by DB2, DM1, DM2, and DB1. Growing microalgae in a nitrate-restricted setting could influence and prompt the reduction of biomass growth to occur which also consequently effect the overall lipid productivity. Previous study reported that lack of nitrate availability in the culture media, not only increases the lipid concentration but also cause a decrease in carbohydrate [151]. This is because, nitrate starvation blocks the starch synthesis as lipid and carbohydrate paths compete for a common carbon precursor. Hence, in this study one stage cultivation was carried out in which the medium contained the desired nitrate concentration, and as the culture grew, its nutrients started depleting, gradually leading to starvation. Nitrate levels must be regulated during the culture process to retain the nutrient content of a medium constant and avoid disruption to the cell

membrane, biomass degradation, and other changes in cell structure, nitrate level to maintain the nutrient contents of a medium stable and thereby prevent damage to the cell membrane, loss of biomass, and other changes in cell composition [152]. Development of lipids and biomass is species-dependent in each of these techniques. In short, all the industrial dairy food processing waste combinations generate higher lipid content than IBG due to a greater phosphorus starvation culture conditions which enhanced prompt an efficient lipid accumulation from the cultivated biomass. The result obtained, indicates that DB1 attain an optimum lipid production among all the other culture medium combinations. A previous research also supports the use of industrial biscuit processing waste in conjunction with BG-11 for microalgae cultivation [153].

3.3.6.2 Protein content

Protein can be characterized as an extremely long chain polyamide. The amides contain nitrate, which comprises about 16% of protein atomic content [154]. During the elemental analysis, almost all of the industrial dairy food processing waste contain amide. Even so, after its combination with BG-11, the overall nitrate content must be considered, particularly for microalgae cultivation. Rahpeyma et al., (2019) reported that microalgae has the ability to store protein in different parts of the cell like cytoplasm, organelles, plastids, cell wall in the cultivation phase and protein extraction occurs by disrupting microalgae cell walls to extract the protein out which can be processed into useful products [155]. The protein content derived from the biomass of microalgae and the highest protein content was identified in DB1 at 227.45 mg/g, followed by DB2 at 225.69 mg/g, and DM1 at 192.99 mg/g. The protein concentration for DM2 and IBG are less compared with the other culture combinations, containing 189.99 mg/g and 182.77 mg/g, respectively as displayed in Table 3.4.

Xie et al., (2017) indicated that there will be slight decline in the protein content if the culture medium consists of excessive nitrate concentration. Nitrate in the medium will gradually

deplete causing nitrate starvation while the biomass growth occurs. Protein content proportionally improved with increase in starvation time, but excessive extension of starvation time results in lower protein content [156]. As per Figure 3.4, the consumption of nitrate was more than 50% for all the organic and inorganic combinations in the first 7 days and from the 8th day onward, starvation time begins to take place. There was no excess nitrate supply to the *Chlorella vulgaris* cells in between the cultivation process, therefore protein accumulation was not inhibited. It was found that DB1's nitrate concentration was the lowest at 420 mg/L, leading to the maximum protein content, although the analysis was conducted during the starvation time frame. The improvement of protein content in DB1 was found to be 24% more as compared to the inorganic medium. Hence, the integration of industrial dairy food processing waste with BG-11 provided adequate nitrate for microalgae growth as well as effective protein accumulation.

3.3.6.3 Carbohydrate content

In majority of a biomass conversion technologies, carbohydrates are the main substrate for production of biofuels such as bioethanol, bio-hydrogen, and biogas. The production of carbohydrate serves two main purposes for microalgae; they act as structural components in the cell walls, and as storage component inside the cell [157]. Carbohydrate as storage compound provide the energy needed for the metabolic processes which allows microalgae to survive in harsh conditions such as temporary survival in dark conditions. The carbohydrate content of biomass depends on the microalgal species and on the conditions of cultivation. Ravindran et al., (2016) reported that microalgae species such as *Porphyridium cruentum* and *Spirogyra* have the ability to generate 40-57% and 33-64% of carbohydrate content respectively [158]. Nevertheless, for the biofuel production to be maximized, high content of carbohydrate has to be combined with the capability of algae species to produce high biomass productivity. Since the combination of industrial dairy waste with inorganic medium enhance

the biomass production, its carbohydrate content is taken into consideration for the selection of optimal combination. The carbohydrate concentration of *Chlorella vulgaris* biomass developed in multiple culture integration is shown in Table 3.4. The largest carbohydrate content was observed in DM2, (425.89 mg/g) followed by DB1 (314.01 mg/g), DM1 (293.09 mg/g), DB2 (235.05 mg/g), and IBG (134.58 mg/g).

As compared to the combination of food compost using BG-11, it is measured that DB1 has 57% more carbohydrate content [122]. This can be due to the high carbon content in the industrial dairy food processing waste as shown in Figure 3.2. CO₂ gas are also the major carbon source that promotes the growth of microalgae photosynthesis cells and allows carbon fixation metabolism. As for this study, organic carbon source was already present in the industrial dairy food processing waste and the supply of industrial CO₂ maximizes the bioaccumulation of carbohydrate in microalgae cells [159]. Besides, nitrate starvation was used to monitor the partitioning of biomass, while the stress conditions coupled with high light intensity, sufficient CO₂ concentration results in high total carbohydrate content. Whereas, for the generation of lipid and protein an environmental stress due to nitrate starvation is adequate. Hence, in this study the carbohydrate content is observed to be higher than the lipid and protein composition from the cultivated *Chlorella vulgaris* biomass. Overall, the result obtained indicates that DB1 attained an optimum combination of carbohydrate, lipid and protein production among all the other culture medium combinations. In near future, monitoring the influence of multi nutrient supply in the culture medium can be anticipated using computer vision and AI algorithms. The use of modern digital technologies such as sensors, automation applications, and the Internet of Things (IoT) can be implemented to allow large scale algae growth monitoring feasible.

3.4 Conclusion

Integration of the industrial dairy food processing waste with IBG culture for the growth of microalgae provides numerous economic and environmental advantages. In comparison to the non-integrated medium, the mixture of DB1 as growth media offers the greatest microalgae production (146.63 mg/L/d), lipid yield (238.20 mg/g), protein (227.45 mg/g), and carbohydrate concentration (314.01 mg/g). As compared to the BG-11 culture media (IBG), the integration of organic medium (DB1) significantly increases biomass production, lipid, protein, and carbohydrate content by 44%, 11%, 20%, and 57% respectively. Overall, this study proves that industrial dairy food processing waste can be integrated with BG-11 medium for microalgae cultivation process with further investigation on modern digital technologies for large scale smart production plant in near future.

CHAPTER 4

MICROALGAE CULTIVATION WITH INDUSTRIAL BISCUIT WASTE REMEDiation BASED ON VISION IMAGING SYSTEMS FOR BIOMASS GROWTH MONITORING

Overview

Continuous automation of conventional industrial operations with smart technology have drawn significant attention. Firstly, the study investigates on optimizing the proportion of industrial biscuit processing waste powder, (B) substituted into BG-11 as a source of cultivation medium for the growth of *Chlorella vulgaris*. Various percentages of industrial biscuit processing waste powder, (B) were substituted in the inorganic medium to analyse the algal growth and biochemical composition. The use of 40B combination was found to yield highest biomass concentration (4.11 g/L), lipid (260.44 mg/g), protein (263.93 mg/g), and carbohydrate (418.99 mg/g) content compared with all the other culture ratio combination. Secondly, the exploitation of colour acquisition was performed onto *Chlorella vulgaris* growth phases, and a novel photo-to-biomass concentration estimation was conducted via image processing for three different colour model pixels. Based on linear regression analysis the red, green, blue (RGB) colour model can interpret its colour variance precisely.

Keywords: Colour acquisition; Colour model; Biomass growth; Dairy waste; Culture medium

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Peter, Kit Wayne Chew, Apurav Krishna Koyande, Sia Yuk-Heng, Huong Yong Ting,

Saravanan Rajendran, Heli Siti Halimatul Munawaroh, Chang Kyoo Yoo, Pau Loke Show*.

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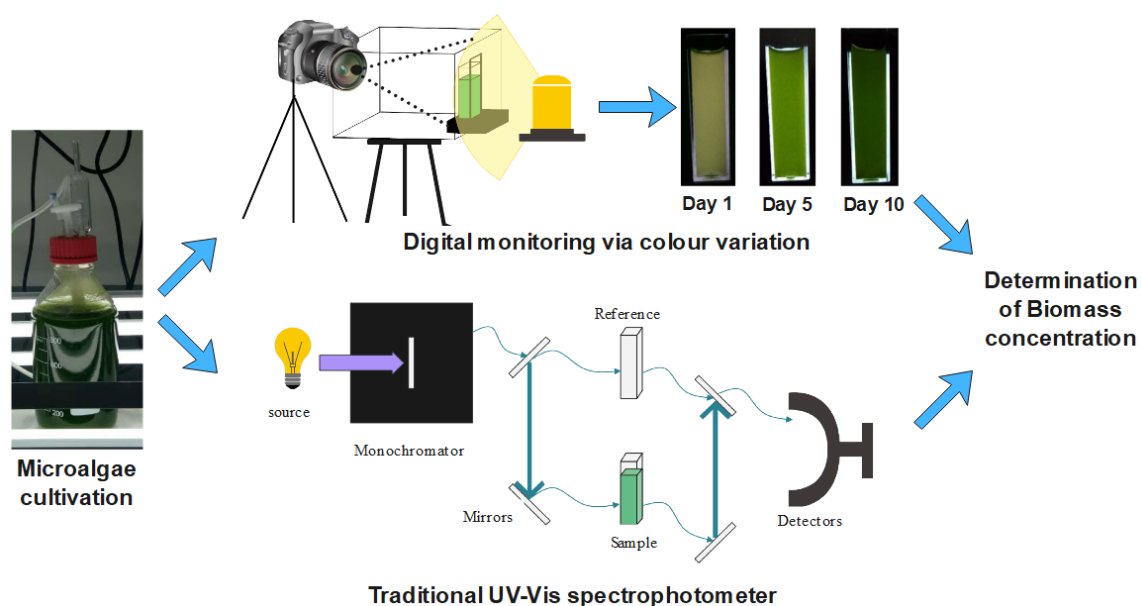


Figure 4.1: Graphical abstract for microalgae cultivation with industrial biscuit waste remediation based on vision imaging systems for biomass growth monitoring.

4.1 Introduction

Microalgae are a photosynthetic eukaryotic microorganism that can develop effectively along with their simple structure that colonizes the habitats of both marine and freshwater. In terms of biomass, each species of microalgae produces different ratios of lipids, carbohydrates and proteins which can be used for human welfare. Interest in the development of useful molecules ranging from therapeutic proteins to biofuels is also being developed by microalgae. In particular, *chlorella vulgaris* can be used commercially as a food colouring ingredient, prescription nutrition ingredient, detox agent for digestive system, antibodies, and so on [160]. It was proposed by clinical trials that supplementation with *chlorella vulgaris* improves hyperlipidemia and hyperglycemia and protect against oxidative stress, cancer and chronic obstructive pulmonary disease [161]. Moreover, compared to fossil fuels, the processing of

biofuels using *chlorella vulgaris* biomass is known as a sustainable biological feedstock. All these advantages can only be acquired by cultivating high quality biomass from *chlorella vulgaris* and by paying close attention to its cultivation condition in order to generate premium quality biomass. microalgal biomass production is influenced by a number of parameters, the most significant of which are nutrient availability, temperature, and light [162]. By generating changes in metabolism, these variables impact the development of microalgae and the composition of the biomass. One of the core priorities of microalgal product production is to choose an effective nutrient medium. The choice of a medium depends largely on many aspects, including the chemical composition of the medium, such as nitrate and phosphorus. There are a variety of media available for microalgae cultivation, but it's important to identify which medium is best for microalgae development. Inorganic medium is commonly used for microalgae production and has recently been studied to partially substitute organic mediums such as agricultural wastewater, vegetable compost waste, Palm Oil Mill Effluent (POME). Thus, this study focuses on the combination of inorganic and industrial biscuit waste powder which is not suitable for human consumption. These waste powders still consist of activation carbon, nitrate and phosphate sources that potentially can be used for the growth of microalgae. Moreover, initial phase of colour model analysis was conducted to address the challenge of digitalizing biomass growth monitoring in order to commercialize microalgae research technologies. By analysing its culture colour strength, the maturing of *chlorella vulgaris* biomass can be accessed via raw eyes, as it shifts colour from cloudy white to light green to dark green biomass culture solution.

The use of robotic operations based on visual instructions in modern manufacturing has increased rapidly in recent years. Visual based technologies attracted many investors attentions in developing modern machine vision and imaging systems towards factory digitalization. Colour models such as RGB (Red, Green, Blue), CMYK (Cyan, Magenta, Yellow, Black), and

HSL (hue, saturation and lightness) plays an important role when it comes to visual based quantification [163]. With the development of science and technology, colour printing industries, textile manufacturing, paint manufacturing and food production industries uses various measuring instruments that describe colours in numbers or provide a physical colour match for quality check [164]. As for the cultivation of microalgae, colour match identification can also be used in detecting the maturing of biomass growth. Now, UV- vis spectrophotometer is used in laboratory scale microalgae cultivation (APHA method). Figure 4.2 illustrate the working mechanism of a classic double beam UV -vis instrument. Spectrophotometer analysis is a technique used for measuring the intensity of electromagnetic radiation at different wavelengths ranges from 800-220nm. Although, this technique can provide extremely accurate readings the major downside of using a UV-vis spectrophotometer is the time it takes to dilute the samples as it can only detect a maximum absorbance value of 4. Thus, the digitalization of microalgae biomass is essential especially in case of large-scale production. In this study, RGB, CMYK, and HSL colour model was evaluate for its colour correlation alongside with the microalgae growth during cultivation process. RGB model combines equal portion of red and green “light” to produce yellow, while the CMYK model combines equal parts of yellow and cyan (blue) “paint” to produce green. These two main colour models are based on the concept of additive and subtractive colours [165]. Besides, HSL colour model is defined as a subset of the RGB model that expresses the colours in terms of hue, saturation and luminosity. These colour models is a mathematical framework that explains how colours may be represented as a collection of integers. A coordinate system is used to define colour models, and each colour in the system is represented by a single point in coordinate space. Thus, RGB, CMYK and HSL were chosen to be compared, to identify which colour model suits the microalgae biomass colour growth monitorisation.

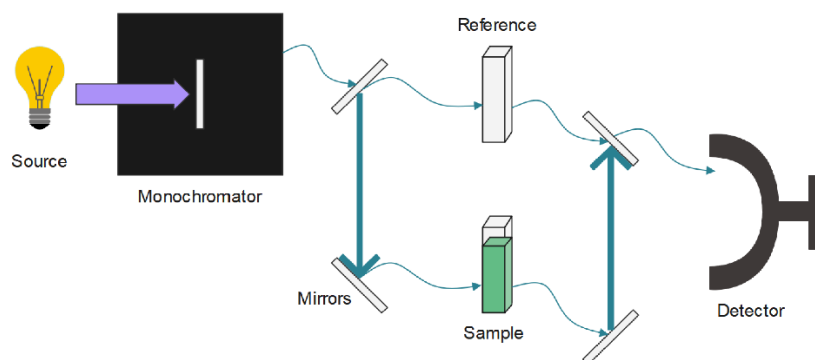


Figure 4.2: Illustration of basic double beam UV-Vis's spectrophotometer

The aim of this research is to select a suitable colour model method that can be used to identify the maturity of microalgae growth during its cultivation phase. Specifically, RGB, CMYK, and HSL colour model is investigated. The colour variation has been observed from various concentration of medium that comprises of industrial biscuit waste powder (B) with BG-11 (100C) to determine the optimized mixing ratio. The novelty of this study is to identify the best colour model indexes that could represent the experimental data results. An accurate colour model representation is essential to conduct digital image processing which is considered as an initial stage for the development of smart automated microalgae growth monitoring using digital image-data process.

4.2 Methodology

4.2.1 Microalgae species and culture medium preparation

The *Chlorella vulgaris* species was collected from a reservoir field in southern Taiwan. The modified BG-11 medium contained of 1.5 g NaNO_3 , 0.03 g K_2HPO_4 , 0.075 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.006 g anhydrous citric acid combined with four stock solutions. The first stock solution made of 2 g Na_2CO_3 ; The second stock solution made of 3.6 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$; The third stock solution made of 0.6 g ferric ammonium citrate and 0.1 g EDTA; The fourth stock solution made of 2.86 g H_3BO_3 , 1.81 g $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, 0.222 g $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 0.3 g $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$, 0.07 g $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 0.04 g $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$. All these four-stock solutions are mixed with 1 L of deionized water. In an indoor photo-bioreactor (PBR), 200 ml of the BG-11 medium was used

for the pre-culturing. The PBR consisted of a 250 ml glass vessel, and the top vessel is covered with a layer of rubber that holds connections for compressed air and CO₂ supply, culture removal stream, and oxygen releasing stream. The PBR was set up at room temperature with constant stirring source from the magnetic stirrer with hotplate (FAVORIT) at 200 rpm with 2.5 cm length magnetic stirrer bar for a 250 ml glass vessel. Then the framework is placed in a rack that holds external horizontal LED light sourced on the back and front of the vessel. LED, 3000K warm white light was used (50/60 Hz) on the PBR wall. Air was purified using (0.45µm filter) and blended with 1.5% of compressed CO₂ at the rate of 200 ml/min. The culture broth was continuously aerated (0.2 vvm, volume gas per volume medium per minute). After 7 days of pre-culture, the subsequent batch cultures, were performed using similar PBR operating parameters in 1L glass vessel for each combination with 4.5 cm length magnetic stirrer bar in agitation. A total of 5 glass vessels were used for one complete set cultivation cycle and the experiment were repeated three times. The average readings were recorded.

4.2.2 Preparation of industrial waste as cultivation medium

The industrial biscuit waste powder was provided by local biscuit manufacturers located in Malaysia. In a 250 ml conical flask, 2 g of industrial biscuit waste powder was combined with 200 ml deionized water. The flasks were fitted in a water bath and rotated for 30 min at 150 rpm at room temperature. This study focusses on the optimization of industrial biscuit waste medium volume with chemically modified BG-11 medium (100C). The industrial biscuit waste powder was mixed at 100 ml (10B) followed by 200 ml (20B), 400 ml (40B), 600 ml (60B) and remaining culture volume mixed with inorganic BG-11 medium up to a total of 1 L culture medium. The control PBR consist of 1 L of BG-11 (100C). Throughout the study, all mixture combinations have a pH range of 6-7.5 which was measured using Mettler Toledo SevenCompact™ pH Meter S210, Brand (Merck).

4.2.3 Black box description setup

Matte black paint was applied to a cuboid shaped box with (5 cm width x 8 cm length), to prevent additional light reflection inside the box. Matte black paint was chosen to avoid the reflection of light to defect the colour vision training for image classification in Custom Vision. Following that, a 25 mm diameter circular hole is punched (adequate to place the digital single-lens reflex, DSLR camera). Parallel to that, a rectangular shaped hole with (10 mm length x 45 mm height) was punched to match perfectly in the cuvettes. Figure 4.3 illustrates the black box setup for the image capturing. Throughout the analysis, Starna Scientific Ltd cuvettes (type 1/Q/10) was used. Moreover, two magnets (1.5 cm width x 3 cm length) were added as the cuvette stand (position both in and out of the black box). The LED white lighting was positioned at a 15 cm gap from the black box. This allows the camera, cuvette sample, and light source to be aligned in a straight line. The distance of the light source was maintained constant at 15 cm, meanwhile the DSLR camera was placed fitted to the box's hole.

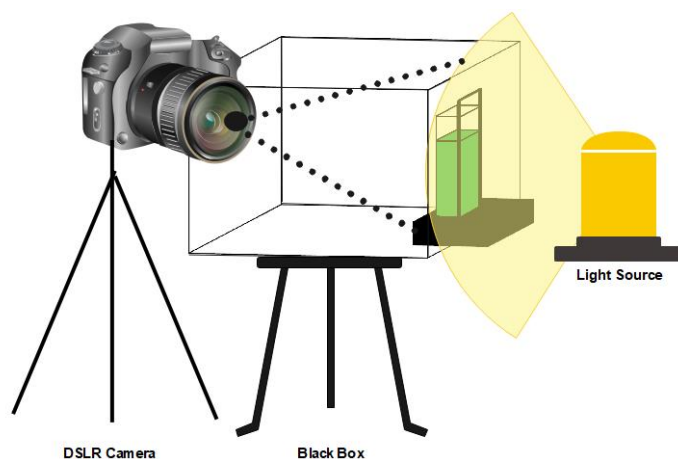


Figure 4.3: Illustration of black box setup for image.

4.2.4 Data collection for image processing

The DSLR camera and images specification set throughout this study are as follows, ISO sensitivity: 1600, 4mm focal length, lens type: macro, lens magnification: f/2.2, image size (pixels): 2352 x 4160. A total of 1500 images were used for data training; all the images were used for data testing and validation. 30 images were taken for each combination mixture every

24 hours for 10 days. The integration of visual studio 2019 platform (colour analysis) and extracting data for linear regression model was used to identify the best fit colour model. RGB, CMYK, and HSL colour model studies was compared in this study. To include consistent regions of interest, all images were cropped to a rectangular shape (8 mm length x 35 mm height) for the colour analysis. A glimpse of the colour variation taken on day 1, day 5, and day 10 during the *chlorella vulgaris* growth are shown in Figure 4.4.

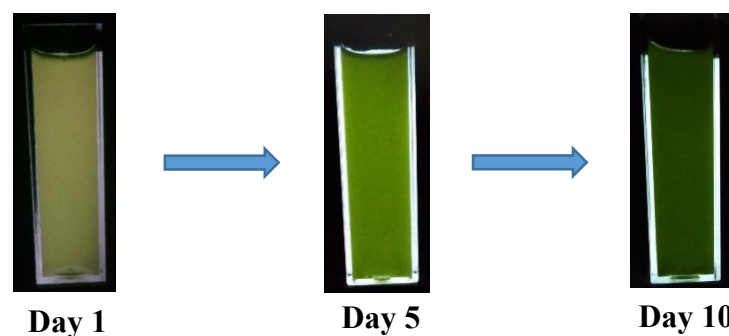


Figure 4.4: Sample images that indicates the colour variation during microalgae cultivation.

4.2.5 Image processing (photo to colour index)

Microsoft Visual Studio (an integrated development environment) was used to develop the image processing and analysis program. The program developed is Windows Forms App which used .NET Framework and C# programming language. The user interface of the program is built using drag and drop toolbox provided. The user interface is written in XAML (Extensible Application Markup Language) and defined the controls such as button, label and image holder in this program. It described the hierarchical relations and properties of the controls. When the program is executed, the function named “MainWindow” is invoked in the file MainWindow.xaml.cs. This function initializes the components defined in MainWindow.xaml. The click event of the button in xaml file is hooked with the function in cs file. The click event of the button opens a dialog to allow the user to pick an image from the computer. After the

image is selected, the image undergoes some image processing steps using Aforge.NET which is a computer vision and artificial intelligence library.

First, the image undergoes gray scaling process and turns into 8bits image which carries intensity information only. The image will then undergo a thresholding to create a binary image which contains black and white only. These processes are taken to make the segmentation step easier. The object of the image is segmented from the background using connected pixels algorithm. The black pixel is considered as background while white pixel is considered as object. The information such as height and width of the white pixels are used to segment the object. The coordinate of the bounding box of the object can be determined from the segmentation step. The coordinates are used to crop the object from the original image with colour information. The magnitude of red, green, blue intensity for each pixel in the centre region is recorded. The average value for each colour is computed and used to calculate the value of index colour. The related results and processed image are displayed on the graphical user interface created by xaml file. The program is further improved to automate the process. BrowseAllFiles and BrowseAllFilesRecordAll are used to loop through the folders and files and generate a folder that contains the processed image (to ensure the image processing step is success) and the result generated. The result will be in the form of a colour index value. Each image that is uploaded creates a colour index value that represents colours numerically.

4.2.6 Evaluation of microalgae cell growth

UV-Vis's spectrophotometer (UV-1800, Shimadzu) was used at wavelength of 680 nm to measure the microalgae biomass growth concentration. The dry cell weight (DCW) of the microalgae biomass was calculated by filtering 5 ml of aliquots of culture via a cellulose acetate membrane filter (25 mm diameter, 0.45µm pore size). All filters were dried for 8 hours at 90 °C. By subtracting the weight of the empty filter from the weight of the full filter, the microalgae DCW was calculated. Then, by calibrating the absorbance, OD₆₈₀ the absorbance

value obtained from the UV-Vis measurement which were translated to biomass concentration. A blank sample of each culture combination was used to reduce the transmission of medium colour in the absorbance readings. Next, the transparent supernatant was extracted from the microalgae culture stock for each mixture variation after centrifugation at 6000 rpm for 15 min. The remaining pellet was freeze dried for 8 hours and used for detailed bio-composition analysis and characterization studies.

Eq. (1) represent the biomass growth productivity, P_b (mg/L/d) and Eq. (2) represent the specific growth rate, μ (d^{-1}):

$$P_b = \frac{W_2 - W_1}{t_2 - t_1} \quad (1)$$

$$\mu = \frac{\ln \frac{W_2}{W_1}}{t_2 - t_1} \quad (2)$$

Where W_1 and W_2 are DCW (g/L) at time t_1 (start) and t_2 (end), respectively.

4.2.7 Evaluation of nitrate and phosphorus concentration of culture combinations

The spectrophotometric approach was used to determine the nitrate content [166]. Every 24 hours, the microalgae specimens were collected out of the PBR and are diluted with deionized water in order to measure the absorbance value using UV-Vis spectrophotometer at 220 nm optical density. The standard curve was constructed using various concentration of pure sodium nitrate ($NaNO_3$). As for the phosphorus content measurement, the atomic absorption spectroscopy (AAS, PerkinElmer A Analyst 400) was used [114]. At day 0, day 6 and day 20 of the cultivation cycle, 20 ml of each medium variety was removed from the PBR and heated with regular mixing to promote phosphorus removal from the mediums. The standard association between absorbance and phosphorus concentration was developed using dibasic ammonium phosphate, $(NH_4)_2HPO_4$ with known concentration. The AAS spectrophotometer

was set to detect light at 213.6 nm, which is the phosphorus resonance axis. The difference in content over the original was used to measure the overall phosphorus concentration removal (%).

4.2.8 Microalgae-based lipid production

Bligh and dryer approach were used to remove the lipid content through microalgae biomass [115]. Chloroform, methanol, and deionized water were mixed with ratio of 1:2:0.8 (w/v) and 50 mg of harvested biomass was submerged in the solution with total volume of 7.6 ml. Then, an ultrasonic water bath (Elma, Germany) was used to sonicate the mixture at 35 kHz frequency for 5 min. Next, the mixture was then sonicated for another 5 min after applying 2 ml of chloroform and filtered water. After sonication, the mixture was stirred vigorously for 30 seconds before centrifuging at 3000 rpm for 5 min. The base layer was poured into a pre-weighted glass vial and was heated in an oven at 80 °C for 30 min. After heating, the vials were placed in a room temperature desiccator and allowed to cool for 5 hours before being weighed. The lipid content was measured as a percentage difference (wt %) of DCW as per Eq. (3).

$$\text{Lipid content (wt\%)} = \frac{\text{Final vial weight} - \text{Initial vial weight}}{50 \text{ mg}} \times 100\% \quad (3)$$

4.2.9 Protein extractions

The microalgae biomass was exposed to cell disruption using ultra sonication to retrieve its protein [116]. Firstly, 0.1g of dried microalgae biomass was combined with 10 ml of deionized water containing 0.5N KOH. The mixture was then vortexed for 60 seconds before being ultra-sonicated for 20 min at a frequency of 35 kHz and centrifuged for 5 min at 7000 rpm. The top layer was used for protein quantification and its remaining pellet are drained. Bradford method was implemented to test the protein content in the extracted microalgae biomass [167]. In a dark setting, 2.5 ml Bradford reagent was mixed with 0.25 ml biomass sample (supernatant layer). The mixture then was analysed at 595 nm using UV-Vis's spectrophotometer. The

standard interaction among absorbance (OD_{595}) and protein concentrations was established using bovine serum albumin reagent (BSA).

