


# SUSTAINABLE CULTIVATION AND HARVESTING STRATEGY FOR *SPIRULINA* BIOREFINERY: INTEGRATING INTERNET-OF-THINGS MONITORING AND CONTINUOUS CULTIVATION

LIM HOOI REN

Department of Chemical and Environmental Engineering,

Faculty of Science and Engineering,

University Of Nottingham Malaysia, Jalan Broga, 43500 Semenyih, Selangor Darul Ehsan, Malaysia

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**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)

Lim Hooi Ren

(ID: 20323109)

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**ABSTRACT**

Overall summary:

*Spirulina* biomass accounts for 30% of the total algae biomass production globally. In conventional process of *Spirulina* biomass production, cultivation using chemical-based culture medium contributes 35% of the total production cost. Moreover, the environmental impact of cultivation stage is the highest among all the production stages which resulted from the extensive usage of chemicals and nutrients. Thus, various types of culture medium such as chemical-based, modified, and alternative culture medium with highlights on wastewater medium is reviewed on the recent advances of culture media for *Spirulina* cultivation. This creates an opportunity for the development of sustainable practices for the cultivation of *Spirulina*.

This thesis explores various aspects of upstream bioprocessing applications of *Spirulina* platensis microalgae. The thesis begins by investigating sustainable water management approaches, including recycling culture medium, and utilizing rainwater resources, for the cultivation of *Spirulina*. Different pre-treatment methods such as microfiltration and UV-light are examined to treat rainwater and recycled medium, and their effects on *Spirulina* growth are evaluated. Furthermore, the study explores the effects of nutrient-deficient media and harvesting ratios on *Spirulina* cultivation, aiming to optimize biomass and phycocyanin production. Additionally, the potential of using spent coffee ground waste as a nutrient source for *Spirulina* cultivation was investigated, along with the feasibility and profitability of large-scale production of *Spirulina* tablets. The thesis also explores the application of real-time monitoring using Internet-of-things (IoT) technology in a *Spirulina* cultivation system. Sensors

are used to monitor important parameters such as absorbance, temperature, light intensity, and water level, with data transmitted to a cloud-based platform for analysis. Overall, this thesis contributes to the understanding of sustainable cultivation practices, real-time monitoring technologies, and economic feasibility assessments in the *Spirulina* industry, paving the way for further advancements and applications in Malaysia.

## LIST OF PUBLICATIONS

### From This Thesis

1. **Lim, H.R.**, Khoo, K.S., Chew, K.W., Chang, C.K., Munawaroh, H.S.H., Kumar, P.S., Huy, N.D. and Show, P.L., 2021. Perspective of *Spirulina* culture with wastewater into a sustainable circular bioeconomy. *Environmental Pollution*, *284*, p.117492. [Q1, IF: 8.9 (2022)]
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3. **Lim, H.R.**, Khoo, K.S., Chew, K.W., Tao, Y., Xia, A., Ma, Z., Munawaroh, H.S.H., Huy, N.D. and Show, P.L., 2023. Upstream bioprocessing of *Spirulina* platensis microalgae using rainwater and recycle medium from post-cultivation for C-phycocyanin production. *Journal of the Taiwan Institute of Chemical Engineers*, p.104986. [Q1, IF: 5.7 (2022)]
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7. Ali, S., Khoo, K.S., **Lim, H.R.**, Ng, H.S. and Show, P.L., 2022. Smart Factory of Microalgae in Environmental Biotechnology. In *Microalgae for Environmental Biotechnology* (pp. 263-292). CRC Press.

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**NOMENCLATURE**

| | |
|---|---|
| ABS: | Absorbance |
| AC: | Alternate current |
| AI: | Artificial intelligence |
| AN: | Ammoniacal nitrogen |
| ANFIS: | Adaptive neuro-fuzzy inference system |
| ANOVA: | Analysis of variance |
| APC: | Allo-phycocyanin |
| BG-11: | Blue green – 11 |
| BLAE: | Banana leaf ash extract |
| BOD: | Biological oxygen demand |
| CAGR: | Compound annual growth rate |
| CAPEX: | Capital expenditures |
| CFTRI: | Central food technological research institute |
| CHNS: | Carbon-Hydrogen-Nitrogen-Sulphur |
| CMOS: | Complementary metal-oxide semiconductor |
| $CO_2$: | Carbon dioxide |
| COD: | Chemical oxygen demand |
| CPC: | C-phycocyanin |
| DC: | Direct current |
| DCW: | Dry cell weight |

EDTA: Ethylenediaminetetraacetic acid

FDM: Frequency-division-multiplexed

GWP: Global warming potential

HASL: Hot air solder leveling

HPLC: High-performance liquid chromatography

HVAC: Heating, ventilation, and air conditioning

I2C: Inter-integrated circuit

IC: Integrated circuit

IoT: Internet of things

IR: Industrial revolution

LCD: Liquid crystal display

LED: Light-emitting diode

MF: Microfiltration

MMFC: Microalgae-microbial fuel cell

NIR: Near-infrared

NPK: Nitrogen, phosphorus, and potassium

OD: Optical density

$OH^-$: Hydroxyl ions

OPEX: Operating expenses

PBR: Photo-bioreactor

PCA: Principal component analysis

| | |
|---|---|
| POME: | Palm oil mill effluent |
| RGB: | Red, green, and blue |
| RNA: | Ribonucleic acid |
| RWHS: | Rainwater harvesting system |
| SBR: | Sequencing batch reactor |
| SHU: | Synthetic human urine |
| SS: | Suspended solids |
| SVM: | Support vector machine |
| TDS: | Total dissolved solids |
| TN: | Total nitrogen |
| TP: | Total phosphorus |
| UI: | User interface |
| USD: | U.S. dollar |
| UV: | Ultraviolet |
| VCCM: | Vermicompost of cow manure |
| VCFS: | Vermicompost of fish sludge |
| WSN: | Wireless sensor network |

# CHAPTER 1

# INTRODUCTION

## 1.1 RESEARCH BACKGROUND

In 2020, there are 7.8 billion of peoples around the world and this numeral will continue to increase on an average of 0.8% yearly. It is expected that the world population could reach up to 9.9 billion by 2050 (Kaneda, Greenbaum and Kline, 2020). Furthermore, food scarcity will become a global issue if the food productivity of the current state of the industries remains the same, the world cannot meet the food demand of the increasing human population (Koba, 2014). The implementation of "*Spirulina*" as the "Food of the Future" is regarded as a solution on this issue (Saranraj and Sivasakthi, 2014). The cultivation of *Spirulina* requires less area compared to conventional farming such as poultry and vegetable farms by around 49 to 132 times (Khoo et al., 2020a). *Spirulina* can be cultivated in a closed photobioreactor system or an open-pond farming and it can double its biomass concentration every 2 – 5 days (Sharoba, 2014; Nguyet et al., 2017). The biomass productivity rate of *Spirulina* can yield within 20 - 400 times more protein compared to food sources such as soybeans, corn and beef (Sharoba, 2014). There are many applications of *Spirulina* in various sectors such as food supplement, animal feed, cosmetics and natural colouring (Oliveira and Ribeiro, 2020). In fact, many countries have begun to ban or limit the production of synthetic colouring due to various adverse health effects such as increased mortality, retardation growth, decreased food intake and fertility rate in the long term (Rajapaksha, Wansapala and Silva, 2017). Consequently, industries have to search for various alternative natural colours in replacing synthetic colours (Scotter, 2011). Microalgal *Spirulina* biomass has been

commercialized for its potent blue pigment namely phycocyanin which can be an alternative replacement for natural colouring in various food application industries. *Spirulina* is among one of the 10 food colouring ingredients that has come under legal scrutiny (Scotter, 2011). Therefore, there is a demand in the production of *Spirulina*, since the market is expected to show an annual growth rate of 10.5% until 2026 based on the report by Allied Market Research (Spirulina Market Outlook - 2026, 2019).

One of the major drawbacks in *Spirulina* cultivation remains in the high cost of the chemical-based culture medium. Up-to-date, chemical-based culture medium such as Zarrouk medium, Conway and Kosaric are being used by many companies for the cultivation of *Spirulina* (Swamy, Murthy and Ravishankar, 2006). However, the overall cost of Zarrouk culture medium is not sustainable in a long run as the approximately cost per litre of culture medium is USD 0.08, which accounts for about 35% of total cost of the algal biomass production (Costa et al., 2019; Yu et al., 2019). Therefore, scientific community has been searching for numerous alternative nutrient sources (i.e. seawater, vermicompost and wastewater) to reduce the cost of these chemical-based culture medium. Wastewater is a promising alternative source of nutrient.

Wastewaters are enriched with nitrogen and phosphorus making them possible to replace the compositions in the chemical-based culture medium. The presence of bacteria in wastewater would promote a microalgae-bacteria consortia system. Previous studies had revealed microalgae-bacteria consortia system can greatly improve the organic and inorganic removal efficiency in wastewater and even double the biomass productivity (Jia and Yuan, 2016; Wicker and Bhatnagar, 2020). The presence of bacteria could provide carbon dioxide ($CO_2$) for the

*Spirulina* growth simultaneously taking in oxygen produced from *Spirulina* and this forms a circulation supply chain of oxygen and $CO_2$. Moreover, *Spirulina* cultivation requires $CO_2$ for photosynthesis during the cultivation stage which can contribute to reducing the carbon footprint in the atmosphere. This application can solve the global problem of wastewater and the global warming issues in reducing the carbon footprint. Acquiring this green technology will allow many companies to reduce their carbon footprint and effluent production, meeting the environmental sustainability while reduce the economically cost of the company. However, there are limitation to utilise wastewater in the *Spirulina* production industries especially in the food and pharmaceutical sectors. One of the major limitations is the uncertain changes of nutrients content in wastewater which may affect the growth and production of *Spirulina*. Unlike chemical-based culture medium, the concentration of nutrients is fixed and supply constantly to the culture medium. Therefore, the growth and production of *Spirulina* biomass are more manageable throughout the production process.

This present work aims to highlight the recent development of culture medium for *Spirulina* cultivation such as chemical-based, modification and alternative culture medium. The discussion on wastewater culture medium for *Spirulina* cultivation and its sustainable circular bioeconomy in the industries will be evaluated. In fact, the recent research, and developments of different types of wastewaters from municipals, aquaculture, and animal effluents were comprehensively review in this study. Nonetheless, the future challenges and prospects on the development of attaining an alternative low-cost culture medium for *Spirulina* cultivation were addressed.

## 1.2 RESEARCH OBJECTIVES

This research work aimed to explore the sustainable cultivation and harvesting strategy for *Spirulina* biorefinery. The objectives of the research were to identify alternative sustainable resources such as rainwater and spent coffee ground. Additionally, the research also aims to integrate IoT monitoring in the *Spirulina* cultivation system and finally evaluate the feasibility and potential profitability of the production of *Spirulina* tablet. The detailed research objectives are as listed below:

- To investigate the growth of *Spirulina* by recycling the culture medium obtained from the post-cultivation process and substituting rainwater resources for the preparation of the culture medium.
- To design and implement a *Spirulina* cultivation system with embedded IoT monitoring technology through the cloud.
- To employ an integrated continuous cultivation and harvesting strategy for the biorefinery of *Spirulina* microalgae.
- To utilize spent coffee grounds as an alternative nutrient source for the cultivation of *Spirulina*.
- To conduct a techno-economic assessment of *Spirulina* cultivation for *Spirulina* tablet production to evaluate the feasibility and potential profitability of this activity in Malaysia.

## 1.3 RESEARCH SCOPE

**Chapter 3: Upstream bioprocessing of *Spirulina* platensis microalgae using rainwater and recycle medium from post-cultivation for C-phycocyanin production**

Two sustainable water management approaches were suggested in this chapter which are recycling culture medium and utilizing alternative rainwater resources. Clean water used to prepare culture medium were replaced with rainwater. Additionally, the recycling of the culture medium after post-cultivation were also investigated. Different pre-treatment methods such as microfiltration (MF), UV-light and combined MF-UV, were applied to treat rainwater and recycled medium before the cultivation of *Spirulina*. Biomass concentration, biomass productivity and phycocyanin accumulation of *Spirulina* were examined to assess the *Spirulina* growth under different pre-treatment conditions of both rainwater and recycled medium.

**Chapter 4: Evaluation of real-time monitoring on the growth of *Spirulina* microalgae: Internet-of-things and microalgae technologies**

Sensors were installed on a 10-L photobioreactor closed system to monitor important parameters such as absorbance, temperature, light intensity, and water level. These sensors were connected to a Lolin NodeMCU ESP8266 V3, microcontroller board based on the ESP8266 Wi-Fi module, used to control, and monitor the system. The microcontroller board was programmed to transmit data from the sensors to an open-source cloud-based software known as ThingSpeak. ThingSpeak is an IoT analytics platform service that allows clients to aggregate, visualize and analyse live data streams in the cloud-based system (i.e.,

ThingSpeak website and ThingSpeak mobile application). This chapter aim to demonstrate the application of real-time IoT monitoring of various parameters in a 10-L *Spirulina* cultivation system.

**Chapter 5: Effect of nutrients-deficient and harvesting ratio strategy for biomass and phycocyanin production from *Spirulina* microalgae**

The Zarrouk culture medium preparation involves the use of various chemicals. Therefore, the effect of nutrient-deficient media on *Spirulina* cultivation were investigated. This is important to determine the specific chemicals that significantly affect the growth of *Spirulina*. The conventional practice is to harvest the entire biomass from the *Spirulina* culture after 10 days of growth. However, the current study explores the effects of different harvesting ratios (e.g., 10%, 20%, and 30%). Additionally, based on previous findings that highlighted $NaNO_3$ has significant impact on the growth and phycocyanin accumulation in *Spirulina*. Therefore, the effect of $NaNO_3$ addition within the concentration of 2.5 g/L, 3.5 g/L and 4.5 g/L were investigated. Finally, the optimised parameters of harvesting ratio and $NaNO_3$ addition are evaluated in a 10-L scale-up cultivation tank. The *Spirulina* growth conditions were assessed through measurements such as biomass concentration, biomass productivity, and phycocyanin accumulation.

**Chapter 6: Phycocyanin production from *Spirulina* with the nutrient replacement of local spent coffee ground waste**

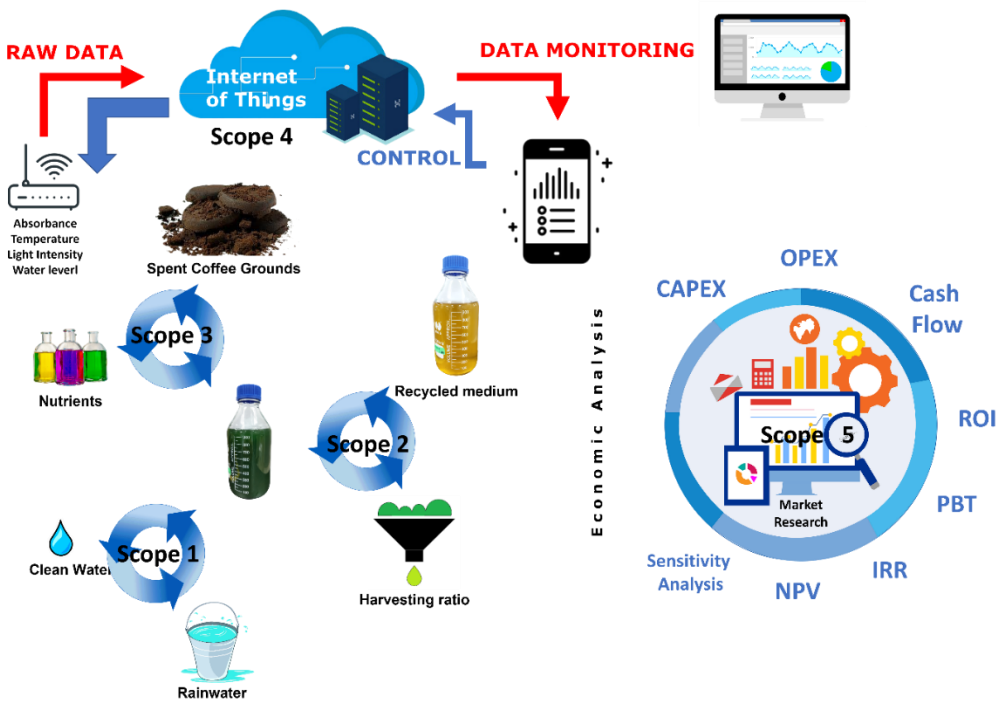
This chapter focuses on utilizing spent coffee ground collected from local shops in Malaysia for the cultivation of *Spirulina*. CHNS analysis was conducted to determine the nitrogen content of the spent coffee grounds. The study also

explores the different concentration (25%, 50%, 75%, 100%) of Zarrouk medium supplemented in spent coffee ground extract on *Spirulina* cultivation. Biomass productivity and phycocyanin accumulation were examined to assess the *Spirulina* growth under different concentration of Zarrouk medium supplemented in spent coffee ground extract. Moreover, the spent biomass after phycocyanin extraction were examined for its remaining protein content which could be potentially used for animal feed production.

**Chapter 7: Techno-economic assessments on the production of *Spirulina* tablet from large scale open pond cultivation of *Spirulina***

This chapter aims to conduct a techno-economic assessment of *Spirulina* cultivation to produce 20 tonnes of *Spirulina* tablet production in order to evaluate the feasibility and potential profitability of this activity in Malaysia. A detailed economic calculation such as capital expenditures (CAPEX) and operating expenses (OPEX) was performed. Additionally, a cumulative cash flow analysis was conducted, based on the projected lifespan of the plant within 10 years, and various financial metrics including payback time, return on investment, net present value and internal rate of return were evaluated. By understanding the technical and economic aspects of *Spirulina* cultivation, it may be possible to identify opportunities for growth and development in the microalgae industry in Malaysia.

## 1.4 THESIS FLOWCHART (RESEARCH CONNECTIVITY AND RELATIONSHIP)



# CHAPTER 2

# LITERATURE REVIEW

## 2.1 CHEMICAL-BASED CULTURE MEDIUM

Chemical-based culture medium has been conventionally used in the industries for microalgae cultivation. Some examples of chemical-based culture media are Zarrouk (Michael, Kyewalyanga and Lugomela, 2019), Central Food Technological Research Institute (CFTRI) (Salunke et al., 2016), Blue Green (BG-11) (Khoo et al., 2019), F/2 (Dineshkumar, Narendran and Sampathkumar, 2016b), Conway (Dineshkumar, Narendran and Sampathkumar, 2016b), Kosaric (Rizal et al., 2017) and Synthetic Human Urine (SHU) (Marrez et al., 2013) mediums that has been formulated by previous researchers. To-date, Zarrouk medium is the common standard medium used for *Spirulina* cultivation for many years due to its decent biomass productivity. Based on a study conducted by Yuan et al. (2018) has revealed the frequent used of Zarrouk medium for the cultivation of *Spirulina* microalgae from eight different companies. Furthermore, many research articles have reported on the maximum biomass dry weight of *Spirulina* cultivation in Zarrouk medium ranging from 0.69 to 6.90 g/L (Michael, Kyewalyanga and Lugomela, 2019; Dineshkumar, Narendran and Sampathkumar, 2016b).

Sodium bicarbonate ($NaHCO_3$) and sodium nitrate ($NaNO_3$) are some of the important common chemicals found in most of the culture medium which act as the carbon and nitrogen sources, respectively. *Spirulina* is an alkaliphile microalgae where it tends to grow better in a high pH culture medium. When $NaHCO_3$ is added into culture medium, it dissociated and formed hydroxyl ions

($OH^-$) which helps to prevents the fluctuation in the cultivation system by sustaining a high pH throughout the cultivation condition (Salunke et al., 2016; Michael, Kyewalyanga and Lugomela, 2019; Soni, Sudhakar and Rana, 2019). This is important to provide an optimum cultivation condition for the growth of *Spirulina* microalgae. On the other hand, $NaNO_3$ is an important nitrogen source for the *Spirulina* cultivation because the presence of nitrogen composition influence the production of biomass and protein accumulation (i.e., higher nitrogen gives more protein content) (Michael, Kyewalyanga and Lugomela, 2019).

Further to that, the main important nutrient sources in a culture medium includes carbon, nitrogen, potassium and phosphorus needed to ensure optimum growth of *Spirulina* (Bandara and Arunakumara, 2020). Carbon sources include $NaHCO_3$ and $Na_2CO_3$; nitrogen source includes $NaNO_3$, $NH_4Cl$ and $KNO_3$; potassium source includes $KNO_3$, $K_2SO_4$, $K_2HPO_4$; phosphorus source includes $NaH_2PO_4$ and $K_2HPO_4$. Despite of the various nutrient sources of medium composition for *Spirulina* cultivation, the cost and composition of cultivation medium are some of the challenging factors for commercially viable production (Pandurangan et al., 2020). The cost of culture medium accounts for about 35% of total cost of the algal biomass production (Yu et al., 2019). Therefore, research includes modification of culture medium and exploring alternative culture medium source such as wastewater are studied to reduce the cost of culture medium for the purpose of economical *Spirulina* production in various application. **Table 2.1** shows a comprehensive evaluation of different type of chemical-based culture medium.