4.2.10 Discovery of carbohydrate content

A modified technique was used to remove carbohydrate compounds from microalgae biomass [168]. The mixture of 50 mg powdered biomass and 0.5 ml 18M H_2SO_4 was kept in a biosafety chamber for 30 min. Then, 4.5 ml of deionized water was included to the mixture and autoclaved the solution at 120 °C for 30 min, followed by 10 min of centrifugation at 6000 rpm. The carbohydrate content of the transparent supernatant was determined using the phenol-sulphuric method [169]. The mixture of 1 ml 18M H_2SO_4 and 0.2 ml of 4% (w/v) phenol with 10 μ l of supernatant was measured using the UV-Vis spectrophotometer using 490 nm. The standard relationship between OD_{490} and carbohydrate content was identified using starch extracts with various concentration.

4.3 Results and discussion

4.3.1 Effect of biscuit powder waste on microalgae biomass yield.

The growth of *Chlorella vulgaris* biomass was monitored to identify the optimum percentage of 100C substitution. There will be variation on the nutrient source as the overall culture medium properties differs according to the industrial biscuit waste powder mixing ratio. The biomass growth was compared with control medium, 100C. Based on Figure 4.5, it is observed that 40B yielded that highest biomass growth compared to all the other medium combinations. This analysis reports that, the maximum biomass concentrations were found to be for 40B at 4.11 g/L, followed by 20B, 100C, 10B and 60B at 3.11 g/L, 2.17 g/L, 1.12 g/L, and 0.91 g/L, respectively. According to the report, the combination of industrial biscuit waste powder and 100C takes just 10 days to enter its stationary growth phase, while 100C alone is still rising on the ninth day. Generally, with 100C culture medium it takes up to 14 – 16 days for the *Chlorella vulgaris* growth to reach its stationary growth phase (Chew et al., 2018a). Despite, the fact that

development continues after the ninth day, the biomass concentration is still lower than the 40B concentration. The primary role of culture medium is to initiate the exponential growth process until the microalgae have adapted to their new medium surroundings and to keep the stationary phase going. Thus, it is important to identify the optimal replacing volume of industrial biscuit waste powder with 100C that enhances the overall microalgae biomass productivity within a short period of time as compared to 100C medium alone.

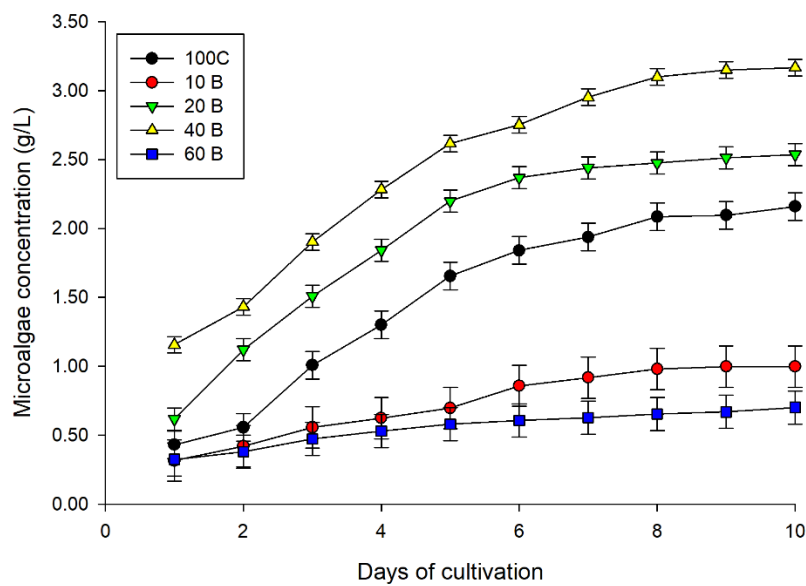


Figure 4.5: Cultivation curve of *Chlorella vulgaris* grown in different combinations of 100C and biscuit industry waste powder culture combinations.

The presence of light source also plays a significant role in the *chlorella vulgaris* biomass growth curve. In this study, 24 hours of LED fluorescence light was shined on both sides of the PBR to avoid biomass loss during the night. In the photosynthesis process, microalgae species needs light as a source of energy to turn CO₂ and water into carbohydrate and oxygen (Peter et al., 2021). Studies confirms that during microalgae formation, more than 50% of the light energy from traditional illumination (sunlight) is not utilized due to the variation of intensity, spectral quality and photoperiod over throughout the day and confirms that LED light has useful wavelength spectrum for the microalgae growth with lesser electric energy compared to fluorescent light (Yeh and Chung, 2009). The colour of medium was the darkest

for 60B relative to all other mixing combinations, which is why it has the shortest growth curve. Hence, in addition to nutrient content, microalgae growth is influenced by light availability. The dilution factor played a role in overall colour intensity as 100C is a colourless medium and the amount of industrial biscuit waste powder was fixed in all medium combination. Since light is known to be one of the most important factors in microalgae growth, the dark colour of the culture media combination might obstruct light from reaching the microalgae, preventing it from growing (Chew et al., 2018a).

Table 4.1: Dry cell weight content (DCW), biomass productivity (P_b) and specific growth rate (μ) of *chlorella vulgaris* grown in various concentration of control BG-11 medium, 100C and industrial biscuit waste powder culture, B.

Culture combination	DCW (g/L)	P_b (mg/L/d)	μ (d ⁻¹)
100C	2.12 ± 0.05	104.38 ± 2.15	0.19 ± 0.012
10B	1.13 ± 0.06	52.61 ± 4.23	0.10 ± 0.010
20B	3.11 ± 0.08	140.09 ± 2.89	0.12 ± 0.028
40B	4.08 ± 0.09	174.99 ± 5.41	0.28 ± 0.063
60B	0.91 ± 0.07	38.95 ± 2.33	0.16 ± 0.015

Table 4.1 lists the biomass productivity and basic growth rate of *chlorella vulgaris* FSP-E at day 10 of its cultivation cycle. It is observed that, 40B medium had the maximum biomass productivity of 174.99 mg/L/d, followed by 20B, 100C, 10B, and 60B at 140.09 mg/L/d, 104.38 mg/L/d, 52.61 mg/L/d, and 38.95 mg/L/d, respectively. Although 10B medium was the lightest colour medium after 100C, the biomass productivity measured was 98 % lower compared to 100C. This is because, growth of microalgae biomass is constrained due to the starvation of nutrients supply in culture medium combinations. While 60% of the industrial biscuit waste powder solution is substituted in the 100C medium, this does not imply that the micronutrient content of the culture medium is high. Table 4.1 also indicates that 40B has the greatest specific growth rate (μ) at 0.28 d⁻¹, followed by 20B, 100C, 10B, and 60B at 0.12 d⁻¹, 0.19 d⁻¹, 0.10 d⁻¹, and 0.16 d⁻¹, respectively. To carry out an optimal cultivation process, a

proper mix of nutrient sources with adequate light penetration is essential. Thus, it is not desirable to entirely substitute the inorganic medium. Other limiting factors such as culture pH, temperature, and CO₂ supply were kept constant during the cultivation process to avoid any additional influences on biomass development. In summary, integration of culture medium plays a significant role for the microalgae growth and its cell compositions with 40B medium combination. Conventionally, the biomass growth analysis was monitored daily with the aid of UV-vis spectrophotometer. This method demands an extra energy and manpower source on a regular basis monitoring. A colour model analysis is needed to attempt a digitalization monitoring device. Thus, further research was done to determine the right colour model.

4.3.2 Colour index analysis – linear regression analysis

Linear regression is a concise algorithm that is commonly used for quantification analysis. The linear algorithm is still significant in determining and constructing functions, but it depends on the training dataset used. In order to construct a linear regression, a dataset with low variance is preferable to the one with high variance. When a dataset has a high variance, an outlier would exist, resulting in lower prediction accuracy [170]. Hence, this study excludes a clustering technique to exclude outlier results. The clustering method is useful for identifying the smallest variations between colour levels in the comparison and recorded images. These traits would be useful in potential inventions for artificial intelligence studies for mobile app development. R² value was used to rank the efficiency of the different calibration regression models. In this study, RGB, CMYK, and HSL colour model was evaluated for its colour index values as per Figure 4.6. RGB colour mode is suitable for digital platforms, whereas CMYK and HSL is used for print items, and computer graphics respectively [171]. Nevertheless, to effectively refine the design, the regression processes behind each colour model must be analysed for each scenario.

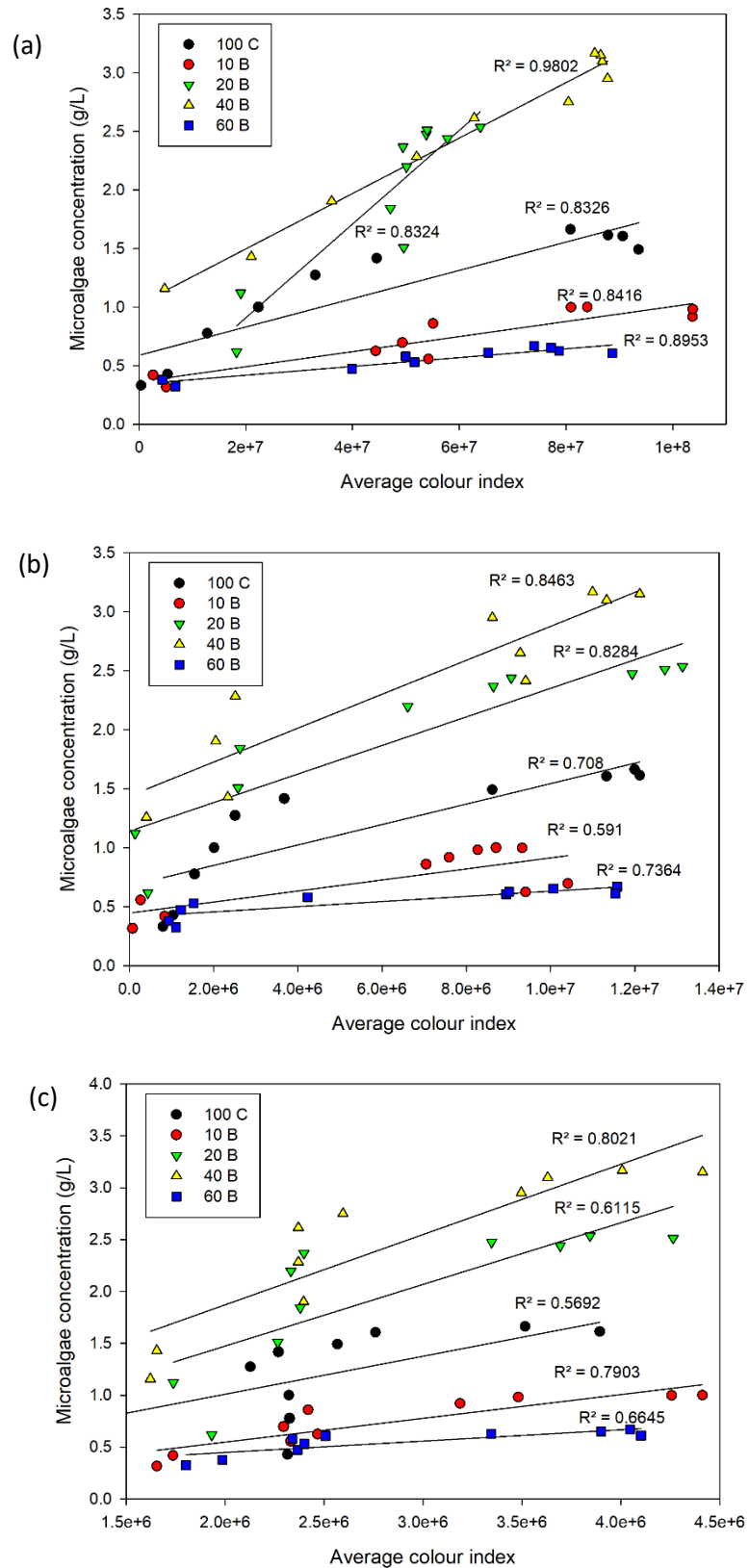


Figure 4.6: Comparisons of average colour indexes (a) RGB (b) CMYK (c) HSL with its corresponding microalgae growth.

Linear regression was measured in Microsoft Excel to provide the simplest image estimation, since it is typically conducted on UV-visible spectrophotometry using a regular calibration curve. For each colour model, 1500 data points of colour index values were grouped into ten categories, and the mean value is shown in the graph for detailed R^2 comparison. According to Table 4.2, the RGB colour index has the highest R^2 value of 0.98 for 40B followed by CMYK, then HSL. This is because, RGB colour model properties is more suitable for digital image process especially for screen display design [172]. As each microalgae cell of *Chlorella vulgaris* matures, its colour switches from light green to dark green, and it is discovered that the RGB colour model can read and interpret its colour variance more precisely than CMYK and HSL. Furthermore, both the experimental results and the image processing study, RGB model indicate that 40B is the best cultivation environment for *Chlorella vulgaris*. Thus, the use of AI in microalgae cultivation is considerably faster and more efficient than the traditional experiment analysis method. It can be inferred that the RGB colour model can be used for the development of mobile applications for estimating biomass growth data using images raster pixel.

Table 4.2: Comparisons of colour model (linear regression analysis)

Analysis		RGB				
		100C	10B	20B	40B	60B
Regression Statistic	R^2	0.8326	0.8416	0.8324	0.9802	0.8953
	Adjusted R^2	0.8176	0.5996	0.7014	0.6392	0.8793
	Standard error	0.2990	0.2760	0.3640	0.0060	0.2970
	Significance F	6.08×10^{-5}	0.0038	0.0012	0.0037	8.54×10^{-5}
ANOVA test	Variable	1.00×10^{-8}	6.00×10^{-9}	4.00×10^{-8}	2.00×10^{-8}	4.00×10^{-9}
	P- value	0.0001	0.0038	0.0012	0.0037	0.0000
	Intercept	0.5897	0.3611	0.1102	1.0222	0.3420
	P - value	1.99×10^{-6}	1.73×10^{-5}	1.72×10^{-5}	2.17×10^{-5}	8.54×10^{-5}
Analysis		CMYK				
Regression Statistic	R^2	0.708	0.591	0.8284	0.8463	0.7364
	Adjusted R^2	0.7603	0.3667	0.7599	0.697	0.6857

	Standard error	0.3270	0.3110	0.3390	0.0050	0.2640
	Significance F	0.0001	0.0104	0.0007	0.0005	3.30×10^{-5}
ANOVA test	Variable	9.00×10^{-8}	5.00×10^{-8}	1.00×10^{-7}	1.00×10^{-7}	2.00×10^{-8}
	P- value	0.0001	0.0104	0.0006	0.0006	0.0003
	Intercept	0.6778	0.4460	1.1394	1.4380	0.4136
	P - value	0.2242	0.0739	0.4729	0.1512	0.7499
Analysis		HSL				
Regression Statistic	R²	0.5692	0.7903	0.6115	0.8802	0.6645
	Adjusted R²	0.5282	0.7633	0.5845	0.9582	0.6245
	Standard error	0.4890	0.2230	0.3400	0.0050	0.2370
	Significance F	0.0034	0.0007	0.0007	0.0003	1.35×10^{-5}
ANOVA test	Variable	4.00×10^{-7}	2.00×10^{-7}	6.00×10^{-7}	7.00×10^{-7}	1.00×10^{-7}
	P- value	0.0034	0.0006	1.28×10^{-7}	0.0003	1.35×10^{-5}
	Intercept	0.2765	0.0875	0.2849	0.5189	0.2317
	P - value	0.3460	0.3194	0.0504	0.1626	0.0017

4.3.3 Nutrient removal efficiencies

During cell development, microalgae require nitrate and phosphorus as essential nutrients for metabolite cycle and biosynthesis [173]. The industrial biscuit waste powder is made up of organic substrate and micronutrient components that promote microalgae development. This study found that the nutrient available in the combination medium is adequate to commence and improve microalgae development, although the nutrition available in the combined medium are being substituted as a fraction in 100C medium. In Figure 4.7, the nitrate concentration data for various molasses mediums demonstrate a decreasing trend across the culture time. Microalgae's ability to effectively absorb nitrogen and phosphorus gives biscuit manufacturers an opportunity in recycling their powder waste into a sustainable energy source. Thus, determining the optimal integration ratio of industrial biscuit waste powder in 100C to enhance microalgae growth rate and bio-product recovery becomes a worthwhile endeavour.

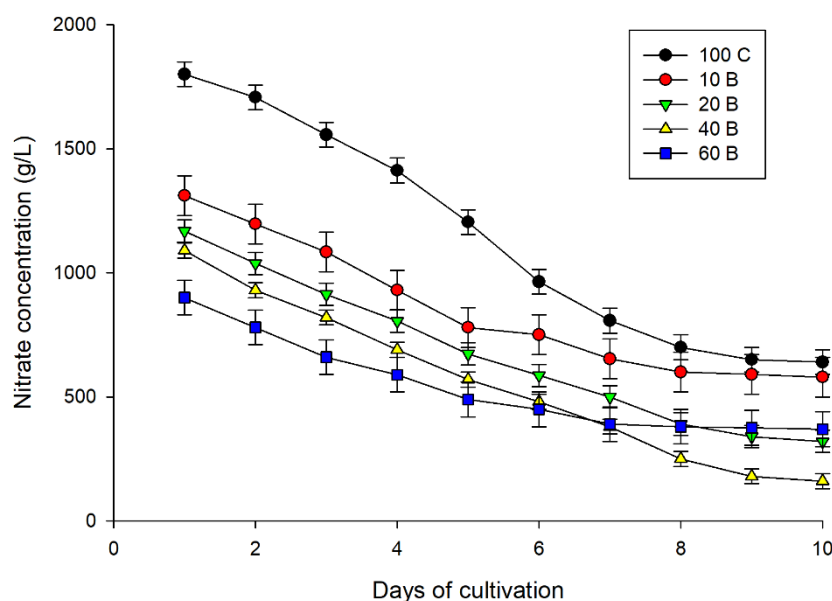


Figure 4.7: Nitrate concentration during the growth of *Chlorella vulgaris* grown in various combinations of BG-11 and biscuit industry waste powder medium, B.

Although the biomass growth in industrial biscuit waste powder integrations was already reaching its stationary phase (10th day), Figure 4.7 indicates that the nitrate content was still decreasing. However, in 100C following the 9th day, the nitrate reduction slows down and tend to reach steady state. This is merely because the microalgal biomass in 100C has attained its maximum growth productivity despite the amount of nutrient available. 40B had the greatest nitrate removal (58%) within the first four days, followed by 100C (49%), 20B (45%), 10B (41%), and 60B (36%), respectively. Despite the high initial nitrate concentration of the inorganic medium, microalgae development is somewhat slower in 100C than in a mixture of organic and inorganic mediums. This is because, integration of industrial biscuit waste powder and 100C speeds up the growth process by providing an adequate nitrate condition, during the initial days of the cultivation. Besides, the nitrate removal stagnant at day 6 for 60B which simultaneously defect the biomass growth. This can be due to the lack of light penetration since the beginning of its culture. Figure 4.7 illustrates that *chlorella vulgaris* cells in 40B consume 86 % of nitrate from their medium, followed by 20B (72 %), 100C (64 %), 10B (56 %), and 60B (52 %) combinations on the 10th day (end of cycle). This removal percentage implies that

the growth phase of a cultivation process is influenced by both light penetration and nutrition availability.

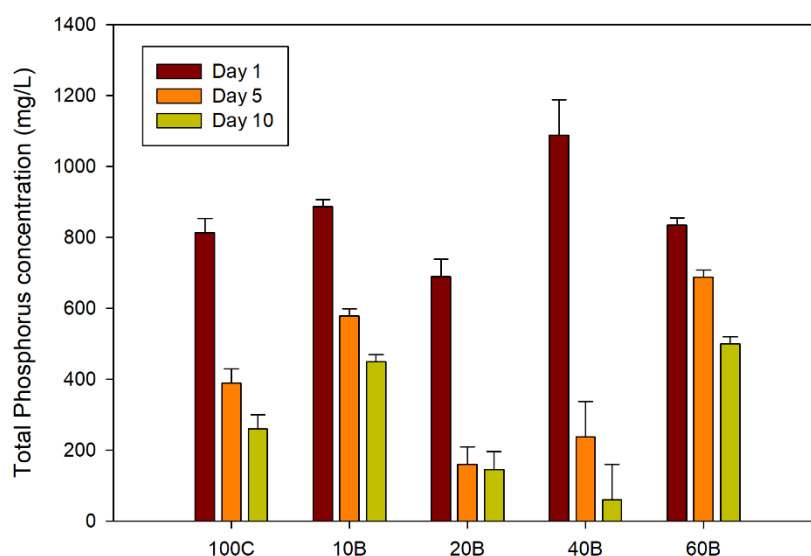


Figure 4.8: Removal percentage of phosphorus content in various combinations of BG-11 and industrial biscuit waste powder medium, B.

In microalgae, polyphosphate is the dominant speciation which makes phosphorus, P an essential nutrient to uptake for the growth process [173]. When the phosphorus supply is abundant, *chlorella* species can accumulate phosphorus as polyphosphate granules and able to accumulate up to 3% P in the biomass [174]. In comparison to 500 different varieties of wheat flour, biscuits have the best availability of phosphorus [175]. This is because phosphorus is the main mineral and most of it is used to source energy and other essential cellular functions, especially in children under the age of ten. Thus, in this report, it was suggested that industrial biscuit waste powder be used as a phosphorus supply for the growth of microalgae. The inclusion of industrial biscuit waste powder to 100C modified the culture medium to have various phosphorus content, as seen in Figure 4.8. As a result, the phosphate evaluation aids in determining the best culture medium for an optimum mixture ratio for the growth of *chlorella vulgaris*.

It is observed that, 40B has the highest initial phosphorus content at 1088 mg/L followed by 60B, 10B, 100C, and 20B at 887 mg/L, 835 mg/L, 813 mg/L, and 689 mg/L respectively. The initial phosphorus content of each mixture culture varies according to the dilution ratio, despite the fact that the mass of industrial biscuit waste powder was set at 2g. Although the initial phosphorus content is high, this does not guarantee that the microalgae can grow well. The intake of nutrients is also affected by the microalgae species as well as other factors such as light penetration, culture temperature, and pH [162]. Thus, identifying the optimal ratio of industrial biscuit waste powder solution to 100C solution is vital. Besides, the phosphorus removal reduction percentage occurs mostly from day 1 to day 5 than from day 6 to day 10 of the cultivation process. This is why the biomass growth was so rapid within the first 5 days. Based on Figure 4.8, *chlorella vulgaris* cells in 40B consume 94 % of phosphorus content from their medium, followed by 20B (79 %), 100C (68 %), 10B (49 %), and 60B (40 %) combinations on the 10th day (end of cycle). It is also observed that, the culture medium contains a high initial phosphorus content for 60B, the algae cell did not react favourable. This is because, excessive phosphorus content decreases the energy supply for microalgae cells to uptake nutrients for optimal microalgae growth [149] Moreover, limitations in phosphorus and nitrate could provide an ideal environment for cell species to produce lipid, protein, and carbohydrate toward the stationary process. Furthermore, the integration of industrial biscuit waste powder with 100C decreases the cultivation time to 10 days due to its sufficient nitrate and phosphorus concentration in the modified culture medium, which increases the growth and bio-composition properties of the cells in a short period of time. To determine the optimal industrial biscuit waste powder, detailed lipid, protein, and carbohydrate analyses are performed.

4.3.4 Biochemical compositions

4.3.4.1 Lipid content

Microalgae biomass have attracted international interest, due to their capacity in biofuel production. The major economic disadvantage of algae species in the past is either they produce high biomass production with low lipid accumulation, or high lipid accumulation with low biomass production [176]. However, with the use of industrial biscuit waste powder, the cultivation process improves both microalgae biomass growth and lipid content simultaneously.

Table 4.3: Biochemical composition of *Chlorella vulgaris* in different combinations of BG-11 and industrial biscuit waste powder medium, B.

Culture combinations	Lipid (mg/g)	Protein (mg/g)	Carbohydrate (mg/g)
100C	206.70 ± 5.3	246.18 ± 3.0	294.90 ± 4.9
10B	196.80 ± 4.1	188.88 ± 0.2	244.66 ± 4.2
20B	220.64 ± 5.6	254.82 ± 4.0	318.10 ± 8.1
40B	260.44 ± 2.4	263.93 ± 3.9	418.99 ± 1.9
60B	165.05 ± 5.5	175.09 ± 0.9	171.88 ± 0.4

Note: Results shown are the sum of three readings ± SD

Based on Table 4.3, the lipid concentration for 40B was greatest at 260.44 mg/g, followed by 20B, 100C, 10B and 60B, at 220.64 mg/g, 206.70 mg/g, 196.80 mg/g, and 165.05 mg/g respectively. The amount of nitrogen and phosphorus in a culture medium is considered to be a significant influence since it has a direct effect on algal growth kinetics, which is closely linked to nutrient uptake and lipid build up [177]. While microalgal cells are exposed to phosphorus deficiency, the biosynthetic pathways are changed, resulting in enhanced lipid synthesis as a response to this stress. This implies that the lipid formation is dependent on the nutrient absorbed proportion rather than the substitution ratio of industrial biscuit waste powder. Thus, the lipid content in 40B was determined to be the maximum since it absorbs 94% of the phosphorus content in the medium, resulting in a stressed environment.

Amongst the most important induction factors for initiating and maximizing lipid accumulation in microalgae is nutrient depletion. In this analysis, nitrate deficiency begins on the eighth day of cultivation and extends until the tenth day. With only two days of starving, there is a significant variation in lipid content, allowing the optimum organic medium replacement ratio to be identified. Previous studies on *Chlorella* species algae revealed that nutrient deprivation for 4 days boosted lipid accumulation to 58.39 mg/g without shortfall in biomass development, making it a possible cost-effective treatment for cheaper biofuels [178]. However, the greatest lipid content obtained in this investigation was 260.44 mg/g for a 40B mixing combination. Furthermore, light penetration influences lipid synthesis. This is due to the fact that microalgae absorb light for photosynthesis and store energy reserves such as lipids. When light intensity is low, microalgal biomass concentration declines, resulting in poor growth and an unfavourable influence on lipid accumulation. Meanwhile, excessive light intensity causes photoinhibition, which damages microalgal photosystems and lowers lipid formation [179]. Hence, appropriate light penetration must be supplied during culture to promote the development of microalgae. (Mandotra et al., 2016) reported that the greatest lipid content of 203.15 9.1 mg/L was obtained when the culture was exposed to a light intensity of 6000 lux [180]. Meanwhile, 260.44 mg/g was accumulated in this investigation for 40B under 6000 lux (LED light). In brief, a growth medium with increased phosphate and nitrate deprivation and appropriate light penetration would yield an effective lipid from cultured biomass.

4.3.4.2 Protein content

Protein and polysaccharides co-production is common in dairy food processing due to its extreme value-added molecules content and lack of toxicity in the production flow [181]. Meanwhile, microalgae protein has a high quality and amino acid profile, making it a good fit for functional foods, health care goods, and animal feed additives [182]. Hence, the industrial biscuit waste powder medium was combined with 100C as the cultivation medium and, the

protein build-up in the *chlorella vulgaris* was investigated. According to Table 4.3, the greatest protein content in 40B at 263.93 mg/g followed by 20B, 100C, 10B, and 60B with protein content of 254.82 mg/g, 246.18 mg/g, 188.88 mg/g, and 175.09 mg/g. The percentage of nitrate absorption in microalgae cell is the key cause of variations in protein formation. Nitrogen is found in biological macromolecules such as proteins, peptides, enzymes, and algae, and it plays a critical role in converting nitrogen (NO_3 , NO_2 , and NH_4^+) to its organic formula through the assimilation mechanism [183]. Based on Figure 4.7, the biomass uptakes 85% of nitrate from the medium during the cultivation process, indicating that the 40B medium mixture achieves the most transformation.

The amount of protein produced by *chlorella vulgaris* grown in control and nitrogen-starved cultures. Protein rise was 7% more significant in 40B nitrogen-starved cultures compared to 100C culture calculated on the tenth day. Light condition during the microalgae cultivation period is another essential factor for protein production in microalgae, as previous studies reveals that increased light irradiance and longer photoperiod results in higher protein production [184]. However, the light penetration in this sample ranges for the first four days depending on the organic medium substitution ratio, despite the LED light intensity remained stable for the entire ten days. As microalgae absorbs light for respiration, cell division, and accumulating nutritive quality, this is where fluctuation in protein content may occur. In comparison to a previous analysis, 16 hours of light and 8 hours of darkness produced 13.25 mg/ml of protein content in *chlorella vulgaris* [185]. Meanwhile, this research uses a 24-hours light source that can produce 263.93 mg/g at maximum for 40B, demonstrating that providing continuous light can provide an ideal environment for bio-composition formation in microalgae cells.

4.3.4.3 Carbohydrate content

Carbohydrates are the main products of photosynthesis via carbon fixation metabolism known as Calvin cycle [186]. These carbohydrates are either stored as backup materials in the plastids (e.g., starch) or become the primary component of cell walls (e.g., cellulose, pectin, and sulfated polysaccharides). Nevertheless, depending on culture conditions including irradiance, nitrate depletion, temperature change, pH transfer, and CO₂ supplementation, the distribution and synthesis of carbohydrates (mainly starch and cellulose) in microalgae can differ significantly [187]. Two key parameters that lead to the development of carbohydrate in *chlorella vulgaris* cells in this study were nitrate starvation and light penetration. The largest carbohydrate value was discovered in 40B, (418.99 mg/g) and followed by 20B (318.10 mg/g), 100C (294.90 mg/g), 10B (244.66 mg/g), and 60B (171.88 mg/g). As compared to the combination of food compost using 100C as inorganic medium, 40B synthesizes 20% more carbohydrate content in *chlorella vulgaris*, FSP-E [188]. This is because environmental tension varies (typically nitrate limitation). Generally, when microalgal cells are exposed to nitrogen depleted culture medium, poor cell growth (or lower biomass productivity) occurs, inhibiting biochemical composition [187]; however, in this research, the *chlorella vulgaris* species was able to increase lipid, protein, and carbohydrate content without compromising cell growth rate. Furthermore, providing adequate illuminance is necessary for microalgal development, as light source may provide energy that is then stored in the microalgal biomass as carbohydrates or lipids [189]. This analysis used a 24-hours LED light supply of 6000 Lux, and light penetration was observed to cause carbohydrate aggregation in microalgae. Previous research has shown that the light intensity from 40 to 400 mol/m²s¹ causes an increase in carbohydrate aggregation from 269.62 mg/g to 343.84 mg/g [190]. Overall, the results show that the medium integration of 40B produces the best combination of lipid, protein, and carbohydrate content of all the medium combinations.

4.4 Conclusion

The combination of industrial biscuit waste powder with control medium, BG-11 (100C) culture for microalgae development has both economic and environmental benefits. This is because, rather than dumping the waste powder, it can be mixed with growth media to promote microalgae biomass with valuable bio-compositions including lipid, protein, and carbohydrate. This study concludes that the combination ratio of 40B as culture medium provides the highest microalgae biomass concentration (4.08 g/L), lipid yield (260.44 mg/g), protein (263.93 mg/g) and carbohydrate content (418.99 mg/g). Furthermore, the primary problem in large-scale microalgae cultivation monitoring has been addressed by presenting image-colour quantification as an alternative to using a UV-vis spectrophotometer. As the *chlorella vulgaris* biomass grows, it shifts colour from cloudy white to light green then to dark green culture solution and with the use of suitable colour model a digital monitorization device can be set up for future development. Image quantification using simple linear regression identifies the appropriate colour model for this investigation with adequate precision and accuracy. Overall, this research suggests that 40% of the industrial biscuit waste powder can be replaced with an inorganic medium for microalgae production, and that the RGB model is the optimal colour model to utilise to monitor *chlorella vulgaris* growth for future automated microalgae growth monitoring development.

CHAPTER 5

LIFE CYCLE ANALYSIS: A COMPARISON OF *CHLORELLA VULGARIS*, FSP-E CULTIVATION PATHWAY FOR INDUSTRIAL SCALE

Overview

Scaling up algal cultures to large volumes for commercial production is a challenging task that involves high capital and operating cost. Hence, the feasibility of a laboratory scale culture process is important to be tested, before expanding the production. This study aims to investigate the scaling up of *Chlorella vulgaris*, FSP-E cultivation by discovering the best culture pathway using life cycle assessment, LCA. The study also emphasizes on the techniques that can be used in large-scale algal operations, for photo-bioreactor cleaning and maintenance. Culture mediums plays a significant role in overall costing for microalgae growth processes, and their supply were addressed during the scaling up cultivation systems in terms of flow mixing, pipeline costing. Cradle to gate LCA technique was used in this study considering every phase in the lifecycle, starting from culture medium, carbon dioxide (CO₂), air, and water supplies for microalgae growth till end-product dried biomass generation, including storage. Three growth media applications were compared between (1) BG-11, a chemical-based medium; (2) a combination of biscuit waste from a dairy processing facility with BG-11; and (3) re-cycling of Path 2's residual culture medium after harvesting. The results indicated that recycling of remaining culture water obtained from dried biomass back into the photo-bioreactor was identified to be the most sustainable *Chlorella vulgaris* production process that can be considered for a commercial facility for bio-fuel production.

Keywords: Life Cycle Analysis, Scaling-up, Biscuit Waste Powder; Environmental; *Chlorella vulgaris*; Cultivation

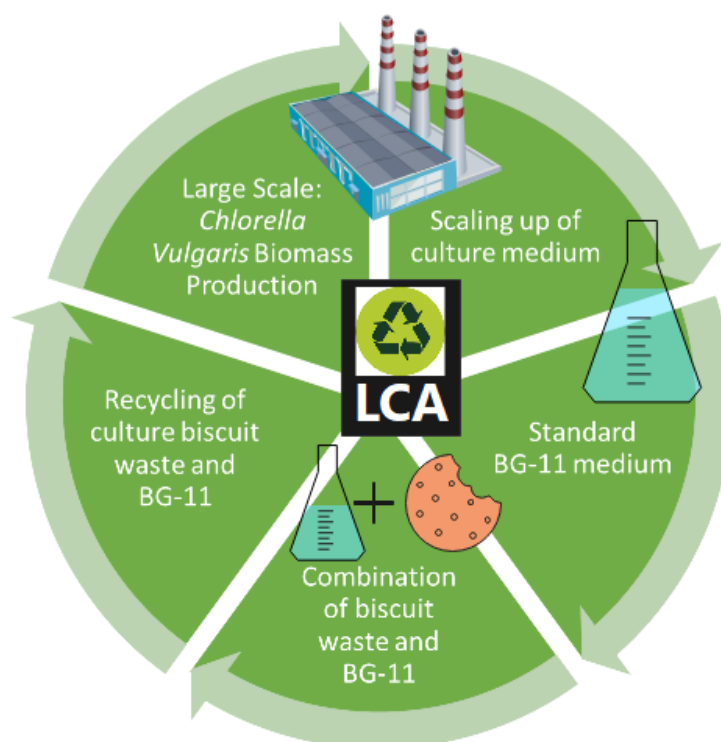


Figure 5.1: Graphical abstract of life cycle analysis for a comparison of *Chlorella vulgaris*, FSP-E cultivation pathway for industrial scale.

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5.1 Introduction

European Commission (2019) acknowledged the relevance of algae for green bio-economy, given to their function in the environment as well as their potential for commercial uses that reduce reliance on land-based fossil fuel commodities such as coal, petroleum and natural gas [191]. This is due to the fact that microalgae have the capacity to convert water, sunlight, and carbon dioxide, CO₂ into valuable compounds such as lipids, protein, carbohydrate which can also be further processed to produce bioenergy in replacing fossil fuel usage [192]. Microalgae

have been widely recognised recently as a viable bioenergy source through lipid extraction due to significant benefits over other bioenergy feedstock, such as a faster growth rate than terrestrial plants and the ability to be cultivated on non-productive land [3]. Therefore, extensive works have been reported primarily on the cultivation of microalgae for energy purposes as an alternative to present fossil-based sources [37,194]. Despite all the discoveries on microalgae-based processes, large-scale microalgae cultivation for commercial generation of biofuels is currently very limited. Photo-bioreactors have a larger volumetric yield than open ponds and therefore are recommended for microalgae production. This is because, photo-bioreactors have been reported to have excellent energy retention, extremely efficient utilisation of the culture area, decreased open air pollution, and variable energy consumption values for mixing and gas/liquid mass transfer [195,196].

It is well established that microalgae may be grown on non-arable area, clean wastewater, which could generate fuel and other useful by-products in the process [197]. Based on land usage and production potential, biodiesel made from microalgae has the potential to replace crude oil diesel [198]. There are many different design configurations for producing microalgae biodiesel, and not all of them yield the same advantages or have the same consequences when laboratory studies were scaled up. Thus, Pérez-López et al.,(2017) proposed that Life Cycle Assessment (LCA) method has the ability to be utilized as a decision-making tool in supply chain management, and operation flow as it can identify the constraints to be tackled during the scaling up of industrial facilities that are sustainable [199]. Besides, a LCA methodology could evaluate, and contrast different configurations based on the cultivation parameters that would be extremely beneficial to the industry as these comparisons can be used for research development and investment purposes. Since, microalgae culture has been identified as a critical phase that has a considerable impact on downstream bio-refinery processes, the LCA framework was used in this study was primarily focused on microalgae cultivation process

configuration. Moreover, an LCA framework has the potential to also compare various cultivation technology by considering environmental differences caused by local climates of the cultivation site.

Microalgae based products has the potential for large-scale use, and various organizations are aiming to strengthen the technology to promote all across the world [200]. As the sector is rapidly evolving, demanding the development of an LCA tool that can ensure the sustainability and viability of the system by producing meaningful insights for decision makers and research experts to expand into pilot scale plant [201]. Based on the literature and lab scale investigation, Cinar et al.,(2020) reported that *Chlorella* and *Spirulina* species were the most utilized in the synthesis of both biopolymers and plastic blends due to the growth in global plastic waste pollution [202]. Hence, an LCA analysis was necessary to determine the best culture path based on the effects of sub-processing technologies on resource demand (land usage), economic feasibility, and environmental impact indicators [203]. It was discovered that inorganic medium (BG-11) can replace 40% of the industrial biscuit waste powder, and that the RGB model is the appropriate colour model to monitor *Chlorella vulgaris* growth for future automated microalgae growth monitoring [204]. Hence, this study focuses on investigating the upscaling process feasibility of industrial biscuit waste powder as an alternative cultivation medium using LCA framework (GaBi Software). Three different scenarios were evaluated to find an effective pilot-scale microalgae cultivation pathway. Previous chapter evaluated the laboratory scale cultivation pathway; however, this section focusses on the pilot scale process flow and its feasibility.

5.2 Methodology

Constructing a LCA framework includes defining life phases based on existing academic and industry information, doing a data gap analysis, evaluating LCA focus, creating functional units, and verifying the framework with industry and academic data. The following is a list of

the significant actions taken in this research from the ISO 14040 series (ISO, 2006a) [205]: (1) Identify the stages of cultivation for process flow model setups; (2) Comparisons of existing LCAs from previous literature in model building and data gap analysis for reliability; (3) Prioritize the LCA goal on replacing of organic culture medium; (4) Define the functional units that will be utilised in all configurations; (5) Establish the process flow variables for configuration design; (6) Construct and define the LCA framework's default case (Inorganic cultivation system); (7) Identify and create a data gathering checklist for the LCA framework, such as microalgae composition, industrial biscuit waste powder characteristics; (8) Build the LCA framework in GaBi, via objects, flows, and parameters feature; (9) Comparison framework scenarios between three possible case studies.

5.2.1 Microalgae description

Chlorella vulgaris was discovered by a Dutch scientist named Martinus Willem Beijerinck (green algae) [206]. *Chlorella* species are diverse and may be found thriving in freshwater, marine, and soil habitats. The *Chlorella* genus has approximately 37 taxonomically acknowledged species [207]. In this study, the *Chlorella vulgaris* species was chosen and acquired from a reservoir field in southern Taiwan. Ahmad et al.,(2020) reported that the diameter of *Chlorella vulgaris* ranges from 2-10 μm spherical in shape [208]. Moreover, common mineral compositions observed in *Chlorella vulgaris* were calcium (399.6 mg/kg), magnesium (93.24 mg/kg), phosphorus (199.8 mg/kg), potassium (319.7 mg/kg), sodium (359.6 mg/kg), and sulphur (186.5 mg/kg), while trace element composition, mg/kg^{-1} included copper (102 mg/kg), iron (11,101 mg/kg), manganese (454 mg/kg), and zinc (64 mg/kg) [209]. Considering their excellent photosynthetic capacities and high growth rates, *Chlorella vulgaris* are regarded as promising biological resources. Thus, *Chlorella vulgaris* is commercially grown for uses in the food sector due to high protein content, as well as for specialized oils in both cosmetic and nutraceutical industries [210]. The composition of algal species changes

depending on the culture environment such as light, temperature, pH, salinity, and medium nutrition levels. In this study the cultures were at $25 \pm 2^{\circ}\text{C}$ under 24 hours (6000 lux LED light) for a consistent growth efficiency throughout the cultivation period.

5.2.2 LCA goal and scope definition

The life cycle assessment (LCA) is a standardized technique for quantifying environmental consequences associated with any product or service across its full life cycle, from raw material acquisition to manufacture, transportation, packaging, and finally the good's end-of-life. However, this evaluation used a cradle-to-gate method (a partial product life cycle from resource supply through product manufacture, including all associated transportation). The purpose of this study was to use LCA to compare the environmental performance of various scenarios and potential consequences of algae farming. LCA allows a thorough evaluation of the investigated system's environmental performance across the whole process chain, from introducing in the industrial biscuit waste powder through microalgae culture medium, bio-flocculation coupled with tangential flow filtration, rotary drum dryer, and algal biomass storage. The main goal of this investigation was to evaluate three alternative cultivation frameworks for *Chlorella vulgaris* large scale production utilising BG-11, as well as its integration potential with organic medium (industrial dairy waste powder) as culture medium and identifying the most sustainable pathway that should be considered in commercialization. The LCA layout was separated into foreground and background processes, which refer to the steps that are directly impacted by the research and the processes that provide energy and materials *via* a standardized market, respectively [211]. In this study, the system boundary is based on a cradle to gate model where foreground systems were classified as disinfection of reactor, formulation of growth medium, algae growth process, and biomass drying; whereas

ground water supply, electricity source, carbon dioxide gas, and air input will be referred as background systems for the production cycle.

5.2.3 Overview of LCA framework flow

The key processes examined in the production chain of this study were cleaning and sterilisation, cultivation of microalgae, bio-flocculation coupled with tangential flow filtration, and rotary drum dryer. Microalgae cultivation can occur in a variety of technical configurations, which can be grouped into two categories: open and closed systems [195]. The existing large-scale industrial growing methods developed microalgae in open ponds using a paddle wheel that circulates microalgae cells, nutrients, and water while continuously being exposed to the environment [212]. However, closed cultivation systems such as photobioreactor (PBRs) could reduce contamination hazards and control parameter management for greater production with high-value to the biomass [213]. Thus, a cylindrical horizontal vessel PBR tank was examined in this study. As microalgae grows in a closed environment, regular tank cleaning and sterilization were considered in this configuration to prevent contamination. This was readily accomplished by utilising automated cleaning systems. After several cycles of cultivation, a biofilm layer is often visible as a slimy coating of sediments on the PBRs wall with a thickness ranging up to hundreds of micrometres, and it can limit the effectiveness of light penetration into the culture medium. These challenges were negligible for laboratory cultivation process, whereas for large scale cultivation plant it has to be considered.

There are several techniques for removing biofilm on the illuminated surface of PBRs, including the use of the cleaning agent material, optimal hydrodynamics, mechanical cleaning, and ultrasonic technology [214]. Öztürk Kerestecioğlu et al.,(2019) discovered that a vertical-column PBRs have excellent mixing capabilities that reduces the inner surface fouling which makes the force cleaning easier [215]. Hence, in this investigation a disinfection agent (Freebac-Clearoxyl) was used as a cleaning agent. A non-toxic disinfectant that eliminates both

organic and microbiologic contamination [216]. The solution is highly effective, as it does not require any chemical agitation, mixing with groundwater with minimal dosage is sufficient enough. Precisely, Freebac-Clearoxyl has a maximum active duration time up to 110 hours with a minimal 5 minutes application time that reduces the downtime of the new cultivation cycle [217]. Moreover, the drained cleaning waters can be added into the wastewater stream at the end of the culture as there will be no hazardous residues left behind. Previous study also proved that the Freebac-Clearoxyl® was used as a cleaning agent for the cultivation of *Nannochloropsis* in natural seawater [199]. Overall, Freebac-Clearoxyl® can reduce the cleaning process cost substantially. To guarantee the absence of competing algae and protozoa, each reactor was cleansed with the chosen disinfection agent and sterilised with tap water prior to inoculation. The system was washed three times with tap water in the case of the cylindrical shaped PBRs. Tap water was injected into the surface of the PBR column in the initial step of the cleaning procedure to eliminate any accumulated algae stains on the wall surface. The PBR column was then rinsed with 3% disinfection agent (including hydrogen peroxide), while the third one utilised 0.5 g/L plastic beads for biofilm removal with a tap water rinse. The first two procedures can be automated using a clean-in-place (CIP) spray system [218]. Subsequently, a vacuum cleaning system was utilised to remove all water for 1-2 hours to avoid any external contamination onto the new fresh medium.

5.2.3.1 Photobioreactor

The 2500 L column PBR require working space of 4.27 m length x 2.44 m width x 2.79 m height) was inoculated for the cultivation of microalgae [219]. *Chlorella vulgaris* in fresh culture medium, industrial biscuit waste powder medium, and carbon dioxide are the primary feeds that will be transported into the vessel throughout the cultivation process. Microalgae can absorb carbon dioxide as carbon source and synthesise valuable compounds at the same time. The flue gas, recovered from power plants can be directly mixed with atmospheric air via the

pipeline stream into the PBRs [220]. The previous laboratory scale findings about the formulation of culture medium and the major source of nutrients for the growing of *Chlorella vulgaris* were retained [204]. The PBRs in this study were made of transparent glass vessels. This is to ensure continuous light penetration, and also ease the inner wall cleaning methods [221]. Moreover, glass vessel tanks can last for more than 50 years and also have the ability to withstand the pressure of a large quantity culture medium in it [222]. Although the PBRs in this study were kept indoor, it is important to select material that could avoid internal corrosions and are highly resistant to heat. LED lights with 6000 lux were installed around the cultivation vessel to keep a 15cm distance from the glass vessel surface for equal distribution of light into the PBR. Four LED light stands were placed around the culture tank, each at a 90° angle to each other's. LED generates monochromatic light that is continuously supplied and only provides the wavelengths of light that are more beneficial for algae growth compared to sunlight [223].

Besides, the culture temperature was maintained in between 25 - 30°C (ambient temperature). A mechanical agitator mixer with 500 rpm speed was installed in all the PBRs tanks, for the stirring of algae culture for optimal biomass growth. Additionally, industrial flue gas (CO₂) was merged with compressed atmospheric air and transported directly into the culture medium at 13.6 m/s (approximately 5mm bubble diameter) [224]. The culture feed was supplied into the PBR vessels by the opening chamber pump at the top of the vessel, while the CO₂ and air were introduced via an intake chamber pump in the middle of the tank. The cultivation vessel's only outflow is placed at the bottom of the vessel, allowing for complete draining if necessary. Total draining is only necessary during tank maintenance or cleaning. Furthermore, after the 7th day of *Chlorella vulgaris* cultivation, a part of the culture will be transported out for bio-flocculation by tangential flow filtering. According to Soomro et al.,(2016) bio-flocculation coupled with tangential flow filtration is the most promising industrial microalgae dewatering

method with the lowest energy consumption [95]. Then, the cultivation vessel will be refilled with an equal volume of culture media. After the 7th day of culture, a semi-continuous operation was conducted in which the biomass was harvested at a fixed dilution rate ranging from 20 to 40 L/day.

5.2.3.2 Transportation

The PBR system requires pipeline connection for all the inlet chambers. In this study, silicon rubber connections were used for lab scale, as the inlet volume is small and the flow pressure from the CO₂ tank (0.5 vvm) is sufficient for the air and CO₂ gas supply. However, bigger diameter pipelines and greater pressure pumps are necessary for large volume cultivation, particularly for the nutrients supply stream. This is due to the possibility of organic nutrients clogging the pipeline's walls. As a result, peristaltic pumps were installed with platinum-cured silicone tubing, allowing culture supplies to be pushed into the PBR system. Peristaltic pumps can be easily controlled without the need of any extra energy, by the implementation of a digital control system on Arduino Uno R3 [225]. This transportation technique was implemented throughout the cultivation process.

5.2.3.3 Dewatering technology

Water comprises most of the composition of cultivated microalgae. One of the most difficult challenges in algal biofuel production is the recovery of lipids from microalgae. Patel et al.,(2021) reported that, a non-polar organic solvent such as hexane is a typical economical commercial solvent that is well-suited to extract lipids in large scale from dry algae [226]. This is because the presence of moisture in algae creates a substantial barrier for the extraction of lipids using a non-polar organic solvent [227]. Thus, prior to lipid extraction, algae are recommended to be dried. Biological dewatering processes are recognised for being low-cost and sustainable separation approaches due to the low energy consumption, lowering energy needs, and lack of synthetic chemical usage [228]. It is necessary to develop an efficient,

cost-effective, and environmentally acceptable harvesting and dewatering process for commercialising microalgae biomass-based biofuels. In regard, Uduman et al.,(2010) discovered that the primary goal of dewatering for biofuels production is to concentrate the dilute microalgae suspension of about 0.02–0.06 % to 5–25 %, which is achievable in a two-step dewatering process where the primary stage is aimed at 2–7 % and the secondary stage produces 15–25 % of microalgae slurry [229]. In terms of energy consumption, CO₂ emissions, and cost, the two-stage microalgae dewatering by bio-flocculation combined with tangential flow filtration, TFF process is the most appealing, with relatively low values for each parameter when compared to centrifugation as a single step and other two-step dewatering systems [95]. Moreover, Weschler et al.,(2014) reported that the tangential flow filtration (TFF) technique could achieve harvest efficiency of up to 89% with only 0.2 kWh/m³ [230]. Hence, in this study the two-step microalgae dewatering by bio-flocculation (co-cultivation of microalgae *via* filamentous fungi) with 99% harvesting efficiency followed by tangential flow filtration [231]. Moving forward, the wet algae were designed to be dried in a rotating drum dryer, resulting in dry powder biomass. Figure 5.2 illustrate the tangential flow filtration working principle. The final drying process used was determined by the size of the operating condition as well as the end use of the dried product. In a study comparing spray drying and drum drying methods for microalgae to produce bio-fuel (high lipid content), the latter was suggested due to greater digestibility, reduced energy consumption, and cheaper investment [232].

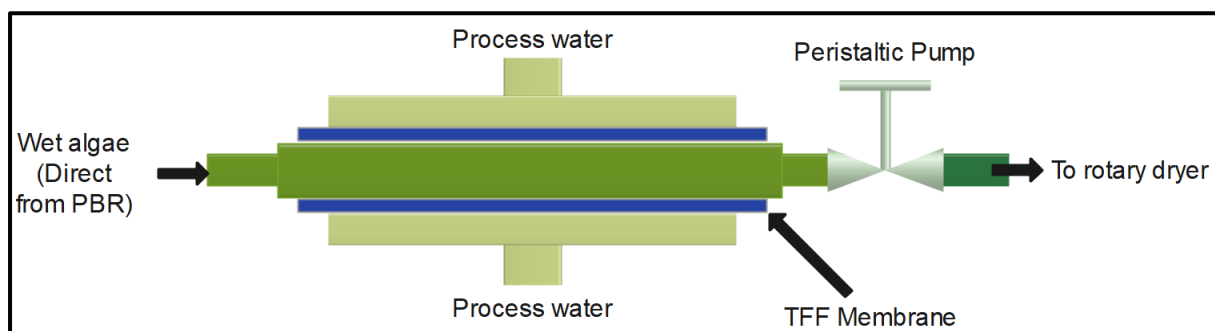


Figure 5.2: Working principal of tangential flow filtration.

5.2.3.4 Biomass storage

Drying of microalgae biomass is widely recognized as an efficient method for extending shelf life and allows storage at room temperature [233]. However, the algal biomass tolerance to time and temperature must be known so that its yield is not influenced by biomass and lipid degradation prior to recovery. In this study, the dried biomass was stored in a storage tank at -20°C to at least reduce the degradation of bio-composition. A cost-benefit study revealed that microalgae storage temperature at -20°C with vacuum packed preservation could sustain a maximum of 20 weeks with 12% loss in bio-composition stability [234]. Since daily biomass output will be substantially high, the biomass extraction and bio-refinery process may be carried out each day, and biomass export can also be considered. The inputs considered for this phase were solely based on the electrical consumption of both chiller and vacuum mode storage tank operation.

5.2.4 Economic assessment

The economic influence of alternative goods or product designs must subsequently be considered in the private sector decision-making settings addressed by LCA. However, neither the internal nor the external economic elements of the decisions are covered by the established LCA approach, nor are they adequately handled by conventional economic analysis. In this study, both the upstream processes (cultivation and drying) of microalgae, were considered as continuous operation throughout the year with clean up time every 12 weeks. The economic strategy was scaled up based on experimental data acquired from a laboratory-based culture from previous studies [204], as well as operational pilot-scale microalgae production units. Manpower is required for everyday operations at the industrial facility, particularly for PBR cleaning or maintenance (e.g., refilling of cleaning agent/inorganic media, maintenance of blocked pipes and defective reactors). The labour working hours were calculated independently from the cultivation hours, as maximum 2 hours of work force is needed for

each cultivation day. Each culture cycle takes fourteen days to complete, splitted as follows: 7 days of downtime for microalgae cultivation and harvesting, including PBR cleaning and maintenance; and another 7 days for starting another microalgae culture batch; for a total of 12 cycles based on a 2500 L industrial continuous PBR (Industrial Plankton) [219]. The study seeks to forecast the influence of various algal production techniques on total annual cost, energy demand, and individual cost contribution.

The capital costs are estimated by multiplying the total cost of equipment by a Lang factor [235]. The Lang factor was used to installation price including costs for piping, pumps, and electrical facilities. The Lang Factor is a widely used metric in industrial engineering to assess a plant's capital and operational expenses. It is an estimated ratio of the entire cost of developing a process inside a plant to the cost of all major technical components. The scaling factor rule was used to extrapolate the purchasing costs to different capacities and scales:

$$\frac{\text{cost } B}{\text{cost } A} = \left(\frac{\text{cost } B}{\text{cost } A} \right)^n \quad (1)$$

Where cost A represent the purchase costs of a unit operation with size or capacity size A and size B; n (scaling factor of the equipment).

The total annual costs, TAC (P_c) for harvesting-dewatering (bio-flocculation) are given by [236,237]:

$$P_c = (0.5I + M) \times C_I + \frac{C_I}{A} + C_e + C_c + C_l + (C_{loss}) \quad (2)$$

Where C_I represent the total investment cost (\$); I (rate of interest, %); M (the % maintenance costs); $\frac{C_I}{A}$ (yearly depreciation, \$/year over depreciation time, A); C_e (the energy costs, \$/year); C_c (costs for consumables, \$/year); C_l (costs for labour, \$/year); C_{loss} (optimal term which represent the loss of algae, \$/ year).

The production costs per kilogram produced algae biomass are:

$$P_{c,algae} = \frac{P_c}{Y_{total}} \quad (3)$$

Where Y_{loss} (total produced biomass in a year, kg/ year).

5.2.5 Life-cycle assessment

GaBi software was designed to assist industries with life cycle assessment, costing, reporting and working environments. GaBi's visualization method allows to freely replace the model sketch with own data or data from the database due to its straightforward user interface, drag-and-drop functionality, hierarchical database management, and comprehensive search capability. GaBi examines the effects of alternative manufacturing, energy sources, distribution, recyclability, and sustainability on each element from a life cycle viewpoint. In particular, GaBi databases protect manufacturers by enabling clients to produce and align their production process before scaling up large scale plant [238].