**Table 2.1** Evaluation on the different types of chemical-based culture medium.

| Culture medium | Cultivation conditions | Maximum Biomass dry (D)/ wet (W) weight & Productivity, P | Biomass composition | References |
|---|---|---|---|---|
| Zarrouk | 14 days, 2 L<br>Light intensity 4.5 klux<br>12/12 h light/dark cycle | D = 6.9 g/L<br>P = 0.235 g/L/d | Protein: 65%<br>Lipid: 6.84%<br>Carbohydrate: 13.62%<br>Fiber: 11.37%<br>Moisture: 9.92%<br>Ash: 9.93% | (Michael, Kyewalyanga and Lugomela, 2019) |
| | 30 days, 20 mL<br>Light intensity 4.5 klux | D = 1.86 g/L | Not provided | (Dineshkumar, Narendran and Sampathkumar, 2016b) |

| | | | |
|---|---|---|---|
| | 12/12 h light/dark cycle | | |
| | 30 days, 250 mL<br>Light intensity 4.5 klux | D = 4.87 g/L | (Marrez et al., 2013) |
| | Open pond (18.63 litre)<br>Closed reactor (17.36 litre)<br>Light intensity 1.5 to 4.5 klux | P: 8.568 g/L/d<br>P: 10.231 g/L/d | (Soni, Sudhakar and Rana, 2019) |
| | 10 days, 1 L | W: 16.6 g/L | Protein: 64.42%<br>$\beta$ -carotene: 1817 $\mu$g/g<br>(Sukanya, Meena and Ravindran, 2020) |

| | | | | |
|---|---|---|---|---|
| JPJM | 15 days, 200 mL | W: 4.32 g/L | Not provided | (Salunke et al., 2016) |
| CFTRI | 12/12 h light/dark cycle | W: 11.33 g/L | | |
| DMSD | | W: 3.33 g/L | | |
| BG11 | 30 days, 20 mL | D = 4.87 g/L | | (Dineshkumar, Narendran and Sampathkumar, 2016b) |
| F/2 | Light intensity 4.5 klux | D = 0.46 g/L | | |
| Conway | 12/12 h light/dark cycle | D = 0.52 g/L | | |
| Kosaric | 18 days, 1.0 L 12/12 h light/dark cycle | D = 0.63 g/L | Chlorophyll *a*: 7.7 mg/L | (Rizal et al., 2017) |
| SHU | 30 days, 250 mL Light intensity 4.5 klux | D = 4.30 g/L | Phycocyanin: 50.63 $\mu$g/mL | (Marrez et al., 2013) |

| | | | | |
|---|---|---|---|---|
| | | | Allo-Phycocyanin: 51.5 $\mu$g/mL<br>Phycoerythrin: 44.13 $\mu$g/mL | |
| Schlösser medium | 8 days, 100 mL | D = 1143 mg/L<br>P = 136 mg/L/d | Total Protein: 18.77 U/mg | (Barros et al., 2020) |
| Aiba & Ogawa 1977 liquid medium | 15 days, 10 L<br>Light intensity 3.0 to 5.0 klux<br>12/12 h light/dark cycle | D = 0.53 g/L | Chlorophyll *a*: 47 $\mu$g/mL<br>Protein: 61.04%<br>Fiber: 13.77%<br>Lipid: 17.66% | (Abdelhay, Moustafa and EL-Metwaly Essa, 2019) |

*Biomass compositions are in w/w% basis

### 2.1.1 MODIFIED CULTURE MEDIUM

Modification of culture medium includes the substitution of chemicals with alternative source such as urea, table sugar, cassava flour, sweet potato flour, taro flour, banana leaf ash extract, corn steep liquor and struvite. Many studies have reported on the substitution of chemicals that act as the main nutrient sources which are carbon (Bandara and Arunakumara, 2020), nitrogen (Rizal et al., 2017) and phosphorus (Markou et al., 2019). An alternative nitrogen source which is urea can be used to replace chemicals that act as nitrogen source for *Spirulina* cultivation. Soni, Sudhakar and Rana (2019) revealed that the modified medium with urea has improved the biomass productivity of *Spirulina* cultivation in both open pond and closed reactor system. It is certain that urea increases the biomass productivity to a certain level, however excessive amount of urea can inhibits the growth of *Spirulina* which would reduce the biomass productivity (Rizal et al., 2017). The challenge remains in the uncertain nitrogen content of urea which varies with different living things, whether it is from human or animals. It is suggested that further studies are needed in optimising the urea concentration in achieving its maximum biomass productivity.

Besides replacing nitrogen source chemicals, researcher had unveiled the substitution of carbon source chemicals with organic medium. A recent study by Bandara and Arunakumara (2020) studied the replacement of carbon source chemicals in Zarrouk medium with organic mediums such as table sugar, cassava flour, sweet potato flour and taro flour which achieve biomass dry weight of 0.75 g/L, 1.17 g/L, 0.86 g/L and 1.03 g/L, respectively. In fact, the estimation cost of $Na_2CO_3$ and $NaHCO_3$ can be reduced up to 91.6% by replacing 100% of the carbon source in the Zarrouk medium with taro flour

(Bandara and Arunakumara, 2020). On the other hand, Khatun et al. (2020) revealed that 50% of $NaHCO_3$ and 75% micronutrient in Kosaric medium can be replaced with banana leaf ash extract without affecting the maximum biomass dry weight. Both Kosaric medium and modified Kosaric medium with banana leaf ash extract achieved maximum biomass dry weight of 0.63 g/L and 0.65 g/L, respectively. Although organic mediums or banana leaf ash extract are the cheaper source, however there are not sustainable in the long term as there are obtained from food source.

The increasing human populations are in a risk of facing food scarcity thus it is not sustainable to utilise these organic mediums or banana leaf ash extract. Instead, more research should focus on utilising waste, wastewater or by-products produce from industrial processes. For example, struvite effluent recovered from a biogas plant treating municipal and agro-industrial waste have been studied in replacing $KH_2PO_4$ of Zarrouk medium (Markou et al., 2019). Struvite has high content of phosphorus which contains about 43.7% phosphorus. The results showed that replacing $KH_2PO_4$ in Zarrouk medium with 700.8 g/L of sterilised struvite has a higher maximum biomass dry weight of 1.0 g/L compared to $KH_2PO_4$ which has a maximum biomass dry weight of 0.8 g/L. By utilising waste sources is a sustainable solution yet certain challenges still remain such as the contamination from waste products which may inhibits the *Spirulina* growth resulting in low biomass productivity. Therefore, it is suggested more research is needed in separate the nutrients from the waste products while adding it into the culture medium. **Table 2.2** presents a comprehensive evaluation on the different type of modified culture medium.

**Table 2.2.** Evaluation on the different types of Modified Culture Medium.

| Culture medium | Modification | Maximum biomass dry (D)/ wet (W) weight and productivity (P) | Biomass composition | References |
|---|---|---|---|---|
| Zarrouk | without the carbon source ($NaHCO_3$) | D = 1.87 g/L<br>P = 114 mg/L/d | Protein: 53.4%<br>Carbohydrate: 16.6%<br>Lipid: 12.5%<br>Phycocyanin: 52.5 mg/g | (Cardias, Morais and Costa, 2018) |
| | • $FeSO_4.7H_2O$, EDTA was completely removed.<br>• NaCl, $MgSO_4.7H_2O$ concentration were reduced<br>• Urea was added. | Open pond<br>P = 11.34 g/L/d | Chlorophyll: 0.80 mg/L/d | (Soni, Sudhakar and Rana, 2019) |
| | | Closed reactor<br>P = 12.28 g/L/d | Chlorophyll: 0.85 mg/L/d | |

| | | | |
|---|---|---|---|
| • Replace carbon source $NaHCO_3$ & $Na_2CO_3$ with table sugar, cassava flour, sweet potato flour and taro flour. | D = 0.747 g/L, 25% Table sugar,<br>D = 1.174 g/L, 50% Cassava flour<br>D = 0.862 g/L, 25% Sweet potato flour<br>D = 1.033 g/L, 100% Taro flour | | (Bandara and Arunakumara, 2020) |
| • Replace phosphorus source with struvite | D = 1.0 g/L | Protein: 67.7%<br>Carbohydrate: 11.0%<br>Lipid: 6.4%<br>Phycocyanin:14.2%<br>Chlorophyll *a*: 2.04%<br>Carotenoids: 0.21% | (Markou et al., 2019) |

| | | | | |
|---|---|---|---|---|
| | • Replace nitrogen source $NaNO_3$ with urea and corn steep liquor | D = 1.44 g/L | Protein: 58-62%<br>Total Carbohydrate: 8-11% | (El-Sayed and El-Sheekh, 2018) |
| Kosaric | • Replace nitrogen source $NaNO_3$ with urea | D = 0.61 g/L, 25% Urea<br>D = 0.90 g/L, 50% Urea<br>D = 0.66 g/L, 75% Urea<br>D = 0.59 g/L, 100% Urea | Chlorophyll *a*: 7.6 mg/L<br>Chlorophyll *a*: 9.0 mg/L<br>Chlorophyll *a*: 7.6 mg/L<br>Chlorophyll *a*: 7.4 mg/L | (Rizal et al., 2017) |
| | • Replace $NaHCO_3$ and micronutrients with banana leaf ash extract (BLAE) | D = 0.63 g/L, 50% BLAE, 75% micronutrient | Chlorophyll *a*: 7.9 mg/L | (Khatun et al., 2020) |

*Biomass compositions are in w/w% basis

### 2.1.2 ALTERNATIVE LOW-COST CULTURE MEDIUM

#### 2.1.2.1 VERMICOMPOST-BASED CULTURE MEDIUM

An alternative to chemical fertiliser may be vermicompost which has been used as an organic fertiliser in agriculture farming. Vermicompost is the product of the decomposition process using earthworms as the drivers of nutrient recovery from organic solid waste such as food waste, fish sludge and cow manure (Rini et al., 2020). Unlike chemical fertiliser which composed mainly of nitrogen, phosphorus, and potassium, vermicompost composed of organic matter, nitrogen and phosphorus. Organic matter consists most of the nutrient in it and its composition in vermicompost varies with the type of waste used for composting. Abdelhay, Moustafa and EL-Metwaly Essa (2019) reported on the cultivation of *Spirulina* in vermicompost of fish sludge (VCFS) and cow manure (VCCM) while comparing with Aiba and Ogawa 1977 liquid medium. VCCM has organic matter, phosphorus, and nitrogen content of 52.96%, 0.63% and 2.76%, respectively which is relatively higher compare to VCFS of 27.52%, 0.23%, and 1.49%, respectively. Therefore, the maximum biomass dry weight of VCCM is 0.55 g/L higher than VCFS of 0.50 g/L and Aiba and Ogawa 1977 liquid medium of 0.53 g/L. The vermicompost of cow manure have shown to improve biomass productivity while the vermicompost of fish sludge does not shown any improvement which could be due to insufficient nutrients for the growth of *Spirulina*. Vermicompost is a more sustainable solution as it utilise waste products in the decomposition process which can be used for the cultivation of *Spirulina*. To the best of author knowledge, the studies reported on utilising vermicompost for *Spirulina* cultivation are scarce. It is suggested to have more studies on the different type of vermicompost such as food waste and

optimising the vermicompost loading on the growth of *Spirulina* in achieving the highest maximum biomass dry weight.

#### 2.1.2.2 SEAWATER-BASED CULTURE MEDIUM

Seawater which covers 96% of the Earth surface contain nutrients such as sodium, chlorides, potassium, calcium and magnesium is another alternative culture medium. Bezerra et al. (2020) reported that the maximum biomass dry weight of seawater medium is 2.17 g/L lower compared to Zarrouk medium of 2.34 g/L. Moreover, the protein and lipid content of 8.0% and 6.7%, respectively are relatively low compared to Zarrouk medium which has protein and lipid content of 52.5% and 12.1%, respectively. However, the carbohydrate content in seawater medium is 68.6% which is relatively high when compare with Zarrouk medium of 22.6%. This could be due to the high salinity and the reduced nitrogen and phosphorus supply causing *Spirulina* experience nitrogen starve condition which led to the increase in carbohydrates and compromising the protein and lipid content (Bezerra et al., 2020). Seawater medium has high salinity which provides the adequate growth of *Spirulina*, but the drawback of this medium is its insufficient in nutrients. Therefore, previous studies have reported on the supplement of Zarrouk nutrients and digestate into seawater medium before conducting the cultivation of *Spirulina* (Bezerra et al., 2020; Markou et al., 2021). The supplement of Zarrouk mediums into seawater medium is not a sustainable solution as the purpose of alternative culture medium is to replace the chemicals of chemical-based culture medium. Instead, the supplement of digestate is a by-product produce from anaerobic digestion of a biogas plant may be a sustainable solution. It is suggested to have more studies on formulating and optimising the seawater medium with waste product (e.g.,

urea and digestate) as the nutrient source in achieving its maximum biomass dry weight for *Spirulina* cultivation. **Table 2.3** provides a comprehensive evaluation on the different type of alternative culture medium.

**Table 2.3.** Evaluation on the different types of Alternative Culture Medium.

| Culture medium | Biomass dry/ wet weight and Productivity, P | Biomass composition | References |
|---|---|---|---|
| Seawater | D = 1.42 g/L | Not Provided | (Dineshkumar, Narendran and Sampathkumar, 2016b) |
| | D = 2.17 g/L<br>P = 0.18 g/L/d | Protein: 8%<br>Carbohydrate: 68.6%<br>Lipid: 6.7% | (Bezerra et al., 2020) |
| Fertiliser | D = 7.5 g/L<br>P = 0.254 g/L/d | Protein: 52.85%<br>Lipid: 6.61%<br>Carbohydrate: 15.29%<br>Fiber: 9.79% | (Michael, Kyewalyanga and Lugomela, 2019) |

| | | | |
|---|---|---|---|
| | | Moisture: 5.45%<br>Ash: 9.55% | |
| | D = 1.82 g/L<br>P = 177 mg/L/d | Chlorophyll: 12.84 mg/L<br>Protein: 45.81%<br>Lipid: 9.41% | (Kumari et al., 2014) |
| Vermicompost | D = 0.50 g/L | Chlorophyll *a*: 32.9 $\mu$g/L<br>Protein: 31.25%<br>Fiber: 8.78%<br>Lipid: 19.62% | (Abdelhay, Moustafa and EL-Metwaly Essa, 2019) |
| | D = 0.55 g/L | Chlorophyll *a*: 46.9 $\mu$g/L | |

| | | | |
|---|---|---|---|
| | | Protein: 66.31%<br>Fiber: 14.96%<br>Lipid: 5.21% | |
| Bean Sprouts Extract + Urea | D = 0.30 g/L | Phycocyanin: 257.12 mg/L | (Adharani et al., 2017; Dianursanti, Taurina and Indraputri, 2018) |
| Tequesquite | D = 763.2 mg/L | Not Provided | (Martínez-Jerónimo, Flores-Hernández and Galindez-Mayer, 2017) |
| My An mineral water | P = 10 g/m$^2$/d | Protein: 68.32%<br>Lipid: 7.32%<br>Ash: 7.46%<br>Chlorophyll *a*: 1.34% | (Nguyet et al., 2017) |

*Biomass compositions are in w/w% basis

## 2.2 IMPLEMENTATION OF WASTEWATER MEDIUM FOR *SPIRULINA* CULTIVATION

The global wastewater production rate has reached 400 $km^3$/year, of 70% account for domestic sector while the remaining 30% accounts for manufacturing sector and non-point sources (Flörke et al., 2013; Mateo-Sagasta, Raschid-Sally and Thebo, 2015; Zhang and Shen, 2019). It is reported that high, lower middle and low income countries on average, 70%, 28% and 8% of the generated wastewater is treated, respectively (Mateo-Sagasta, Raschid-Sally and Thebo, 2015). Moreover, Van Drecht et al. (2009) have predicted a rapid increase in global sewage emissions, an increase of 87.5 - 142.2% of nitrogen per year and 84.6 – 138.5% of phosphorus per year from 2000 to 2050. This is due to the combined effect of increasing human population and rapid development of urbanisation resulting in intensive aquaculture and poultry farming to meet the food demand of human population. Therefore, there is a need for further development on the utilisation of wastewater for various application such as *Spirulina* cultivation. Recent studies have reported on the cultivation of *Spirulina* in many types of wastewater which consists of municipal wastewater (Djaghoubi et al., 2015; Lee et al., 2020), aquaculture wastewater (Cardoso et al., 2021), swine wastewater (Lu et al., 2020), olive oil milling wastewater (Markou, Chatzipavlidis and Georgakakis, 2012), distillery wastewater (K and M, 2018; Krishnamoorthy, Manickam and Muthukaruppan, 2019), confectionary wastewater (El-Kassas, Heneash and Hussein, 2015), saline wastewater (Mata et al., 2020), brine wastewater (Duangsri, 2011) (Duangsri and Satirapipathkul, 2011), tapioca wastewater (Hadiyanto, Christwardana and da Costa, 2023), tofu wastewater (Hadiyanto, 2018), rubber wastewater (Hadiyanto et al., 2020), paper mill wastewater (Asthary et al., 2019) and dye wastewater (Ismail

et al., 2021). **Table 2.4** summarises the different types of wastewaters for *Spirulina* cultivation.

**Table 2.4.** Evaluation on different types of wastewater for *Spirulina* cultivation.

| Type of Wastewater | Biomass dry weight (D)/ Productivity, (P) | Biomass composition | Removal efficiencies (R%) | References |
|---|---|---|---|---|
| Municipal | D = 262.50 mg/L | Protein: 46.02% | N: 81.51%<br>P: 80.52% | (Zhai et al., 2017) |
| Aquaculture | D = 0.22 g/L<br>P = 0.03 g/L/d | | $NH_3$: 19.8%<br>$NO_2^-$: 100%<br>$NO_3^-$: 98.7%<br>$PO_4^{3-}$: 94.8% | (Nogueira et al., 2018) |
| | D = 0.5 g/L<br>P = 0.613 g/L/d | Lipids: 8.8%<br>Protein: 38.3%<br>Carbohydrate: 41.4% | COD: 30%<br>$NH_4^+$: >99%<br>P: 47.8% | (Riaño, Hernández and García-González, 2012) |

| | | | | |
|---|---|---|---|---|
| | | Ashes: 11.6% | | |
| Swine | D = 204.6 mg/L | | Total P: 41.6%<br>COD: 84.3% | (Mezzomo et al., 2010b) |
| | D = 1.28 g/L<br>P = 74.22 mg/L/d | Chlorophyll: 0.134 mg/L/d | Total N: 85.86%<br>Total P: 78.69%<br>$NH_4$: 100%<br>COD: 70.02% | (Lu et al., 2020) |
| Olive oil mill | D = 1.5 g/L | Chlorophyll: 1.9 $\mu$g/L | Phenols: 80% | (S. Mostafa et al., 2019) |
| Distillery | D = 8.11 g/L | | TDS: 60 – 70%<br>COD: 60 – 70% | (Krishnamoorthy, Manickam and Muthukaruppan, 2019) |

| | | | | |
|---|---|---|---|---|
| Desalination | D = 0.87 g/L | Phycocyanin: 2% | | (Matos, da Silva and Sant'Anna, 2021) |
| Tapioca | P = 55.38 mg/L/d | | COD: 67% | (Hadiyanto, Christwardana and da Costa, 2023) |
| Anaerobically Digested Dairy Manure | P = 0.08 g/L/d | Phycocyanin: 4.90% | | (Taufikurahman et al., 2020) |
| Cow Effluent | D = 1.05 g/L | Protein: 572.9 mg/g<br>Carbohydrate: 179.4 mg/g<br>Chlorophyll *a*: 9.20 mg/g<br>Carotene: 1.95 mg/g | | (Çelekli et al., 2016) |

| | | | | |
|---|---|---|---|---|
| Real Human Urine | D = 0.81 g/L | Protein: 35.4%<br>Lipid: 19.8% | $NH_4^+$: 97%<br>TP: 96.5%<br>Urea: 85 - 98% | (Chang et al., 2013) |

### 2.2.1 TECHNOLOGY INVOLVED TO ADDRESS THE CHALLENGE FACE BY WASTEWATER MEDIUM

The major challenge faced by utilising wastewater medium for *Spirulina* cultivation is the composition of nutrients in real wastewater varies widely and that can significantly influence the biomass, growth and subsequent nutrients removal by microalgae (Zhai et al., 2017). Beside the presence of high nitrate and phosphate concentration, the presence of toxic substances (i.e. ammonia, chromium and phenol) may inhibit the growth of *Spirulina* when exceeded a certain amount (Magro et al., 2012; Markou, Chatzipavlidis and Georgakakis, 2012; Lee et al., 2020). On the other hand, swine wastewater is toxic for *Spirulina* cultivation because the coloration of the wastewater causes sunlight penetration problems in the culture medium, decreasing the cyanobacterium growth (Mezzomo et al., 2010b). In a nutshell, three challenges of utilising wastewater have been addressed here: the uncertainty of nutrient concentration in wastewater, toxicity of wastewater and the colour of wastewater.

Therefore, this has demanded the current researchers to explore and propose many interesting solutions to these challenges such as dilution, supplements and coupling with wastewater treatment technology. However, the *Spirulina* biomass productivity is still relatively low as compared to conventional chemical-based culture medium such as Zarrouk medium (0.69 - 6.9 g/L). The maximum biomass dry weight of *Spirulina* cultivation in wastewater reported between the range of 0.2 - 1.5 g/L (Zhai et al., 2017; Nogueira et al., 2018; S. Mostafa et al., 2019). Studies reported on the various solution to support *Spirulina* growth and enhance the biomass productivity of *Spirulina* cultivation in wastewater will be discussed in later section.

#### 2.2.1.1 DILUTION OF WASTEWATER

Dilution of wastewater is necessary to reduce the toxic effect of various inhibitory substance and colouring of wastewater that may inhibit the growth of *Spirulina* especially when utilising wastewater from municipal or animal. Inhibitory substances such as heavy metals, herbicides, pesticides, detergents, household cleaning products and personal care product are commonly found in wastewater (Larsdotter, 2006). Animal wastewater are reported to be the ideal nitrogen sources and containing essential nutrients such as sulphate, calcium, magnesium, potassium, iron and chloride (Ghofar et al., 2019). Cheunbarn and Peerapornpisal (2010) reported that when 10% *Spirulina platensis* was inoculated into swine wastewater, the growth of *Spirulina* was only a short period before all the algae died. This is because the cell concentration inoculated was low, the *Spirulina* could not adapt to the environment quickly and died too early when transferred from the commercial medium to swine wastewater. Therefore, Cheunbarn and Peerapornpisal (2010) begin its experiment with 20% *Spirulina platensis* inoculation in swine wastewater. Moreover, the presence of excessive ammonia in wastewater, exceeding 42 mg/L concentration may be toxic to many microalgae (Ratana et al., 2010). Lu et al. (2019) reported that *Spirulina* has an ammonium tolerant level of 127.5 mg/L. Exceeding the tolerant level may result in low phosphate removal efficiency (Mezzomo et al., 2010b). This occurred due to the inhibition of *Spirulina* growth influenced by the toxicity of ammonia; thus, no phosphorus was removed. Despite the confliction of nitrogen assimilation, it is certain that by diluting wastewater, it reduces the concentration of ammonia and may favour the other nutrients removal efficiency such as nitrite, nitrate and phosphate.

Although dilution of wastewater may help reduce the toxicity and colour of wastewater allowing the growth of *Spirulina*, the concentration of organic and ammonium pollutant will decrease as well. As a result, the biomass composition of *Spirulina* cultivated in diluted wastewater may be affected as well. Riaño, Hernández and García-González (2012) studied the different loading of organic and ammonium in two different photobioreactor. The lipid contents in biomass doubled from approximately 9% - 18% when the organic and ammonium loading of aquaculture wastewater increase by 10-fold in the photobioreactors, and the ammonium removal of soluble phosphorus increased from 47% to 67%. In a nutshell, dilution of wastewater may support the growth of *Spirulina*, improve other nutrient removal efficiency but on the short side, due to less nutrients presence in diluted wastewater, the biomass productivity of bioactive compounds such as lipids content may be reduced. Low biomass productivity means that a vast area of land would be needed for cultivation system and ancillary facilities and extend the distances between cultivation position and resources (Chen, Zhao and Qi, 2015). Further research is needed in eliminating the negative impacts of wastewater instead of reducing it by dilution method, one method is to couple *Spirulina* cultivation with wastewater treatment technology which will be discuss in later section.