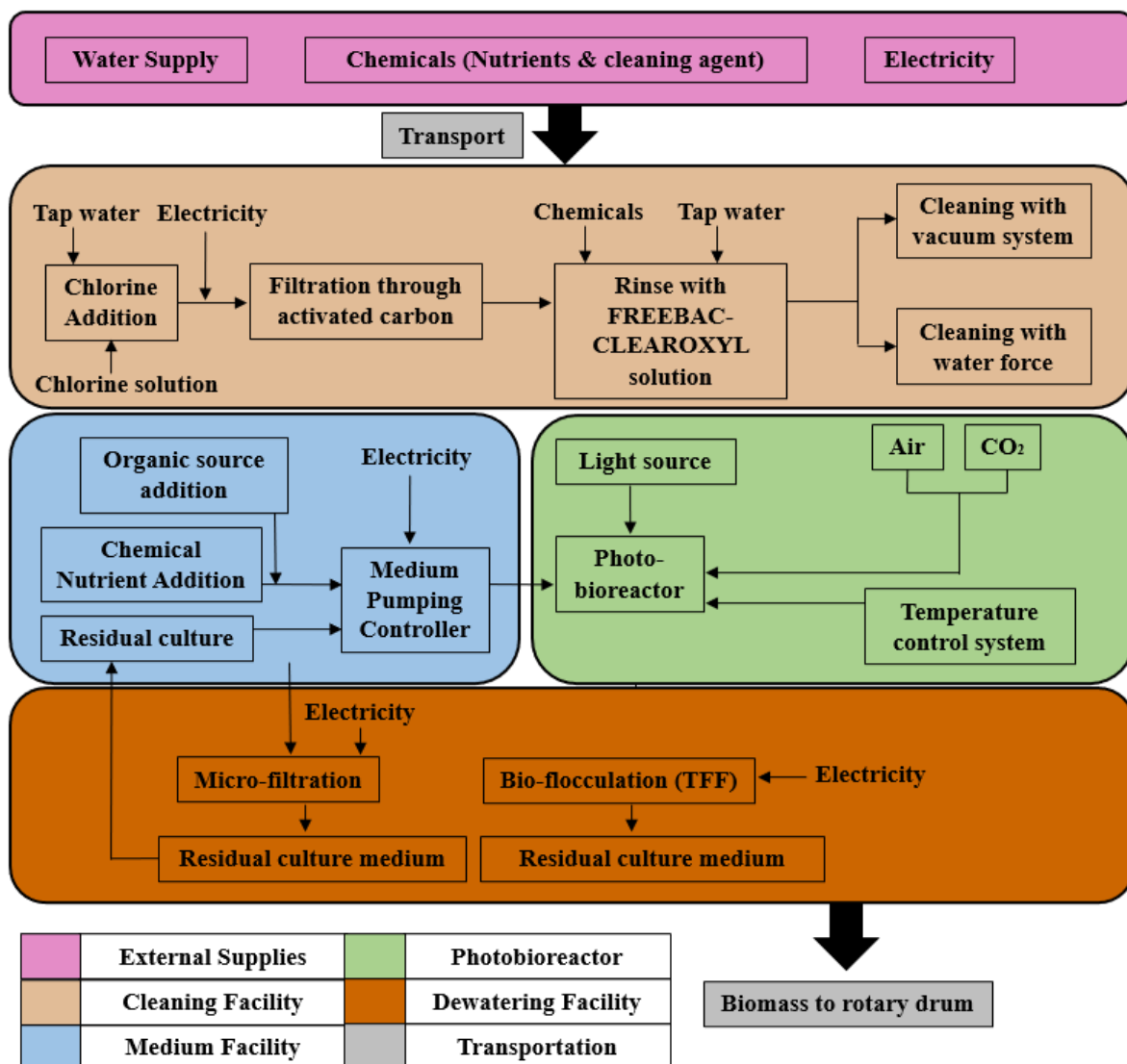


Figure 5.3: Pilot plant layout for a microalgae cultivation facility.

The database employs a flowchart technique that is easily adaptable to the development of an LCA framework. In this study, the evaluation was conducted utilizing GaBi ts: Education database 2020. The functional unit used in this study was considered as 1kg of *Chlorella vulgaris* dry biomass ready for harvesting in semi continuous mode. The boundaries include building facilities, motor engines usage, column distribution and land required for cradle to gate. Figure 5.3 depicts the production system, which indicate the process units that were considered in this study.

5.3 Results and discussion

5.3.1 Pilot scale microalgae cultivation facilities

Productivity, energy demand, and building materials of cultivation systems were acquired for the LCA inventory in the study was attained from previous laboratory study [204]. According to Araújo et al.,(2021) the most recent production units of algae have been identified in France, Ireland, and Spain, while the most microalgae units have been in Germany, Spain, and Italy [191]. This is primarily due to large-scale production locations that provide steady and appropriate environmental conditions throughout the development phase [239]. The main concern for industrial algal production is the need to design a cultivation technique with novel solutions to overcome the operations of large scale PBR by lowering the existing high infrastructure and logistical costs associated with an enhanced biomass yield. Thus, the location site chosen in this study was European, EU as shipping of dried biomass to all over the world will also be convenient. Moreover, Europe plays a central role in renewable energy technologies, especially in establishing institutional support mechanisms to promote the green energy market [240]. According to a global roadmap organized by the International Renewable Energy Agency (IRENA), the European Union is expected to substantially increase the proportion of renewable energy from about 17% to over 70% in their total energy usage which is 50% more than others regions such as India and the United States [241]. In regard, hydro power electricity source was selected as energy inputs of the LCA framework.

5.3.1.1 Scale up parameters

The sustainability factor is becoming extremely important in clinical research especially at initial phase of development. While novel materials are being created at the laboratory scale, one challenge that frequently arises is to demonstrate their “sustainability” comparison to existing process materials. Scaling up a laboratory process flow is a critical and fundamental element when it comes to LCA studies. Typically, when a process has been proven and refined

in the laboratory, it is up scaled in a series of phases before the actual plant is built as the practical scale up process will involve large amount of investment model. As a result, employing lab data, LCA technology may be used to scale up the synthesis of innovative materials. Similarly, this approach started on the laboratory scale and gives a technique for simulating industrial scale production using existing data with requiring literature understandings of large-scale microalgae cultivation behaviour. For instance, the processes in the laboratory are not directly linked to one another via tubing pipeline, and the vessels and equipment utilized are not equivalent to the reactors and machineries of a commercial facility. Hence, extrapolating from LCI data will not be feasible.

In this article, an engineering-based scale-up approach for simulating algal processes and conducting LCA investigations on a commercial scale using lab protocol, publications, and a patented document. The objective is to fill a gap by offering this framework that specially focus on using industrial biscuit waste powder for scaling up lab operations for LCA purposes and generating results that are comparable to a future potential industrial production. A five-step method was followed in the scale-up framework; (1) Actual laboratory data collection for process flow build up, inclusion of previous similar study research papers, and a patented document; (2) Generate a basic plant flow diagram that covers all of the processes which includes reactors, connection pipeline, and major equipment that can be used for industrial scale cultivation; (3) Each functional units, were scaled up in accordance with the framework's method; (4) The linking and integration of all the associated process stages' input and output data is then included; (5) LCA study was generated for proposed scale up framework to gather logical and systematically assembled data from laboratory. The scale up data presented in Table 5.1, was generated by the LCA model itself. When the layout model was completed, GaBi could balance its flowrates and generates an active project.

Table 5.1: Comparison of inventory table for lab scale microalgae cultivation using industrial biscuit waste powder and scaled up pilot scale model via LCA model.

Parameters	Lab - scale	Scale up (Option 1)
Electricity		
Source	Socket power	Hydropower
Daylight (LED), kwh/year	5.28	2,434
Stirrer, kwh/year	5.52	2,658
Total Voltage supply, kwh/year	5.76	3,658 (Including generator)
Water		
Source	Deionised water	Deionised water
Working Volume, L	10	14, 000
Volume per PBR, L	1	2500
Number of PBR required	10	6
Carbon Dioxide + Air		
Industrial Carbon dioxide flow, volume gas per volume medium per minute (vvm)	0.2	25792
Air source	Air pump	Compressed air, 7 bars
Combined air flowrate, m ³ /h	0.2 – 0.4	25,000 – 40,000
Air pump energy/ Pipeline, kwh/year	0.556	1,390
Biscuit Waste Powders		
Mass per litre, g	2	2.80 x10 ⁵
Biscuit Powder Compositions (wt%)		
Carbon content		52.4
Oxygen content		32.1
Nitrate content		25.6
Chlorine content		0.25
Sulphur content		0.22
Phosphorus content		0.07
Pre-culture microalgae (FSP-E)		
Estimated volume per litre culture, (ml)	15	2.10 x 10 ⁶
Compositions of Pre-culture (wt %)		
Calcium		399.6
Magnesium carbonate		93.24
Manganese		454
Zinc phosphate		64
Copper		102
Iron		11,101
Phosphorus		199.8
Potassium		319.7
Selenium		0.5
Sodium		359.6
Sulphur		186.5
Photo-bioreactor (modified – BG-11), g		
Sodium nitrate	1.5	5.83 x 10 ⁴
Dipotassium hydrogen phosphate	0.03	1.17 x 10 ³
Magnesium sulphate	0.075	2.92 x 10 ³

Citric acid	0.006	2.33×10^2
Sodium carbonate	0.02	7.78×10^2
Calcium chloride	0.036	1.40×10^3
Ferric ammonium citrate	0.006	2.33×10^2
Ethylenediaminetetraacetic acid, EDTA	0.001	3.89×10^1
Boric acid	0.00572	2.22×10^2
Manganese chloride	0.00362	1.41×10^2
Zinc sulphate	0.00044	1.71×10^1
Sodium molybdate dihydrate	0.0006	2.33×10^1
Copper (II) sulphate pentahydrate	0.00014	5.44
Cobalt (II) nitrate hexahydrate	0.00008	3.11
Silicon minerals	2.5	9.72×10^4
Oxygen		
Oxygen gas flowrate, LPM	1.33×10^3	3.94×10^{13}
TFF membrane filter		
Biomass liquid, kg	3	116667
Water outlet flowrate, g/min	0.002	4140
Freeze dryer		
Biomass solid, g/day	1.55	11,400
Grinding (storage)		
Energy for grinding (Storage), kWh/year	5.28	365
Final output microalgae biomass powder, g	1.5	11,100

Note: (FU: 1 kg DW extracted biomass in a slurry comprising 22% DW)

5.3.2 Case studies

Once the baseline *Chlorella vulgaris* cultivation was established using BG-11 (option 1), integration of industrial biscuit waste powder was introduced (option 2), followed by the recycling of remaining culture water obtained from dried biomass back into the photo-bioreactor (option 3). Table 5.2 describes the parameters considered in the scenario LCA modelling for commercial plant. All three approaches mentioned in this paper were evaluated in the lab, and its corresponding data were used for large scale feasibility testing. The growth time of *Chlorella vulgaris* was set at 7 days for all three options.

Table 5.2: Process Flow Options for Configuration Design.

Cultivation system	Option 1	Option 2	Option 3
Algae strain	<i>Chlorella vulgaris</i> , FSP-E		
Study scale range	14, 000 Litres/PBR working volume (estimated 6 PBRs)		
CO₂ source	Flue gas from ammonia production (NH ₃)		
Air source	Compressed air, 7 bars		
Electricity source	Hydropower energy		
Nutrient source	BG-11	Industrial biscuit waste powder	

Water source	Tap water	Tap water + recycled medium
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The selection of an appropriate nutrient is critical in creating an optimum method for *Chlorella vulgaris* biomass. Wong et al.,(2017) discovered that various different nutrient modification to the medium recipe had a direct influent on the cell content, including fat, carbohydrate, protein, chlorophyll, and pigments [242]. Hence, artificial medium with established chemical composition was utilised to promote varied nutritional requirement of specific algae species development. The modified BG-11 medium was shown to accelerate the development of *Chlorella vulgaris* at an optimum rate and also able to produce high lipid and fat content during the nutrient deprivation stage [243]. In regard the modified BG-11 medium was mixed with industrial biscuit waste powder in option 2 and option 3 and the LCA model indicates that option 3 was the best medium combination in terms of process waste output.

In laboratory experiments, the medium will be kept without stirring for at least 24 hours at the end of a culture cycle to allow biomass settling and 20% bottom culture will be extracted out. On the other hand, the leftover 80% culture will be discarded which is inapplicable for a pilot scale plant. As a result, LCA recommended to recycle the culture medium back into PBRs, and it was observed that 40-50% of the leftover culture after bio-flocculation, tangential flow filtration may be combined with fresh water without affecting the overall performance of algae production. The LCA model was designed according to an actual existing 2500 L industrial continuous PBR (Industrial Plankton). These PBRs were able to generate 400-600g dry biomass per day with a maximum production time of 12 weeks [219]. The LCA model, it was confirmed that a total of eight cycle can performed using the industrial biscuit waste powder. The second major concern was the carbon dioxide source. For laboratory scale, a compressed industrial CO₂ gas was supplied, whereas the LCA model specifically used CO₂ source which was in the database (Flue gas from ammonia production (NH₃). This is because, European

union has a total capacity for industrial ammonia production of roughly 21 million tonnes, with an estimated average of 2.9 tonnes CO₂ emission [244]. The LCA model proved viable with all the characteristics stated for large scale, and its layout was depicted in Figure 5.4.

Several assumptions were made to develop the LCA model for large scale *Chlorella vulgaris* biomass production. The first assumption was the power needed for stirring of PBRs, membrane filtration, and freeze dryer. The stirring motor in a 2,500L PBR requires 4.65 kWh, while spray cleaning needs 7.35 kWh [219]. The system's power consumption for membrane filtration and rotary drum dryer was set at 2.23kWh/m³ [245] and 33.6kWh (evaporates 18.2 kg of water for every kilogram of dry algal product), respectively [232]. Secondly, the cultivation site was an indoor facility like the laboratory testing. Cultivation in large outdoor facilities implies having little control over environmental conditions such as weather condition, atmospheric temperature, and sunlight source. Hence, this LCA study was designed as an indoor culture, with all building blocks operating at a range of 25°C – 30°C. Lastly, in the proposed scenario, the growing system is linked to a dairy facility, and waste powder supply does not require any further transportation. Based on comparisons, option 3 was the best cultivation pathway among the three scenarios due to process sustainability (minimum waste generation). Overall, the proposed LCA model confirmed that large-scale growth of *Chlorella vulgaris* utilizing biscuit powder waste is possible, with 400 – 600g of dry algae biomass generated per day for a working capacity of 14, 000 litres whereas, 0.5 – 1g of dry algae biomass were generated in a lab scaled 1 litre photobioreactor.

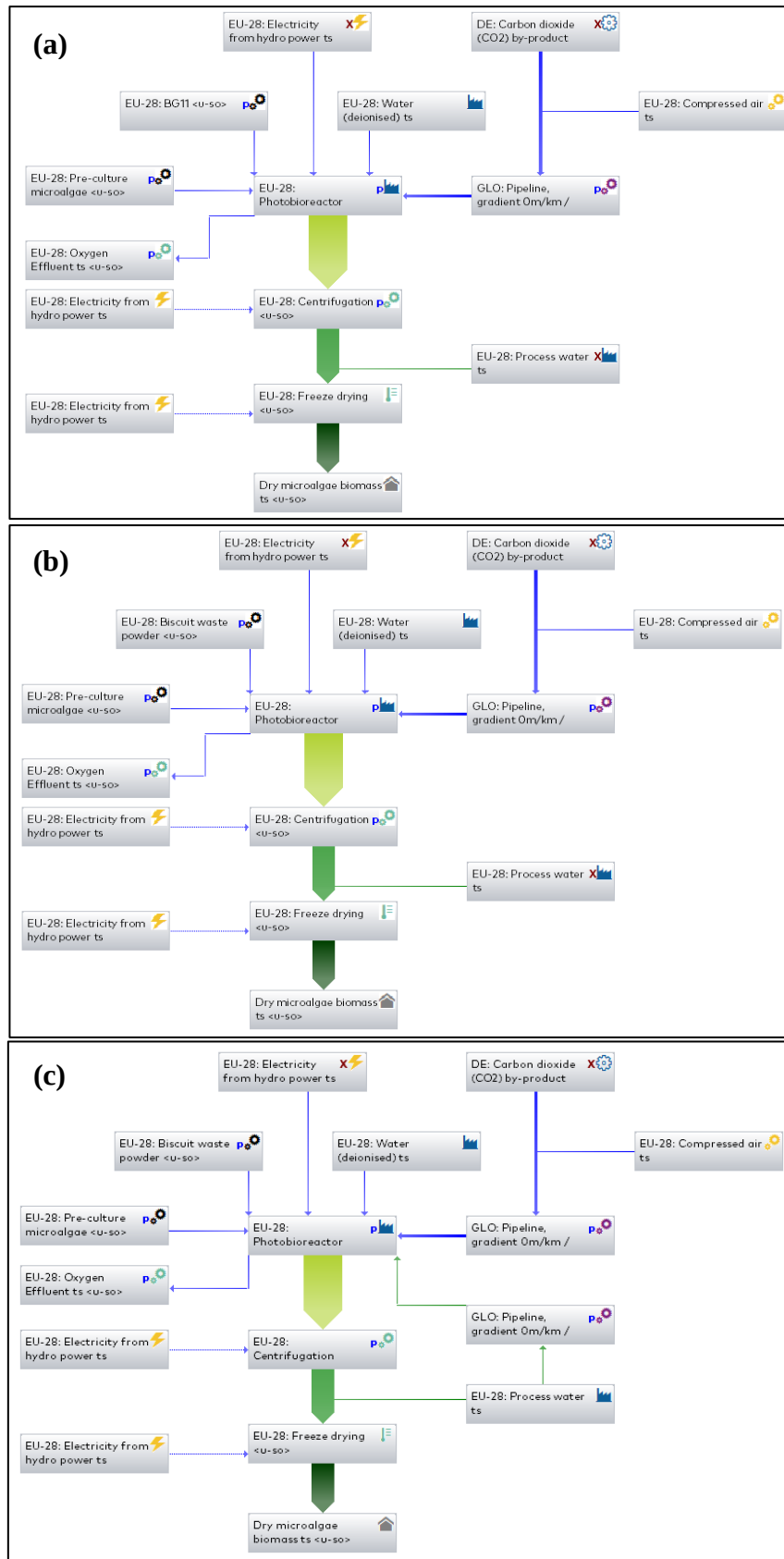


Figure 5.4: *Chlorella vulgaris* cultivation layout with (a) option 1; (b) option 2; (c) option 3 from GaBi software.

5.3.3 Economic analysis

A detailed economic study was carried out to analyse the effects of microalgae production and characteristics on microalgae costs. Different assumptions, such as the price of raw materials and equipment, are liable to change because of market fluctuations and uncertainties. This brief economic study, on the other hand, might give further insights into the most crucial aspects relating to commercial scale microalgae production. The upstream operations in this study include the continual cultivation and harvesting of microalgae throughout the year. The production model was generated using lab-scale experimental data. For the economic study of the growing facility, several assumptions were taken into consideration based on the location site (European region). Throughout the year, biomass production was examined, and after each cycle, all photo-bioreactors were treated to cleaning and maintenance. Each cycle had a length of 10 days, which was split as follows: 7 days of microalgae cultivation, 3 days of harvesting downtime, including PBR cleaning and maintenance, and 7 days to start another microalgae culture, for a total of 12 weeks of PBR operation. The duration of each cycle was set at 7 days since the combination of biscuit waste and BG-11 may attain its maximum growth potential in 7 days [204].

Table 5.3 displays the capital investment as well as the unit cost of consumables for upstream microalgae biomass production. The processing facility was located near the cultivation facility, and hence, transportation was not needed.

Table 5.3: Capital investment, unit cost of supplies for microalgae biomass production.

Assumptions considered		References
Rate of interest including land tax, I (%)	5	[246]
Water loss for bio flocculation method for 10 g/L algae produced (2,500L/PBR), m ³ /year	134,140	[247]
Annual CO ₂ capture, ton/year*	102.13	[248]
Algae Market Price, \$/kg	10 - 15	[249]
CO ₂ storage per ton, \$/ton	37	[250]

Cost for BG-11 for 1,000 L medium, \$	18,360	[251]	
Electricity cost (C _e), \$/kwh*	12,325.34	[252]	
Tap water cost, \$/m ³ *	4.84	[253]	
Direct capital cost, \$			
Reactor construction	2.28 x 10 ⁴	[254]	
Circulation pump	6.29 x 10 ³		
Process control (piping & fitting)	2.10 x 10 ⁴		
Infrastructure for cultivation (Light stand,CO ₂ chamber)	6.56 x 10 ⁴		
Infrastructure for harvesting	1.66 x 10 ⁴		
Tangential Flow Filtration	3.10 x 10 ⁵	[255]	
Field laboratory	2.11 x 10 ³	[256]	
Installation	6.46 x 10 ³		
Total Capital Investment	4.51 x 10 ⁵		
Operating capital cost (Unit cost of supplies)			
Options	1	2	3
Chemicals (Culture medium)/2500L (Litre)	275,400	110,160	44,064
Land rental, \$/ha. year*	7,104		
CO ₂ capture & storage cost	3,778.81		
Culture consumables, C _c	295.65		
Engineering & supervision (Employee expenditure), C _i	3,227.90		
Maintenance, M	199.91		
Loss of algae, C _{loss} , \$/kg	3,353,500		
Water supply cost, \$/m ³	123,154	104,616	93,957
Cost for harvesting-dewatering: bio-flocculation (P _c), \$/kg	8.49 x 10 ⁸	8.48 x 10 ⁸	8.44 x 10 ⁸
Production costs per kg of algae biomass produced (P _c), \$/kg	1.44	1.40	1.37
Total Operating Cost, \$	3.78 x 10 ⁶	2.15 x 10 ⁵	1.38 x 10 ⁵

Note: * = Supplies costing were based on Netherlands

The breakdown of direct capital expenses of a large-scale microalgae production facility were retrieved from similar previous studies [257–259]. Regardless of the process flow alternatives, the infrastructure of the microalgae production plant remains the same, with an estimated capital cost of \$ 451,000. Moving ahead, the operational cost is the most important variable influencing the total profitability of a manufacturing facility since it includes the continuous expenditures acquired from day-to-day operations. According to the LCA framework demonstrated in Figure 5.4, the electricity consumption needed for all three alternatives is

identical. The hydropower-based facility supplied an approximate of 12325.34 kwh/day of electricity. Option 3 also requires minimal quantity of inorganic culture medium since the process flow re-cycles the discarded culture medium back into the production facility. Moreover, Eq. (2) was applied to calculate the harvesting-dewatering cost, and it was shown that the most significant element in optimising the dewatering system was energy costs, C_e , and expenses of algae loss, C_{loss} each year. This is because a concise balance between the additional pump and piping installation as well as the algae recovery output will be essential. Overall, option 3 was discovered the least water-consuming microalgae production process.

5.4 Limitations

Expanding microalgae cultivation from a lab-scale unit to a commercial one is a challenge due to the difficulty in maintaining growth variables. This is why most large-scale cultivations produce less produces less overall biomass productivity than what is predicted from the laboratory data. When scaling up microalgae culture, one of the most significant problems to address is the operational mode. This is due to the fact that for laboratory size culture, a batch scale cultivation is generally carried out because the difference in cleaning time and costing for continuous versus batch is not significant. However, semi-continuous cultivation is preferred for large-scale cultures to reduce time and cost of scaled up photo-bioreactors [257]. Hence, instead of harvesting the entire culture volume once the required biomass concentration (g/l) is attained, just a portion of the culture volume is harvested and replaced with either fresh or recycled medium, as proposed in the study. Nutrient injection to replace nutrients absorbed by harvested algae, as well as other losses, must be carefully controlled to avoid either depletion of a vital nutrient or progressive build-up of a specific nutrient to amounts that restrict development. Moreover, blending culture medium, and providing homogeneous light environment for algal cells is a straightforward method for small-scale cultures. Consistent

mixing in large photo-bioreactors is challenging, particularly to minimise fouling in glass walls.

The circulation and mixing in large scale culture units differs significantly from those in mini reactors from laboratory scale. The longer the channels, the longer the area of laminar flow with negligible vertical mixing especially at the bottom corner edges [260]. This indicates that cells in the centre of the photo-bioreactors column get little or no light for extended periods of time, but cells on the surface of the water column are exposed to high irradiances. In order to reduce uneven mixing from occurring, flow rectifiers are commonly added to strive to balance out the flow around bends [258]. The ongoing photo-bioreactor scale up initiatives faces plenty of hydrodynamic challenges to attain an optimal algae production. However, computational fluid dynamics (CFD) has shown to be a useful technique for forecasting the complicated fundamental phenomena in photo-bioreactors that can be scaled up commercially [261]. Since this study focuses on the cultivation of indoor photo-bioreactors, it is critical to emphasize the requirement for strict control of oxygen build up and biofouling, as well as the necessity for routine disinfection spray. The use of organic carbon in transparent photo-bioreactors creates more harmful contamination conditions. The technical difficulty in sanitizing any closed photo-bioreactors has the capability to influence the algae growth productivity [262]. Clean-In-Place (CIP) techniques have been proposed in this study as an alternative to ensure highly efficient sanitation.

Seasonal weather conditions with daily shifts in atmospheric temperature have the potential to influence the microalgae growth productivity. *Chlorella vulgaris* grows well at temperatures ranging from 20 to 40 °C, making it a suitable alternative for indoor culture [263]. Furthermore, in an indoor culture facility, the light source can be kept in ideal condition throughout the algal growth process. However, the barrier with an indoor culture would be the necessity of energy supply for continuous power generation, as well as the added cost required for an oxygen

supply capture chamber. Finally, monitoring algae development is one of the challenges to tackle. The use of a UV-Vis spectrophotometer is adequate for laboratory algae development; however, this technique is not viable and time-consuming for commercial scale algae production. Hence, the Internet of Things (IoT) can be used to create a digital platform utilising a smart phone. This could be accomplished by building a mobile app for data gathering and visualisation.

5.5 Conclusion

The production of bio-products from microalgae demands the synthesis of large-scale biomass with the appropriate biochemical composition. By utilising sustainable and organic growth medium route options for large-scale microalgae biomass production, this study addressed one of the key economic challenges. Life cycle analysis was used to assess the feasibility and technical elements of alternative upstream process configurations. The use of LCA has been effective in the industrial scale scaling up of microalgae culture systems that generate 600 g/d dried microalgae biomass. It was discovered that the use of food processing waste as culture media (Option 3) was the best approach for a large-scale operation. The approach and results from this work can be helpful in determining optimum plant setups for commercial implementation of microalgae cultivation with sustainable medium that can be considered for a commercial facility for bio-fuel production.

CHAPTER 6

A DIGITAL PLATFORM FOR MICROALGAE GROWTH MONETARIZATION VIA MOBILE APPLICATION

Overview

There is a great demand for a clean, economical, and long-term energy source, due to the depletion of fossil fuels. This gives the algal production-based biofuel a wider energy source business standpoint, as Industry 4.0 is widely being explored for producing sustainable energy. Large scale production of microalgae biomass for biofuel production is quite a challenge due to several factors such as costly photo-bioreactor, sustainable medium for optimal growth, and time-consuming algae growth monitoring procedures. Moreover, the conventional UV-Vis Spectrophotometry approaches are excessively time consuming, costly, and inconvenient, preventing stakeholders from pursuing large-scale algae cultivation. As a result, this study made use of modern digital technologies such as sensors, automation applications, and the Internet of Things (IoT) in coding a mobile application that can be used to overcome the challenge of using tedious lab-based UV-Vis's analysis especially for large scale plants. Moreover, mobile applications run on a small hand-held mobile device that is portable, easy to use, and accessible from any location. The Java programming language, which was applied to produce the application framework, was covered in this section. The goal of this study was to develop a mobile application (FindAlgae) with a smart algae growth monitoring by analyzing colour differences in a single click.

Keywords: Artificial Intelligence, JAVA language, Mobile Application, Azure, Commercialization

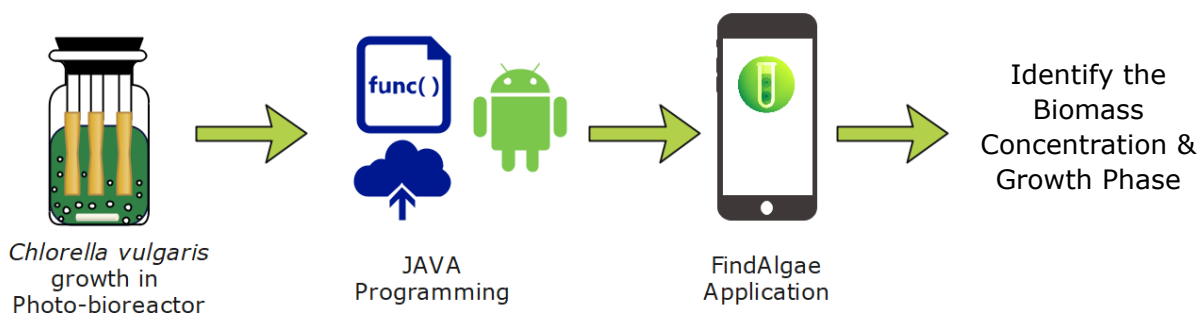


Figure 6.1: Graphical abstract for a digital platform for microalgae growth monetarization via mobile application.