#### 2.2.1.2 ENHANCEMENT OF *SPIRULINA* CULTIVATION IN WASTEWATER MEDIUM

Though wastewater usually contains high in nitrogen and phosphorus content and due to methods, such as dilution of wastewater may dilute or decrease the content of nitrogen and phosphorus. Moreover, wastewater such as swine, municipal, POME or complex wastewater from monosodium glutamate factory

(Jiang et al., 2015) contains high content of ammonia, while other form of nutrients such as carbon and phosphorus are less in proportion. This would cause the nutrients proportion in wastewater to be insufficient or inappropriate for the growth of *Spirulina*. Therefore, it is necessary to add some important nutrients beforehand to improve the biomass productivity of algae (Cheunbarn and Peerapornpisal, 2010). Subsequently, it is important to understand the nutrient (C, N, P) assimilation mechanism of microalgae in the wastewater treatment. Normally, microalgae assimilate inorganic carbon in the form of $CO_2$ and $HCO_3^-$ in the photosynthesis process represent by Equation (2.1) (Larsdotter, 2006).

$$6H_2O + 6CO_2 + \text{light} \rightarrow C_6H_{12}O_6 + 6O_2 \qquad (2.1)$$

Besides, inorganic nitrogen and phosphorus are macro-nutrients which are required in high amount for microalgae growth in the preferred form of nitrite ($NO_2^-$), nitrate ($NO_3^-$), ammonium ($NH_4^+$) and $H_2PO_4^-$, $HPO_4^{2-}$, respectively (Whitton et al., 2015). In addition, the preference of nitrogen species assimilation by microalgae follows this order $NH_4^+ > NO_3^- > NO_2^-$ (Whitton et al., 2015). Both inorganic nitrogen and phosphorus are transported across the cell membrane in their respective preferred form, nitrogen assimilated into amino acids for the formation of proteins while phosphorus is assimilated into nucleotides following phosphorylation for the synthesis of ribosomal RNA (Whitton et al., 2015). Carbon, nitrogen and phosphorus are important nutrients required for the optimum growth of *Spirulina*. However, C:N:P compositions vary in different type of wastewater. The presence of high N:P ratio of 30:1 and low N:P ratio of 5:1 in wastewater would cause phosphorus and nitrogen be a limiting factor for the growth of microalgae, respectively (Larsdotter, 2006). The

optimum N:P mass ratio reported for microalgae cultivation is 7.2:1 (Zhai et al., 2017). Further to that, Nur (2014) have also shown to alter the C:N:P ratio in POME wastewater by supplementing bicarbonate, urea and triple super phosphate as source of carbon, nitrogen and phosphorus, respectively to achieve the optimum C:N:P ratio of 56:9:1 for the growth of *Spirulina*.

Many studies have reported that supplementation has significantly improved the growth of *Spirulina*, biomass productivity and nutrients removal compare to without supplementation of wastewater (Chang, Lee and Den, 2013; Çelekli et al., 2016; Lu et al., 2020). Besides adding supplements into wastewater, there are studies reported concentrate wastewater such as dairy wastewater and lac wastewater is used as supplements in chemical-based culture medium which shows better performance compared to chemicals supplemented into wastewater medium (Kulkarni, Auti and Saraf, 2016; Pumas and Pumas, 2016; Pereira et al., 2019). However, the limitation of supplements remains in the extend of biomass productivity enhancement. Supplements can only extend the maximum biomass productivity to a certain extent, exceeding the amount would result in the inhibition of *Spirulina* growth. Therefore, it is not a long-term sustainable solution. **Table 2.5** shows a comprehensive evaluation on the different type of supplements in wastewater medium.

**Table 2.5.** Evaluation on the different types of supplements in wastewater medium.

| Type of Wastewater | Supplementation | Biomass dry/ wet weight and Productivity, P | Biomass composition | Removal efficiencies (R%) | References |
|---|---|---|---|---|---|
| Swine | 8.0 g/L $NaHCO_3$, 1.5 g/L of $NaNO_3$ | $17.8\times10^4$ cells $mL^{-1}$ | Protein: 55.88% | COD: 23%<br>BOD: 45%<br>$NO_3^-$: 49%<br>$NH_4^+$: 92%<br>$PO_4^{3-}$: 67% | (Mezzomo et al., 2010b) |
| | 4.5 g/L $NaHCO_3$, 0.2 g/L urea | P = 12.0 g/L/d | Protein: 57.9%<br>Total Fatty Acid: 4.18% | TN: 34 mg/L/d<br>TP: 4 mg/L/d | (Ratana et al., 2010) |

| | | | | |
|---|---|---|---|---|
| | | Linoleic acid: 0.70%<br>$\gamma$ -Linolenic acid: 1.12%<br>Phycocyanin: 19.5% | | |
| 0.05 g/L ferrous sulphate | P = 98.67 mg/L/d | Protein: 55.15%<br>Carbohydrate: 12.32%<br>Lipids: 11.49%<br>Iron: 2.03 mg/g<br>Chlorophyll: 0.156 mg/L/d | COD: 78.15%<br>$NH_3$-N: 100%<br>TP: 83.39%<br>TN: 91.34% | (Lu et al., 2020) |
| 1.0 g/L $NaHCO_3$ | D = 1.70 g/L<br>P = 103.33 mg/L | Lipids: 8.52% | TN: 91.24%<br>TP: 87.44% | (Lu et al., 2021) |

| | | | | | |
|---|---|---|---|---|---|
| | | | | $NH_4^+$: 100% | |
| | 1.0 g/L Sodium acetate | D = 2.18 g/L<br>P = 135.33 mg/L | Lipids: 8.63% | TN: 85.72%<br>TP: 87.02%<br>$NH_4^+$: 100% | |
| Municipal | 300 mg/L Glucose | D = 322.1 mg/L | Chlorophyll a: 9.93 mg/g | N: 97.58%<br>P: >99% | (Zhai et al., 2017) |
| Synthetic human urine | 200 mg/L Sodium acetate | D = 1.75 g/L | Protein: 60.2%<br>Lipid: 17.9% | $NH_4^+$: ~100% | (Chang et al., 2013) |
| Olive-oil mill | 1 g/L $NaNO_3$, 5 g/L $NaHCO_3$ | D = 1696 mg/L | Carbohydrate: 33.64%<br>Lipids: 16.91% | COD: 65.53%<br>Carbohydrate: 88.41% | (Markou, Chatzipavlidis and Georgakakis, 2012) |

| | | | | | |
|---|---|---|---|---|---|
| | | | Protein: 31.52%<br>Chl: 1.02% | Phenols: 100%<br>P: 100% | |
| Cow Effluent | 0.5 g/L $NaNO_3$ | D = 1.26 g/L | Protein: 673.3 mg/g<br>Carbohydrate: 172.4 mg/g<br>Chlorophyll a: 18.8 mg/g<br>Carotene: 4.68 mg/g<br>Malondialdehyde: 63.60 mg/g<br>Proline: 79.30 mg/g | Not Provided | (Çelekli et al., 2016) |
| | 0.5 g/L $NaNO_3$, 0.5 g/L NaCl | D = 0.99 g/L | Protein: 661.1 mg/g<br>Carbohydrate: 177.5 mg/g | Not Provided | |

| | | | | | |
|---|---|---|---|---|---|
| | | | Chlorophyll *a*: 11.33 mg/g<br>Carotene: 5.75 mg/g<br>Malondialdehyde: 66.51 mg/g<br>Proline: 80.50 mg/g | | |
| Dairy manure | 16.8 g/L $NaHCO_3$, 25 g/L NaCl | P = 0.08 g/L/d | Phycocyanin: 4.90% | Not Provided | (Taufikurahman et al., 2020) |
| Aquaculture | 25% Zarrouk medium | D = 1.17 g/L<br>P = 0.20 g/L/d | Protein: 65.73%<br>Phycocyanin: 16.60 mg/mL<br>Polyunsaturated fatty acid: 38.20%<br>$\gamma$-Linolenic: 23.29% | $SO_4^{2-}$: 94.01%<br>$PO_4^{3-}$: 93.84%<br>Br: 96.77%<br>COD: 90% | (Cardoso et al., 2021) |

| | | | | | | |
|---|---|---|---|---|---|---|
| Desalination | 25% medium | Zarrouk | D = 1.14 g/L<br>P = 0.05 g/L/d | Carbohydrate: 52.29%<br>Lipids: 12.79%<br>Ash: 2.69% | $NO_3^-$: 96.99%<br>$PO_4$: 83.11%<br>Zn: 96.43%<br>TDS: 24.89%<br>$SO_4$: 29.65% | (Mata et al., 2020) |

### 2.2.1.3 CULTIVATION FACTORS AFFECTING THE *SPIRULINA* GROWTH IN WASTEWATER

Further to that, other factors such as colour of light, light intensity (Bahman et al., 2022), temperature (Venkata Subhash et al., 2014) and initial *Spirulina* biomass concentration (K and M, 2018) have some effects on the enhancement of *Spirulina* cultivation in wastewater. The optimum temperature for cultivation of *Spirulina* was 30 °C (Venkata Subhash et al., 2014). Moreover, Bahman et al. (2022) revealed that blue light (5800 lux intensity) is more energetic and efficient for photosynthesis compared to the other colours which gives the highest biomass (5.45 g/L) and protein concentration (3 g/L) after 8 days of cultivation. However, the colour of wastewater varies in different type of wastewater, thus the optimum light intensity for different type of wastewater may differs as well. Finally, scalability is also an important factor in *Spirulina* cultivation, many studies conducted experiments in lab scale with volume from 1 to 10 L. The performance of lab scale photobioreactor and industrial scale photobioreactor may differs in biomass productivity. Krishnamoorthy, Manickam and Muthukaruppan (2019) revealed that the maximum biomass concentration and productivity of 50 L photobioreactor (8.11 g/L, 0.115 g/L/d) is slightly higher compared to 500 L photobioreactor (7.45 g/L, 0.094 g/L/d). Thus, when scale up from lab scale to industrial scale, there would be a slight decrease in biomass productivity performance. This is because at larger scale, maintaining a constant cultivation condition (i.e., light, temperature) remains a challenge. Further research is needed in optimisation of biomass productivity prior to the development of wastewater utilisation for *Spirulina* cultivation.

#### 2.2.1.4 PRE-TREATMENT OF WASTEWATER: COMBINATION WITH TREATMENT TECHNOLOGIES

Although the dilution or supplements in wastewater supports the growth of *Spirulina*, it is unable to eliminate the negative impacts of wastewater. This causes insufficient or inappropriate nutrients in wastewater which often lead to *Spirulina* experience nitrogen starves during cultivation. The biomass composition is usually relatively high in carbohydrate content which is suitable for the application of biofuels or fertiliser (Kumar et al., 2020). However, the utilisation of wastewater medium in *Spirulina* cultivation for the application of food supplements, functional food, nutraceuticals, and pharmaceutical products has yet to be reported. This could be due to the perception of hygiene and the presence of toxic heavy metals which is harmful towards human health. *Spirulina* has a unique quality to detoxify (neutralise) or chelate the toxic minerals, this is a unique characteristic of *Spirulina* that is not yet confirmed in any other type of microalgae (Ghofar et al., 2019). To reduce or eliminate the negative impacts of wastewater utilisation for *Spirulina* cultivation, researchers have tried to pre-treat wastewater, incorporate other wastewater treatment technology such as adsorption technology, ion exchange membrane technology, $CO_2$ scrubber and a novel method of microalgae-microbial fuel cell (MMFC) (Chang et al., 2013; Markou, Vandamme and Muylaert, 2014; Chang et al., 2016; Hadiyanto, Christwardana and da Costa, 2023).

Frequent studies have been reported on the coupling of adsorption technology with *Spirulina* cultivation using natural zeolite, synthetic zeolite and granular $\gamma$-alumina adsorbent. Markou, Vandamme and Muylaert (2014) used natural zeolite to adsorb the ammonia from the anaerobically digested poultry manure

wastewater. The ammonia enriched zeolite was then placed in modified Zarrouk medium (without $NaNO_3$) to replace the nitrogen source. This strategy isolates the ammonia from the wastewater excluding the suspended solids, the dissolved coloured compounds and any other possible contaminant of the wastewater. However, the drawback of natural zeolite is its low phosphorus sorption capability. Therefore, Markou et al. (2015) enhanced the low phosphorus sorption capability of natural zeolite by chemically pre-treating with $Ca(OH)_2$ as the modifying agent. Besides, Lu et al. (2019) have also reported on the use of synthetic zeolite shows a better performance than natural zeolite to mitigate ammonium toxicity in wastewater. Moreover, the biomass composition (e.g., protein, lipid, chlorophyll *a*, chlorophyll *b* and carotenoid) was higher compared to *Spirulina* cultivated with wastewater diluted with freshwater. Further to that, Lee et al. (2020) have also studied the pre-treatment of municipal wastewater with granular $\gamma$-alumina adsorbent by adsorbing the nitrate and phosphate nutrients presence in the wastewater. The adsorbent is place in the culture medium to allow the nutrients to desorb into the medium for the cultivation of *Spirulina*. However, the drawback of the adsorbent was unable to fully desorb the nutrients, *Spirulina* cannot utilise the nutrients recovered by the adsorbents completely for its growth. Therefore, further research is required in enhancing the nutrients adsorbing and desorbing efficiency of the adsorbent. On the other hand, Chang et al. (2016) designed an annular photobioreactor where an ion exchange membrane is place in between the municipal wastewater and culture medium of *Spirulina*. Nutrients such as nitrogen and phosphorus in wastewater continuously permeated into microalgae cultures through the ion exchange

membrane for microalgae growth. Thus, avoiding the direct mixing of algae cells with wastewater by separating them into two chambers.

Other technology coupling with *Spirulina* cultivation has been reported such as $CO_2$ scrubber, submerged anaerobic membrane bioreactor and sequencing batch reactor. In the studies of Chang, Lee and Den (2013) diluted dairy wastewater was used as a scrubbing liquid in an alkaline absorption process for $CO_2$ capturing from flue gases. Thus, producing a $CO_2$ (which act as a carbon source) enriched diary wastewater and was directly used for cultivation of *Spirulina*. Park, Labrecque and Lavoie (2013) pre-treated municipal wastewater in a submerged anaerobic membrane bioreactor before using it for *Spirulina* cultivation. Besides, pre-treating the municipal wastewater, Chavan and Mutnuri (2019) collected municipal wastewater after secondary treatment in a sequencing batch reactor (SBR) treatment process. This shows that current wastewater treatment plant which employ SBR system can couple with the application of *Spirulina* cultivation producing value-added product, in turn generating income for the company.

Microalgae-microbial fuel cell is a relatively new and promising technology coupling with the cultivation of *Spirulina*. Hadiyanto, Christwardana and da Costa (2023) design a MMFC system where tapioca wastewater was placed at the anode while *Spirulina* cultivation was placed at the cathode. The COD removal efficiency in wastewater was 67% and biomass productivity of *Spirulina* cultivation reported was 55.38 mg/L/d with specific growth of 0.172 $d^{-1}$. This approach has not only treated wastewater and produce *Spirulina* biomass but also generate green electricity. The application of MMFC is relatively new and the studies on *Spirulina* cultivation utilising MMFC

technology is scarce. Therefore, there are many potential and further research is needed to enhance its efficiency and applications. **Figure 2.1** summarise the pros and cons of the development of wastewater utilisation for the cultivation of *Spirulina*.

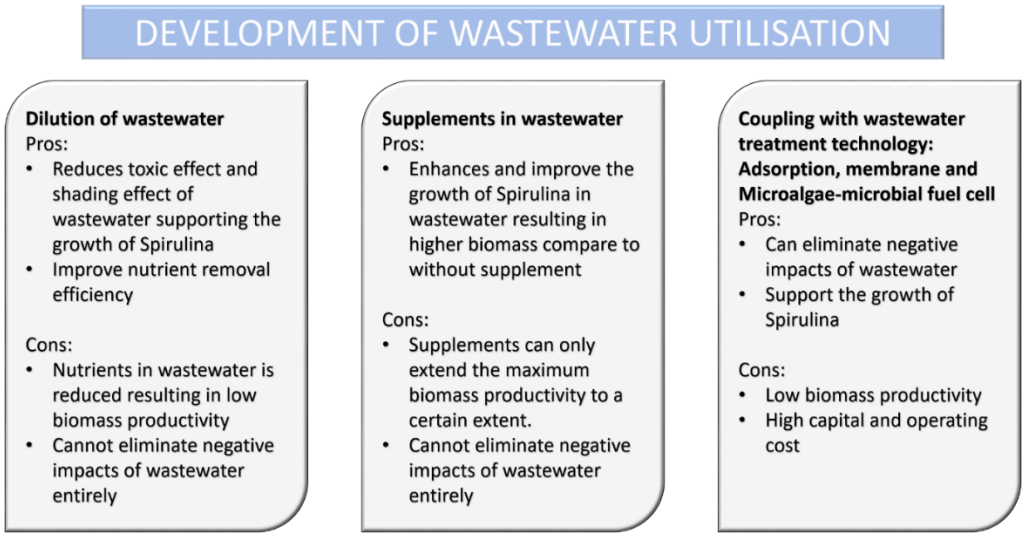



**Figure 2.1** Development of wastewater utilisation for the cultivation of *Spirulina*

## 2.3 A SUSTAINABLE CIRCULAR BIOECONOMY APPROACH

A circular economy is an economic system where products and services are traded in closed loops or "cycles" with the aim to create a system that allows for the long life, optimal use, refurbishment, remanufacturing and recycling of products and materials (Reno, Regaldo and Gagneten, 2020). The cultivation of *Spirulina* can produce many value-added products and are mainly cultivated in Zarrouk medium or other chemical-based culture medium. However, the cost and environmental impact of chemical-based culture medium is a major drawback. By replacing chemical-based culture medium with wastewater

medium would help to close the loop and formulate a circular bioeconomy while reducing the environmental impact from both chemical-based culture medium and wastewater. As there are many types of wastewaters, the three main type of wastewater which had been studied widely includes municipal wastewater, aquaculture wastewater and animal wastewater will be evaluated on the opportunity available in achieving sustainable circular bioeconomy.

Municipal wastewater or also known as sewage wastewater are wastewater coming from household, industries or a mixture of it. They are typically wastewater generated by human population. Sewage pollution has been a very serious issues to the environment such as polluting the rivers. In Malaysia, according to the Environmental Quality Report (2018) published by the Department of Environment Malaysia, a total of 638 Malaysia rivers were monitored, 50% categorised as Clean, 17% categorised as slightly polluted and 33% categorised as polluted river. The report also shows that the whole Malaysia is producing about 650 tonnes/day of biological oxygen demand (BOD), 830 tonnes/day of suspended solids (SS) and 200 tonnes/day of ammoniacal nitrogen (AN). Sewage treatment is the largest contributor, 37% of the BOD and 79% of AN and the second largest contributor, 36% of SS in the river.

Further to that, aquaculture is a rapid development industry, there are recent development in moving from coastal aquaculture farming to land-based intensive farming. According to Food and Agriculture Organisation 2020 report shows aquaculture production from 1986 to 2018, the production of marine aquaculture production increases from 6.3 to 30.8 million tonnes of fish while inland aquaculture production increases from 8.6 to 51.3 million tonnes of fish. Inland aquaculture accounts 62% of the aquaculture fish production. This is

because coastal aquaculture farming is frequently affected by weather and prone to diseases which may affect the farming business. On the other hand, land-based intensive farming is protected from weather and less prone to diseases, thus farming business is more stable. Due to the increase of fish production from land-based intensive farming, wastewaters rich in organic and inorganic compounds resulting from fish excreta and decomposition of unconsumed feeding ration can generate many negative impacts to aquatic environments, making them susceptible to eutrophication (Benedito et al., 2013; Nogueira et al., 2018). The load of waste is directly proportional to the fish production (Turcios and Papenbrock, 2014). Consequently, further research is needed to treat and utilise the wastewater generated by aquaculture with more efficient and economic.

Animal wastewaters are generated from livestock production such as pig, chicken, cattle and sheep. Many studies have been reported on the cultivation of *Spirulina* in swine wastewater. This is because the water consumption in pig farm is high compared to other livestock. The water consumption of sheep is 2 to 10 L per day per head, cattle is 25 to 100 L per day per head and pig is 7 to 113 L per day per head (Sakadevan and Nguyen, 2017). Moreover, according to Food and Agriculture Organisation, the production of pork and chicken have increased rapidly in China, Thailand and Viet Nam during the 1990s. About 42% of nitrogen and 90% of phosphorus that flows into the South China Sea from the Pearl River basin in Guangdong Province, the Chao Phrya River basin in Thailand and the Red River and Dong-Nai River basin in Viet Nam, comes from swine wastewater. Instead of allowing these nutrients to flow into the South

China Sea polluting the environment, this serves as good opportunity to utilise these nutrients for the cultivation of *Spirulina*.

Not to mention that there are many sources of wastewater coming from different industries which will continue increase such as POME wastewater is another source which is increasing in Malaysia. Malaysia is the second largest palm oil contributors and has achieved 19.92 million tons of palm oil production and exported 16.65 million tons of palm oil in 2017 (Kushairi and Nambiappan, 2017; Tan and Lim, 2019). About 1 tons of fresh fruit bunch produces 0.66 tons of palm oil mill effluent (Indriyati, 2008; Nur and Buma, 2019). Therefore, there are many opportunities and resources in utilising wastewater such as municipal wastewater, aquaculture wastewater, animal wastewater and other source of wastewater for *Spirulina* cultivation instead of allowing the nutrients to be discharged into the land, river and sea causing pollution and disrupting the ecosystem.

With the current and development of *Spirulina* cultivation incorporating other technologies such as adsorption, ion exchange membrane and MMFC. It is notable that the practicality of utilising wastewater as a culture medium for *Spirulina* cultivation is developing. By cultivating *Spirulina* in wastewater as the culture medium, organic and inorganic pollutants in wastewater can be reduced thus this improves the quality of wastewater prior to discharge to environment. In addition, the biomass produced during *Spirulina* cultivation in wastewater can be valorised into many value-added products such as clean water, bioactive compounds, biofuels and even generating green electricity (Chew et al., 2021). Also, $CO_2$ is essential for the cultivation of *Spirulina* for its photosynthesis process. Thereby, this mitigates the $CO_2$ in the atmosphere while

producing oxygen to the environment. It is notable that for every kg of algal biomass can absorb around 1.83 kg of $CO_2$ (Khoo et al., 2020a). According to the International Energy Agency, the total world production of dry algal biomass is estimated at about 10,000 tons per year with half of the yield being produced in mainland China (Huo et al., 2011; Ye et al., 2018). Thus, it can be estimated that about 18,300 tons of $CO_2$ is removed from the atmosphere every year by producing algal biomass. Further to that, incorporating the technology MMFC can generate green electricity. However, this technology is still in its infant phase, the electricity generation is low (14.47 mW $m^{-2}$), further research is needed in this area. If this application and implementation is successful in the future, green electricity can be produced while conducting wastewater treatment, *Spirulina* cultivation and reduce carbon footprint in the atmosphere at the same time.