6.1 Introduction

The world is on the verge to encounter a paradigm shift of industries due to the elevation of the use of artificial intelligence, machine learning, and deep learning. Over the decades, the modern production of microalgae to generate bio-products, and biofuels has risen tremendously. According to the National Renewable Energy Laboratory, bio-products would have a 25% market share in the international chemical sector by 2025 [264]. This prompts the demand of up-scaling algae production in an economical and sustainable pathway. On this note, the industrial revolution 4.0 can break down traditional barriers and allow for sustainable growing conditions with quick testing on biomass growth. In previous years, photo-bioreactors are widely investigated on its design structure, in terms of sustainable and economical aspects [265]. The emerging of technology onto photo-bioreactors has developed a constant maximum biomass yield by monitoring microalgae growth with an array of interconnected sensors, adjusting conditions as needed, and identifying ideal growth parameters through machine learning [266]. Many industrialists and researchers had ventured into emerging the microalgae growth processes in closed reactors using artificial intelligence studies [112,267]. However, implying a digital algae growth monitoring system with the biomass concentration identification is yet to be discovered. Implementation of artificial intelligence able to overcome the tough challenge, by identifying the best time to harvest the algae before it degraded. This

discovery will be very helpful for large scale algae cultivation, as ideally it is not feasible to measure the algae growth using UV-Vis photospectrometer manually daily. Moving forward, Internet of Things (IoT) were widely implemented to form smart factories that enables the sharing of data associated with specific key elements such as a cyber-physical system, a mechanical device system run by computer-based algorithms. IoT, generates cloud computing uses offsite network hosting and data backup. Moreover, cognitive computing technology develops a platform that incorporates artificial intelligence into the daily lives of many individuals [268].

People have become adapted to using computers and digital applications in the modern age of information and communication systems. However, the use and development of mobile applications is a relatively new growing industry. This is since mobile applications run on a small hand-held mobile device that is portable, easy to use, and available from any location. Hence, this research focuses on developing a mobile application that could ease the microalgae biomass growth. This is because, one of the greatest challenges of up scaling microalgae cultivation process is knowing the right time to harvest the algae before it degrades and its daily biomass monitoring. The current UV- Vis Spectrophotometry methods are too tedious, costly and time consuming which prevents stake holders in venturing into large scale algae growth [112]. Thus, the development of mobile application will reduce the cost and time in monitoring large-scale algae growth. As the algae cell matures, the digital growth monitoring application determines the harvesting phases by capturing the colour intensity. This section is a tailor-made description of the functionalities and tasks the code segments for the mobile application development. The content refers to the content available on the FindAlgae Code in (APPENDIX III).

6.2 JAVA programming (mobile application layout)

Mobiles applications are written in many programming codes. In this study, android apps are programmed using the Java programming language. Java is a general-purpose programming language that is used to create apps in the Android Studio environment with the Android Software Development Kit (SDK). Java can be run in two ways: in a browser window or in a virtual machine that doesn't require the use of a browser which gives a flexibility tends to mean a lot when it comes to re-using code and updating software. Java is the official language of Android development, which means it receives the most support from Google and is used to create most apps on the Play Store. Moreover, Java Programming codes has key features: (1) Object-oriented programming; (2) it works on all systems; (3) it has APIs for connectivity; (4) it is simple to learn and interpret; (5) Access to hundreds of open-source frameworks; (6) Easy access to professional assistance from Android networks; (7) Superior Integrated Development Environments, IDEs make coding simple and error-free. Figure 6.2 describes the screen navigation for FindAlgae. In case a photo with filter is used, or a photo that is out of the algae colour an error indication will be shown in the results screen (after clicking the analyse button in screen 4).

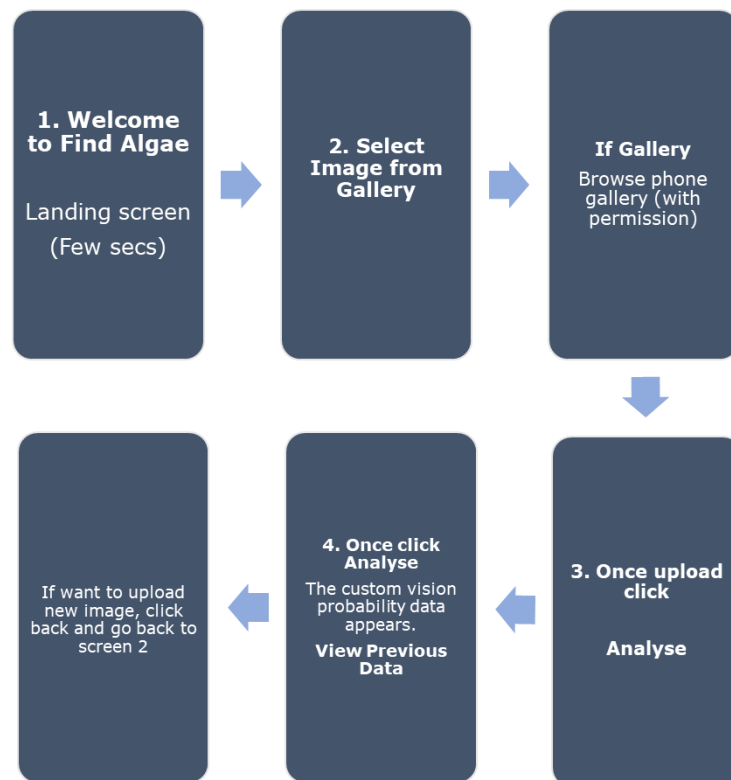


Figure 6.2: User interface of FindAlgae application

6.3 FINDALGAE programming functions (workflow)

There were four stages to the experiment's design. Various criteria and parameters were specified prior to the implementation of applications. Custom vision service is a cloud-based technology that was used to quickly train, deploy, and improve the microalgae growth image classifiers. This enable developers to build the intelligent edge from Microsoft Cognitive Services that will be added on the mobile model export. Once image classification was completed, the java programming begins. The breakdown of the design flow was presented in Table 6.1.

Table 6.1: Content workflow for FindAlgae mobile application development.

Design Flow	Details
Upload image to app	User needs to be able to upload an image by selecting and upload from gallery
Send image to analyze culture score from custom vision	Convert the uploaded image to a format that can be sent to the custom vision platform to analyze
View previous culture info after processing the image	Keep a record of recent tested image output for user to know previous data

Publish the Application to Play store	User should be able to download through Google play store
---------------------------------------	---

6.3.1 Import statement

Complex programs are usually developed by large organizations and made available for use as free of service in the open-source platforms. These programs help other developers achieve complex tasks in a much simpler way by just importing these programs into their development task. Therefore, effectively bringing in all the complex work they have done, making it easier for us to use it, whenever it is required, without having to redo the complex tasks of creating the base programs. The imports have been brought in from standard Java libraries, Google libraries and Microsoft custom vision libraries which will be used further down in the program. The imports from Microsoft and Google are available on the Microsoft and Google Documentations on Custom Vision documentation and Google Firebase Documentation respectively.

```
import android.Manifest;
import android.annotation.SuppressLint;
import android.app.ProgressDialog;
import android.content.DialogInterface;
import android.content.Intent;
import android.content.pm.PackageManager;
import android.graphics.Bitmap;
import android.net.Uri;
import android.os.AsyncTask;
import android.os.Build;
import android.os.Bundle;
import android.os.StrictMode;
import android.provider.MediaStore;
import android.util.Log;
import android.view.View;
import android.widget.Button;
import android.widget.ImageView;
import android.widget.Toast;
```

Figure 6.3: Example of Import statement [Full Codes in APPENDIX III]:

6.3.2 Pre-startup initialization

Initialization does the work of creating certain objects, such as buttons, lists, credentials, etc. which are required to be created for the application to startup. These would be prerequisites for the application to turn on. The prerequisite initializations fall into the MainActivity class, which simply is the name assigned for the opening screen. While classes, are how object oriented programming works on a fundamental level. The details of which are more oriented towards a computer science module and not of much relevance to the context of the experiment and core deliverables of the thesis.

6.3.3 On create initialization

Once the program has started up, there are certain initializations done which will be used during the working of the application. The significance of the initializations done at the “OnCreate” is beneficial for better application performance before starting. Therefore, which seems suitable to be initialized after starting up is defined.

```
@Override
protected void onCreate(Bundle savedInstanceState) { // when the application is starts and is ready to use
    super.onCreate(savedInstanceState);
    setContentView(R.layout.activity_main);
    StrictMode.ThreadPolicy policy = new StrictMode.ThreadPolicy.Builder().permitAll().build();
    StrictMode.setThreadPolicy(policy);
    mAuth = FirebaseAuth.getInstance();
    GoogleSignInOptions gso = new GoogleSignInOptions.Builder(GoogleSignInOptions.DEFAULT_SIGN_IN)
        .requestIdToken(getString(R.string.default_web_client_id))
        .requestEmail()
        .build();
    // Build a GoogleSignInClient with the options specified by gso.
    mGoogleSignInClient = GoogleSignIn.getClient( activity, this, gso);
    //assign values to variables
    imageView=findViewById(R.id.image_view);
    Uploadbtn=findViewById(R.id.upload);
    buttonrequest = findViewById(R.id.button);
    Logout = findViewById(R.id.logout);
    Project_id = "aaead50a-95cd-403c-a3c2-9b774399e43b";
    storage = FirebaseStorage.getInstance();
    storageReference = storage.getReference();
    StorageReference httpsReference = storage.getReferenceFromUrl( fullUrl: "https://firebasestorage.googleapis.com/v0/b/findalgae.
```

Figure 6.4: Example of on create initialization [Full Codes in APPENDIX III].

6.3.4 Predefined functions

A core concept in programming is to ensure certain codes don't need to be typed again and again. To enable this, usually required tasks are coded into function as required by the use case. These functions are later called in the program whenever required, so that the function call is

executed in simple one-line codes, than doing the whole hard work of making the function again. Similar concept to why import statements are used.

6.3.4.1 Sign in functions & firebaseauth with google

The function is self-explanatory, as expected, it does the work of signing into the Google account for the user. To use the Google Firebase features needed in the application. This will be directly linked to the google account signed in by user in order to access google in their device.

This function performs the task of calling previous function for picking image from the gallery if the permission was giving by the user.

```
@Override
public void onRequestPermissionsResult(int requestCode, @NonNull String[] permissions, @NonNull int[] grantResults) {
    super.onRequestPermissionsResult(requestCode, permissions, grantResults);
    switch (requestCode){
        case PERMISSION_CODE: {
            if (grantResults.length > 0 && grantResults[0] ==
                PackageManager.PERMISSION_GRANTED) {
                //if permission was granted
                pickImageFromGallery();
            } else {
                //Permission was denied
                Toast.makeText( context: this, text: "Permission Denied", Toast.LENGTH_SHORT).show();
            }
        }
    }
}
```

Figure 6.5: Example of onRequest permission function [Full Codes in APPENDIX III]

6.3.4.2 Upload image function

Similarly, the task of the function is self-explanatory. However, it does an additional task in relation to the concentration analysis. How the function gets about to uploading the image can be elaborated into a few steps as below.

1. If the user has selected an image, the application will create an image file in Google Firebase storage and place it inside a folder
2. The image file will be named “Pick”; an arbitrary name.
3. The function displays progress of the upload.
4. Once the upload is completed into firebase storage, the function gets the URL link for the image that was uploaded. This link is used to analyze the culture concentration.

5. Once the link is retrieved, it will start the “longrunning” function. Which will be elaborated in subsequent sections.
6. Where relevant, the function will display to the user a success or failure message.

6.3.4.3 Long running task function

A long running task is a function named as fit for purpose. Its sole responsibility is to work on tasks that would usually take some time to finish. Such as working with the internet, where network speeds may vary and loading times may be longer. This is done so that the application doesn't frustrate the user by being stuck. To ensure a smooth operation of the tasks put into this function, the function performs its work in the background, while keeping the user informed on progress.

In the FindAlgae context, the tasks put into this function are as follows:

1. Prepare an http call (just like a regular browser does when we enter a website and press enter)
2. Gets the response from the website (azure custom vision link)
3. Saves the response into a variable, which is later displayed in the analyze function

6.3.4.4 Pick image from gallery & open analyze function

The selection of images will be based on the user's permission to access their device's images. Upon acceptance, user will be able to select the image was required to analyse. Moreover, the open analyze function navigates the application to the second page where the results from the concentration analysis will be displayed for the user. When the second page is opened, it assigns the data from page 1 into the list of data in page 2 which is to be displayed to the user. As in below image.

6.3.4.5 Request storage permission function

The function requests the permission for storage from the user's phone.

```

private void requestStoragepermission(){
    if (ActivityCompat.shouldShowRequestPermissionRationale( activity: this, Manifest.permission.READ_EXTERNAL_STORAGE)){
        new AlertDialog.Builder( context: this)
            .setTitle("Permission needed")
            .setMessage("This permission is needed to store the data")
            .setPositiveButton( text: "ok", new DialogInterface.OnClickListener() {
                @Override
                public void onClick(DialogInterface dialog, int which) {
                    ActivityCompat.requestPermissions( activity: MainActivity.this, new String[] {Manifest.permission.RE
                }
            })
            .setNegativeButton( text: "cancel", new DialogInterface.OnClickListener() {
                @Override
                public void onClick(DialogInterface dialog, int which) { dialog.dismiss(); }
            })
            .create().show();
    } else{
        ActivityCompat.requestPermissions( activity: this, new String[] {Manifest.permission.READ_EXTERNAL_STORAGE}, Storage.

```

Figure 6.6: Example of request storage permission function [Full Codes in APPENDIX III]

6.3.4.6 On activity result function (run time)

This function does the work of calling the upload image function when the user has selected an image from the gallery. Prior to uploading, it also retrieves the information needed to upload the image into Google Firebase Storage. With all the predefined functions set up, the application is supposed to react in certain ways when the user interacts with the application. Such as click of buttons. The working tasks associated with the actions the user may take are elaborated below. In the application, each instance a user may touch the screen is referred to as an “onClick” event. Therefore, whenever user made a click, the application will do certain things. Each onClick even is described. When the analyse button is clicked, it calls the open analyse function. When the upload button is clicked, based on the permission provided, it will call the Pick image from gallery function. When the logout button is clicked, the application will logout the firebase credentials used in the instance.

6.4 Future development

Automation is the process of eliminating errors in a process by integrating and regulating the tracking of variation and adjustment action. There are various stages of automation, each requiring the technology to have a specific degree of intelligence. Simpler, repetitive operations

are typically easier to automate than complicated procedures that need a fresh selection for each output. Thus, the opportunity for automating the microalgal bioproducts manufacturing chain is highly dependent on the technologies and methods employed in each step of the process. Algae-based biofuel manufacturing confronts significant challenges especially for large scale application: (1) inefficient photosynthetic solar energy collection; (2) inappropriate growth conditions; and (3) high cultivating and downstream processing energy and economic expenses. Firstly, the best conditions for microalgae culture, lab-based multi-parameter improvement have to be established. This will demand the use of technology and procedures to investigate the wide range of feasible production settings. Peter et al., (2021) reveals that the red, green, blue colour model can be used to study the maturity of algae growth without undergoing the tedious UV-Vis Spectrophotometer analysis [112]. In near future, the implementation of machine learning can be integrated with the colour model analysis for instance live algae growth monitoring, for example a closed-circuit television, CCTV based algae growth monitoring, or a colour sensor indication system can be considered. Moreover, improvisation can also be done in including various type of algae species and there are also other factors that can be considered such as temperature control, pH maintenance, and sustainable light supply automation.

Provided appropriate culture conditions have been identified, photobioreactor innovation must be enhanced to guarantee that they can be consistently applied at an equitable capital and operational cost. According to study, artificial intelligence may also be used to automate the modelling and control of pH regulation in a microalga tubular photobioreactor by reducing CO₂ losses [269]. Now, the on-off switching processors are used with system dynamics and disturbances not considered during the controller design. Finally, after microalgae have been effectively produced, downstream techniques must be designed to maximize extraction value while reducing energy consumption. Centrifugation is commonly used in laboratories to

remove water quickly, but it is very energy consuming, making it impractical for bioenergy applications on a large scale, although it is beneficial in extracting high value such as polyunsaturated fatty acids (PUFAs), phycobiliproteins, enzymes and toxins [92]. However, combining centrifugation with other harvesting methods, such as flocculation, as a fundamental pre-concentration phase can significantly improve electrical output while lowering production costs. Machine learning and technology may be utilized by constructing a spiral plate technology that allows for digitalization of the spinning speed [237]. Hence, new innovations in the microalgae sector would be extremely beneficial to the environment and product quality, particularly in large-scale biofuel production.

6.5 Conclusion

Mobile application development frameworks, without a doubt, are changing the way we design modern mobile applications. In this study, android studio apps were used to model the mobile application. This chapter has shown what Android is and how it works with XML in combination with Java Programming. It has described on the main function that was used to build the FindAlgae mobile application framework. In short, algae culture growth images will be captured and stored in the mobile's gallery. These images will be uploaded to the FindAlgae application, and data analysis will be extracted from custom vision service and produce corresponding results which consist of a range of algae concentration alongside with the growth stage. The use of Artificial Intelligence and Machine Learning in mobile application development, as well as the integration of Virtual Reality in personalization, user experience, and the usage of smart chat bots, will be central concerns in near future. With the implementation of technology in microalgae-based processes automation.

CHAPTER 7

SEMI-BATCH *CHLORELLA VULGARIS* CULTIVATION: CUSTOM VISION MODEL FOR BIOMASS GROWTH MONITORING

Overview

There is a great demand for a clean, economical, and long-term energy source, due to the depletion of fossil fuels. Large-scale production of microalgae biomass for biofuel production is likely attributable to several challenges, including the high cost of photobioreactors, the need for a sustainable medium for optimum development, and time-consuming algal growth monitoring techniques. Firstly, the research aims at improving the strategy of recycling culture media for semi-batch cultivation of *Chlorella vulgaris*. As compared to all other culture ratio combinations, 40RB combination yielded the greatest biomass growth (4.52 g/L), lipid (317.40 mg/g), protein (280.57 mg/g), and carbohydrate (451.37 mg/g) content. Next, custom vision was applied to *Chlorella vulgaris* maturing stages, and a unique digital architecture framework was developed. The iteration model delivers result interpretation with an accuracy of more than 92 % of every data set based on the trained Model Performance. This concludes that the digital mobile app (FindAlgae) able to produce data as accurate to 92% like the lab testing results.

Keywords: Semi batch; culture medium recycling; cultivation; colour model; biomass growth

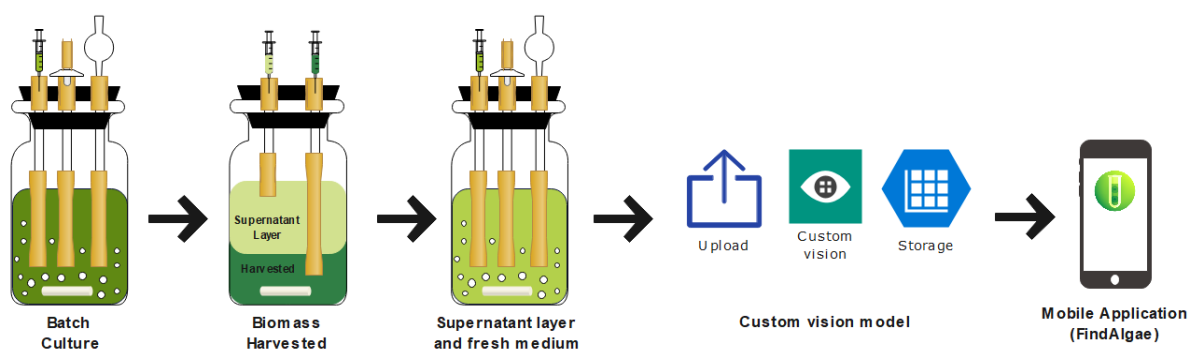


Figure 7.1: Graphical abstract for semi-batch *Chlorella vulgaris* cultivation using custom vision model for biomass growth monitoring.

7.1 Introduction

Microalgae biomass are emerged as a novel, sustainable, and clean energy source for the synthesis of third-generation biofuels [270]. According to the US Energy Information Administration [271], non-renewable energy resources such as petroleum are expected to reach maximum output in 2019, followed by a downward trend, particularly for crude oil supply. Gielsen et al., (2019), reported that the energy consumption has risen dramatically, with global energy demand expected to rise by more than 85% by 2040 [240]. Hence, alternate energy sources were constantly studied based on the existing climate, as the rising global population, industrialization and increasing need for transportation occurred. Biodiesel was recognized as one of the most promising alternative fuels. Biodiesel was described as mono-alkyl esters produced from vegetable oils, animal fats, or waste cooking oil that have a long chain of fatty acids [272]. As compared to conventional petroleum-based diesel, biodiesels are characterized as biodegradability with higher Cetane number, higher flash point, and lower exhaust pollutants [273]. However, the production of biodiesel from crops has a major controversy due to the food versus fuel competition.

To address these issues, microalgae-based biomass is proposed as a possible feedstock for the generation of sustainable and renewable biodiesel. Omar et al., (2021) discovered that microalgae with high biomass productivity and lipid content, are favorable for biodiesel generation [274]. From an environmental standpoint, microalgae growth combined with CO₂ fixation and wastewater bio-treatment could develop the cultivation system into a viable green energy generator without competing food crops for arable land and water [275]. Based on biomass productivity, microalgae species such as *Botryococcus*, *Chlamydomonas*, and *Chlorella* could generate 15-300 times more oil for biodiesel generation than conventional crops [276]. Microalgae also have short harvesting cycle (ranging from 1-10 days depending on the culture condition), allowing continuous harvests with higher biomass yield as compared

to traditional crops that are harvested once or twice a year [277]. Hence, microalgae cultivation is recognized as a modern biotechnologist advancement and it is projected that by 2036, the global market for microalgae-based biomass would have the capacity to produce 5000 tons of dry biomass per year with a revenue of USD 1250 million [278].

For large scale microalgae cultivation process, photo-bioreactor is the most preferred system as it saves land area and allow the culture to grow in a closed environment with less exposure to external contamination [279]. Nevertheless, the capital and operational costs for photo-bioreactors are significantly high due to the fabrication of high-volume tank and control system for nutrient medium, carbon supply, and light source [280]. Hence, growing microalgae in a semi-continuous culture mode is the most cost-effective cultivation system compared to batch mode for large biomass output within a short period of time. The semi - batch culture mode is preferred as it prevents the downtime at the end of the culture, and also minimizes the limitation in terms light penetration during stationary phase. However, excessive addition of fresh culture medium may result in a rise in medium osmotic pressure, which may damage the photosynthetic mechanism of microalgae [281]. Thus, in this study *Chlorella vulgaris* cultivation was cultivated in semi-batch mode with nutrient recycling and the fresh medium supply ratio was optimized.

Moreover, industrial revolution 4.0 can break down traditional barriers and allow for sustainable growing conditions with quick testing on biomass growth. In previous years, photo-bioreactors are widely investigated on its design structure, in terms of sustainable and economical aspects [272]. The emerging of technology onto photo-bioreactors has developed a constant maximum biomass yield by monitoring microalgae growth with an array of interconnected sensors, adjusting conditions as needed, and identifying ideal growth parameters through machine learning [282]. However, implementation of artificial intelligence able to overcome the tough challenge, by identifying the best time to harvest the algae before

it degraded. The current UV- Vis's methods are too tedious, costly and time consuming which prevents stake holders in venturing into large scale algae growth. Hence, this study also includes the application of custom vision service in monitoring the algae growth via image classifiers.

7.2 Methodology

7.2.1 Semi-batch culture medium flow

The *Chlorella vulgaris* species was isolated in Taiwan and obtained from National Cheng Kung University (NCKU). The fresh medium and PBR setup was created as described in a recent work [153]. Following the initial batch culture of 7 days, the succeeding cycles were done immediately using the recycled medium with comparable PBR operating settings. Once the harvesting day reached, the stirring is stopped, and a portion of the culture medium was extracted out for centrifugation process. Meanwhile, the remaining culture medium (in this case 20%, 40%, 60%, and 80%) was recycled back into a fresh culture medium, making it back to a total of 1L nutrient medium. The photobioreactors and fresh media were placed in a horizontal laminar flow cabinet equipped with ultraviolet (UV) light (AHC-5A1, Esco Micro Pte. Ltd., Singapore) during the transformation of medium to avoid any environmental contamination into the culture. The harvesting day was fixed to be after 7th day of the culture and each round was considered as a cycle [153]. The biomass growth was compared with mixing ratio of 20%, 40%, 60%, and 80% as 20RB, 40RB, 60RB, and 80RB respectively.

7.2.2 Azure bot architecture

Windows Azure compute service runs application on a Windows Server foundation. The Azure platform has the following components such as Azure Compute, Azure Storage, Fabric Controller, SQL Azure, Azure Platform AppFabric, Content Delivery Network, and Connect. Azure IoT portal natively supports virtualized containers that can hold firmware to applications which allows better associated hardware dependent IoT device. The workflow includes three

primary steps: (1) Image classification model on Microsoft powered Custom Vision; (2) Azure IoT hub driven by Edge Virtual Machines; (3) Deployment application to be distributed to Edge gateway and IoT devices. Figure 7.2 depicts the general workflow, prerequisites, service configuration, and settings utilized in this experimental scheme

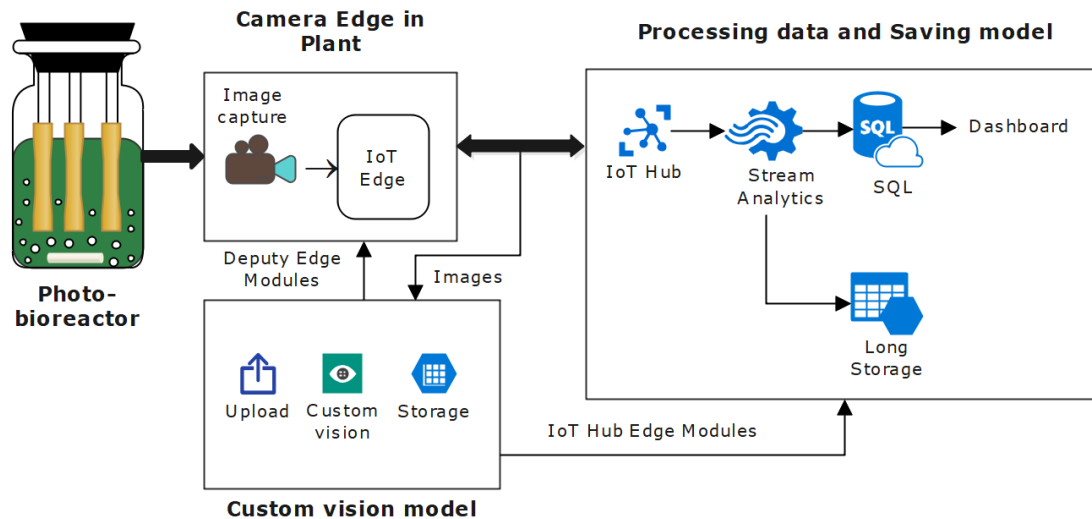


Figure 7.2: Digital architecture system of Algae growth monitoring.

7.2.3 Azure custom vision services

Azure custom vision is an Artificial Intelligence service that allows computers to mimic capabilities of the human brain such as learning, understanding, and recognizing patterns without needing to be explicitly coded for that as we generally do with algorithms. Machine learning is a subset of AI that train a computer system to make predictions based on the available data. In this study, 1000 images were used per tag in the initial training set with 300 pixels on the shortest edge. Three tags were created classified as initial phase (0.41g/L – 1.59 g/L); growth phase (1.60g/L – 2.50g/L); and harvest phase (more than 2.5g/L). Thus, a total of 3000 images were uploaded and tagged to allow the custom vision service to train the images. Then the classifier uses all the current images to create a model that identifies the visual qualities of each tag. The training process would take about 30 minutes where simultaneously the performance about the training process is displayed. Lastly, the Custom Vision Service uses the images that were submitted for training to calculate precision and recall, using a process

called k-fold cross validation [283]. Precision, recall, and average precision are measurements that displays the effectiveness of a classifier model [284]. The model was trained using images obtained in previous study [153].

7.2.4 Determination of microalgae cell growth and bio-composition

The dry cell weight, DCW of the *chlorella vulgaris* was measure as described in a recent work [153] using a UV-Vis's spectrophotometer (UV-1800, Shimadzu) at wavelength of 680 nm. Eq. (1) and (2) indicate the cell growth rate, P_b (mg/L/d), and the growth performance, respectively μ (d^{-1}):

$$P_b = \frac{X_2 - X_1}{t_2 - t_1} \quad (1)$$

$$\mu = \frac{\ln \frac{X_2}{X_1}}{t_2 - t_1} \quad (2)$$

Where X_1 and X_2 are dry cell weight (g/L) at time t_1 (initial) and t_2 (final)

The microalgae samples were analyzed every day for the bio-composition measurement. UV-Vis spectrophotometer at 220 nm optical density was used for nitrate detection while The AAS spectrophotometer was adjusted to 213.6 nm for phosphorus removal detection as shown in the previous study [153]. The concentrations of lipids, proteins, and carbohydrates were measured as described in a previous works [153]. The lipid content is expressed as a percentage difference (wt %) of DCW in Eq. (3). Furthermore, the protein concentration of the extracted microalgae biomass was determined using the Bradford method [285]. A standard correlation between absorbance (OD_{595}) and protein concentrations was established using the bovine serum albumin analysis. The carbohydrate content of the transparent supernatant was determined using the phenol-sulphuric method [279]. The conventional relationship between OD_{490} and total carbohydrates was observed using starch extracts of various concentrations.

$$\text{Lipid content (wt\%)} = \frac{\text{Final vial weight} - \text{Initial vial weight}}{50 \text{ mg}} \times 100\% \quad (3)$$

7.2.5 Statistical data analysis

The one-way ANOVA with two-tailed test in IBM SPSS Statistics version 26 was used to interpret data on total protein, lipid, and carbohydrate, biomass development rate, nitrate, and phosphorus level. In this study, descriptive statistical analysis is provided as mean $\pm$ standard deviation using Sigma Plot V.10. The data given in this investigation are the mean of three runs. The summation of variances was assessed using a significance level of $P \leq 0.05$ in all statistical analyses of the data. Microsoft Azure IoT portal, was designed to satisfy the initial experiment setup demands.

7.3 Findings and discussions

7.3.1 Semi-batch culture medium flow

Chlorella vulgaris was cultivated in a closed photobioreactor to determine the best medium replacement ratio for semi-batch operation. Since the overall culture medium properties fluctuate depending on the preceding culture mixing ratio, there will be slight deviation in the nutrient supply. The biomass growth was compared with mixing ratio of 20RB, 40RB, 60RB, and 80RB. In comparison to all the other media mixing combinations, Figure 7.3 shows that 40RB yielded the highest biomass development. Optimum biomass content was recorded for 40RB at 4.52 g/L on the seventh day, followed by 60RB, 20RB, and 80RB at 3.45 g/L, 3.15 g/L, and 2.77 g/L respectively. The first cycle of culture growth was observed to be near to each other since its initial growth condition was already optimized in previous study [153]. There was a noticeable difference in the microalgae development after combining new medium with part of the existing culture from day seven. Figure 7.3 indicates that combining new media with previous media takes just three days to display significant growth and reach its stationary growth phase on the fifth day of the second cycle, whereas fresh medium alone continues to

grow on the sixth day of the first cycle. While cultivated in its optimum culture medium combinations, Peter et al., (2021) revealed that *Chlorella vulgaris* growth can take up to 7- 10 days to reach its stationary growth phase [286], hence the microalgae growth cycle was only observed for 7 days in this study. Up-scaling of microalgae production can be made economically achievable, if the growth method was advanced from batch to semi-batch, since recycling the culture medium is recommended to avoid the demand of fresh water which will increase the cost overall operation cost. Thus, determining the ideal culture medium recycling volume that increases total microalgae biomass production for a longer length of time is critical.

Another round of cultivation was carried out using the same mixing ratio combination to validate the growth pattern of cycle 2. As for the third cycle, the optimum biomass concentration attained was at 2.11 g/L for 40RB, followed by 2.05 g/L, 1.85 g/L, and 1.32 g/L for 60RB, 20RB, and 80RB respectively. Based on Figure 7.3, the optimal biomass growth was found to be slightly decreasing as the number of cycles increased. This is because the medium's colour changes to dark green more rapidly due to the presence of re-cycled culture as compared to the first cycle. Imran et al.,(2021) also reveals that microalgae require light energy to convert carbon dioxide and water into glucose and oxygen during photosynthesis [287]. Even though both sides of the PBR were exposed to 24 hours of LED fluorescent light, the culture's initial colour thickness differed. The colour medium for 80RB recycled medium was reported to be the darkest, followed by 60RB, 40RB, and 20RB. This is because, high more volume of recycled culture medium leads to a high intense green biomass in culture. However, the microalgae development is not only influenced by its light distribution intensity but also the nutrient content [288].

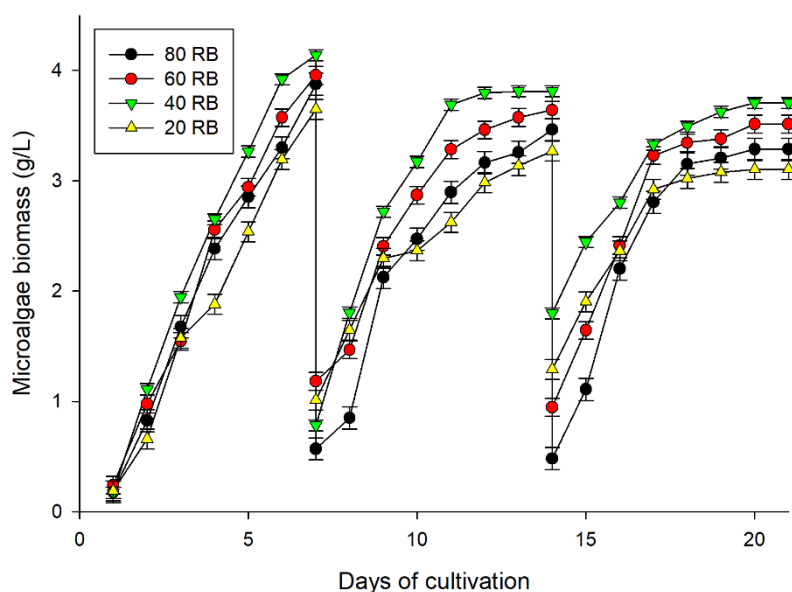


Figure 7.3: Semi-batch Experimental *Chlorella vulgaris* Development

Table 7.1 reports the cell growth rate and productivity of *Chlorella vulgaris* at day 7 of all three of its cultivation cycles. The 40RB medium had the highest biomass productivity for all three cycles at 165.50 mg/L/d (first cycle), 118.45 mg/L/d (second cycle), and 75.71 mg/L/d (third cycle) respectively. Furthermore, it has been observed that as the number of cycles advances, the biomass productivity decreases. This is due to a rapid depletion of the recycled medium's nutrition supply and a restriction in light distribution. An accurate combination of nutrition supplies, as well as adequate light penetration, is required to carry out an effective growing process. Thus, full replacement of the inorganic medium is not recommended. In this study the light distribution was kept constant throughout the monitoring period, since controlling the light parameters for large scale microalgae production would be more expensive and time consuming. Table 7.1 shows that combining 40RB from the previous culture cycle enhances microalgae development for at least 21 days continuously without downtime. Previous study reported that a batch culture (fresh culture) yields 174.99 mg/L in about 10 days, however in this study with recycled medium, roughly about 4.52 mg/L was produced on the 7th day [153].

In summary, semi-batch culture is preferred for large-scale microalgae production as it is more cost-effective and energy-efficient, with optimal biomass output [272].

Table 7.1: Characteristics of *Chlorella vulgaris* grown in IBG and industrial dairy waste culture mixtures.

Culture Mixtures	20RB	40RB	60RB	80RB
Cycle 1, C1				
DCW (g/L)	3.15 ± 0.05	4.52 ± 0.08	3.45 ± 0.05	2.77 ± 0.04
P_b (mg/L/d)	139.56 ± 4.30	165.50 ± 2.85	151.12 ± 5.41	134.03 ± 2.11
μ (d⁻¹)	0.34 ± 0.01	0.56 ± 0.03	0.52 ± 0.06	0.44 ± 0.01
Cycle 2, C2				
DCW (g/L)	2.55 ± 0.05	3.91 ± 0.01	2.10 ± 0.15	2.52 ± 0.02
P_b (mg/L/d)	92.11 ± 4.10	118.45 ± 2.25	107.67 ± 5.40	95.99 ± 2.11
μ (d⁻¹)	0.54 ± 0.04	0.65 ± 0.05	0.50 ± 0.01	0.48 ± 0.02
Cycle 3, C3				
DCW (g/L)	2.05 ± 0.05	2.11 ± 0.01	1.85 ± 0.05	1.32 ± 0.04
P_b (mg/L/d)	68.90 ± 4.30	75.71 ± 2.11	64.67 ± 5.21	54.34 ± 2.11
μ (d⁻¹)	0.45 ± 0.05	0.66 ± 0.03	0.68 ± 0.02	0.41 ± 0.01

7.3.2 Image classifier model performance metrics

Conventionally, a UV-Vis spectrophotometer was used to track biomass increase daily. This method necessitates the use of additional energy and labor on a regular basis for surveillance. Thus, the image classifier model was examined to develop a digital platform for microalgae growth monitoring. The accuracy and performance metrics of the model was observed and one such records from the runs are presented in Figure 7.4. For all the inputs, images of algae growth phases were fed to the classifier model. The Custom Vision service calculates three metrics (1) Precision indicates the percentage of the class predictions that were correct; (2) Recall indicates the percentage of class predictions that were correctly identified; (3) Average precision (AP) measures model performance by computing the precision and recall at different thresholds. The model predicted a precision and recall accuracy of 92.5% followed by an average precision of 97.1%. It is however very important to publish the model as this information were uploaded to build the Python application for future studies.

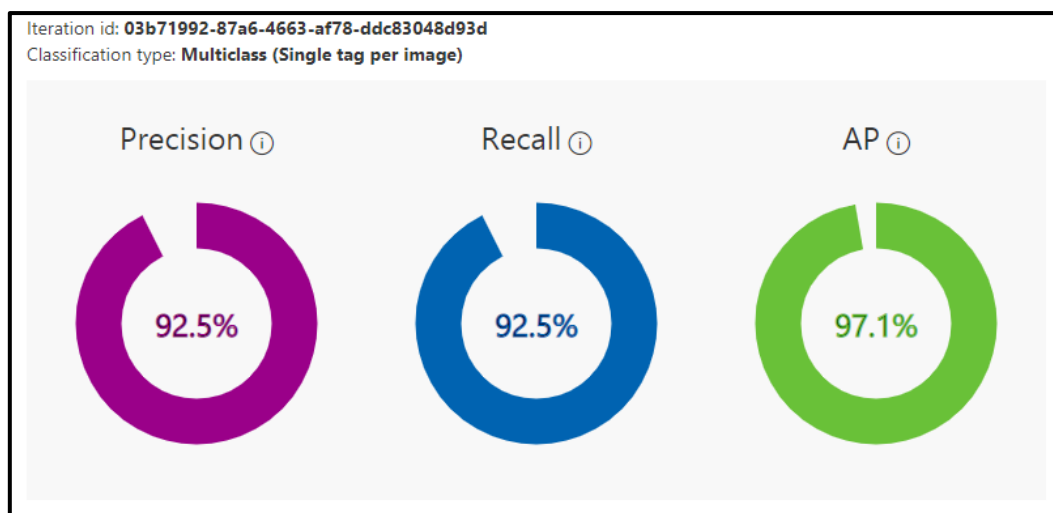


Figure 7.4: Overall trained Model Performance using Custom Vision Service

In this study, the probability threshold was adjusted at 90%, as it represents the accuracy of the image tagging. It is noticed that, as the probability threshold increases, the model will tend to be more accurate as it will only classify when the model is more confident in the tag. As a result, the precision of the model will be higher. However, 100% probability threshold is not advisable in this case, as the recall begins to decrease. The recall of the model will decrease because the model is strictly tagging photos it is more confident in, which makes plenty of other images go undetected. On the other hand, decreasing the probability threshold will increase the decrease the precision of the model, but the model will have a much higher recall as it classifies more photos. Hence, the optimized probability threshold discovered in this study was at 90% and kept constant throughout. Quick tests were carried out daily with an image of the algae growth and the comparisons of experimental algae growth monitoring. It is discovered that custom vision service was able to replace the traditional method of UV-Vis based algae monitoring to a single click, one iteration model training by producing an accuracy of more than 92% of every data set. The green image represents the image upload into the model (via FindAlgae application). Then the model predicts an output based on the database generated by custom vision itself. The predictions results are shown in terms of probability (percentage). However, the highest percentage prediction probability will only be displayed in

the mobile display. Figure 7.5 illustrated an example of a quick test conducted on the 5th day of the cultivation process.

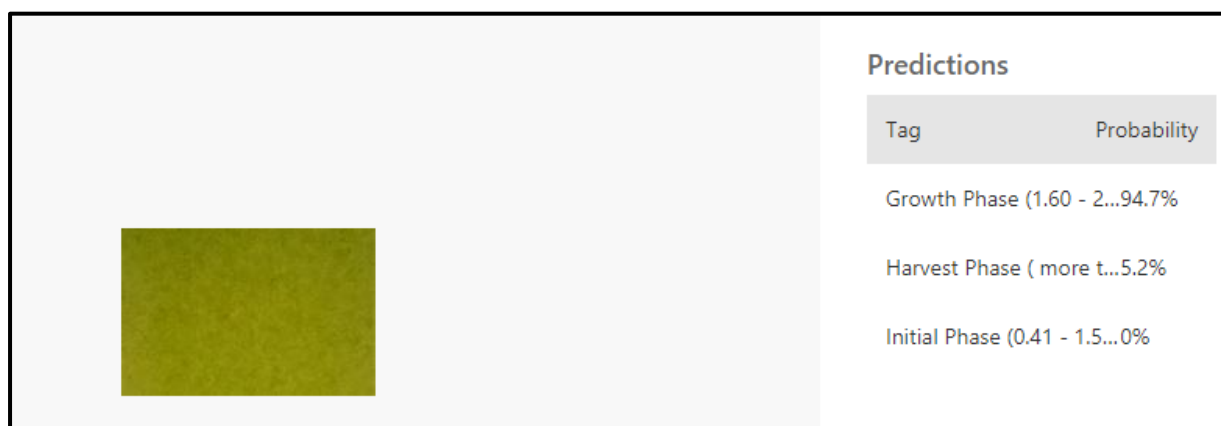


Figure 7.5: Model Prediction of a quick test conducted on the 5th day of the cultivation process.

7.3.3 Nutrient reduction

Microalgae require nitrogen and phosphate as key nutrients for the metabolite cycle and biogenesis during cell growth [289]. The 40RB combination of fresh industrial biscuit waste have been proven to provide an adequate nutrient that improvise the microalgae growth in batch scale [153]. Panahi et al.,(2021) proves that the nitrate content declines when it is absorbed by the microalgae cells throughout the cultivation phase [243]. However, this study discovered that when a fresh medium is added, the nitrate content will increase again. This means that the respective culture combination can be utilize for a semi-batch cultivation system to boost microalgae growth while lowering overall processing costs.

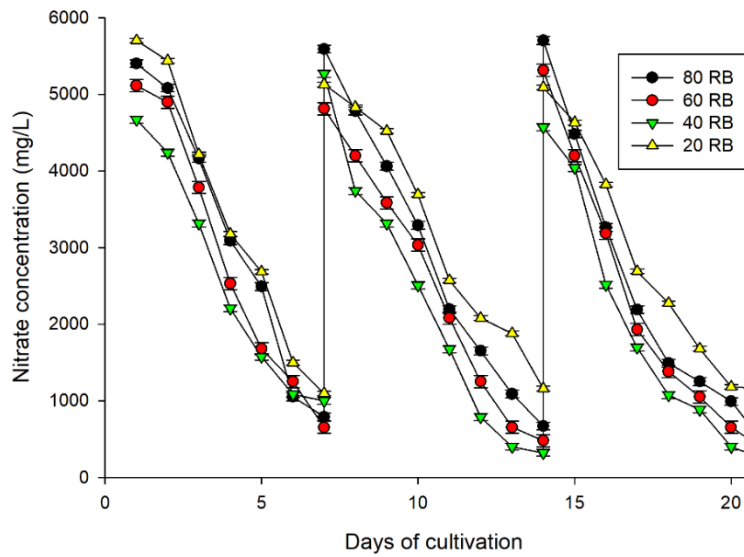


Figure 7.6: Nitrate concentration for the semi-batch *Chlorella vulgaris* cultivation.

Figure 7.6 illustrates that the first cycle's initial nitrate concentration is more persistent than the other two cycles. This is because the first cycle uses entirely fresh medium; however, when recycled and fresh medium are mixed, the initial nutrients content fluctuates since the recycled medium have minimum nutrient whereas the fresh medium is filled with nutrient. Thus, the nutrients are not distributed. Hence, stirring process was introduced to promote homogenous mixing. That is why, from the 2nd day onwards the nutrient curve does not fluctuate. Moreover, whenever a sufficient organic medium is substituted with BG-11, the pH value is also affected by the fluctuations in nutrient content [290]. Nonetheless, the nitrate compound is homogenized after 2 days, and each recycled medium combination demonstrates a significant difference. Despite having varied fresh medium volumes in the culture, it has been reported that nitrate concentration for the growth phase decreases as the number of cycles increases. As a result, it has been established that a high nutrient concentration does not assure optimal microalgae growth. However, the perfect combination of nutrients and medium is very important. Additionally, it has been discovered that as the number of cycles rises, the *chlorella vulgaris* species absorbs more nitrate content to achieve optimal growth. This is

because, the frequent use of recycled media into the PBR, resulted in high nitrate starvation during the cultivation process that trigger the algae species to consume all the nitrate source.

Figure 7.6 also indicated that the nitrate concentration was still reducing although the biomass development had reached a saturation point on the 12th day (cycle 2). This suggests that the medium is prompting *chlorella vulgaris* cells to synthesis optimum bio-chemical composition in a nitrate-depleted environment. A previous study found a similar trend, with cells able to produce the maximum carbohydrate, protein, and lipid productivities whenever nitrate concentrations are low while cell maturation is constant [291]. However, on the 21st day (cycle 3), the nitrate content in the 40RB mixing ratio medium remained consistent, which correlates with its cell culture growth. This is quite significant because, despite the abundance of nutrients, the microalgal biomass in 40RB has reached its maximal growth productivity. According to Figure 7.6, 60RB has the greatest nitrate removal at (87 %), followed by 80RB at (85%), 20RB at (81%), and 40RB at (79%) respectively for the first batch cycle.

However, upon the second cycle the nitrate reduction rate changes where 40RB has the greatest nitrate reduction removal at 94%, followed by 60RB at (90%), 80RB at (88%), and 20RB at (77%) respectively. The variation in nutritional concentration between a fresh media and a recycled medium causes this shift. However, Loftus et al., (2021) have shown that, though including industrial biscuit medium boost the development phase in the early stages, other aspects like as light dispersion are equally important [292]. In this study, as compared to the second cycle with recycled medium integration, the first cycle has a more continuous light source onto the culture during its initial phase. Based on Figure 7.6, it also proven that 40RB have the best nitrate concentration in the culture medium to generate an optimum semi-batch biomass growth for 21 days.

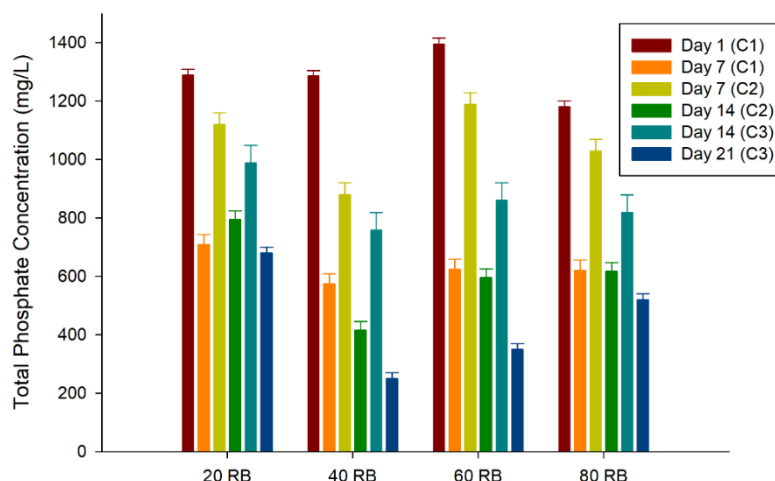


Figure 7.7: Phosphate variations for the semi-batch *Chlorella vulgaris* cultivation.

Polyphosphate is the most abundant speciation in microalgae, making phosphorus (P) an important resource for growth [173]. The major mineral, which is mostly consist in biscuit processing powders are phosphorus and previous research has found that biscuit manufacturing waste powders can be drawn as a feed source for microalgae development [286]. However, while using industrial biscuits manufacturing waste as a recycled medium, the cultures potentially possess different levels of phosphorus, as shown in Figure 7.7. Hence, the phosphate analysis assists in identifying the appropriate recycled medium for *chlorella vulgaris* growth in a semi batch mode. The phosphate content was measured every 7 days in this study to see if there were any variations between the initial fresh medium cycle and the recycled medium cycle. Day 7 (C1) is measured at the end of the cycle 1 (before adding fresh medium), meanwhile day 7 (C2) is measured in the beginning of cycle 2 (after adding fresh medium + recycled medium).

The highest initial phosphorus concentration for the first batch cycle was at 1395 mg/L in 60RB, followed by 1288 mg/L, 1285 mg/L, and 1180 mg/L in 20RB, 40RB, and 80RB, respectively. A high phosphorus concentration at the beginning does not guarantee optimal microalgae production. In this study, it is observed that the rise in phosphorus during the initial phase reduces as the number of cycle increases. This is because the integrated of recycled

medium consists lower phosphate content, compared to the fresh medium. Thus, determining the optimal recycled medium to fresh modified industrial biscuit waste medium ratio is essential. The consumption of phosphate was highest for 40RB during the first and second cycles, at 55% removal, and at 67% removal during the third cycle. On the other hand, the phosphate removal reduction for the other recycled medium combinations is in the range of 25% to 45%, which has an influence on biomass growth. Based on Figure 7.7, 60RB had the greatest beginning phosphorus content and had 48% of the phosphorus removed, the algal cells did not respond well to biomass development. This is because when there is an excess of phosphorus in the water, the microalgae cells to acquire minerals for effective microalgae development is diminished [293]. In addition, during stationary phase, both phosphate and nitrate limitations would create an optimal surrounding for *Chlorella vulgaris* to generate lipid, protein, and carbohydrate.

7.4 Bio-composition analysis

7.4.1 Microalgae-based lipid production

Microalgae biomass has drawn global interest due to its potential as a biofuel source. Findings reveal that using industrial biscuit manufacturing waste powder for culture promotes both microalgae biomass production and lipid content at the same time [153]. This study, on the other hand, presented a semi-batch cultivation approach based on recycled culture medium, which resulted in a more frequent high yield lipid synthesis. According to Table 7.2, the highest lipid content was found in 40RB for the first cycle, at 317.40 mg/g, followed by 60RB, 20RB, and 80RB, at 313.60 mg/g, 310.20 mg/g, and 308.00 mg/g indicating that the 40RB recycled medium provides optimum stress that induces lipid production. As for the following cycles, it was observed that the lipid generation reduces. This signifies that lipid deposition is influenced by the nutrients ingested throughout the culture medium's development phase as well as the nutrient deficiency stage [294]. Nutrient restriction is the major factors for

developing and promoting lipid production. In this investigation, nitrate insufficiency develops on the sixth day of culture and continues through the second and third cycles. Since the recycle medium is already lacking in nitrate and phosphate, and only a portion of the new medium is consumed, the nutritional deficiency persists.

Previous research found that nutrient restriction for two days enhanced lipid generation to 260.44 mg/g without reducing biomass growth, indicating that it might produce biofuel at a low cost [153]. However, the highest lipid concentration in this study was 317.40 mg/g for a 40C1 ratio with less than 24 hours of nutritional deprivation. Light penetration impacts lipid production because microalgae collect radiance for photosynthesis and capture bio-compound such as lipids. Microalgal biomass concentration drops when light intensity is low, leading to poor growth and a negative impact on lipid accumulation [295]. Meanwhile, too much light induces photoinhibition, which harms microalgal photosystems and reduces lipid production. As a result, adequate light distribution must be provided during culture to encourage microalgae growth. However, when recycling media is utilized, the light dispersion changes, notably in cycles 2 and 3. Thus, it is observed that 40RB ratio mixing accumulated under 6000 lx (LED light) yielded the most lipid synthesis. In summary, a culture media deficient in phosphate and nitrate with enough light distribution would develop an efficient lipid from the algae cells.

Table 7.2: Bio-composition analysis for the semi-batch *Chlorella vulgaris* cultivation.

Culture Combination	Lipid (mg/g)	Protein (mg/g)	Carbohydrate (mg/g)
Cycle 1, C1			
20C1	310.20 ± 5.5	207.30 ± 2.1	326.61 ± 2.3
40C1	317.40 ± 3.2	280.57 ± 1.5	451.37 ± 0.1
60C1	313.60 ± 2.2	230.41 ± 4.2	365.39 ± 3.1
80C1	308.00 ± 4.5	205.25 ± 5.1	302.25 ± 1.8
Cycle 2, C2			
20C2	229.19 ± 3.1	201.98 ± 1.5	224.18 ± 1.8
40C2	286.36 ± 1.3	266.56 ± 2.3	388.70 ± 0.1
60C2	272.22 ± 2.2	213.88 ± 0.6	229.31 ± 1.3
80C2	208.44 ± 4.2	202.56 ± 2.5	218.05 ± 2.0

Cycle 3, C3			
20C3	117.95 ± 3.5	199.63 ± 0.8	179.96 ± 3.3
40C3	132.82 ± 2.6	254.35 ± 1.1	279.09 ± 2.1
60C3	114.06 ± 2.2	204.40 ± 2.2	231.38 ± 0.3
80C3	121.99 ± 1.3	194.92 ± 1.3	170.27 ± 0.4

*Note: Data present are the mean of three runs ±SD

7.4.2 Microalgae protein extraction

Protein and polysaccharide formation is prominent in dairy industry due to their molecular content in the process cycle [296]. Nevertheless, protein has a high amino acid content and is suitable for meals, pharmaceutical products, and livestock feed [297]. As a result, the efficiency of protein production for the recycling ratio of industrial biscuit manufacturing waste coupled with BG-11 was studied. Based on Table 7.2, the optimal protein concentration in 40RB at 286.36 mg/g followed by 60RB, 20RB, and 80RB with protein value of 272.22 mg/g, 229.19 mg/g, and 208.44 mg/g. The proportion of nitrate uptake in *chlorella vulgaris* cells is the primary source of protein production. Nitrates is discovered in cellular macromolecules such as proteins, polysaccharides, enzymes, and algae, and it is required for the absorption mechanism that changes nitrates to its basic formula [298]. According to Figure 7.6, the algae cells absorb 94 % of the nitrate throughout culture. Table 7.2 lists the quantity of protein generated by *Chlorella vulgaris* grown in standard and nitrogen deficiency cultures.

Light conditions during the microalgae growing phase are also important for protein development in microalgae, as prior study has indicated that greater light source and extended photoperiod leads in higher protein synthesis [299]. In this study, light penetration varied throughout the first two days of cycles 2 and 3, depending on the integration ratio, even though the LED light distribution remained constant throughout the culture period. However, for the first batch cycle all the PBR has a constant light distribution. Microalgae consumes light for metabolism, cell division, and nutritive value buildup, protein content fluctuates [151]. In comparison to a prior study, *chlorella vulgaris* generated 263.93mg/g protein concentration after 24 hours of light exposure [153]. Meanwhile, this study shows that with 40RB of recycled

culture media, the protein content may be kept at an optimal level (280.57 - 254.35 mg/g of protein content).

7.4.3 Evaluation of microalgae carbohydrate content

In photosynthesis, carbohydrate is the primary product *via* carbon fixation metabolism [300]. These starches are either kept in the plastids (starch) as backup supplies of the cell membranes. Nonetheless, the distribution and synthesis of carbohydrates in microalgae can vary dramatically relying on culture circumstances such as light, nitrate exhaustion, temperature shift, pH shift, and CO₂ supply [301]. In this work, nitrate restriction and light distribution were two crucial criteria that led to the formation of carbohydrate in *Chlorella vulgaris* cells. It was observed that, 40RB (451.37 mg/g) had the greatest carbohydrate value, followed by 60RB (365.39 mg/g), 20RB (326.61 mg/g), and 80RB (302.25mg/g). Moreover, it is observed that the carbohydrate content reduces rapidly when there is a combination of recycled medium. This is due to the extremely low nitrate content at the end of the culture cycles. When microalgal cells are subjected to nitrogen-depleted growth medium, it typically exhibits biomass yield, that restricts their biochemical profile [302].