*Spirulina* biomass contains high content of micronutrients and macronutrients; the chemical composition of *Spirulina* biomass composed of 60 - 70% proteins, carbohydrates, vitamins like provitamin A, vitamin C, vitamin E, minerals such as iron, calcium, chromium, copper, magnesium, manganese, phosphorus, potassium, sodium and zinc, fatty acids, and pigments such as chlorophyll a, phycocyanin and carotenes (Soni, Sudhakar and Rana, 2017). Likewise, *Spirulina* biomass may be used for biogas production due to its low lipid content and high carbohydrate content make it an attractive candidate for anaerobic digestion (Chavan and Mutnuri, 2019). After harvesting the *Spirulina* biomass from wastewater medium, the wastewater may be discharged into the river without any secondary pollution. Thereby, utilising wastewater as a cultivation medium for *Spirulina* can solve the environmental pollution issues, produce value added product and even generate green electricity. **Figure 2.2** shows a

brief process flow of algae production from upstream to downstream process when chemical-based culture medium is replaced with wastewater medium in achieving a sustainable circular bioeconomy.

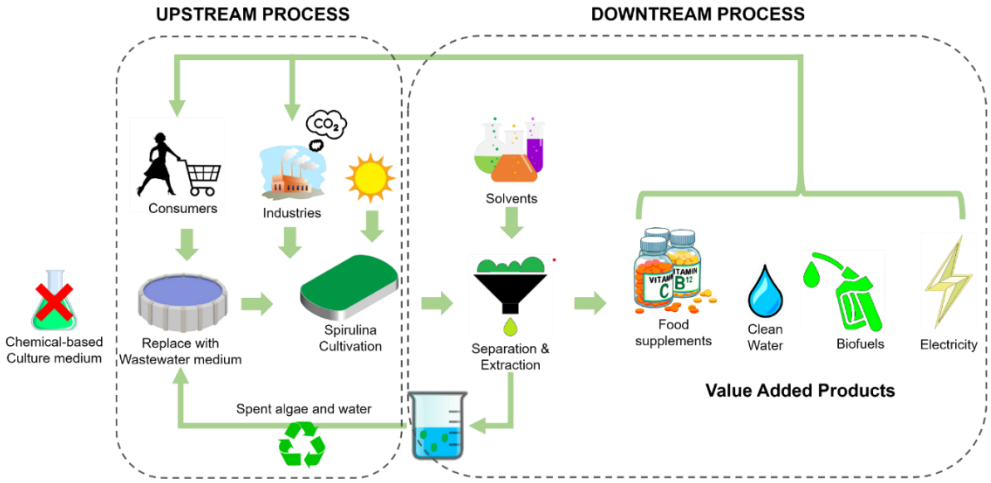



**Figure 2.2** The process flow of *Spirulina* cultivation from upstream to downstream process (adopted from Pienkos, Laurens and Aden (2011))

## 2.4 CHALLENGES AND PROSPECTS

The impact of microalgae cultivation stage has the highest environmental impacts among all production stages resulting from the extensive use of chemicals, nutrients, and energy (Ye et al., 2018). Rodríguez et al. (2018) evaluated the environmental performance of biogas production from *Spirulina* through life cycle assessment using experimental and simulation results. The results shown that high consumption is required in the cultivation stage, which also leads to increase in the global warming potential (GWP) category. Rodríguez et al. (2018) suggested a potential alternative could be the use of nutrients from wastewater. The development of chemical-based culture medium is quite established as there are many different types of formulation available in

the industries, but the cost and environmental impact is the main drawback. Therefore, there is a need to focus the research area in investigating various enhancement techniques, modification and alternative source of culture medium such as wastewater to reduce the cost of culture medium for *Spirulina* cultivation. The modification of chemical-based culture medium in replacing chemical source (e.g., carbon, nitrogen and phosphorus) is not established and requires more research in finding alternative source and evaluate the cost efficiency and environmental impact on the application in the industry. Furthermore, the current research and development area of alternative source of culture medium have been on wastewater. Many studies have reported on the cultivation of *Spirulina* in various type of wastewater. Therefore, there is also needed to explore the possibility of other alternative source of culture medium for *Spirulina* cultivation such as vermicompost medium.

Wastewater requires more research in the practicality use as a culture medium for various industry application such as food supplements, natural colouring, cosmetics, and pharmaceuticals. There are many types of wastewaters and the challenge remains in the different characteristics of various wastewater that may affect the growth of *Spirulina* and the productivity of *Spirulina* biomass. The current development has focused too much in supporting the growth of *Spirulina* in wastewater and enhancing the biomass productivity, it does not eliminate the negative impact of wastewater completely on the growth of *Spirulina*. Therefore, there should be a change in the concept of utilising wastewater by coupling with wastewater treatment technology such as adsorption, membrane and MMFC which had been studied for the application for *Spirulina* cultivation. These methods have proven to eliminate all negative effects of wastewater such as

toxic effects and colour that may inhibit the growth of *Spirulina*. However, the cost of adsorption and membrane technology remain a drawback. For instance, the cost of membrane is around $100,000 for treating wastewater with a flow rate of 10 to 20 gallons per minute, while the cost of zeolites may range within $1960 to $48,000 for treating 1000 gallons of water (Albatrni, Qiblawey and El-Naas, 2021). Although adsorption technology which highly depends on zeolite may be regenerate and reused many times, but it will eventually reach its end-of-life. In addition, it is reported that about 840,000 membranes per year ended up in incineration and landfill (Yusuf et al., 2020). Thus, the management of spent zeolite and membrane is very important and will greatly impact on the sustainability of the *Spirulina* cultivation process. A sustainable alternative to zeolites is the use of bio-char adsorbent which are derived from waste source such as banana peels (Te et al., 2021) and palm kernel shell (Wan Mahari et al., 2020). Su et al. (2020) had shown that bio-char successfully achieved 67% of ammonia removal from aquaculture wastewater with a final ammonia concentration of 0.42 mg/L. Therefore, it is possible to utilise the adsorbed ammonia as nutrients for the cultivation of *Spirulina*. Further research is needed to assess on the cost-effective and sustainability of bio-char and membrane technology on the application *Spirulina* cultivation with wastewater medium. Lastly, MMFC is a further development of microalgae-microbial wastewater treatment. It is yet to be practical in the industry because the electricity generation and biomass productivity reported are still low. Further research is needed in increasing the electricity generation and biomass productivity.

Further to that, maintaining optimum nutrients content in wastewater has been a major challenge to many researchers for many years. However, with the

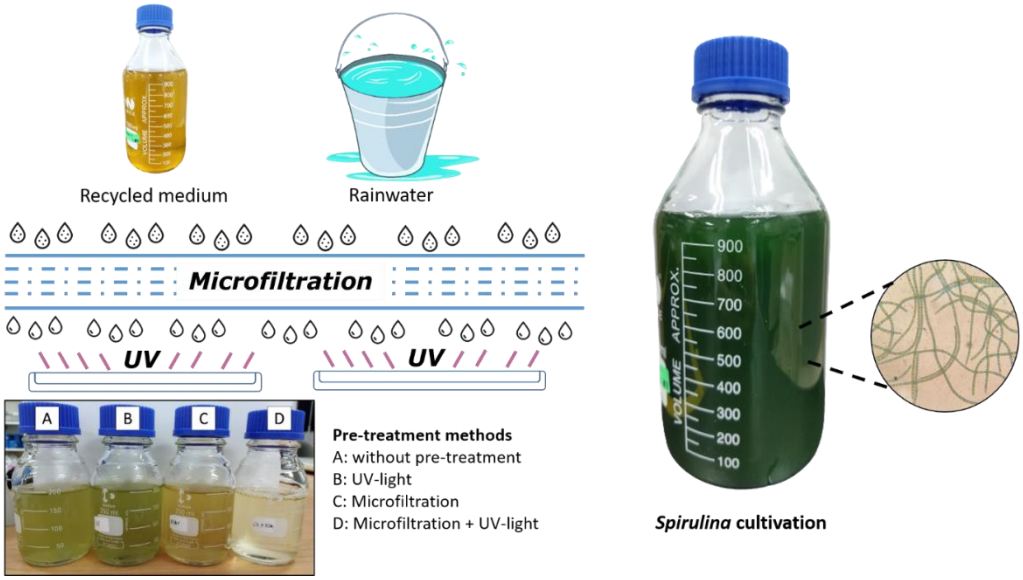
development of Internet of Things (IoT), it is possible to monitor and control nutrient content in wastewater with an IoT integrated water treatment system (Martínez et al., 2020). Sensors are place in the system to monitor cultivation environment in wastewater while transferring real time data to operators. In case of insufficient nutrients, system will automatically add the require nutrients into the system. To the best of author knowledge, no study has been reported on the application of IoT with wastewater medium and *Spirulina* cultivation. This could open a new research area in the microalgae cultivation research society.

## 2.5 CONCLUSIONS

The current development of chemical-based culture medium has been established as there are many different types of chemical-based culture medium available in the industry. However, the limitation of these chemical-based culture medium remains at their cost and environmental impact. The research area should focus more in developing alternative low-cost culture medium such as wastewater rather than enhancing the productivity of *Spirulina* biomass. The literature studies have shown the possibility of *Spirulina* cultivation in wastewater. However, the negative impacts of wastewater on *Spirulina* cultivation remains a challenge such as the uncertainty of nutrients content, toxicity, and colour of wastewater. Therefore, more research and development are needed in overcoming these challenges such as investigating the potential coupling of wastewater treatment technology with *Spirulina* cultivation and assessing the practicality in the industries. As the development of the practicality of utilising wastewater for *Spirulina* cultivation continues, it will benefit the society, industries, and environment in achieving a sustainable circular bioeconomy.

## CHAPTER 3

# UPSTREAM BIOPROCESSING OF *SPIRULINA* PLATENSIS MICROALGAE USING RAINWATER AND RECYCLE MEDIUM FROM POST-CULTIVATION FOR C-PHYCOCYANIN PRODUCTION




This chapter consists of thesis-version of work published in the Journal of the Taiwan Institute of Chemical Engineers.

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## 3.1 ABSTRACT

The cultivation of microalgae represents a water-intensive process, prompting the need to reduce its water footprint and explore alternative water sources. Therefore, this research work aims to investigate the growth of *Spirulina* by recycling the culture medium obtained from the post-cultivation process and substituting rainwater resources for the preparation of the culture medium. The effect of pre-treatment methods, specifically microfiltration and UV-light was investigated. These methods were applied to treat the recycled medium and rainwater resources used for *Spirulina* cultivation. Biomass concentration, biomass productivity and phycocyanin accumulation of *Spirulina* were examined to assess the *Spirulina* growth under different pre-treatment conditions. After three cultivation cycles in the recycled medium, biomass productivity decreased from 0.2246 to 0.0802 g/L/d which might be due to high salinity and biological pollutants that inhibit *Spirulina* growth. However, different pre-treatment methods using microfiltration and UV-light on the recycling medium were found to be effective in increasing biomass productivity from 0.1395 to 0.1861 g/L/d. Additionally, the use of rainwater for *Spirulina* cultivation was explored. Applying pre-treatment methods to the rainwater, biomass productivity was further improved from 0.3211 to 0.3837 g/L/d. These findings suggest that the use of pre-treatment methods and alternative water sources can improve the biomass productivity of *Spirulina* and potentially reduce the environmental impact throughout the microalgae cultivation process.

***Keywords:*** *Spirulina*; rainwater; pre-treament; recycle medium; phycocyanin

## 3.2 INTRODUCTION

*Spirulina* contributes the largest production among many other microalgae species, which accounts for about 30% of the total annual 10,000 tons of dry-weight algae biomass production globally in the microalgae industries (Lim et al., 2021; Amorim et al., 2021). *Spirulina* has been commercially exploited in the food industry, agriculture, perfumery, medicine, pharmaceuticals and environmental applications (Soni et al., 2021). This is due to its various benefits such as anticancer, antiviral, antioxidant and anti-allergic agent properties that exhibit in a pigment-protein complex known as phycocyanin found in *Spirulina* (Soni et al., 2021; Bai et al., 2011). However, the high production cost of microalgae biomass remains unfavourable, therefore several research outputs have utilized wastewater effluents as an alternative cultivation medium and simultaneously help to bioremediate wastewater treatment plants (Djaghoubi et al., 2015; Lu et al., 2019). On the other hand, several arise challenges such as cultivation contamination, growth inhibition and high dilution cost are needed to be addressed (Xie et al., 2022).

A study has reported the use of recycling systems and high-density cultures to increase the efficiency of water recycling for the cultivation of microalgae (Pugazhendhi et al., 2020). Recycling systems can significantly reduce water usage but also result in lower biomass concentration due to algicidal products from biological pollutants (e.g. zooplankton, bacteria, fungus and virus) in the culture medium inhibiting the microalgal photosynthesis (Yu et al., 2019). To address this issue, various pre-treatment methods that include filtration and UV-radiation have been investigated as effective techniques to treat wastewater before using it as a culture medium for the cultivation of microalgae (Tejido-

Nuñez et al., 2019; Cho et al., 2011). Qin et al. (2014) showed that exposing dairy wastewater to UV-light for 15 min increases *Chlorella* biomass concentration from 0.861 g/L (untreated) to 1.218 g/L. On the other hand, Ramsundar et al. (2017) reported that filtration is the most feasible pre-treatment for the bacterial reduction in municipal wastewater, showing promising *Chlorella* biomass productivity of 77.14 mg/L/d. These methods can also be applied to pre-treat recycled medium. Recycling medium is an important water management strategy towards achieving sustainable water utilization. However, it is important to also explore alternative water sources to further improve water use efficiency.

To comprehend these findings, Farooq et al. (2015) revealed that the production of 1 litre microalgal biodiesel requires approximately 3 tonnes of water. Yang et al. (2011) also reported that the production of 1 kg of microalgal biodiesel, 3726 kg of water, 0.33 kg of nitrogen and 0.71 kg of phosphate are required if freshwater is used without recycling. Further to that, Yang et al. (2011) conducted a life cycle assessment and showed that the recycling of harvested water and replacing it with sea or wastewater decreases water usage by 84% (3129 kg) and 90% (3353 kg), respectively. Thus, the idea of using alternative water resources (i.e. harvested rainwater) demonstrates an economically feasible solution for large-scale industries. Microalgae industries can benefit from mounting a rainwater harvesting system (RWHS) to collect rainwater as a water source. For instance, a catchment area of 10,000 $m^2$ can provide a potential rainwater collection of 23,000 $m^3$ annually (Hafizi Md Lani, Yusop and Syafiuddin, 2018). Hashim et al. (2013) developed a simulation that studied the installation of RWHS for a community of 200 houses with an average total daily

water demand of 160 $m^3$, achieving significant water savings of up to 58%. Harvested rainwater does not only provide a water source for the cultivation of microalgae, it can also be utilized as cooling water throughout the process preparation of microalgae-based biofuels. For instance, thermal conversion technologies (e.g. combustion, gasification, and pyrolysis) may contribute significant amounts of heat during the conversion process that can be effectively dissipated by utilizing harvested rainwater as cooling water (Khoo et al., 2020b).

The objective of this research work aims to investigate the growth of *Spirulina* by recycling the culture medium obtained from the post-cultivation process and substituting rainwater resources for the preparation of the culture medium. To the best of the author's knowledge, this research work demonstrates the feasibility of using rainwater resources for the cultivation of *Spirulina* microalgae. Further to that, different pre-treatment (e.g. microfiltration (MF), UV-light and combined treatment (MF-UV)) of rainwater resources or recycled medium were evaluated to understand its effects on the growth of microalgae, dry biomass concentration and phycocyanin accumulation of *Spirulina* microalgae. This research work establishes a concept of sustainable microalgae cultivation by utilizing alternative rainwater resources and recycling the cultivation medium, which can potentially reduce the upstream processing cost of *Spirulina* microalgae.

## 3.3 MATERIALS AND METHODS

### 3.3.1 MICROALGAE STRAINS

Fresh *Spirulina platensis* was provided by Biolina Corporation Sdn. Bhd (Malaysia). To ensure the purity of the strain, the centrifuge washing technique and streak plating technique was conducted based on the method of Parvin, Zannat and Habib (2007) with some modifications to isolate the *Spirulina* strain from the fresh medium. The isolated *Spirulina* strain is then maintained and kept in a petri dish that was prepared with Zarrouk medium agar. **Figure 3.1** showed the morphology of *Spirulina platensis* under a microscope (PB3730, Microtech) with a magnification of 10x and 40x. The sample was prepared by dropping a few droplets of *Spirulina* microalgae samples onto a glass slide and seen under a microscope.

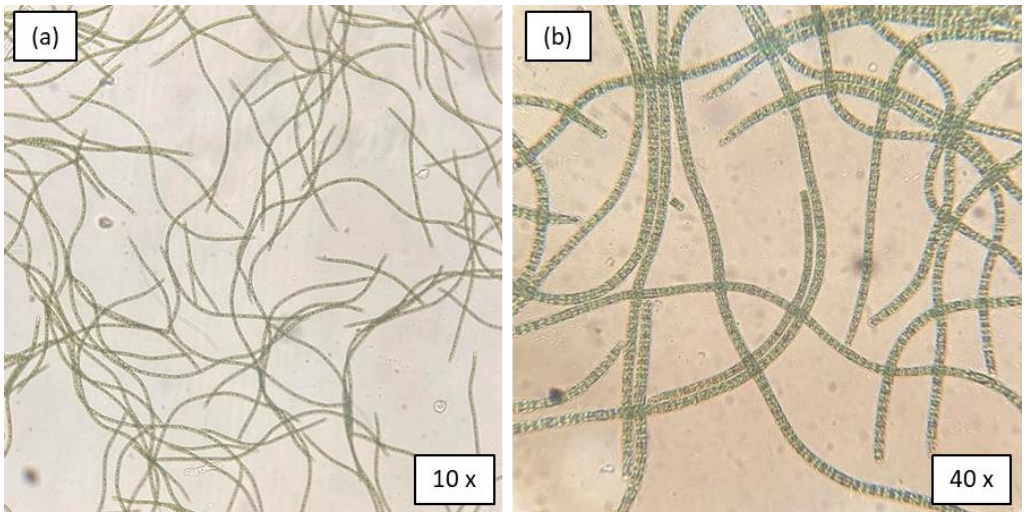



**Figure 3.1** Morphology of *Spirulina platensis* strain under a microscope (a) 10x magnification and (b) 40x magnification

### 3.3.2 SELECTION OF MICROALGAE CULTIVATION MEDIUM

The selection of microalgae cultivation medium including modified BG-11 medium (Rippka et al., 1979) and Zarrouk medium (Zarrouk, 1966) with minor modification was used to determine the biomass concentration and C-phycocyanin accumulation of *Spirulina* microalgae. Previous study has reported the cultivation of *Spirulina* in BG-11 medium and Zarrouk medium. However, the biomass concentration in BG-11 medium (~6.0 g/L) is lower as compared to Zarrouk medium (~7.0 g/L) (Qaisar, Panhwar and Perwez, 2016). Therefore, this study aims to modify the BG-11 medium, which requires lesser chemical usage to achieve comparable biomass concentration to that of Zarrouk medium. **Table 3.1** shows the composition of the modified BG-11 medium and Zarrouk medium. All the chemicals were of analytical grade and were prepared by measuring each chemical listed in **Table 3.1** with a weighing balance and mixing them using deionised water.

**Table 3.1** Modified BG-11 and Zarrouk medium composition

| **Modified BG-11 medium** | **(g/L)** | **Zarrouk medium** | **(g/L)** |
|---|---|---|---|
| Sodium bicarbonate ($NaHCO_3$) | 18 | $NaHCO_3$ | 18 |
| Sodium nitrate ($NaNO_3$) | 1.5 | $NaNO_3$ | 2.5 |
| Magnesium sulfate heptahydrate ($MgSO_4.7H_2O$) | 0.075 | $MgSO_4.7H_2O$ | 0.2 |
| Calcium chloride dihydrate ($CaCl_2.2H_2O$) | 0.036 | $CaCl_2.2H_2O$ | 0.04 |
| Dipotassium hydrogen phosphate ($K_2HPO_4$) | 0.03 | $K_2HPO_4$ | 0.5 |
| Sodium carbonate $Na_2CO_3$ | 0.02 | Sodium chloride (NaCl) | 1 |
| Citric acid | 0.006 | Potassium sulfate ($K_2SO_4$) | 1 |
| Ferric ammonium citrate | 0.006 | $FeSO_4.7H_2O$ | 0.01 |
| Ethylenediaminetetraacetic acid (EDTA) | 0.001 | EDTA | 0.08 |
| Micronutrient | 1 mL/L | Micronutrient | 1 mL/L |

Micronutrient: $H_3BO_3$: 2.86 g/L, $MnCl_2.4H_2O$: 1.81 g/L, $ZnSO_4.7H_2O$: 0.222 g/L, $Na_2MoO_4.2H_2O$: 0.3 g/L, $CuSO_4.5H_2O$: 0.07 g/L, $Co(NO_3)_2.6H_2O$: 0.04 g/L

### 3.3.3 CULTIVATION OF MICROALGAE: EXPERIMENTAL SETUPS ON PRE-CULTURED AND BATCH CULTURE

#### 3.3.3.1 TYPE OF CULTURE MEDIUM: ZARROUK MEDIUM AND BG-11 MEDIUM

The *Spirulina* strains were inoculated and pre-cultured in 250 mL of Schott bottle with Zarrouk medium and modified BG-11 medium for 10 days until it reached the late log phase before subjecting for batch culture. After reaching late log phase growth, the pre-cultured *Spirulina* microalgae is transferred into batch culture using 1 litre of Schott bottle in a closed photobioreactor system containing Zarrouk medium or modified BG-11 medium. The batch culture was placed onto a magnetic stirrer to ensure uniform mixing and to prevent the *Spirulina* biomass from settling at the bottom of the close photobioreactor system. The cultivation mode of *Spirulina* microalgae was conducted under photoautotrophic growth conditions in which the light intensity was installed on the opposite side of the bottles and adjusted to 3000 lux using a light meter (UT383, UNI-T) (Chew et al., 2018b).

#### 3.3.3.2 EVALUATION OF THE VARIOUS TYPE OF AERATION SYSTEMS FOR THE CULTIVATION OF *SPIRULINA* MICROALGAE

Different types of aeration systems (i.e. aerated air, carbon dioxide and mixture of aerated air and carbon dioxide (ratio 1:1 volume)) were investigated in the batch culture of *Spirulina* microalgae. The airflow rate was maintained at 400 cc/min using an airflow regulator (Dwyer, Malaysia) throughout the experiment. The biomass concentration and C-phycocyanin accumulation in *Spirulina*

microalgae were evaluated daily using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan). The biomass concentration of the culture was determined by optical density (OD) measurement at a wavelength of 688 nm (Chen et al., 2016). Further to that, the UV-Vis spectrophotometer had a limitation in that can only read absorbance values up to 4. Thus, all samples had to be diluted 10 times throughout the experiment to ensure consistency and by taking its average to ensure the accuracy of the reading.

#### 3.3.3.3 EVALUATION ON RECYCLED MEDIUM FOR SUBSEQUENT *SPIRULINA* MICROALGAE CULTIVATION

The term "recycled medium" refers to the supernatant recovered by centrifuged from post-cultivation of *Spirulina* after 10 days. Based on the experimental setup, the recovered medium volume is usually lesser than 1-L due to water evaporation and sample collection for absorbance measurement. The composition of recycle medium contains water, nutrients and residual biomass. To prevent wastage of nutrients, the number of cycles of recycling medium for *Spirulina* cultivation was investigated. The recycled medium was recovered after 1$^{st}$ cycle of *Spirulina* cultivation (for 10 days). An amount of 1 litre of modified BG-11 chemicals were added and dissolved in the recycled medium and top-up with deionised water until the volume reached 1 litre for the subsequent batch culture while maintaining the pH range within 9.0 to 10.0. It is then subjected to another cycle of cultivation. This is repeated until the *Spirulina* growth curve does not increase continuously for 10 days of cultivation for each cultivation cycle. **

### 3.3.3.4 PRE-TREATMENT METHODS ON RECYCLED MEDIUM

It is noted that biomass productivity decreases during the 2nd cultivation cycle of recycle medium. Therefore, it was suggested to pre-treat the recycled medium before using it for the subsequent cultivation of *Spirulina* microalgae. After the 1st cultivation cycle of all 4 bottles of 1-L *Spirulina*, the recycled medium was recovered and subjected to different pre-treatment methods (e.g., microfiltration, ultraviolet (UV) light, combined treatment). Microfiltration uses a qualitative filter paper with a pore size of 5 to 13 µm (FiltraTech, France) to filter suspended solids from the recycled medium. The UV-light treatment uses an aquarium UV sterilizer lamp (UV-011) with a power of 11 W placed to pre-treat the recycled medium for 10 min. Three different pre-treatment conditions were investigated including microfiltration treatment (MF), UV-light treatment (UV) and combined treatment (microfiltration and UV-light treatment (MF-UV)). A control condition (without pretreatment) was employed for comparison purposes. For combined treatment (MF-UV), the recycled medium was initially treated with microfiltration and followed by UV-light treatment. After pre-treating the recycled medium, An amount of 1 litre of modified BG-11 chemicals were added and mixed uniformly before inoculating the *Spirulina* microalgae into the medium

### 3.3.3.5 CULTIVATION OF *SPIRULINA* MICROALGAE USING RAINWATER AS AN ALTERNATIVE WATER RESOURCE

Exploring alternative water resources is necessary as recycling medium from post-cultivation may not be a sustainable long-term water management strategy, hence the use of rainwater resources is suggested for the cultivation of *Spirulina* microalgae. The water resource used to prepare the culture medium for the

cultivation of *Spirulina* microalgae was replaced using rainwater resources. Similar pre-treatment methods used on recycle medium including MF, UV and combined treatment (MF-UV) were also applied to alternative rainwater resources for the cultivation of *Spirulina* microalgae. Although there are other pre-treatment technologies (i.e. adsorption, ion-exchange membrane) but it is not economically sustainable to treat rainwater. The study aimed to evaluate the impact of alternative rainwater resources subjected to different pre-treatment methods for the *Spirulina* biomass concentration and phycocyanin accumulation. Similar operating conditions obtained earlier from the closed photobioreactor system were employed for the subsequent studies. Similarly, an amount of 1 litre of modified BG-11 chemicals were added and mixed uniformly to the pre-treated rainwater before inoculating into the cultivation of *Spirulina* microalgae. The operating parameters of this research study were summarised in **Table 3.2**.

**Table 3.2** Operating parameters of *Spirulina* cultivation for the production of phycocyanin

| No. | Operating parameter | Variables |
|---|---|---|
| **1** | Type of culture medium | Zarrouk medium, modified BG-11 medium |
| **2** | Type of aeration | Air, carbon dioxide, mixture of air and carbon dioxide |
| **3** | No. of cycles on recycled medium | 1st cycle (fresh medium), 2nd cycle, 3rd cycle |
| **4** | Pre-treatment methods on recycled medium * | Control, microfiltration (MF), ultraviolet (UV) light and combined treatment (MF-UV) |
| **5** | Pre-treatment methods on rainwater medium ** | Control, microfiltration (MF), ultraviolet (UV) light and combined treatment (MF-UV) |

* Recycle medium without pre-treatments

** Rainwater medium without pre-treatments

### 3.3.4. QUANTIFICATION OF BIOMASS CONCENTRATION OF *SPIRULINA* MICROALGAE CULTIVATED IN RECYCLED MEDIUM AND RAINWATER RESOURCE

The 1-L batch cultivation of *Spirulina* was monitored for 10 days. According to the preliminary experimental test, the growth curve of *Spirulina* was observed to reach the late log phase after the $10^{th}$ day, its absorbance value is within 6 to 8. After 10 days of growth, the 1-L of matured *Spirulina* was mixed uniformly and a portion of the microalgae biomass was subjected to dilution in several concentrations (e.g. 20%, 40%, 60%, 80% and 100%). The absorbance for each of the diluted concentrations (i.e. microalgae biomass) was quantified using a UV-Vis spectrophotometer. The diluted biomass samples were centrifuged (6500 rpm for 5 min) to remove the supernatant (Koyande et al., 2019). To further remove the excessive moisture content in the wet biomass, the samples were subjected to freeze-drying (0.021 mbar for 48 hours) in a freeze dryer (Alpha 1-2 LDplus, Martin Christ). The weight of each freeze-dried biomass sample was recorded and a standard calibration curve of dry biomass weight against absorbance was plotted.

#### 3.3.4.1 *SPIRULINA* MICROALGAE BIOMASS PRODUCTIVITY

The biomass productivity ($P_b$) and specific growth rate ($\mu$) were calculated based on the following **Equation (3.1)** and **Equation (3.2) (**Chew et al., 2018a**)**, respectively:

$$P_b \text{ (mg/L/day)} = (N_2-N_1)/(t_2-t_1) \quad (3.1)$$

$$\mu \text{ (d}^{-1}\text{)} = \ln (N_2/N_1)/(t_2-t_1) \quad (3.2)$$

where, $N_1$ and $N_2$ are biomass concentration (g/L) at time $t_1$ and $t_2$, respectively.

### 3.3.4.2 PHYCOCYANIN QUANTIFICATION

Two types of phycocyanin were analysed which are C-phycocyanin (CPC) and Allo-phycocyanin (APC). In brief, phosphate buffer (50 mL) was added to a weighted amount (0.45 g) of the freeze-dried *Spirulina* biomass before subjecting it to the extraction process. The utilization of phosphate buffer as the extraction solvent was due to its ability to stabilize the pigment-protein complex molecules which are sensitive when subjected to intrinsic conditions (i.e. high temperature or pressure) during the extraction process (G-Bioscience, 2019). To facilitate the release of phycocyanin, the centrifuge tube containing the *Spirulina* biomass and phosphate buffer solvent is subjected to ultrasonic treatment (Elma, Germany) at 35 kHz for 5 min to break the cell wall of microalgae biomass (Chew et al., 2019). The pre-treated samples were centrifuged and the supernatant was collected for the quantification of CPC and APC concentration.

The concentration of CPC and APC were calculated based on **Equation (3.3)** and **Equation (3.4),** respectively (Chia et al., 2020; Chew et al., 2019).

$$CPC\ (mg/mL) = \frac{OD_{615} - 0.474 \times OD_{652}}{5.34} \quad (3.3)$$

$$APC\ (mg/mL) = \frac{OD_{652} - 0.208 \times OD_{615}}{5.09} \quad (3.4)$$

Where $OD_{615}$ and $OD_{652}$ are the optical density wavelength at 615 nm and 652 nm, respectively.

The purity of CPC and APC was calculated based on **Equation (3.5)** and **Equation (3.6) (**Chia et al., 2020**;** Chew et al., 2019**)**.

$$Purity_{CPC} = \frac{OD_{620}}{OD_{280}} \quad (3.5)$$

$$Purity_{APC} = \frac{OD_{652}}{OD_{280}} \quad (3.6)$$

Where, $OD_{280}$, $OD_{620}$, and $OD_{652}$ are the optical density wavelength at 280 nm, 625 nm and 652 nm, respectively.

### 3.3.5 STATISTICAL ANALYSIS

All cultures were performed in duplicate and average values were reported. The values were then expressed in terms of mean and standard deviation. Subsequently, the data were subjected to a one-way analysis of variance (ANOVA) using Microsoft Excel software to assess any significant differences between the groups with a p-value of less than or equal to 0.05.

## 3.4 RESULTS AND DISCUSSIONS

### 3.4.1 EFFECTS OF CULTURE MEDIUM ON *SPIRULINA* CULTIVATION

The cultivation of *Spirulina platensis* microalgae in modified BG-11 and Zarrouk medium has exhibited exponential biomass productivity of 0.1896 ± 0.0021 and 0.1964 ± 0.0020 g/L/day, respectively. Initially, the preliminary cultivation of *Spirulina* microalgae cultivated in BG-11 (without $NaHCO_3$) was unable to reach the stationary growth phase which may be due to the pH condition of the culture medium within the range of pH 7 to 8, which is not suitable for the growth of *Spirulina* (Shi et al., 2016). Therefore, the cultivation of *Spirulina* microalgae in BG-11 medium was modified by adding 18 g of sodium bicarbonate ($NaHCO_3$) to increase the pH of the culture medium within the range of pH 9 to 10. Besides, the biomass concentration of modified BG-11 and modified Zarrouk medium were found to be 2.920 ± 0.001 g/L and 2.908 ±

0.001 g/L, respectively as shown in **Figure 3.2**. The biomass concentration of *Spirulina platensis* in the modified BG-11 medium is comparable with the modified Zarrouk medium. However, Dineshkumar, Narendran and Sampathkumar (2016a) reported that the *Spirulina platensis* was able to cultivate in BG-11 medium with a lower biomass concentration of 1.45 g/L as compared to Zarrouk medium (1.86 g/L). *Spirulina* can be cultivated in BG-11 medium by adjusting its pH to a range of 8.8-9.0 as demonstrated by Dineshkumar et al. (2016). Similarly, the addition of 18 g of $NaHCO_3$ increases the pH of BG-11 medium between 9-10 and provides a carbon source, thereby achieve a biomass concentration close to Zarrouk medium. Moreover, the modified BG-11 medium has also shown a higher accumulation of CPC (141.1 mg/g) and APC (61.49 mg/g) as compared to the Zarrouk medium (CPC: 61.35 mg/g; APC: 44.63 mg/g). It is also worth to note that the amount of chemicals used for modified BG-11 medium is lesser compared to Zarrouk medium. Therefore, modified BG-11 medium is selected as the medium in this research to cultivate *Spirulina platensis*.

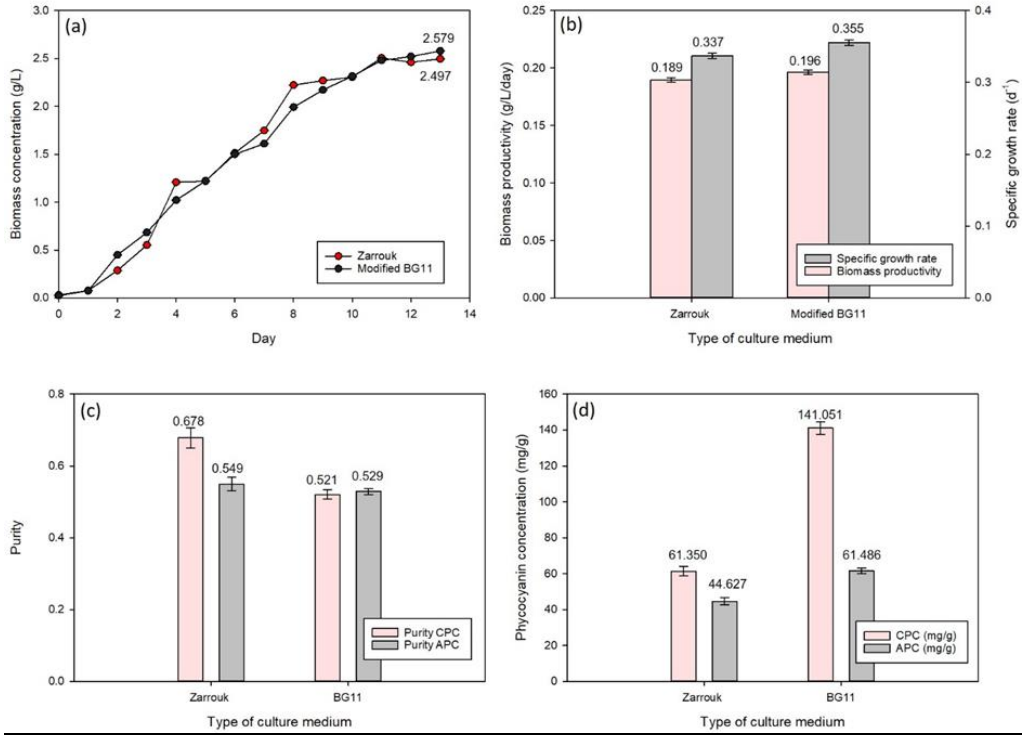



**Figure 3.2** (a) Biomass concentration, (b) biomass productivity and specific growth rate of *Spirulina* microalgae in Zarrouk and modified BG-11 culture medium, (c) Effects of culture medium on phycocyanin purity and (d) Effects of culture medium on phycocyanin concentration

### 3.4.2 EFFECTS OF AERATED AIR, CARBON DIOXIDE AND A MIXTURE OF AIR/CARBON DIOXIDE ON *SPIRULINA* CULTIVATION

The parameter that alters the type of aeration (i.e. aerated air, carbon dioxide and a mixture of aerated air/carbon dioxide (ratio 1:1 volume)) was conducted to determine the aeration effects on the growth of *Spirulina* microalgae. Aerated air was supplied by an air compressor while carbon dioxide was supplied by a $CO_2$ tank. While the mixture of aerated air/carbon dioxide was regulated by an air flowmeter and mix evenly with a ratio 1:1 volume. As shown in **Figure 3.3,**

the biomass concentration of aerated air, carbon dioxide and a mixture of aerated air/carbon dioxide on the 9$^{th}$ day is 1.624 ± 0.001, 0.0500 ± 0.001 and 0.1100 ± 0.001 g/L, respectively. However, the *Spirulina* microalgae do not exhibit any biomass concentration when subjected to carbon dioxide and a mixture of aerated air and carbon dioxide. This could be possibly due to an alteration of pH condition toward slightly acidic in the culture medium caused by the excessive carbon dioxide supply. Several studies have also shown that the increase in $CO_2$ ratio exceeding 15% will inhibit the growth of microalgae (Tan et al., 2015; Chiu et al., 2008). Based on literature studies, when the concentration of $CO_2$ increases to a certain level, the pH value in the cytoplasm region will decrease which eventually reduces the carbonic anhydrase activity within the organelles of microalgae and inhibit their growth ability (Miyachi, Iwasaki and Shiraiwa, 2003). To the best of the author's knowledge, *Spirulina* is an alkaliphile microalga that favours towards pH of 9.5 to 11 throughout its cultivation condition (Lim et al., 2021). On the other hand, Chen et al. (2016) have reported that an optimal value of carbon dioxide (6.5%) would be beneficial for the growth of *Spirulina* microalgae to achieve a biomass productivity rate of 0.17 g/L/day, along with 5-fold higher $CO_2$ removal efficiency. However, it is also suggested to monitor the pH throughout the cultivation process by using a $CO_2$ feeding control to facilitate the aeration process. As good as it sounds, several considerations in terms of economic cost and sustainability involving the usage of chemicals and advanced equipment are needed to ensure such a system is practicable in the microalgae biorefinery process. As a result, aerated air was selected for subsequent parameters due to its optimal biomass concentration of *Spirulina* microalgae cultivated in a modified BG-11 medium.

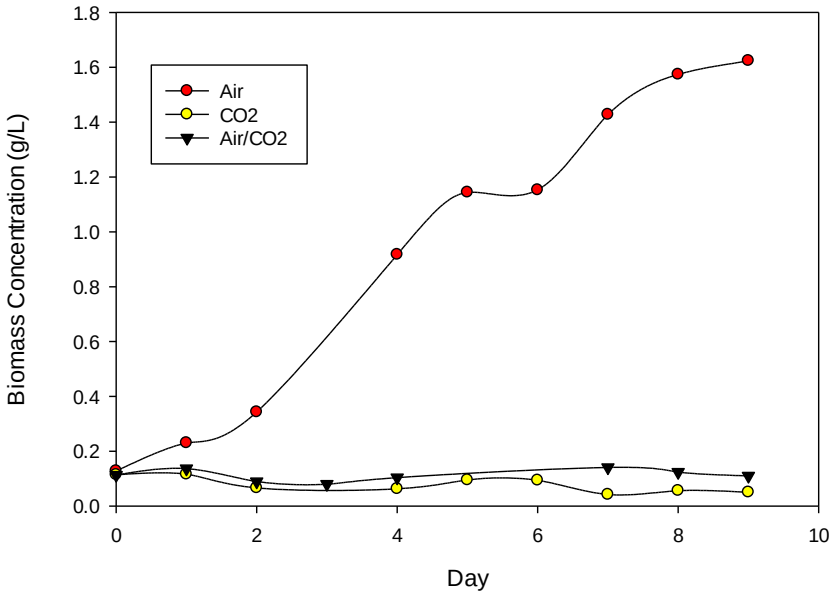



**Figure 3.3** Effects of various aeration types on the *Spirulina* cultivation in modified BG-11 medium

### 3.4.3 EFFECTS OF THE NUMBER OF CYCLES ON THE RECYCLED MEDIUM OF *SPIRULINA* CULTIVATION

The 1st cultivation cycle of *Spirulina* microalgae is cultivated in fresh modified BG-11 medium for 10 days to achieve a biomass concentration of 2.273 ± 0.001 g/L. *Spirulina* biomass was then harvested from the culture medium through centrifugation. A total of 855 mL of supernatant (recycle medium) was recovered, while 145 mL of water could have been lost due to evaporation and sample analysis. The recovered supernatant was recycled to prepare the culture medium for the subsequent cultivation of *Spirulina* microalgae. The recycled medium was added with fresh deionised water, fresh modified BG-11 chemicals and inoculated with fresh *Spirulina* for each consecutive batch cultivation. Similarly, the 2nd cultivation cycle was cultivated for the next 10 days achieving

a biomass concentration of 1.445 ± 0.003 g/L. A total of 825 mL of supernatant was recovered, while 175 mL of water could have been lost due to evaporation and sample analysis. The recycle medium was recycled again following the aforementioned steps for the 3$^{rd}$ cultivation cycle. The 3$^{rd}$ cultivation cycle was cultivated for 10 days while achieving the highest biomass concentration of 1.696 ± 0.001 g/L on Day 6. The biomass concentration and its cultivation cycle are shown in **Figure 3.4(a)**. **Figure 3.4(b)** shows both biomass productivity and specific growth rate decreased from 0.2246 ± 0.0033 to 0.0802 ± 0.0020 g/L/d and 0.4430 ± 0.0064 to 0.1337 ± 0.0001 $d^{-1}$, respectively with increasing cultivation cycle. On the other hand, it can be seen in **Figure 3.4(c)** that the purity of both CPC and APC decreases from 0.6349 ± 0.0119 to 0.4735 ± 0.0078 and 0.7170 ± 0.0064 to 0.5039 ± 0.0077, respectively as the number of cultivation cycle increases. Furthermore, the concentration of APC decreases from 89.961 ± 1.686 to 50.875 ± 0.838 mg/g as the number of cultivation cycles increases as shown in **Figure 3.4(d).** The concentration of CPC varies between the concentration range of 140.412 ± 2.632 and 153.427 ± 3.150 mg/g, as the number of cultivation cycles increases. Although the CPC concentration remains the same, the biomass concentration had been decreased as the number of cultivation cycles increased. $NaHCO_3$ in the amount of 18 g/L was added at each repetition, pH and salinity increased enormously, which led to a decrease in biomass concentration and productivity as shown in **Figure 3.4(b)** (Al Mahrouqi et al., 2015). Salinity is defined as the amount of dissolved salt in a body of water. It is worth noting that the 3$^{rd}$ cultivation cycle started at a higher initial biomass concentration. This is caused by high salinity, when the salinity of the growth medium increases, the density of the medium also increases. This causes the

density of the microalgae cells to become closer to the density of the growth medium, making it more difficult to separate them by centrifugation. Therefore, some of the *Spirulina* microalgae from previous batch cultivation remains and the 3rd cultivation cycle started at a higher initial biomass concentration. Yu et al. (2019) reported a similar trend and that the recycled medium without any pre-treatments inhibited the photosynthesis of *Spirulina platensis* due to algicidal products from biological pollutants in the culture medium resulting in lower biomass concentration (Yu et al., 2019). Although it has demonstrated that it is possible to recycle medium by adding fresh chemicals, this is not a long-term sustainable solution. The biomass concentration will decrease as the number of cultivation cycles increases due to pH, salinity or biological pollutants. Therefore, this research has further investigated different pre-treatment methods such as MF, UV and combined treatment (MF-UV) on recycle medium in the following section. Statistical ANOVA analysis shows that the number of cultivation cycles has a significant effect ($p < 0.05$) on biomass concentration, biomass productivity, specific growth, purity and concentration of CPC and APC.