Meanwhile, the *Chlorella vulgaris* strain was able to boost lipid, protein, and carbohydrate content while maintaining microalgae development. Although the recycled medium has a lower carbohydrate content than the first batch cycle, it is still suitable for large-scale production due to the expense of the re-starting batch cycle cultivation approach. Furthermore, appropriate lighting is required for microalgal growth since light sources can generate energy that is subsequently captured in the algal cells as starches [303]. Light distribution was discovered to produce starch accumulation in algal cells, and this investigation makes use of a 6000 Lux LED light supply that runs 24 hours a day. Previous studies have showed that light intensities ranging from 40 to 400 mol/m²s⁻¹ enhance carbohydrate accumulation with the range of 269.62 mg/g to 343.84 mg/g [304]. Overall, 40RB recycle medium combination generates the optimum

surroundings for lipid, protein, and carbohydrate concentration for a semi-batch cultivation system. Utilizing recycled media with smart algae growth monitoring device, can reduce the cost of *chlorella vulgaris* cultivation system especially for large scale production.

7.5 Conclusion

There is a great demand for a clean, economical, and long-term energy source, due to the depletion of fossil fuels. This gives the algal production-based biofuel a wider energy source business standpoint, as Industry 4.0 is widely being explored. The conventional UV-Vis Spectrophotometry approaches are excessively time consuming, costly, and inconvenient, preventing stakeholders from pursuing large-scale algae cultivation. As a result, modern digital technologies, and Internet of Things (IoT) were implemented. This study also shows that semi-batch cultivation with recycling culture medium of 40RB ratio could yield the maximum biomass growth (4.52g/L), lipid yield (317.40 mg/g), protein content (280.57 mg/g), and carbohydrate concentration (451.37 mg/g). Ultimately, this study proposes that 40RB of industrial biscuit manufacturing waste fresh medium combined with 40RB recycled media can be applied for semi-batch biomass production, and that a custom vision service is best used to detect *Chlorella vulgaris* development for smart data microalgae growth surveillance.

CHAPTER 8

CONCLUSION

This thesis mainly focuses on the production process of *Chlorella vulgaris* and the potential techniques on digitalization of microalgae growth monitoring. First, the application of industrial dairy waste was used as an alternative of inorganic medium (BG-11). It was discovered that the industrial dairy biscuit waste powders enhanced the *Chlorella vulgaris* growth more than inorganic medium, BG-11. Moreover, this approach will be able to lower the operational costs for large-scale microalgae growth. Further testing was conducted to justify and test the replacement efficiency and feasibility. The combination of industrial biscuit waste powder with control medium, BG-11 culture for microalgae development has both economic and environmental benefits. This is because, rather than spending money for the disposal of waste powder, it can be mixed with growth media to promote microalgae biomass with valuable bio-compositions including lipid, protein, and carbohydrate. Next, the study optimizes the microalgae cultivation with industrial biscuit waste remediation based on vision imaging systems for biomass growth monitoring. The optimal culture medium combination for the development of microalgae was identified to be 40% industrial dairy biscuit waste powders combined with 60% BG-11. On the other hand, RGB model also indicated the best cultivation environment for *Chlorella vulgaris* at 40% industrial dairy biscuit waste powder mixing ratio with 60% BG-11. Thus, the use of artificial intelligence in microalgae cultivation is considerably faster and more efficient than the traditional experiment analysis method. It can be inferred that the RGB colour model can be used for the development of mobile applications for estimating biomass growth data using images raster pixel.

Moving forward, the scaling up of algal cultures to large volumes for commercial production is a challenging task that involves high capital and operating cost. Thus, the feasibility of a laboratory scale culture process was tested, before expanding the production of *Chlorella*

vulgaris using industrial waste powder as nutrient source in large scale. The use of LCA has been effective in the industrial scale scaling up of microalgae culture systems that generate 600 g/d dried microalgae biomass. It was identified that the use of re-cycle biscuit processing waste as culture media was the best approach for a large-scale operation. Next, the fourth objective of this thesis was to develop a mobile application (FindAlgae) for microalgae growth monetarization based on vision imaging system. In brief, the algae culture growth images will be captured and stored in the mobile's gallery. These images will be uploaded to the FindAlgae application, and data analysis will be extracted from custom vision service and produce corresponding results which consist of a range of algae concentration alongside with the growth stage. Overall, the mobile application (FindAlgae) was successfully developed, however it is only limited to android users. The lesson learnt here will be that more intense data collection and coding knowledge should be applied in merging android and apple (iCloud) based devices. Lastly the thesis was wrapped up by evaluating the mobile application (FindAlgae) and optimization of the recycling of culture medium in semi-batch cultivation. Ultimately, it was discovered that the semi-batch cultivation with 40% recycled industrial biscuit manufacturing waste fresh medium combined 60% fresh medium with yielded the maximum biomass growth and that the iteration model (FindAlgae) delivers result interpretation with an accuracy of more than 92 % of every data set based on the trained Model Performance.

Digitalization is the technique of reducing mistakes in a process by incorporating and regulating the tracking of variation and adjustment action. There are numerous levels of digitalization, each needing a specific level of intelligence from the technology. Algae-based biofuel manufacturing confronts significant challenges especially for large scale application: (1) inefficient photosynthetic solar energy collection; (2) inappropriate growth conditions (pH and temperature control); and (3) high cultivating and downstream processing energy and economic expenses. In near future, the implementation of machine learning can be integrated

with downstream extraction of microalgae bio-chemical that will convince more stakeholders into microalgae-based production. Innovation in microalgae-based system would be immensely increase the interest of stakeholders in investing into large scale plant to produce microalgae biomass which can be beneficial to the environment as well.

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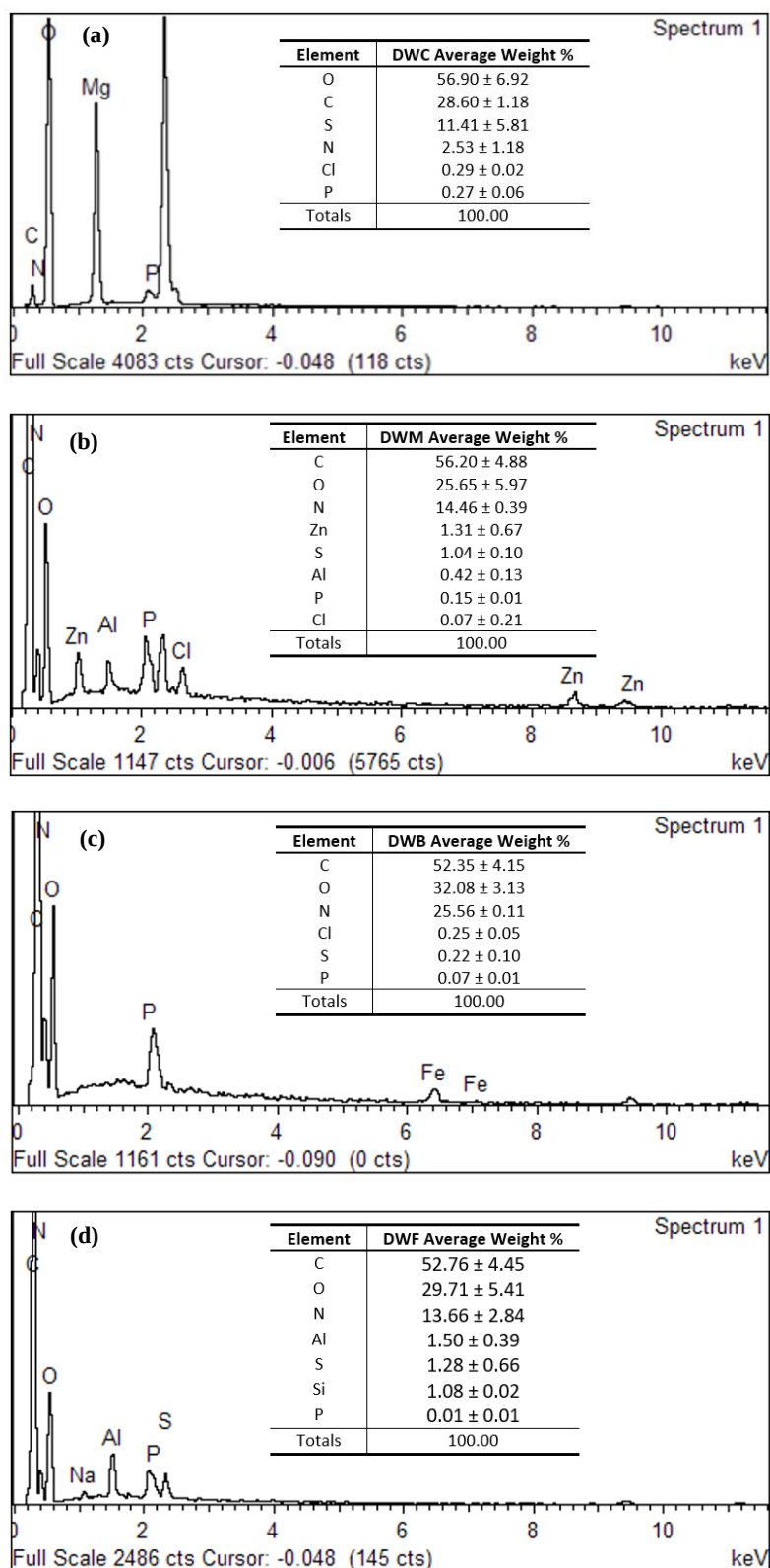
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- [304] Ho S, Chen C, Chang J. Bioresource Technology Effect of light intensity and nitrogen starvation on CO₂ fixation and lipid / carbohydrate production of an indigenous microalga *Scenedesmus obliquus* CNW - N. *Bioresour Technol* 2012;113:244–52. <https://doi.org/10.1016/j.biortech.2011.11.133>.

APPENDICES

APPENDIX I: CHAPTER 2



Appendix Figure 1: Energy-dispersive X-ray spectroscopy detected for industrial dairy food processing waste (a) DM1 (b) DM2 (c) DB1 (d) DB2

APPENDIX II: CODINGS FOR COLOUR MODEL ANALYSIS - CHAPTER 3

I. ColourModelConversion/.gitattributes

```
#####  
####  
  
# Set default behavior to automatically normalize line endings.  
  
#####  
####  
  
* text=auto  
  
#####  
####  
  
# Set default behavior for command prompt diff.  
  
#  
  
# This is need for earlier builds of msysgit that does not have it on by  
# Default for csharp files.  
  
# Note: This is only used by command line  
  
#####  
####  
  
#*.cs    diff=csharp  
  
#####  
####  
  
# Set the merge driver for project and solution files  
  
#  
  
# Merging from the command prompt will add diff markers to the files if there  
# are conflicts (Merging from VS is not affected by the settings below, in VS  
# the diff markers are never inserted). Diff markers may cause the following  
# file extensions to fail to load in VS. An alternative would be to treat  
# these files as binary and thus will always conflict and require user  
# intervention with every merge. To do so, just uncomment the entries below  
  
#####  
####  
  
#*.sln    merge=binary
```

```

#*.csproj merge=binary
#*.vbproj merge=binary
#*.vcxproj merge=binary
#*.vcproj merge=binary
#*.dbproj merge=binary
#*.fsproj merge=binary
#*.lsproj merge=binary
#*.wixproj merge=binary
#*.modelproj merge=binary
#*.sqlproj merge=binary
#*.wwaproj merge=binary

#####
####

# behavior for image files

#

# image files are treated as binary by default.

#####
####

#*.jpg binary
#*.png binary
#*.gif binary

#####
####

# diff behavior for common document formats

#

# Convert binary document formats to text before diffing them. This feature
# is only available from the command line. Turn it on by uncommenting the
# entries below.

#####
####

#*.doc diff=astextplain
#*.DOC diff=astextplain

```

```
##*.docx diff=astextplain
##*.DOCX diff=astextplain
##*.dot diff=astextplain
##*.DOT diff=astextplain
##*.pdf diff=astextplain
##*.PDF diff=astextplain
##*.rtf diff=astextplain
##*.RTF diff=astextplain
```

II. ColourModelConversion/.gitignore

```
## Ignore Visual Studio temporary files, build results, and
## files generated by popular Visual Studio add-ons.

##

## Get latest from https://github.com/github/gitignore/blob/master/VisualStudio.gitignore
# User-specific files
*.rsuser
*.suo
*.user
*.userosscache
*.sln.docstates

# User-specific files (MonoDevelop/Xamarin Studio)
*.userprefs

# Mono auto generated files
mono_crash.*

# Build results
[Dd]ebug/
[Dd]ebugPublic/
[Rr]elease/
[Rr]eleases/
```

x64/

x86/

[Ww][Ii][Nn]32/

[Aa][Rr][Mm]/

[Aa][Rr][Mm]64/

bld/

[Bb]in/

[Oo]bj/

[Oo]ut/

[Ll]og/

[Ll]ogs/

Visual Studio 2015/2017 cache/options directory

.vs/

Uncomment if you have tasks that create the project's static files in wwwroot

#wwwroot/

Visual Studio 2017 auto generated files

Generated\ Files/

MSTest test Results

[Tt]est[Rr]esult*/

[Bb]uild[Ll]og.*

NUnit

*.VisualState.xml

TestResult.xml

nunit-*.xml

Build Results of an ATL Project

[Dd]ebugPS/

[Rr]eleasePS/

dlldata.c

Benchmark Results

BenchmarkDotNet.Artifacts/

.NET Core

project.lock.json

project.fragment.lock.json

artifacts/

ASP.NET Scaffolding

ScaffoldingReadMe.txt

StyleCop

StyleCopReport.xml

Files built by Visual Studio

*_i.c

*_p.c

*_h.h

*.ilk

*.meta

*.obj

*.iobj

*.pch

*.pdb

*.ipdb

*.pgc

*.pgd

*.rsp

*.sbr

*.tlb

*.tli

*.tlh

*.tmp

*.tmp_proj

*_wpftmp.csproj

*.log

*.vspcc

*.vsscc

.builds

*.pidb

*.svclog

*.scc

Chutzpah Test files

_Chutzpah*

Visual C++ cache files

ipch/

*.aps

*.ncb

*.opendb

*.opensdf

*.sdf

*.cachefile

*.VC.db

*.VC.VC.opendb

Visual Studio profiler

*.psess

*.vsp

*.vspx

*.sap

Visual Studio Trace Files

*.e2e

TFS 2012 Local Workspace

\$tf/

Guidance Automation Toolkit

*.gpState

ReSharper is a .NET coding add-in

_ReSharper*/

*.[Rr]e[Ss]harper

*.DotSettings.user

TeamCity is a build add-in

_TeamCity*

DotCover is a Code Coverage Tool

*.dotCover

AxoCover is a Code Coverage Tool

.axoCover/*

!.axoCover/settings.json

Coverlet is a free, cross platform Code Coverage Tool

coverage*.json

coverage*.xml

coverage*.info

Visual Studio code coverage results

*.coverage

*.coveragexml

NCrunch

_NCrunch_*

.*crunch*.local.xml

nCrunchTemp_*

MightyMoose

.mm.

AutoTest.Net/

Web workbench (sass)

.sass-cache/

Installshield output folder

[Ee]xpress/

DocProject is a documentation generator add-in

DocProject/buildhelp/

DocProject/Help/*.HxT

DocProject/Help/*.HxC

DocProject/Help/*.hhc

DocProject/Help/*.hhk

DocProject/Help/*.hhp

DocProject/Help/Html2

DocProject/Help/html

```

# Click-Once directory
publish/

# Publish Web Output
*.[Pp]ublish.xml
*.azurePubxml
# Note: Comment the next line if you want to checkin your web deploy settings,
# but database connection strings (with potential passwords) will be unencrypted
*.pubxml
*.publishproj

# Microsoft Azure Web App publish settings. Comment the next line if you want to
# checkin your Azure Web App publish settings, but sensitive information contained
# in these scripts will be unencrypted
PublishScripts/

# NuGet Packages
*.nupkg
# NuGet Symbol Packages
*.snupkg
# The packages folder can be ignored because of Package Restore
**/[Pp]ackages/*
# except build/, which is used as an MSBuild target.
!**/[Pp]ackages/build/
# Uncomment if necessary however generally it will be regenerated when needed
#!**/[Pp]ackages/repositories.config
# NuGet v3's project.json files produces more ignorable files
*.nuget.props
*.nuget.targets

# Microsoft Azure Build Output

```

```
csx/
*.build.csdef

# Microsoft Azure Emulator
ecf/
rcf/

# Windows Store app package directories and files
AppPackages/
BundleArtifacts/
Package.StoreAssociation.xml
_pkginfo.txt
*.appx
*.appxbundle
*.appxupload

# Visual Studio cache files
# files ending in .cache can be ignored
*.[Cc]ache
# but keep track of directories ending in .cache
!?*.[Cc]ache/

# Others
ClientBin/
~$*
*~
*.dbmdl
*.dbproj.schemaview
*.jfm
*.pfx
*.publishsettings
```

orleans.codegen.cs

Including strong name files can present a security risk

(<https://github.com/github/gitignore/pull/2483#issue-259490424>)

*.snk

Since there are multiple workflows, uncomment next line to ignore bower_components

(<https://github.com/github/gitignore/pull/1529#issuecomment-104372622>)

bower_components/

RIA/Silverlight projects

Generated_Code/

Backup & report files from converting an old project file

to a newer Visual Studio version. Backup files are not needed,

because we have git ;-)

_UpgradeReport_Files/

Backup*/

UpgradeLog*.XML

UpgradeLog*.htm

ServiceFabricBackup/

*.rptproj.bak

SQL Server files

*.mdf

*.ldf

*.ndf

Business Intelligence projects

*.rdl.data

*.bim.layout

*.bim_*.settings

*.rptproj.rsuser

*- [Bb]ackup.rdl

*- [Bb]ackup ([0-9]).rdl

*- [Bb]ackup ([0-9][0-9]).rdl

Microsoft Fakes

FakesAssemblies/

GhostDoc plugin setting file

*.GhostDoc.xml

Node.js Tools for Visual Studio

.ntvs_analysis.dat

node_modules/

Visual Studio 6 build log

*.plg

Visual Studio 6 workspace options file

*.opt

Visual Studio 6 auto-generated workspace file (contains which files were open etc.)

*.vbw

Visual Studio LightSwitch build output

**/*.HTMLClient/GeneratedArtifacts

**/*.DesktopClient/GeneratedArtifacts

**/*.DesktopClient/ModelManifest.xml

**/*.Server/GeneratedArtifacts

**/*.Server/ModelManifest.xml

_Pvt_Extensions

Paket dependency manager

.paket/paket.exe

paket-files/

FAKE - F# Make

.fake/

CodeRush personal settings

.cr/personal

Python Tools for Visual Studio (PTVS)

__pycache__/

*.pyc

Cake - Uncomment if you are using it

tools/**

!tools/packages.config

Tabs Studio

*.tss

Telerik's JustMock configuration file

*.jmconfig

BizTalk build output

*.btp.cs

*.btm.cs

*.odx.cs

*.xsd.cs

OpenCover UI analysis results

OpenCover/

Azure Stream Analytics local run output

ASALocalRun/

MSBuild Binary and Structured Log

*.binlog

NVidia Nsight GPU debugger configuration file

*.nvuser

MFractors (Xamarin productivity tool) working folder

.mfractor/

Local History for Visual Studio

.localhistory/

BeatPulse healthcheck temp database

healthchecksdb

Backup folder for Package Reference Convert tool in Visual Studio 2017

MigrationBackup/

Ionide (cross platform F# VS Code tools) working folder

.ionide/

Fody - auto-generated XML schema

FodyWeavers.xsd

III. ColourModelConversion/DateConvertSql.sln

Microsoft Visual Studio Solution File, Format Version 12.00

Visual Studio Version 16

VisualStudioVersion = 16.0.30621.155

MinimumVisualStudioVersion = 10.0.40219.1

Project("{FAE04EC0-301F-11D3-BF4B-00C04F79EFBC}") = "DateConvertSql",
"DateConvertSql\DateConvertSql.csproj", "{AA5E2D44-5170-4F39-9C26-
F220DC91A143}"

EndProject

Global

GlobalSection(SolutionConfigurationPlatforms) = preSolution

Debug|Any CPU = Debug|Any CPU

Release|Any CPU = Release|Any CPU

EndGlobalSection

GlobalSection(ProjectConfigurationPlatforms) = postSolution

{AA5E2D44-5170-4F39-9C26-F220DC91A143}.Debug|Any CPU.ActiveCfg
= Debug|Any CPU

{AA5E2D44-5170-4F39-9C26-F220DC91A143}.Debug|Any CPU.Build.0 =
Debug|Any CPU

{AA5E2D44-5170-4F39-9C26-F220DC91A143}.Release|Any
CPU.ActiveCfg = Release|Any CPU

{AA5E2D44-5170-4F39-9C26-F220DC91A143}.Release|Any CPU.Build.0 =
Release|Any CPU

EndGlobalSection

GlobalSection(SolutionProperties) = preSolution

HideSolutionNode = FALSE

EndGlobalSection

GlobalSection(ExtensibilityGlobals) = postSolution

SolutionGuid = {DE0E683E-6D85-4D6C-A44B-7383B77E29DB}

EndGlobalSection

EndGlobal

IV. ColourModelConversion/DateConvertSql/App.config

```
<?xml version="1.0" encoding="utf-8" ?>

<configuration>

    <startup>

        <supportedRuntime version="v4.0" sku=".NETFramework,Version=v4.7.2" />

    </startup>

</configuration>
```

ColourModelConversion/DateConvertSql/ColorModel.cs

```
using
System;

    using System.Collections.Generic;
    using System.Linq;
    using System.Text;
    using System.Threading.Tasks;

    namespace DateConvertSql
    {
        public class ColorModel
        {
            public class HSLColor
            {
                public float Hue;
                public float Saturation;
                public float Luminosity;

                public HSLColor(float H, float S, float L)
                {
                    Hue = H;
                    Saturation = S;
                    Luminosity = L;
                }

                public static HSLColor FromRGB(Byte R, Byte G, Byte B)
                {
                    float _R = (R / 255f);
                    float _G = (G / 255f);
                    float _B = (B / 255f);

                    float _Min = Math.Min(Math.Min(_R, _G), _B);
                    float _Max = Math.Max(Math.Max(_R, _G), _B);
                    float _Delta = _Max - _Min;
```

```

float H = 0;
float S = 0;
float L = (float)((_Max + _Min) / 2.0f);

if (_Delta != 0)
{
    if (L < 0.5f)
    {
        S = (float)(_Delta / (_Max + _Min));
    }
    else
    {
        S = (float)(_Delta / (2.0f - _Max - _Min));
    }

    if (_R == _Max)
    {
        H = (_G - _B) / _Delta;
    }
    else if (_G == _Max)
    {
        H = 2f + (_B - _R) / _Delta;
    }
    else if (_B == _Max)
    {
        H = 4f + (_R - _G) / _Delta;
    }
}

//convert to 239,240,240
H = H / 6f * 239f;
if (H < 0)
    H += 239;
//H = H * 60f;
//if (H < 0)
//    H += 360;
//H=H / 360 * 239;
S *= 240f;
L *= 240f;
return new HSLColor(H, S, L);
}

}

public class CMYKColor

```

```

    {
        public float Cyan;
        public float Magenta;
        public float Yellow;
        public float Key;//black

        public CMYKColor(float c, float m, float y, float k)
        {
            Cyan = c;
            Magenta = m;
            Yellow = y;
            Key = k;
        }
        public static CMYKColor FromRGB(Byte R, Byte G, Byte B)
        {
            float Rc = R / 255f;
            float Gc = G / 255f;
            float Bc= B / 255f;
            float k = 1 - Math.Max(Math.Max(Rc, Gc), Bc);
            float c = (1 - Rc - k) / (1 - k);
            float m = (1 - Gc - k) / (1 - k);
            float y = (1 - Bc - k) / (1 - k);
            c *= 100f;
            m *= 100f;
            y *= 100f;
            k *= 100f;
            return new CMYKColor(c,m,y,k);
        }
    }
}
}

```

ColourModelConversion/DateConvertSql/DateConvertSql.csproj

```

<?xml
version="1
.0"
encoding=
"utf-8"?>

  <Project ToolsVersion="15.0"
xmlns="http://schemas.microsoft.com/developer/msbuild/2003">
    <Import
Project="$(MSBuildExtensionsPath)\$(MSBuildToolsVersion)\Microsoft.C
ommon.props"

```

```

Condition="Exists('$(MSBuildExtensionsPath)\$(MSBuildToolsVersion)\
Microsoft.Common.props')" />
  <PropertyGroup>
    <Configuration Condition=" '$(Configuration)' == "
">Debug</Configuration>
      <Platform Condition=" '$(Platform)' == " ">AnyCPU</Platform>
      <ProjectGuid>{AA5E2D44-5170-4F39-9C26-
F220DC91A143}</ProjectGuid>
      <OutputType>Exe</OutputType>
      <RootNamespace>DateConvertSql</RootNamespace>
      <AssemblyName>DateConvertSql</AssemblyName>
      <TargetFrameworkVersion>v4.7.2</TargetFrameworkVersion>
      <FileAlignment>512</FileAlignment>
      <AutoGenerateBindingRedirects>true</AutoGenerateBindingRedirects>
      <Deterministic>true</Deterministic>
    </PropertyGroup>
    <PropertyGroup Condition=" '$(Configuration)|$(Platform)' ==
'Debug|AnyCPU' ">
      <PlatformTarget>AnyCPU</PlatformTarget>
      <DebugSymbols>true</DebugSymbols>
      <DebugType>full</DebugType>
      <Optimize>>false</Optimize>
      <OutputPath>bin\Debug\</OutputPath>
      <DefineConstants>DEBUG;TRACE</DefineConstants>
      <ErrorReport>prompt</ErrorReport>
      <WarningLevel>4</WarningLevel>
    </PropertyGroup>
    <PropertyGroup Condition=" '$(Configuration)|$(Platform)' ==
'Release|AnyCPU' ">
      <PlatformTarget>AnyCPU</PlatformTarget>
      <DebugType>pdbonly</DebugType>
      <Optimize>true</Optimize>
      <OutputPath>bin\Release\</OutputPath>
      <DefineConstants>TRACE</DefineConstants>
      <ErrorReport>prompt</ErrorReport>
      <WarningLevel>4</WarningLevel>
    </PropertyGroup>
    <ItemGroup>
      <Reference Include="Microsoft.VisualBasic" />
      <Reference Include="PermutationLibrary, Version=2.2.0.0,
Culture=neutral, processorArchitecture=MSIL">
        <HintPath>..\packages\PermutationLibrary.2.2.0\lib\net45\PermutationLibr
ary.dll</HintPath>
      </Reference>
      <Reference Include="System" />

```

```

    <Reference Include="System.Core" />
    <Reference Include="System.Xml.Linq" />
    <Reference Include="System.Data.DataSetExtensions" />
    <Reference Include="Microsoft.CSharp" />
    <Reference Include="System.Data" />
    <Reference Include="System.Net.Http" />
    <Reference Include="System.Xml" />
  </ItemGroup>
  <ItemGroup>
    <Compile Include="ColorModel.cs" />
    <Compile Include="Program.cs" />
    <Compile Include="Properties\AssemblyInfo.cs" />
  </ItemGroup>
  <ItemGroup>
    <None Include="App.config" />
    <None Include="packages.config" />
  </ItemGroup>
  <Import Project="$(MSBuildToolsPath)\Microsoft.CSharp.targets" />
</Project>

```

ColourModelConversion/DateConvertSql/Program.cs

```

using
Syste
m;

using System.Collections.Generic;
using System.IO;
using System.Linq;
using System.Text;
using System.Threading.Tasks;
using Microsoft.VisualBasic.FileIO;
using HSLColor= DateConvertSql.ColorModel.HSLColor;
using CMYKColor= DateConvertSql.ColorModel.CMYKColor;

namespace DateConvertSql
{
    class Program
    {
        static List<int> red = new List<int>();
        static List<int> green = new List<int>();
        static List<int> blue = new List<int>();

        static void Main(string[] args)
        {
            //SqlQuery();
            while (true)