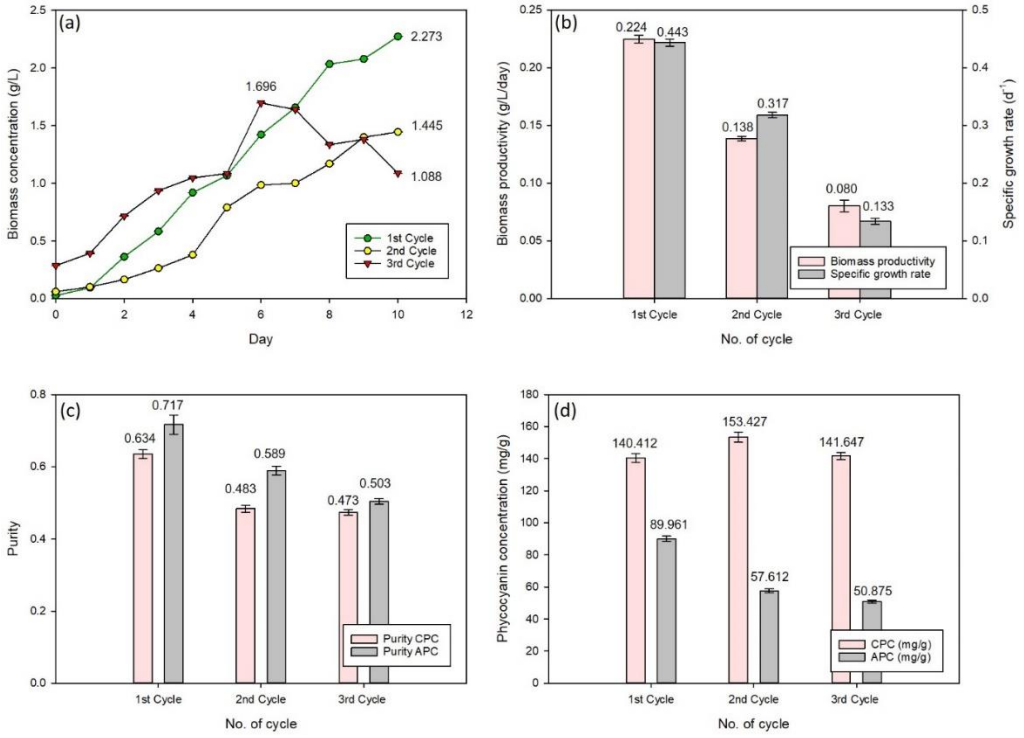



**Figure 3.4** (a) Biomass concentration, (b) biomass productivity and specific growth rate of *Spirulina* cultivation in different cycles of recycle culture medium, (c) effect of recycling medium on the purity of phycocyanin and (d) effect recycling medium on phycocyanin concentration

### 3.4.4 EFFECTS OF PRE-TREATMENT METHOD ON RECYCLE MEDIUM OF *SPIRULINA* CULTIVATION

Recycle medium was recovered from the 1st cycle of *Spirulina* cultivation and was subjected to three different types of pre-treatment methods: MF only, UV only and combined treatment (MF-UV). Three different types of pre-treatment methods were compared to a control without any pre-treatment methods. Microfiltration was used to remove contaminants of microalgae residual from

the culture medium, while UV light was used to disinfect bacteria or microorganisms present in the culture medium (Yu et al., 2019). As seen in **Figure 3.5**, the recycled medium which was subject to combined treatment (MF-UV), the recycled medium is much clearer as compared to the others. Therefore, microalgae industries can implement technologies such as microfiltration and UV-light to pre-treat recycle medium from their post-cultivation and recycle medium for subsequent cultivation again.

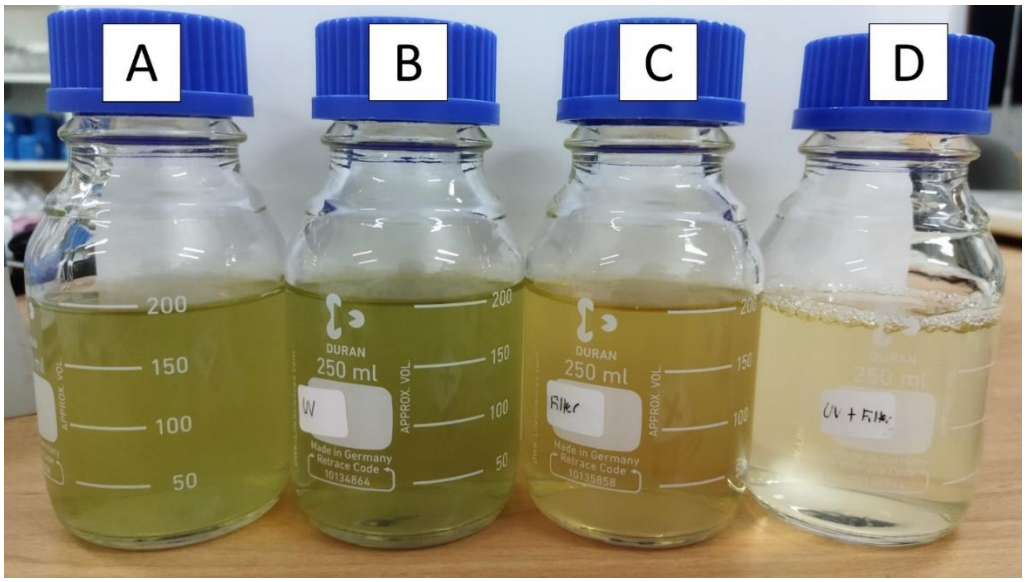



**Figure 3.5** Different pre-treatment methods on recycled medium (A) without pre-treatment (B) UV-light treatment (C) microfiltration treatment (D) combined treatment (MF-UV)

Recycle medium with pre-treatment methods have exhibited higher biomass concentration and productivity as compared to the control shown in **Figure 3.6(a)** and **Figure 3.6(b)**. The pre-treatment methods of control, MF, UV and combined treatment (MF-UV) yield a biomass concentration of 2.245 ± 0.041, 2.489 ± 0.022, 2.691 ± 0.003 and 3.121 ± 0.140 g/L, respectively**.** Whereas the

biomass productivity of control, MF, UV and combined treatment (MF-UV) are 0.1398 ± 0.0077, 0.1540 ± 0.0052, 0.1665 ± 0.0011 and 0.1861 ± 0.0018 g/L/day, respectively. Microfiltration is used to effectively remove impurities, such as bacteria or dead *Spirulina* cells, from the medium, which can hinder the growth of the *Spirulina* culture. Additionally, UV-light treatment is employed to eliminate any remaining harmful microorganisms that may not have been filtered out during the culture medium purification process. Removing these impurities and harmful microorganism, increase the availability of nutrients, reduce competition for resources and reduce the risk of contamination, which can lead to an increase in biomass productivity. Thus, combined treatment (MF-UV) is shown to have the highest biomass concentration and productivity.

**Figure 3.6(d)** showed that control achieved the highest CPC concentration (160.8 ± 4.715 mg/g) as compared to MF (141.1 ± 3.468 mg/g), UV (122.5 ± 0.2968 mg/g) and combined treatment (MF-UV) (132.1 ± 1.088 mg/g). Despite the fact that the recycled medium (with pre-treatment methods) has a lower phycocyanin concentration compared to the control, the biomass concentration obtained was higher compared to the control. This depends on the final product, whether is phycocyanin or biomass concentration favoured. Selection of the appropriate pre-treatment methods will depend on the end goal of the cultivation process. These findings are of great significance as they enable the microalgae industries to decrease nutrient waste and water consumption in *Spirulina* cultivation by recycling medium. Future research can be done in optimizing the pre-treatment methods to improve phycocyanin concentration in recycle medium. Additionally, exploring the effects of different nutrient compositions in the recycled medium on both biomass concentration and phycocyanin

accumulation. Statistical ANOVA analysis shows that the pre-treatment methods on recycle medium have shown a significant effect ($p < 0.05$) on biomass concentration, biomass productivity and phycocyanin concentration of *Spirulina* cultivation but it does not have a significant effect ($p > 0.05$) on the purity of phycocyanin shown in **Figure 3.6(c)**.

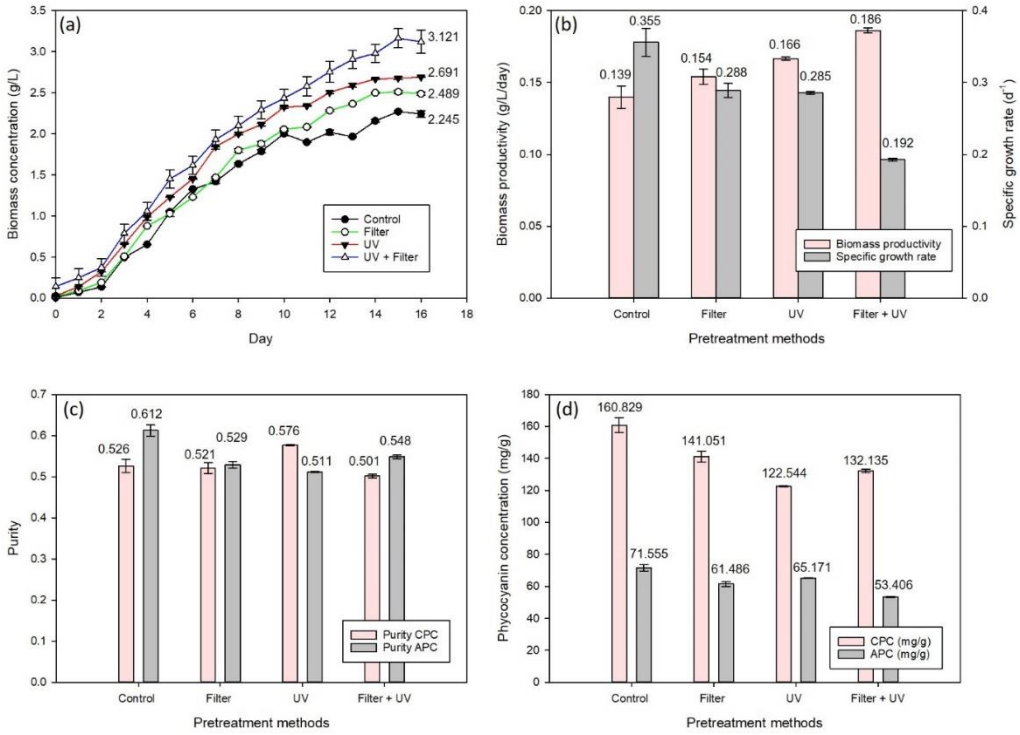



**Figure 3.6** (a) Biomass concentration, (b) biomass productivity and specific growth rate of *Spirulina* cultivation with different pre-treatment methods in recycled medium, (c) effect of pre-treatment methods on the purity of phycocyanin in recycled medium and (d) effect of pre-treatment methods on phycocyanin concentration in the recycled medium

### 3.4.5 EFFECTS OF PRE-TREATMENT METHOD ON RAINWATER FOR *SPIRULINA* CULTIVATION

Studies have reported that the quality of the rainwater in Malaysia is categorised as class II (Nizam et al., 2021; Frichot, Rubiyatno and Talukdar, 2021). While the rainwater resource was deemed suitable for external and domestic use, it necessitated additional treatment before being deemed safe for drinking purposes. The total coliform bacteria levels were higher prior to treatment and decreased to a safe level according to the National Water Quality Standard for Malaysia (DOE, 2020). Therefore, this study evaluates the cultivation of *Spirulina* microalgae using alternative rainwater resources with different pre-treatment including MF, UV and combined treatment (MF-UV). The pre-treatment methods of control, MF, UV-light and combined treatment (MF-UV) achieved biomass concentrations of 3.355 ± 0.109, 3.506 ± 0.013, 3.217 ± 0.024 and 3.981 ± 0.028 g/L, respectively as shown in **Figure 3.7(a)**. The biomass productivity of control, MF, UV and combined treatment (MF-UV) were 0.3211 ± 0.0160, 0.3359 ± 0.0185, 0.3070 ± 0.0477 and 0.3837 ± 0.0201 g/L/day, respectively shown in **Figure 3.7(b)**. These results were compared against *Spirulina* cultivated in a rainwater medium (without pre-treatment) as the control. Combined treatment (MF-UV) shown to have both high biomass concentration and biomass productivity. **Figure 3.7(d)** showed that MF pre-treatment has the highest CPC concentration (130.2 ± 2.348 mg/g) as compared to control (83.04 ± 3.643 mg/g), UV (88.78 ± 1.177 mg/g) and combined treatment (MF-UV) (105.5 ± 1.629 mg/g).

The overall results showed that UV-light treatment resulted in lower biomass concentration and phycocyanin accumulation as compared to other pretreatment

methods. A study reported that UV-light treatment is not sufficient to eliminate indigenous bacteria and protozoa in municipal wastewater resulting in low *Chlorella* biomass concentration (0.5 g/L) as compared to microfiltration treatment (0.67 g/L) (Cho et al., 2011). On the other hand, study have reported the presence of UV-tolerant environmental bacteria in rainwater which is collected from roofs resulting UV-light treatment only is insufficient to treat rainwater (Kim et al., 2016). MF treatment can remove viruses and bacteria from rainwater (Alkhudhiri, 2021). MF treatment has shown higher biomass productivity as compared to UV treatment and exhibits the highest phycocyanin accumulation of 130.2 ± 2.348 mg/g. Whereas, combined treatment (MF-UV) treatment has shown the highest biomass productivity (0.3837 ± 0.0201 g/L/day) as compared to other treatments. Based on these findings, the selection of appropriate pre-treatment conditions will be highly dependent on the requirement of the final product (i.e. high phycocyanin accumulation or high biomass concentration) and subjected to optimization during the cultivation process. The results of this study have shown significant implications for the microalgae industry whereby the use of rainwater by installing rainwater harvesting system can be a viable alternative water resource for the cultivation of *Spirulina* microalgae. Nonetheless, a limitation of this study is that such system is only effective in tropical countries such as Malaysia where there are rainy seasons. Statistical ANOVA analysis shows that the pre-treatment methods on recycle medium have shown a significant effect ($p < 0.05$) on biomass concentration, biomass productivity and phycocyanin concentration of *Spirulina* cultivation but it does not have significant effect ($p > 0.05$) on the purity of phycocyanin shown in **Figure 3.7(c)**.

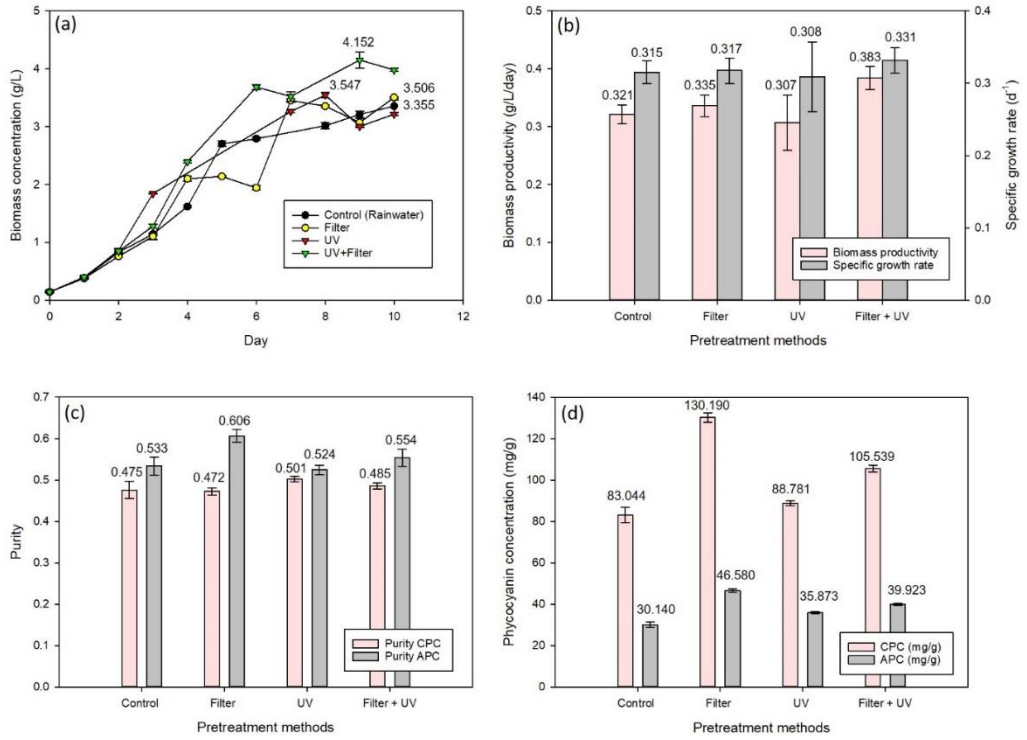



**Figure 3.7** (a) Biomass concentration, (b) biomass productivity and specific growth rate of *Spirulina* cultivation with different pre-treatment methods in rainwater medium, (c) effect of pre-treatment methods on the purity of phycocyanin in rainwater medium and (d) effect of pre-treatment methods on phycocyanin concentration in rainwater medium

### 3.4.6 ECONOMICAL COST ASSESSMENT ON DIFFERENT PRE-TREATMENT METHODS OF RECYCLED MEDIUM AND RAINWATER RESOURCE

Pre-treatment methods can lead to reduced water utility costs while maintaining biomass productivity and biomass quality. **Table 3.3** presented an estimated cost of pre-treatment methods for recycled medium and alternative rainwater resources based on a small-scale volume of 1 L. The results showed that while UV-light pre-treatment is less expensive than microfiltration, it is inadequate for

achieving a satisfactory biomass concentration. On the other hand, microfiltration treatment alone is sufficient, but the combined treatment (MF-UV) is recommended as this approach can effectively eliminate most bacteria and viruses, thereby enhancing the safety and quality of the culture medium. Further research should be studied on the cost-effectiveness analysis of these pre-treatments in large-scale *Spirulina* cultivation.

**Table 3.3** Estimated pre-treatment cost per litre of recycled medium and rainwater resource

| **Type of pre-treatments** | **Estimated cost** |
|---|---|
| **MF** | Cost of FiltraTech Qualitative Filter Paper (100 pieces), pore size 5-13 µm: RM 21.00<br>RM 21.00 ÷ 100 = RM 0.21 per litre |
| **UV-light** | Power: 11 W, Treatment time: 10 min<br>$0.011\ kW \times \frac{1}{6}\ h \times RM\ 0.40\ per\ kWh$<br>$= RM\ 0.00073\ per\ litre$ |
| **Combined treatment (MF-UV)** | Total cost: RM 0.21 + RM 0.00073 = RM 0.21073 per litre |

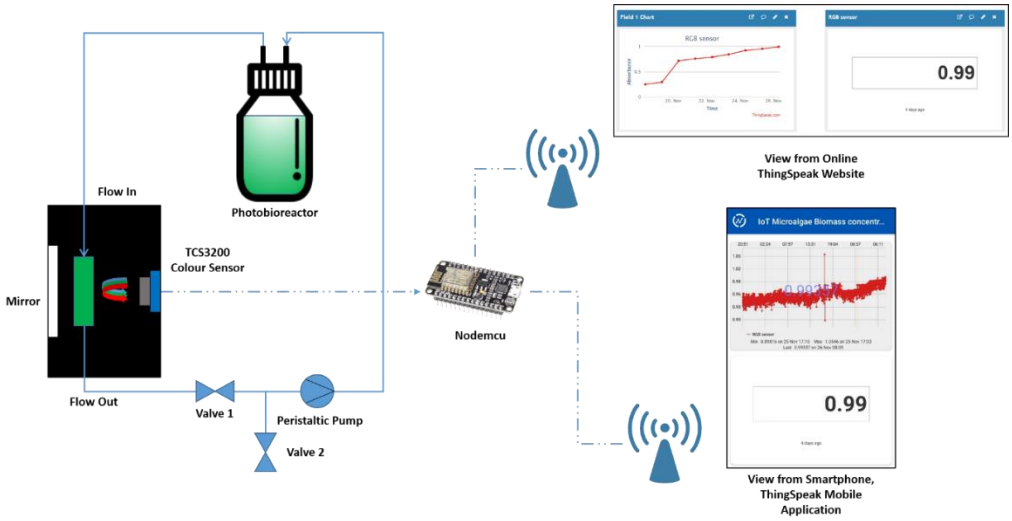
*Cost of electricity in Malaysia: RM 0.40 per kWh (Foong et al., 2023)

## 3.5 CONCLUSION

This research work investigated the growth of *Spirulina* by recycling medium obtained from post-cultivation and substituting rainwater resources for the preparation of the culture medium. Modified BG-11 medium was selected as the medium in this research to cultivate *Spirulina platensis*. Furthermore, *Spirulina* grows well with the supply of air only. Also, pre-treatment methods of microfiltration and UV-light have been shown to improve the biomass and phycocyanin productivity of *Spirulina*. These findings are important for the microalgae industries by providing important data and information in terms of recycling their spent medium after post-cultivation. Moreover, this research also would like to propose microalgae industries to utilize rainwater resource by installing rainwater harvesting systems for subsequent microalgae cultivation. This would greatly reduce the water utility cost in the microalgae process.

## CHAPTER 4

## EVALUATION OF REAL-TIME MONITORING ON THE GROWTH OF *SPIRULINA* MICROALGAE: INTERNET-OF-THINGS AND MICROALGAE TECHNOLOGIES




This chapter consists of thesis-version of work published in the IEEE Internet of Things Journal.

Lim, H.R., Khoo, K.S., Chew, K.W., Teo, M.Y.M., Ling, T.C., Alharthi, S., Alsanie, W.F. and Show, P.L., 2023. Evaluation of Real-Time Monitoring on the Growth of *Spirulina* Microalgae: Internet-of-Things and Microalgae Technologies. *IEEE Internet of Things Journal*.

## 4.1 ABSTRACT

Microalgae farming is still in its infancy phase to implement internet-of-things (IoT) technologies. *Spirulina* microalgae was selected to be investigated in this study because it is the most commercially viable microalgae for large-scale production. There are various methods to measure the biomass concentration of microalgae. However, the conventional methods are time-consuming, laborious, or expensive. Therefore, this research aims to integrate IoT with a biomass concentration sensor (TCS3200), to monitor the biomass concentration of *Spirulina* in real-time and remotely through the cloud-based system (ThingSpeak). The TCS3200 sensor was programmed and set up to measure the absorbance of the *Spirulina* culture in a 10-L cultivation tank. The data was collected and stored in the cloud Thingspeak platform. Besides, additional parameters such as temperature, light intensity, and water level were monitored to gain a comprehensive understanding of the entire cultivation process. These sensors were all controlled by a Lolin NodeMCU ESP8266 microcontroller. The TCS3200 sensor was found to be better suited for measuring low biomass concentrations, while a UV-Vis spectrophotometer was able to measure higher biomass concentrations after the sample was diluted. Real-time monitoring of these parameters allows for timely adjustments to be made to the cultivation conditions to optimize the growth and productivity of the *Spirulina* culture and detect any problems or abnormalities in the process. The use of IoT technologies in the cultivation of microalgae can improve the efficiency and sustainability of the process and provide valuable data for further research and development.

*Keywords:* Internet of Things; *Spirulina*; microalgae; sensors, IR 4.0

## 4.2 INTRODUCTION

In response to the Fourth Industrial Revolution (IR 4.0), the future of agriculture is expected to evolve into high-tech industries where most of the systems are coupled with artificial intelligence and big data (Hussmann, 2018). Many agriculture farmers have begun implementing IR 4.0 technologies such as internet-of-things (IoT) and WSN (Wireless Sensor Network) which can help to save freshwater resources (Lim et al., 2022; Akhter and Sofi, 2022). In fact, the potential of IoT has provided a monitoring platform (i.e., storage and transportation of food) for farms to achieve maximum yield of crop production and helps to prevent any possible crop diseases. (Wietzke et al., 2018; Kim, Lee and Shin, 2018). However, microalgae farming is still in its infancy phase to implement such high technologies. This is primarily due to the complexity of the microalgae cultivation process, which includes factors such as temperature, light, pH, and nutrient levels, among others. The dynamic nature of these factors makes it challenging to integrate IoT technologies into the cultivation process. For example, monitoring and controlling temperature, light, and pH levels in real-time, automating the delivery of nutrients, and tracking the growth of microalgae in large-scale cultivation systems, are some of the scenarios that are currently being researched to implement IoT technologies in microalgae farming.

Precise quantification of microalgae biomass concentration is a crucial aspect of the upstream processing stage in microalgae farming, which is considered the most important step in the process. There are different methods to measure the biomass concentration of microalgae such as cell counting (counting chamber or haemocytometer), spectrophotometer, weighting, and cell density (flow cytometry or particle counter) each with its own set of advantages and

limitations (Di Caprio, 2020; Chew et al., 2019). Cell counting, while considered the most accurate method, is also labour-intensive and susceptible to errors. Light absorbance, while simple and rapid, may not always provide accurate results. Flow cytometry and particle counting, while accurate, are also costly and can only be used for single-celled microalgae. Determination by dry weight and ash-free dry weight, while reliable, is also time-consuming. According to Lu et al. (2017), optical density is the preferred method for measuring microalgal biomass at low concentrations, while dry weight is more suitable for the determination of microalgal biomass at high cell concentrations.

The Internet of Things (IoT) technology has the potential to revolutionize the way we measure microalgae biomass concentration. This research work aims to integrate Internet-of-Things with the biomass concentration sensor. Studies have shown that the implementation of IoT can lead to significant benefits in microalgae farming, including reduced maintenance cost, decreased equipment downtime, reduced risks to safety, health, and the environment, and lower capital investment (Lim et al., 2022). Nguyen and Rittmann (2018) have shown to develop an infrared turbidity sensor connected to an Arduino ATmega microcontroller and auxiliary power relays. The system is adjustable and can provide key operational data in real time and also log it with time stamping. It is also relatively inexpensive, costing less than $250, and the code is available for public use. The turbidity sensor is reliable to measure a typical operating optical density between 0.5 and 4.5. Sandnes et al. (2006) also develop Near-infrared (NIR) light transmittance sensors that can be used to measure the density of a large-scale microalgal culture in real-time and to control the density of the culture by automatically injecting fresh culture medium. The system was also

programmed to operate in a semi-continuous production mode, which recorded diurnal growth patterns that provided information about the microalgal culture's response to environmental conditions. The system was also able to maintain the culture at an optimal population density and sustain high biomass production levels. These studies demonstrate the potential of IoT technology to monitor biomass concentration in real time, but they have yet to bring these data to the cloud. By integrating IoT monitoring technology, data can be monitored through the cloud from anywhere, providing greater flexibility and ease of access.

The objective and novelty of this research work are to design and implement a *Spirulina* cultivation system with embedded IoT monitoring technology through the cloud. To the best of the author's knowledge, there are no prior studies that have explored this specific application. A TCS3200 sensor was designed to measure and monitor microalgae biomass concentration for 24 h throughout the *Spirulina* cultivation process. The data can be monitored through an open-source cloud-based software known as ThingSpeak. ThingSpeak is an IoT analytics platform service that allows clients to aggregate, visualize and analyse live data streams in the cloud-based system. The TCS3200 sensor was tested in a 1-L *Spirulina* cultivation and later scale up and test run in a 10-L photobioreactor embedded with other IoT sensors such as temperature, water level and light intensity.

## 4.3 MATERIALS AND METHODS

### 4.3.1 MATERIALS

TCS3200 sensor, BH1750FVI digital ambient light sensor, water level sensor, Lolin NodeMCU ESP8266 V3, 6 V 1 A Power Supply AC/DC Adapter 100-240V for Arduino NodeMCU board, Male to Male Breadboard Dupont Jumper Wire – 30 cm, Female to Male Breadboard Dupont Jumper wire – 30 cm, Female to Female Breadboard Dupont Jumper wire – 30 cm; the above materials were purchased from SG Robot Technology Enterprise, Malaysia. DS18B20 digital thermometers were purchased from MRE Robotedu Enterprise, Malaysia. Peristaltic pump and 12 V 1 A Power Supply AC/DC Adapter 100-240V were purchased from Dynacom Electronics, Malaysia.

The materials used in this research were carefully selected to enable the monitoring and control of the water level, light intensity and temperature in a specific application. The TCS3200 sensor is a colour sensor that detects light in the visible spectrum, which is programmed to measure the absorbance of the *Spirulina* culture. The BH1750FVI digital ambient light sensor is used to measure the intensity of ambient light in Lux unit. The water level sensor is used to detect the level of water in the cultivation tank. The Lolin NodeMCU ESP8266 V3 is a microcontroller board based on the ESP8266 Wi-Fi module, used to control and monitor the system. The 6 V 1 A Power Supply AC/DC Adapter 100-240V for Arduino NodeMCU board is used to provide power to the microcontroller. The Male to Male, Female to Male, and Female to Female Breadboard Dupont Jumper Wires measuring 30 cm is used to connect the various components of the system. The DS18B20 digital thermometer is used to measure the temperature of the *Spirulina* culture in the cultivation tank. The

peristaltic pump is used to pump the *Spirulina* culture and the 12 V 1 A Power Supply AC/DC Adapter 100-240V is used to provide power to the peristaltic pump.

### 4.3.2 MICROALGAE STRAINS AND CULTIVATION MEDIUM

*Spirulina platensis* was source from Biolina Sdn. Bhd., Malaysia. The *Spirulina* strain was kept in a petri dish that was prepared with Zarrouk medium agar (Dineshkumar, Narendran and Sampathkumar, 2016a). The chemicals for the Zarrouk medium were prepared by mixing and dissolving it in deionised water. A 10-L cylindrical cultivation tank with a diameter of 120 mm and a height of 1500 mm was used to cultivate the microalgae. An air compressor (ACO-308, HAILEA, China) and a nano-airstone (VN-132, Yek Fong Aquarium Accessories Sdn Bhd, Malaysia) were placed inside the cultivation tank to supply the necessary aeration for the culture. Nano-airstone was used because Zhu and Wakisaka (2019) reported that nano-bubble water significantly increased the growth of two kinds of microalgae, *Haematococcus lacustris* and *Botryococcus braunii*, by up to 44% and 26%, respectively. Additionally, 4 tubes of LED light were installed surrounding the cultivation tank to provide an optimal light source (approximately 2000 lux) for the cultivation of the *Spirulina*.

### 4.3.3 TCS3200 SENSOR SETUPS

**Figure 4.1** illustrated the black box consists of a TCS3200 sensor, a cuvette and a mirror. It is set up in a black box to prevent external light from surrounding interfering with the TCS3200 sensor as it is sensitive to light. TCS3200 is composed of configurable silicon photodiodes and a current-to-frequency converter on a single monolithic CMOS integrated circuit. The light-to-

frequency converter reads an 8 x 8 array of photodiodes that consists equal distribution of blue filters, green filters, red filters and no filters. Furthermore, the TCS3200 has built-in LED lights (shown in **Figure 4.1**), and the light produced will pass through the cuvette which contains the continuously flowing sample and reflect by the mirror. The reflected light will be received by the photodiodes and converted into signals (0 or 1) which will be read by the Lolin NodeMCU ESP8266 V3 board. Then the data will be sent to the ThingSpeak server through the internet which can be viewed in real-time on the ThingSpeak website (https://thingspeak.com/channels/1997968) or mobile application as shown in **Figure 4.2**.

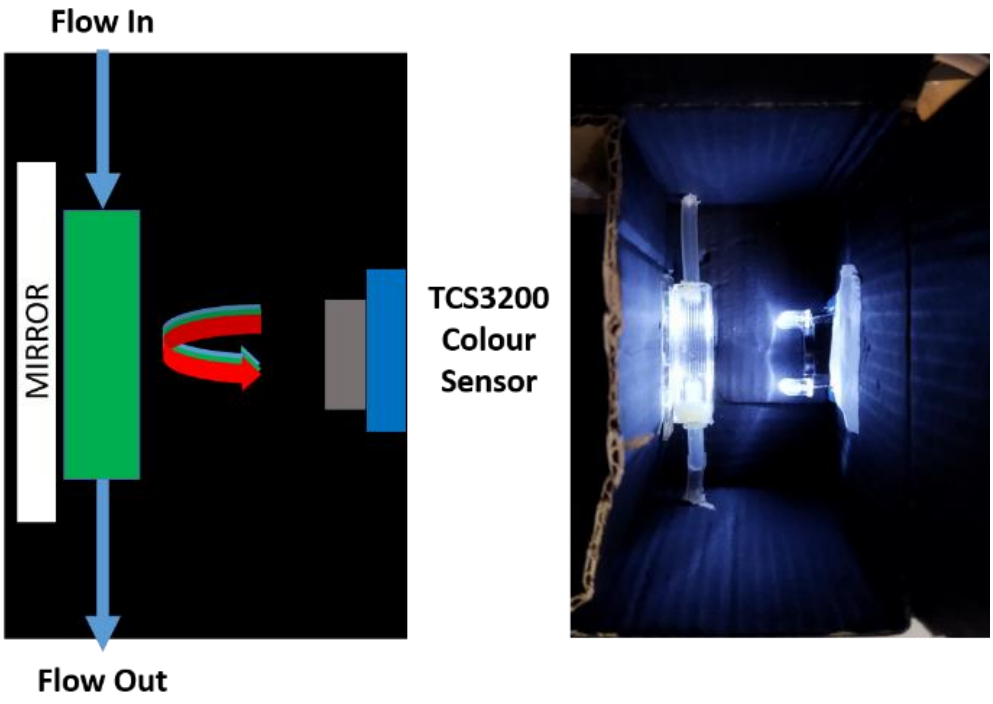



**Figure 4.1** TCS3200 sensor actual setup in the black box

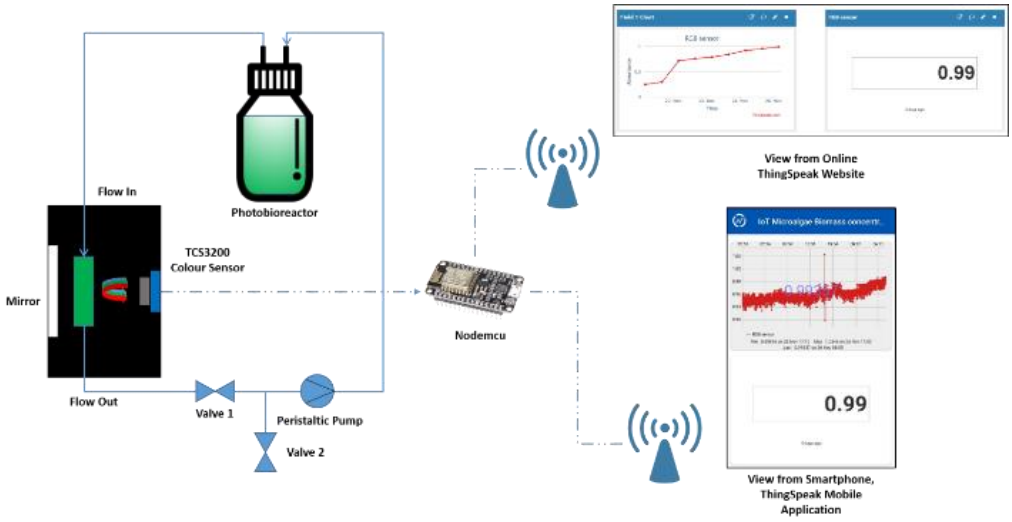



**Figure 4.2** TCS3200 sensor schematic setup

### 4.3.4 TCS3200 SENSOR PRINCIPLE AND CALIBRATION

TCS3200 consists of photodiodes that are blue, green and red filters. Therefore, the signal received is in red, green and blue composition. $L$ represents the relative luminance and it is calculated as a weighted sum of RGB colour component shown in (1) (Benavides et al., 2015).

$$L = 0.2126R + 0.7152G + 0.0722B \qquad (1)$$

The luminance measurement $L$ is linked to the intensity of light $I$ transmitted through the sample and reflected back to the sensor (Benavides et al., 2015). The calibration of TCS3200 was done by recording the relative luminance, $L$ in the blank (deionized water) and set the $I_0$ value. Then the absorbance (ABS) was calculated following the Beer-Lambert law as shown in (2) (Benavides et al., 2015).

$$ABS_{RGB} = -\ln\left(\frac{I}{I_o}\right) \qquad (2)$$

Where $I_0$ is the incident intensity and $I$ is the transmitted intensity.

### 4.3.5 TEMPERATURE SENSOR

The DS18B20, depicted in **Figure 4.3a**, functions as a digital thermometer capable of measuring temperatures in Celsius within the range of -55°C to +125°C (Nayyar and Puri, 2016). It exhibits a high level of accuracy, with deviations limited to ±0.5°C when operating between -10°C and +85°C. Notably, it possesses an alarm capability that allows users to define upper and lower trigger points, which are stored in a non-volatile manner.

In terms of communication, the DS18B20 employs a 1-Wire interface, which necessitates only one data line and a ground connection to establish communication with a central microprocessor. This configuration offers the added advantage of enabling direct power supply from the data line, thereby eliminating the requirement for an external power source. Furthermore, each DS18B20 possesses a unique 64-bit serial code, enabling the concurrent use of multiple DS18B20s on a single 1-Wire bus. This feature proves particularly advantageous in applications such as HVAC controls, temperature monitoring in buildings, equipment, or machinery, as well as process monitoring and control systems. Another noteworthy attribute of the DS18B20 is its programmable resolution, which can be adjusted within the range of 9 to 12 bits.

### 4.3.6 LIGHT SENSOR

BH1750FVI (shown in **Figure 4.3b**) is an ambient light sensor integrated circuit (IC) with an inter-integrated circuit (I2C) bus interface making it easier to connect with microcontroller (Beyaz and Gül, 2022). It has a wide detection range of light intensity with high resolution, capable of measuring light intensity from 1 to 65535 lx (Lijun and Yang, 2017). It operates between 2.4 V to 3.6 V

and works with 0.12 mA low current consumption. It is designed to measure ambient light levels and is often used to adjust the backlight power of LCD screens and keypads on mobile phones.

### 4.3.7 WATER LEVEL SENSOR

The water level sensor (shown in **Figure 4.3c**) measured the amount of water exposure using a series of exposed parallel wires. The sensor comprises ten exposed copper traces, with five designated as power traces and five as sense traces. These traces are arranged in an interleaved manner, such that each power trace is separated by one sense trace. Under normal conditions, the power and sense traces are not electrically connected. However, upon immersion in water, the traces become bridged and creates a variable resistor, wherein the resistance changes based on the extent of exposure to water. The resistance of the sensor varies inversely with the depth of immersion in water. As the sensor is immersed in a greater amount of water, its conductivity improves, resulting in a lower resistance. Conversely, when the sensor is exposed to less water, its conductivity diminishes, leading to a higher resistance. The sensor produces an output voltage that is directly proportional to the resistance. By measuring this voltage, it becomes possible to ascertain the water level. The sensor operates with a working voltage of 5 V DC and can be directly connected to microprocessors or microcontroller (Malche and Maheshwary, 2017). The water sensor module has a user-friendly design with a half-moon slip handle depression and a detection area of 40 mm x 16 mm. It is made using the FR4 double-sided HASL production process and has a mounting hole size of 3.0 mm. It can operate within a temperature range of 10% to 90% relative humidity, from 0°C to 30°C, without condensation. It weighs 3 g and has dimensions of 65 mm x 20 mm x 8 mm.

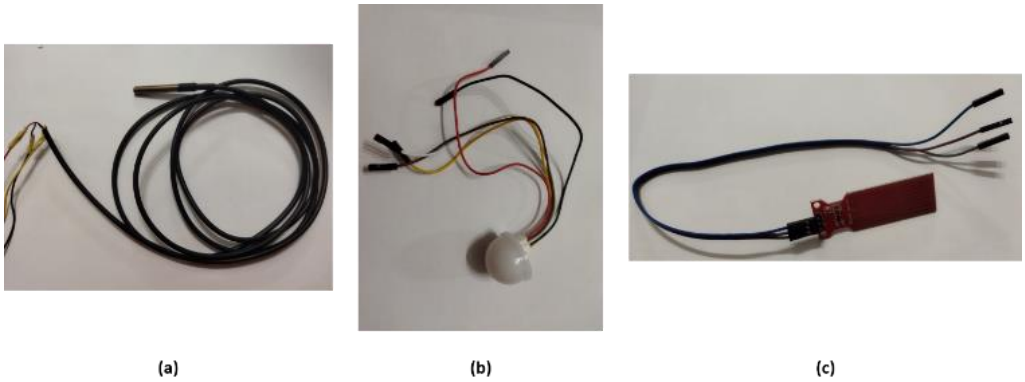



**Figure 4.3** Type of sensors. (a) DS18B20 digital thermometer, (b) BH1750FVI digital ambient light sensor IC, (c) Water level sensor

### 4.3.8 Growth and Absorbance Measurements

A preliminary experiment was conducted in 1-L Schott bottle. The growth curve typically reaches the late-log phase after 10 days, and the absorbance value is usually between 6 to 8. The cultivation of *Spirulina* in a 10-L cultivation tank was monitored for at least 10 days by collecting biomass samples every day. The absorbance of the *Spirulina* culture was measured with a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan) at 688 nm (Chen et al., 2016). However, the UV-Vis spectrophotometer has a limitation that can only read absorbance value up to 4. Therefore, all biomass samples collected from the 10-L cultivation tank were diluted 10 times and the average was taken to ensure consistency and accuracy.

## 4.4 RESULTS AND DISCUSSION

### 4.4.1 COMPARISON OF ABSORBANCE MEASUREMENT WITH UV-VIS SPECTROPHOTOMETER AND TCS3200 SENSOR

A TCS3200 sensor was programmed and set up to measure the absorbance of the *Spirulina* culture in a 10-L cultivation tank for 24 h from 16$^{th}$ to 28$^{th}$ December 2022. These data can be accessed and viewed through the website https://thingspeak.com/channels/1997968. **Figure 4.4a** shows a ThingSpeak field chart that provides an overview of real-time absorbance monitoring with the TCS3200 sensor. **Figure 4.4b** shows a ThingSpeak widget that can view the numeric display of the absorbance. Meanwhile, **Figure 4.4c** shows manual monitoring of absorbance with a UV-Vis Spectrophotometer. Samples were collected every day. It can be observed that the TCS3200 sensor can only measure up to an absorbance value of around 1.7, while the UV-Vis spectrophotometer can measure absorbance values up to around 3.0 and above. The UV-Vis spectrophotometer can measure higher absorbance because the samples collected were diluted with distilled water in the lab. However, the continuous flow of *Spirulina* culture passing through the TCS3200 sensor was not subjected to water dilution, which limits its ability to accurately measure high biomass concentrations. The TCS3200 sensor is therefore better suited for measuring low biomass concentrations.

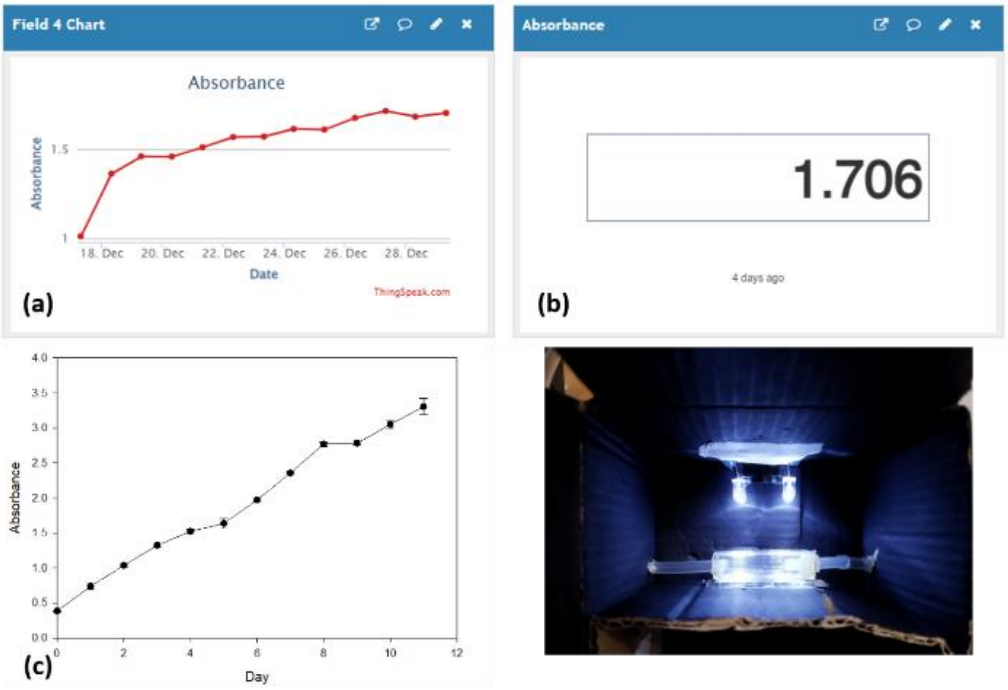



**Figure 4.4** Real-time and remote monitoring of absorbance in 10-L *Spirulina* cultivation tank (a) ThingSpeak field chart display of absorbance (b) ThingSpeak widget, numeric display of absorbance (c) Manual monitoring of absorbance with UV-Vis Spectrophotometer

Absorbance is a measure of the amount of light absorbed by a solution. It is commonly used to assess the concentration of biomass or biomolecules, such as proteins and pigments in a sample. Real-time monitoring of absorbance in a 10-L *Spirulina* cultivation tank is important because it provides insight into the growth condition of the *Spirulina* culture. It also allows for timely adjustments to be made to the cultivation conditions, such as the temperature, pH, and nutrient levels, to optimize the growth and productivity of the *Spirulina* culture. It can also help to detect any problems or abnormalities in the cultivation process, such as contamination or nutrient imbalances, and allow for timely intervention to prevent major setbacks.

*Spirulina* is a type of cyanobacterium that is widely cultivated for its high protein and pigment content, and it is used as a dietary supplement and natural colourant in a variety of products. Real-time monitoring of absorbance in a *Spirulina* cultivation tank is therefore important for ensuring the quality and consistency of these products. Overall, real-time monitoring of absorbance in a 10-L *Spirulina* cultivation tank is a valuable tool for understanding and controlling the growth and health of the culture, and it is essential for maximizing biomass productivity and minimizing risks in the cultivation process.