```

```

    {
        ColourIndex();
        Console.WriteLine("Press enter to continue");
        Console.ReadLine();
    }

}

private static void ColourIndex()
{
    var path = @"C:\Users\User\Downloads\result.txt";
    string[] lines = File.ReadAllLines(path);
    foreach (string line in lines)
    {
        IndexToRgb(int.Parse(line));
    }

    Console.WriteLine($"Count:{red.Count}");
    //RgbToHSL();
    //RgbToCmyk();
    #region grb
    for (int i=0;i<red.Count;i++)
    {
        Console.WriteLine($"{RgbToIndex(green[i],red[i],blue[i])}");
    }
    #endregion
    red.Clear();
    green.Clear();
    blue.Clear();
}

public static int RgbToIndex(int MostSignificantColor, int
MediumSignificantColor, int LeastSignificantColor)
{
    return (MostSignificantColor * 256 * 256) + (MediumSignificantColor
* 256) +
        LeastSignificantColor; //zero based
}

public static int RgbToIndex_Average(int r, int g, int b)
{
    //permutation with repetition
    //return ((r & 0x0ff) << 16) | ((g & 0x0ff) << 8) | (b & 0x0ff);
    return ((r + 1) * (g + 1) * (b + 1)) - 1;
}

```

```

public static void RgbToHSL()
{
    for (int i = 0; i < red.Count; i++)
    {
        var hsl = HSLColor.FromRGB((byte)red[i], (byte)green[i],
(byte)blue[i]);
        Console.WriteLine($"RGB:{red[i]},{green[i]},{blue[i]},
HSL:{{(int)hsl.Hue}},{{(int)hsl.Saturation}},{{(int)hsl.Luminosity}}");
    }
    for (int i = 0; i < red.Count; i++)
    {
        var hsl = HSLColor.FromRGB((byte)red[i], (byte)green[i],
(byte)blue[i]);
        var hslIndex = (hsl.Hue * 241 * 241) + (hsl.Saturation * 241) +
hsl.Luminosity;
        Console.WriteLine($"{{(int)hslIndex}}");
    }
}

public static void RgbToCmyk()
{
    for (int i = 0; i < red.Count; i++)
    {
        var cmyk = CMYKColor.FromRGB((byte)red[i], (byte)green[i],
(byte)blue[i]);
        Console.WriteLine($"RGB:{red[i]},{green[i]},{blue[i]},
CMYK:{{(int)cmyk.Cyan}},{{(int)cmyk.Magenta}},{{(int)cmyk.Yellow}},{{(int)cmy
k.Key}}");
    }
    for (int i = 0; i < red.Count; i++)
    {
        var cmyk = CMYKColor.FromRGB((byte)red[i], (byte)green[i],
(byte)blue[i]);
        //var cmykIndex = (cmyk.Cyan * 101*101*101) + (cmyk.Magenta *
101*101) + (cmyk.Yellow*101)+cmyk.Key;
        var cmykIndex = (cmyk.Yellow * 101*101*101) + (cmyk.Key *
101*101) + (cmyk.Cyan*101)+cmyk.Magenta;
        Console.WriteLine($"{{(int)cmykIndex}}");
    }
}
/// <summary>
/// This function change the index computed from RGB values to R,G,B
values
/// </summary>
/// <param name="OriIndex"></param>
public static void IndexToRgb(int OriIndex)

```

```

{
    int index = OriIndex;
    int R, G, B;
    ///65536
    //index = (AverageR * 256 * 256) + (AverageG * 256) + AverageB;
    if (index >= 65536)
    {
        R = index / 65536;
        index -= R * 65536;
        if (index >= 256)
        {
            G = index / 256;
            B = index - G * 256;
        }
        else
        {
            G = 0;
            B = index;
        }
    }
    else
    {
        R = 0;
        if (index >= 256)
        {
            G = index / 256;
            B = index - G * 256;
        }
        else
        {
            G = 0;
            B = index;
        }
    }

    red.Add(R);
    green.Add(G);
    blue.Add(B);
    Console.WriteLine($"RGB:{R},{G},{B},Index:{OriIndex}");
}

private static void SqlQuery()
{
    var path = @"C:\Users\User\Downloads\siaedit.csv";
    using (TextFieldParser csvParser = new TextFieldParser(path))
    {

```

```

csvParser.CommentTokens = new string[] { "#"};
csvParser.SetDelimiters(new string[] { ","});
csvParser.HasFieldsEnclosedInQuotes = true;
// Skip the row with the column names
csvParser.ReadLine();
string nickname = "";
List<string> title = new List<string>();
List<string> first_name = new List<string>();
List<string> last_name = new List<string>();
List<string> school = new List<string>();
List<string> url = new List<string>();
List<string> country = new List<string>();
List<string> residence = new List<string>();
List<string> interest = new List<string>();
List<string> reason = new List<string>();
int LineNo = 0;
int userid = 2;
int id = 136;
using (System.IO.StreamWriter file =
    new
System.IO.StreamWriter(@"C:\Users\User\Downloads\query.txt"))
{
    while (!csvParser.EndOfData)
    {

        // Read current line fields, pointer moves to the next line.
        string[] fields = csvParser.ReadFields();
        first_name.Add(fields[3]);
        title.Add(fields[2]);
        last_name.Add(fields[4]);
        school.Add(fields[5]);
        country.Add(fields[7]);
        residence.Add(fields[8]);
        interest.Add(fields[9]);
        reason.Add(fields[10]);
        url.Add(fields[6]);
        string query = "INSERT INTO wp_bp_xprofile_data VALUES("
+ id + ",3," + userid + "," +
            nickname + ",0);";
        id++;
        //Console.WriteLine(query);
        file.WriteLine(query);
        query = "INSERT INTO wp_bp_xprofile_data VALUES(" + id
+ ",1," + userid + "," +
            first_name[LineNo] + ",0);";
        id++;
    }
}

```

```

        //Console.WriteLine(query);
        file.WriteLine(query);
        query = "INSERT INTO wp_bp_xprofile_data VALUES(" + id
+ ",4," + userid + "," + title[LineNo] +
        ",0)";
        id++;
        //Console.WriteLine(query);
        file.WriteLine(query);
        query = "INSERT INTO wp_bp_xprofile_data VALUES(" + id
+ ",2," + userid + "," +
        last_name[LineNo] + ",0)";
        id++;
        //Console.WriteLine(query);
        file.WriteLine(query);
        query = "INSERT INTO wp_bp_xprofile_data VALUES(" + id
+ ",9," + userid + "," +
        school[LineNo] + ",0)";
        id++;
        //Console.WriteLine(query);
        file.WriteLine(query);
        query = "INSERT INTO wp_bp_xprofile_data VALUES(" + id
+ ",10," + userid + "," +
        country[LineNo] + ",0)";
        id++;
        //Console.WriteLine(query);
        file.WriteLine(query);
        query = "INSERT INTO wp_bp_xprofile_data VALUES(" + id
+ ",14," + userid + "," +
        residence[LineNo] + ",0)";
        id++;
        //Console.WriteLine(query);
        file.WriteLine(query);
        query = "INSERT INTO wp_bp_xprofile_data VALUES(" + id
+ ",18," + userid + "," +
        interest[LineNo] + ",0)";
        id++;
        //Console.WriteLine(query);
        file.WriteLine(query);
        query = "INSERT INTO wp_bp_xprofile_data VALUES(" + id
+ ",19," + userid + "," +
        reason[LineNo] + ",0)";
        id++;
        //Console.WriteLine(query);
        file.WriteLine(query);
        query = "INSERT INTO wp_bp_xprofile_data VALUES(" + id
+ ",20," + userid + "," + url[LineNo] +

```

```

        "",0);";
        id++;
        //Console.WriteLine(query);
        file.WriteLine(query);
        userid++;
        LineNo++;
    }
}

}

//string[] lines =
System.IO.File.ReadAllLines(@"C:\Users\User\Downloads\Date.txt");
//int id=2;
//for(int i=0;i<lines.Length;i++)
//{
//    string query = "UPDATE `wp_users` SET `user_registered` =
STR_TO_DATE('' + lines[i] + '' , '%d %m %Y, %h:%i%p') WHERE
ID="+id+";";
//    id++;
//    Console.WriteLine(query);
//}
}

}

}

```

ColourModelConversion/DateConvertSql/packages.config

```

<?xml
version="1.0"
encoding="utf-
8"?>

    <packages>
        <package id="PermutationLibrary" version="2.2.0" target
Framework="net472" />
    </packages>

```

ColourModelConversion/DateConvertSql/Properties/AssemblyInfo.cs

```

using
System.Reflection;

using System.Runtime.CompilerServices;
using System.Runtime.InteropServices;

```

```

// General Information about an assembly is controlled through the
following
// set of attributes. Change these attribute values to modify the
information
// associated with an assembly.
[assembly: AssemblyTitle("DateConvertSql")]
[assembly: AssemblyDescription("")]
[assembly: AssemblyConfiguration("")]
[assembly: AssemblyCompany("")]
[assembly: AssemblyProduct("DateConvertSql")]
[assembly: AssemblyCopyright("Copyright © 2020")]
[assembly: AssemblyTrademark("")]
[assembly: AssemblyCulture("")]

// Setting ComVisible to false makes the types in this assembly not
visible
// to COM components. If you need to access a type in this
assembly from
// COM, set the ComVisible attribute to true on that type.
[assembly: ComVisible(false)]

// The following GUID is for the ID of the typelib if this project is
exposed to COM
[assembly: Guid("aa5e2d44-5170-4f39-9c26-f220dc91a143")]

// Version information for an assembly consists of the following
four values:
//     Major Version
//     Minor Version
//     Build Number
//     Revision
//
// You can specify all the values or you can default the Build and
Revision Numbers
// by using the '*' as shown below:
// [assembly: AssemblyVersion("1.0.*")]
[assembly: AssemblyVersion("1.0.0.0")]
[assembly: AssemblyFileVersion("1.0.0.0")]

```

APPENDIX III: : CHAPTER 5

JAVA PROGRAMMING CODE

```
package com.example.findalgae;

import android.Manifest;

import android.annotation.SuppressLint;

import android.app.ProgressDialog;

import android.content.DialogInterface;

import android.content.Intent;

import android.content.pm.PackageManager;

import android.graphics.Bitmap;

import android.net.Uri;

import android.os.AsyncTask;

import android.os.Build;

import android.os.Bundle;

import android.os.StrictMode;

import android.provider.MediaStore;

import android.util.Log;

import android.view.View;

import android.widget.Button;

import android.widget.ImageView;

import android.widget.Toast;

import androidx.annotation.NonNull;

import androidx.annotation.Nullable;

import androidx.appcompat.app.AlertDialog;

import androidx.appcompat.app.AppCompatActivity;

import androidx.core.app.ActivityCompat;

import androidx.core.content.ContextCompat;

import com.google.android.gms.auth.api.identity.SignInClient;

import com.google.android.gms.auth.api.signin.GoogleSignIn;

import com.google.android.gms.auth.api.signin.GoogleSignInAccount;
```

```

import com.google.android.gms.auth.api.signin.GoogleSignInClient;
import com.google.android.gms.auth.api.signin.GoogleSignInOptions;
import com.google.android.gms.common.SignInButton;
import com.google.android.gms.common.api.ApiException;
import com.google.android.gms.tasks.OnCompleteListener;
import com.google.android.gms.tasks.OnFailureListener;
import com.google.android.gms.tasks.OnSuccessListener;
import com.google.android.gms.tasks.Task;
import com.google.common.io.ByteStreams;
import com.google.firebase.auth.AuthCredential;
import com.google.firebase.auth.AuthResult;
import com.google.firebase.auth.FirebaseAuth;
import com.google.firebase.auth.FirebaseUser;
import com.google.firebase.auth.GoogleAuthProvider;
import com.google.firebase.storage.FirebaseStorage;
import com.google.firebase.storage.OnProgressListener;
import com.google.firebase.storage.StorageReference;
import com.google.firebase.storage.UploadTask;

import
com.microsoft.azure.cognitiveservices.vision.customvision.prediction.CustomVisionPredicti
onClient;

import
com.microsoft.azure.cognitiveservices.vision.customvision.prediction.CustomVisionPredicti
onManager;

import
com.microsoft.azure.cognitiveservices.vision.customvision.prediction.models.ImagePredictio
n;

import
com.microsoft.azure.cognitiveservices.vision.customvision.prediction.models.Prediction;

import
com.microsoft.azure.cognitiveservices.vision.customvision.training.CustomVisionTrainingCl
ient;

```

```

import
com.microsoft.azure.cognitiveservices.vision.customvision.training.CustomVisionTrainingM
anager;

import com.microsoft.azure.cognitiveservices.vision.customvision.training.Trainings;
import com.microsoft.azure.cognitiveservices.vision.customvision.training.models.Project;
import org.apache.http.HttpEntity;
import org.apache.http.HttpResponse;
import org.apache.http.client.HttpClient;
import org.apache.http.client.methods.HttpPost;
import org.apache.http.client.utils.URIBuilder;
import org.apache.http.entity.StringEntity;
import org.apache.http.impl.client.HttpClients;
import org.apache.http.util.EntityUtils;
import org.json.JSONArray;
import org.json.JSONObject;
import java.io.ByteArrayInputStream;
import java.io.ByteArrayOutputStream;
import java.net.URI;
import java.util.ArrayList;
import java.util.UUID;

```

```

public class MainActivity<ActivityMainBinding> extends AppCompatActivity {
    //Initializing the needed variables and buttons, etc
    private static final int RC_SIGN_IN = 1000;
    private int Storage_permission_code = 1;
    final static String trainingApiKey = "2a8f552d0ebc4465af4ff8057ae5e22f";
    final static String predictionApiKey = "1494ed7c3743488385434eb00b1d6444";

```

```

        final static String endpoint_pred =
"https://eastus.api.cognitive.microsoft.com/customvision/v3.0/Prediction/aaead50a-95cd-
403c-a3c2-9b774399e43b/classify/iterations/Iteration6/image";

        final static String endpoint = "https://findalgae.cognitiveservices.azure.com/";

        final static String PredictionResourceID = "/subscriptions/f7351ef2-7f84-4d19-96b5-
f86972da412a/resourceGroups/FindAlgae/providers/Microsoft.CognitiveServices/accounts/Fi
ndAlgae";

        Uri selectedImage;

        ImageView imageView;

        Button btnOpen;

        Button Uploadbtn;

        Button buttonrequest;

        Button logout;

        static String message = "Azure Data";

        static ArrayList<String> Pdata1 = new ArrayList<>();

        byte [] byteArray;

        static String Project_id;

        CustomVisionPredictionClient predictor;

        ByteArrayInputStream inputStream;

        Bitmap captureImage;

        FirebaseStorage storage;

        StorageReference storageReference;

        String URILLOCATION;

private static final int IMAGE_PICK_CODE = 1000;

        private static final int PERMISSION_CODE = 1001;

        private GoogleSignInClient mGoogleSignInClient;

        private FirebaseAuth mAuth;

        private Object SignInButton;

        private ActivityMainBinding binding;

        @Override

        protected void onCreate(Bundle savedInstanceState) { // when the application is starts and
is ready to use

```

```

super.onCreate(savedInstanceState);

setContentView(R.layout.activity_main);

StrictMode.ThreadPolicy policy = new
StrictMode.ThreadPolicy.Builder().permitAll().build();

//login.findViewById(R.id.sign_in_button);

StrictMode.setThreadPolicy(policy);

//SignInButton = (android.widget.Button)findViewById(R.id.sign_in_button);

mAuth = FirebaseAuth.getInstance();

GoogleSignInOptions gso = new
GoogleSignInOptions.Builder(GoogleSignInOptions.DEFAULT_SIGN_IN)
    .requestIdToken(getString(R.string.default_web_client_id))
    .requestEmail()
    .build();

// Build a GoogleSignInClient with the options specified by gso.
mGoogleSignInClient = GoogleSignIn.getClient(this, gso);

//assign values to variables
imageView=findViewById(R.id.image_view);
//btnOpen=findViewById(R.id.bt_open);
Uploadbtn=findViewById(R.id.upload);
buttonrequest = findViewById(R.id.button);
logout = findViewById(R.id.logout);
Project_id = "aaead50a-95cd-403c-a3c2-9b774399e43b";
//public Project getProject(UUID.fromString(Project_id));
storage = FirebaseStorage.getInstance();
storageReference = storage.getReference();

StorageReference httpsReference =
storage.getReferenceFromUrl("https://firebasestorage.googleapis.com/v0/b/findalgae.appspot
.com/o/images%2F675e2028-497d-4c8a-bad6-aa5bed2c7050?alt=media&token=5a3bce8d-
aae7-4340-bb13-6339693f357d");

signIn();

// Authenticate

CustomVisionTrainingClient trainClient = CustomVisionTrainingManager

```

```

.authenticate("https://findalgae.cognitiveservices.azure.com/customvision/v3.0/training/",
trainingApiKey)

    .withEndpoint(endpoint);

Trainings trainer = trainClient.trainings();
Project project = trainer.getProject(UUID.fromString(Project_id));
logout.setOnClickListener(new View.OnClickListener() {
    @Override
    public void onClick(View view) {
        FirebaseAuth.getInstance().signOut();
    }
});

//handle button click activity for upload button
Uploadbtn.setOnClickListener(new View.OnClickListener() {
    @Override
    public void onClick(View v) {
        // handle runtime permission
        if (Build.VERSION.SDK_INT >= Build.VERSION_CODES.M){
            if(checkSelfPermission(Manifest.permission.READ_EXTERNAL_STORAGE)
                ==PackageManager.PERMISSION_DENIED){
                //Permission Not yet granted
                String[] permissions =
{Manifest.permission.READ_EXTERNAL_STORAGE};

                //showing popup for runtime permission
                requestPermissions(permissions,PERMISSION_CODE);

            }
            else {
                // Permission already granted
                pickImageFromGallery();
            }
        }
    }
});

```

```

    }
    else {
        // when version is less than marshmellow
        pickImageFromGallery();
    }
}

});

//Requesting camera permission
if (ContextCompat.checkSelfPermission(MainActivity.this,
    Manifest.permission.CAMERA) !=
PackageManager.PERMISSION_GRANTED){
    ActivityCompat.requestPermissions(MainActivity.this,
        new String[]{
            Manifest.permission.CAMERA
        }, 100);
}

// call analyze function and navigate to screen
buttonrequest.setOnClickListener(new View.OnClickListener() {
    @Override
    public void onClick(View v) {
        openAnalyze();
    }
});
}

// this class function should be where the data update from azure should happen
@SuppressWarnings("DefaultLocale")
public void openAnalyze() {
    Intent intent = new Intent(this, AnylzedScreen.class);
    //new LongRunningTask().execute();
    intent.putExtra("message", message);
    intent.putExtra("array", Pdata1);
}

```

```

        intent.putExtra("image", byteArray);
        startActivity(intent); // open page 2
    }
    private void pickImageFromGallery() {
        //method to pick image from gallery
        Intent intent = new Intent(Intent.ACTION_PICK);
        intent.setType("image/*");
        startActivityForResult(intent, IMAGE_PICK_CODE);
    }
    private void requestStoragepermission(){
        if (ActivityCompat.shouldShowRequestPermissionRationale(this,
Manifest.permission.READ_EXTERNAL_STORAGE)){
            new AlertDialog.Builder(this)
                .setTitle("Permission needed")
                .setMessage("This permission is needed to store the data")
                .setPositiveButton("ok", new DialogInterface.OnClickListener() {
                    @Override
                    public void onClick(DialogInterface dialog, int which) {
                        ActivityCompat.requestPermissions(MainActivity.this, new String[]
{Manifest.permission.READ_EXTERNAL_STORAGE}, Storage_permission_code);
                    }
                })
                .setNegativeButton("cancel", new DialogInterface.OnClickListener() {
                    @Override
                    public void onClick(DialogInterface dialog, int which) {
                        dialog.dismiss();
                    }
                })
                .create().show();
        } else{

```

```

        ActivityCompat.requestPermissions(this, new String[]
{Manifest.permission.READ_EXTERNAL_STORAGE}, Storage_permission_code);

    }

}

@Override

public void onRequestPermissionsResult(int requestCode, @NonNull String[] permissions,
@NonNull int[] grantResults) {

    super.onRequestPermissionsResult(requestCode, permissions, grantResults);

    switch (requestCode){

        case PERMISSION_CODE: {

            if (grantResults.length > 0 && grantResults[0] ==

                PackageManager.PERMISSION_GRANTED) {

                //if permission was granted

                pickImageFromGallery();

            } else {

                //Permission was denied

                Toast.makeText(this, "Permission Denied", Toast.LENGTH_SHORT).show();

            }

        }

    }

}

@Override

public void onActivityResult(int requestCode, int resultCode, @Nullable Intent data) {

    super.onActivityResult(requestCode, resultCode, data);

    if (requestCode == 100) {

        //get capture image

        captureImage = (Bitmap) data.getExtras().get("data");

        //Set capture image to image view

        imageView.setImageBitmap(captureImage);

    }

}

```

```

        ByteArrayOutputStream outputStream = new ByteArrayOutputStream();
        captureImage.compress(Bitmap.CompressFormat.JPEG,50,outputStream);

        //final ByteArrayInputStream inputStream = new
        ByteArrayInputStream(outputStream.toByteArray());

        byteArray = outputStream.toByteArray();

        CustomVisionTrainingClient trainClient = CustomVisionTrainingManager

.authenticate("https://findalgae.cognitiveservices.azure.com/customvision/v3.0/training/",
trainingApiKey)

        .withEndpoint(endpoint);

        Trainings trainer = trainClient.trainings();

        Project project = trainer.getProject(UUID.fromString(Project_id));

        CustomVisionPredictionClient predictor = CustomVisionPredictionManager

        .authenticate(endpoint_pred, predictionApiKey)

        .withEndpoint(endpoint_pred);

        try {

            ImagePrediction results =
predictor.predictions().classifyImage().withProjectId(project.id())

                .withPublishedName("Iteration6").withImageData(byteArray).execute();

            for (Prediction prediction : results.predictions()) {

                System.out.println(String.format("\t%s: %.2f%%", prediction.tagName(),
prediction.probability() * 100.0f));

                //insert the array update values here

                message = prediction.tagName();

                Pdata1.add(message);

            }

            catch (Exception e) {

                System.out.println(e.getMessage());

                e.printStackTrace();

            }

        }

        if (resultCode==RESULT_OK && requestCode == IMAGE_PICK_CODE){

```

```

//setting uploaded image to view
selectedImage = data.getData();

//get url
//String URILLOCATION = selectedImage.toString();
imageView.setImageURI(data.getData());

if (requestCode == RC_SIGN_IN) {
    Task<GoogleSignInAccount> task =
GoogleSignIn.getSignedInAccountFromIntent(data);

    try {
        // Google Sign In was successful, authenticate with Firebase
        GoogleSignInAccount account = task.getResult(ApiException.class);
        firebaseAuthWithGoogle(account);
    } catch (ApiException e) {
        // Google Sign In failed, update UI appropriately
        Toast.makeText(this, e.getMessage(),Toast.LENGTH_SHORT).show();
    }
}

uploadImage();
}

}

private void firebaseAuthWithGoogle(GoogleSignInAccount account) {
    AuthCredential credential = GoogleAuthProvider.getCredential(account.getIdToken(),
null);

    mAuth.signInWithCredential(credential)
        .addOnCompleteListener(this, new OnCompleteListener<AuthResult>() {
        @Override
        public void onComplete(@NonNull Task<AuthResult> task) {
            if (task.isSuccessful()) {
                // Sign in success, update UI with the signed-in user's information
                FirebaseUser user = mAuth.getCurrentUser();
            } else {

```

```

        // If sign in fails, display a message to the user.
        Toast.makeText(MainActivity.this, "Sorry, auth failed",
Toast.LENGTH_SHORT).show();

    }

}

});

}

private class LongRunningTask extends AsyncTask<Void, Void, Void>{
    ProgressDialog progressDialog = new ProgressDialog(MainActivity.this);
    @Override
    protected void onPreExecute() { progressDialog.show();
    }
    @Override
    protected Void doInBackground(Void... voids) {
        HttpClient httpClient = HttpClient.createDefault();
        try
        {
            URIBuilder builder = new
URIBuilder("https://eastus.api.cognitive.microsoft.com/customvision/v3.0/Prediction/aaead5
0a-95cd-403c-a3c2-9b774399e43b/classify/iterations/Iteration6/url");
            URI uri = builder.build();
            HttpPost request = new HttpPost(uri);
            //request.setHeader("Content-Type", "multipart/form-data");
            request.setHeader("Content-Type", "application/json");
            request.setHeader("Prediction-key", predictionApiKey);
            String json = "";
            // 3. build jsonObject
            JSONObject jsonObject = new JSONObject();

            //jsonObject.accumulate("url","https://firebasestorage.googleapis.com/v0/b/findalgae.appspot
.com/o/images%2Fpick?alt=media&token=1d7e4ace-4485-427b-845a-d3b3560e1722");

```

```

        jsonObject.accumulate("url",URILOCATION);//

        // 4. convert JSONObject to JSON to String
        json = jsonObject.toString();

        // Request body
        StringEntity reqEntity = new StringEntity(json);
        request.setEntity(reqEntity);

        HttpResponse response = httpClient.execute(request);
        HttpEntity entity = response.getEntity();

        String result = EntityUtils.toString(response.getEntity());
        if (entity != null)
        {
            JSONObject jo = new JSONObject(result);
            JSONArray ja = jo.getJSONArray("predictions");
            JSONObject predictionsjs = ja.getJSONObject(0);
            String predictiontag = predictionsjs.getString("tagName");
            Pdata1.add(predictiontag);
            message = predictiontag;
            //System.out.println(predictiontag);
            //System.out.println(EntityUtils.toString(entity));
        }
    }

    catch (Exception e)
    {
        System.out.println(e.getMessage());
    }

    return null;
}

@Override
protected void onPostExecute(Void aVoid) {

```

```

        progressDialog.dismiss();
    }

}

private void uploadImage()
{
    if (selectedImage != null) {

        // Code for showing progressDialog while uploading
        ProgressDialog progressDialog
            = new ProgressDialog(this);
        progressDialog.setTitle("Uploading...");
        progressDialog.show();
        // Defining the child of storageReference
        StorageReference ref = storageReference
            .child(
                "images/"
                + "pick");
        // adding listeners on upload
        // or failure of image
        ref.putFile(selectedImage)
            .addOnSuccessListener(
                new OnSuccessListener<UploadTask.TaskSnapshot>() {
                    @Override
                    public void onSuccess(
                        UploadTask.TaskSnapshot taskSnapshot)
                    {
                        // Image uploaded successfully
                        // Dismiss dialog
                        progressDialog.dismiss();
                    }
                }
            );
    }
}

```

```

        ref.getDownloadUrl().addOnCompleteListener(new
OnCompleteListener<Uri>() {
            @Override
            public void onComplete(@NonNull Task<Uri> task) {
                URILOCATION = task.getResult().toString();
                System.out.println(URILOCATION);
                new LongRunningTask().execute(); // talking to azure and
analyzing and get the answer back
            }
        });
        Toast
            .makeText(MainActivity.this,
                "Image Uploaded!!",
                Toast.LENGTH_SHORT)
            .show();
    }
})
.addOnFailureListener(new OnFailureListener() {
    @Override
    public void onFailure(@NonNull Exception e)
    {
        // Error, Image not uploaded
        progressDialog.dismiss();
        Toast
            .makeText(MainActivity.this,
                "Failed " + e.getMessage(),
                Toast.LENGTH_SHORT)
            .show();
    }
})
.addOnProgressListener(

```

```

        new OnProgressListener<UploadTask.TaskSnapshot>() {
            // Progress Listener for loading
            // percentage on the dialog box
            @Override
            public void onProgress(
                UploadTask.TaskSnapshot taskSnapshot)
            {
                double progress
                    = (100.0
                        * taskSnapshot.getBytesTransferred()
                        / taskSnapshot.getTotalByteCount());
                progressDialog.setMessage(
                    "Uploaded "
                        + (int)progress + "%");
            }
        });
    }
}

private void signIn() {
    Intent signInIntent = mGoogleSignInClient.getSignInIntent();
    startActivityForResult(signInIntent, RC_SIGN_IN);
}

private void handleSignInResult(Task<GoogleSignInAccount> completedTask) {
    try {
        GoogleSignInAccount account = completedTask.getResult(ApiException.class);
        // Signed in successfully, show authenticated UI.
        //updateUI(account);
    } catch (ApiException e) {
        // The ApiException status code indicates the detailed failure reason.
        // Please refer to the GoogleSignInStatusCodes class reference for more information.
    }
}

```

```
        //Log.w(TAG, "signInResult:failed code=" + e.getStatusCode());
        //updateUI(null);
    }
}
}
```