#### 4.4.1.1 PERISTALTIC PUMP

The peristaltic pump was used to transfer the *Spirulina* cultures from the 10-L cultivation tank to a measurement device (TCS3200 sensor). The *Spirulina* culture pass through a black box, where the TCS3200 sensor measures the absorbance of the cultures. However, peristaltic pumps have a limited lifespan and are not designed to run continuously for long periods. The pump uses a silicon tube and turning mechanism inside, which can cause friction on the tube and result in cracks and leaks after about 2 days of nonstop running. To extend the lifespan of a peristaltic pump, 2 peristaltic pumps were running on an alternating schedule, running each pump for 6 hours each and switching them. This approach can be effective in small-scale operations, but it is not practical for industrial-scale operations. In these cases, a more durable and reliable type of pump, such as a centrifugal pump, is typically used. Mitsuhashi et al. (1995) observed an oxygen production limitation due to shear stress, microscopic observations of *Spirulina* revealed that increases in shear stress cause it to be destroyed. It was seen that at 0.2 Pa of shear stress, the length decreased rapidly compared to when shear stress was at 0.5 Pa, where the length was nearly

constant at 50 gm (Mitsuhashi et al., 1995). Thus, a centrifugal pump can be designed well to handle transferring of *Spirulina* cultures and media. Centrifugal pumps are designed to handle large volumes of fluid and can run continuously for extended periods without experiencing wear and tear. As a result, they are a more suitable choice for industrial-scale microalgae operations.

### 4.4.2 TEMPERATURE SENSOR

A DS18B20 digital thermometer was installed in the 10-L *Spirulina* cultivation tank and monitored for 10 days, from 16$^{th}$ to 28$^{th}$ December 2022. These data can be accessed or viewed through this website https://thingspeak.com/channels/1997968. **Figure 4.5a** shows a ThingSpeak field chart which can only view 8000 data in a single chart because this is a free license version. **Figure 4.5b** shows a ThingSpeak widget that can view the numeric display of the temperature. To provide a comprehensive overview of the data, the data was extracted and plotted on a graph (**Figure 4.5c**), which allows for a more detailed view. The temperature fluctuated between 30-33 ℃, except on 20$^{th}$ December 2022, when the temperature reached a minimum of approximately 28 ℃. This deviation in temperature is likely due to the heavy rainfall that occurred on that day. As the research was conducted in a tropical country, such as Malaysia, that has seasonal changes, this deviation can be attributed to the weather conditions. Based on analysing **Figure 4.5c**, there is a trend where the temperature is the lowest in the morning around 0900 to 1200 and highest in the evening 1800 to 2100. However, there are LED lights installed around the 10-L cultivation tank, there is a tendency that some of the heat from the LED light could have contributed to the temperature of the cultivation tank.

Temperature is a crucial factor in the cultivation of *Spirulina*, as it can affect the growth and productivity of the culture. Although some microalgae strains can tolerate slightly higher or lower temperatures, the optimal temperatures for *Spirulina* cultivation typically range from 28-30°C (Danesi et al., 2011; Kumar, Kulshreshtha and Singh, 2011). By continuously monitoring the temperature in the cultivation tank using a digital thermometer, cultivators can ensure that the culture is maintained within this optimal range. This can help to optimize the growth of the *Spirulina* culture and improve its productivity and yield (Danesi et al., 2011). Real-time and remote monitoring of temperature can also facilitate more efficient and convenient cultivation of *Spirulina*. With the ability to access the temperature data remotely, cultivators do not have to be physically present at the cultivation site to check the temperature. Instead, they can monitor the temperature remotely using a computer or mobile device, which can save time and resources and allow cultivators to focus on other tasks.

In addition to real-time and remote monitoring of temperature, further research could be conducted by incorporating a control system into the IoT monitoring system (Dan et al., 2015). This control system could be used to automatically adjust the temperature of the cultivation tank to maintain it within the optimal range for *Spirulina* growth. The control system could be programmed to turn on or off the heating or cooling devices, such as heating pads or fans, to adjust the temperature as needed. This could help to further optimize the cultivation of *Spirulina* and improve its productivity and yield by ensuring that the culture is maintained at the optimal temperature at all times. Overall, incorporating a control system into the IoT monitoring system could provide an additional level

of control and automation in the cultivation of *Spirulina*, and may be a valuable area for further research and development.




**Figure 4.5** Real-time and remote monitoring of temperature in 10-L *Spirulina* cultivation tank (a) ThingSpeak field chart display of temperature (b) ThingSpeak widget, numeric display of temperature (c) An overview of temperature monitoring for 10 days

### 4.4.3 Light Intensity Sensor

A BH1750FVI digital ambient light sensor was used to monitor the light intensity on the surface of a 10-L *Spirulina* cultivation tank for a period of 10 days, from 16$^{th}$ to 28$^{th}$ December 2022. The light intensity data can be accessed on the open-source website https://thingspeak.com/channels/1997968. **Figure 4.6a** shows a ThingSpeak field chart with a limited capacity of 8000 data points due to the use of a free license. **Figure 4.6b** displays a ThingSpeak widget with

a numeric display of the light intensity. To get a better overall view of the data, the light intensity data were extracted and plotted on a graph, as shown in **Figure 4.6c**.

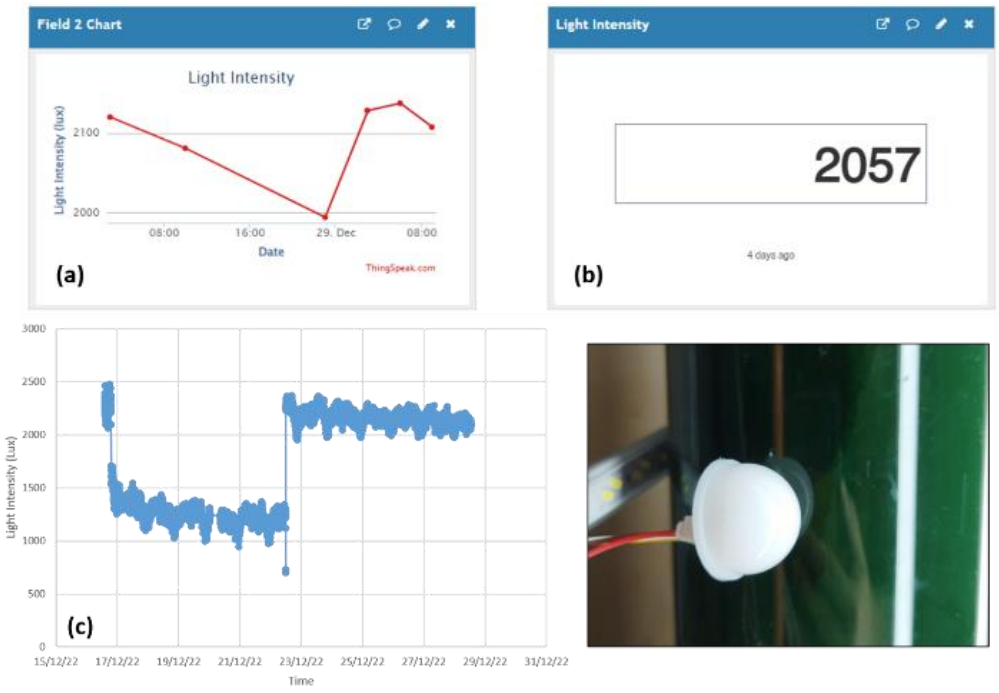



**Figure 4.6** Real-time and remote monitoring of light intensity in 10-L *Spirulina* cultivation tank (a) ThingSpeak field chart display of light intensity (b) ThingSpeak widget, numeric display of light intensity (c) An overview of light intensity monitoring for 10 days

Initially, only two LED lights were installed from 16$^{th}$ to 22$^{nd}$ December 2022, resulting in light intensity levels fluctuating between 1000-1500 lux. On 22$^{nd}$ December 2022, an additional two LED lights were installed with a total number of four LED lights. This resulted in an increase in light intensity to a range fluctuating between 2000-2500 lux. The cultivation tank was located indoors to

reduce external factors such as the sun that may affect the light sensor measurement.

*Spirulina* is a photosynthetic organism, that can convert light energy into chemical energy. Therefore, light intensity is an important factor to provide the culture with sufficient light to optimize its growth and productivity. Kumar, Kulshreshtha and Singh (2011) reported the optimal light intensity for *Spirulina* growth rate, cell production with maximum accumulation of chlorphyll and phycobilliproteins was found to be at 2,000 lux light intensity. However, the carotenoid content was highest at 3,500 lux, suggesting that higher light intensities can increase carotenoid accumulation for photoprotection (Kumar, Kulshreshtha and Singh, 2011). Real-time monitoring the light intensity in the cultivation tank using a light sensor ensure that the culture is receiving an adequate amount of light. This can help to optimize the growth of the *Spirulina* culture and improve its productivity and yield depending on whether growth rate, chlorophyll or carotenoid is favour in the production.

### 4.4.4 WATER LEVEL SENSOR

A water level sensor was installed in the 10-L *Spirulina* cultivation tank and monitored for about 10 days, from 16$^{th}$ to 28$^{th}$ December 2022. **Figure 4.7a** shows a ThingSpeak field chart which can only view the latest 8000 data in a single chart of the water level. **Figure 4.7b** shows a ThingSpeak widget that can view the numeric display of the water level. To have an overview of all the data collected, the water level data were extracted and plotted on a graph which can have a better view of it (**Figure 4.7c**). Monitoring the water level in a *Spirulina* cultivation tank is important for several reasons, including tracking the water evaporation rate over time. Water evaporation is a natural process that occurs

when water molecules in the air are absorbed by the atmosphere, especially Malaysia is located in a tropical climate zone, which generally experiences high temperatures and humidity that can lead to a high evaporation rate, resulting in a decrease in the water level (Mohammad Harmay and Choi, 2022). By monitoring the water level in the *Spirulina* cultivation tank, cultivators can track the rate of water evaporation and adjust the water level as needed to ensure that it is maintained at an optimal level for the culture.

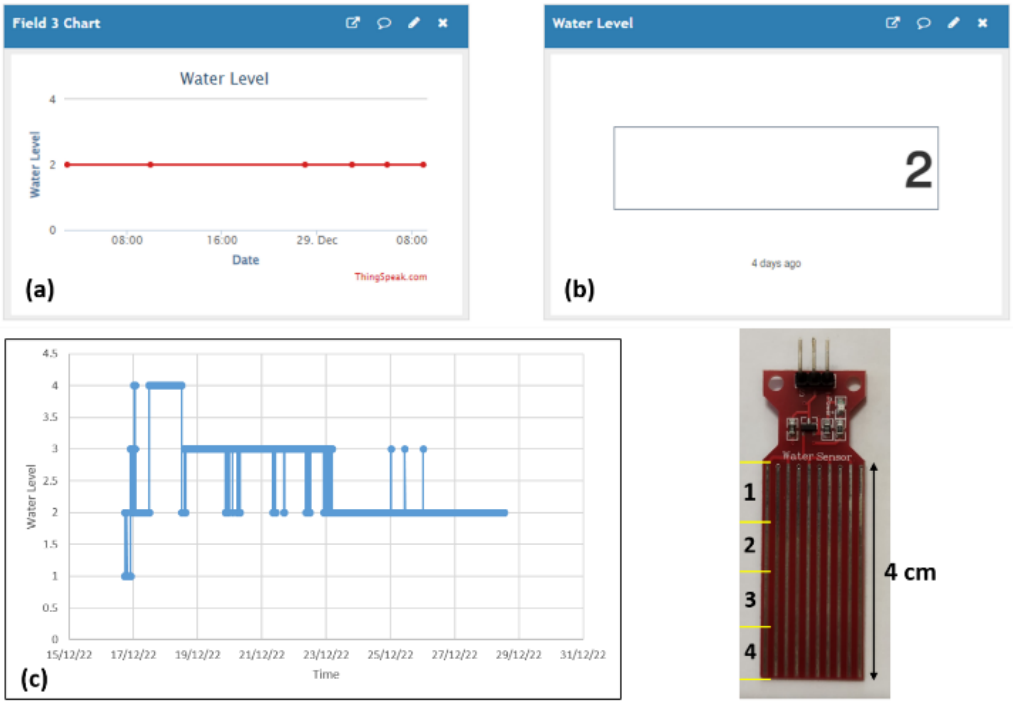



**Figure 4.7** Real-time and remote monitoring of water level in 10-L *Spirulina* cultivation tank (a) ThingSpeak field chart display of water level (b) ThingSpeak widget, numeric display of water level (c) An overview of water level monitoring for 10 days

The water level sensor is 4 cm long and is divided into 4 parts, each of which corresponds to a range of analogue values. The sensor is programmed to read

the analogue value that corresponds to the current water level, and this value is sent to a Nodemcu board. The board then uses this value to determine the water level and sends a corresponding data signal to the ThingSpeak platform. When the water in the container is at the maximum level, the water level sensor will send an analogue signal to the Nodemcu board. The board will then interpret this signal and determine that the water level is at "level 1". The board will then send a data signal, "1" to ThingSpeak to indicate this. As the water level in the cultivation tank decreases, the sensor will continue to send analogue signals to the Nodemcu board that correspond to the current water level. For example, when the water level drops to the next level, the sensor will send an analogue signal that corresponds to "level 2" and the board will send a data signal, "2" to the ThingSpeak platform. This process will continue until the water level reaches the minimum level, “level 4”.

It is important to note that analogue sensors tend to be less stable than digital sensors, which can impact the accuracy and reliability of their output (Jayanta, 2020). In this experiment, the water level sensor was immersed in the *Spirulina* culture for an extended period, causing some *Spirulina* to grow and attach to the sensor. This may have affected the measurement of the water level. Additionally, the aeration provided to the tank resulted in some bubbling that may have wet the water level sensor, potentially impacting its measurement as well. Despite these limitations, the water level sensor can still provide useful information to the operator about the amount of water that has evaporated and allow for timely action to be taken.

### 4.4.5 THINGSPEAK MOBILE APPLICATION

The ThingSpeak Mobile Application interface displayed in **Figure 4.8** allows for the real-time monitoring of various important factors in a 10-L *Spirulina* cultivation tank, including temperature, light intensity, water level, and absorbance, from any location around the world. This capability can be particularly valuable for microalgae industrial players who may have multiple cultivation tanks and need to monitor them remotely. Moreover, these sensors can also be applied in open pond microalgae farming. However, to ensure the measurement accuracy of the sensors, multiple sensors need to be placed around the open pond or ensure adequate flow circulation to evenly distribute microalgae biomass in the open pond.

The current study had demonstrated the capability of this mobile application interface in monitoring a single cultivation tank. However, microalgae industrial players can work with local IoT service providers to integrate this mobile application interface for the real-time monitoring of multiple tanks. This would allow for more efficient and effective management of the cultivation process, potentially leading to improved yields and profitability for the company. In the future, it may be possible to further improve upon this mobile application interface by adding more features or capabilities, such as the ability to remotely adjust cultivation conditions or receive alerts for any issues that may arise.

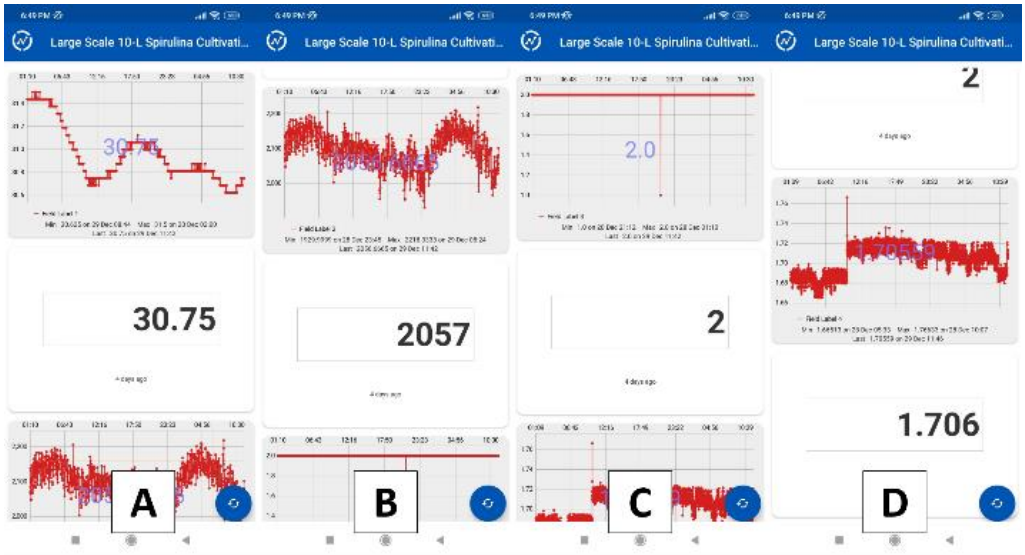



**Figure 4.8** ThingSpeak mobile application interface that can view (A) temperature, (B) light intensity, (C) water level and (D) absorbance

### 4.4.6 IoT Architecture

IoT architecture can be divided into 4 different type of layers which are perception layer, transport layer, processing layer and application layer shown in **Figure 4.9** (Malche and Maheshwary, 2017). The perception layer represents the sensors used in the current study, such as absorbance sensor, temperature sensor, light intensity sensor and water level sensor. The transport layer is where data is transmitted wirelessly from multiple devices to the cloud data center. This includes the use of wireless sensor network, 3G, 4G and the Internet (Malche and Maheshwary, 2017). Processing layer represents the ThingSpeak platform that helps in standardizing communication protocols, device discover and managing data flow between the microcontrollers and applications (ThingSpeak mobile application). The application layer can be represented by the ThingSpeak mobile application where it provides a user-friendly interface to

access and interpret the collected data. Users can visualize the data in tables or graphs, allowing them to gain insights and make informed decisions based on the information presented (Adam, 2022). The key difference between IoT architecture and traditional architecture lies in the transport layer. Unlike traditional architectures, IoT architecture enables wireless transmission of data from multiple devices to the cloud data center, eliminating the need for physical connections between devices and data storage centers.

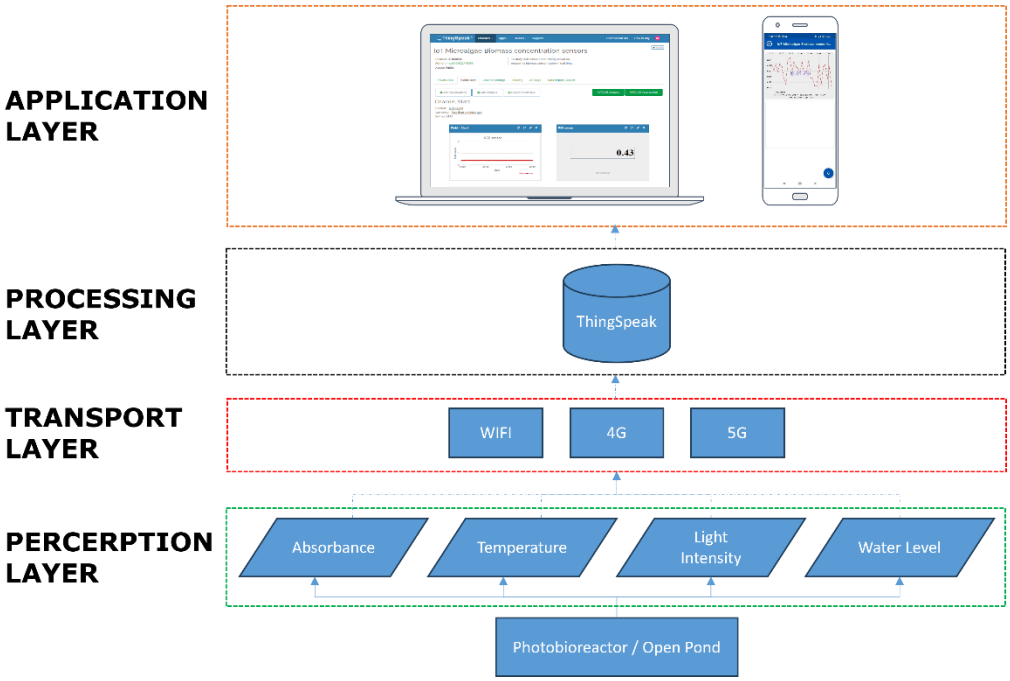



**Figure 4.9** The Internet-of-Things architecture within the cultivation system.

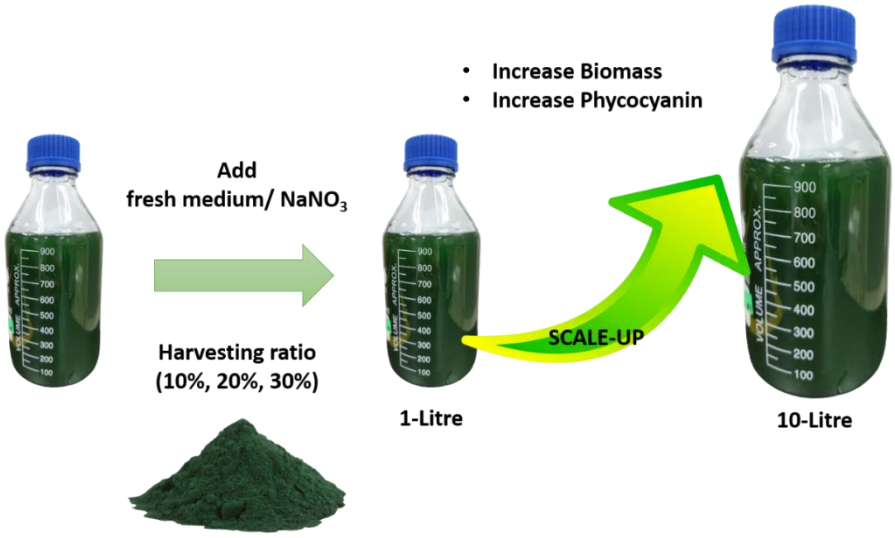
## 4.5 CONCLUSION

The research work has successfully implemented IoT monitoring technology in a *Spirulina* cultivation system. The study demonstrates the monitoring of *Spirulina* cultivation condition such as absorbance, temperature, light intensity and water level can be monitored through an open-source software known as ThingSpeak. Temperature sensor and light sensor were functioning accordingly,

however, absorbance (TCS3200) sensor and water level sensor have some limitation. TCS3200 sensor can only measure up to an absorbance value of around 1.7, while the UV-Vis spectrophotometer can measure absorbance values up to around 3.0 and above. On the other hand, the water level sensor was immersed in the *Spirulina* culture for an extended period, causing *Spirulina* to grow and attach onto the sensor. This may have affected the measurement of the water level. Moreover, the current study has also demonstrated the monitoring of *Spirulina* culture condition through the ThingSpeak mobile application interface. These findings are significant for the development of IoT-based approaches within the *Spirulina* microalgae industry. By implementing IoT technologies, microalgae industries can take advantage of Industry 4.0 and the benefits it offers, such as increased automation and data-driven decision-making.

# CHAPTER 5

# EFFECT OF NUTRIENTS-DEFICIENT AND HARVESTING RATIO STRATEGY FOR BIOMASS AND PHYCOCYANIN PRODUCTION FROM *SPIRULINA* MICROALGAE



## 5.1 ABSTRACT

Recent research has been focusing on issue related to contamination, nutrient availability and strain selection but there have been inefficient methods on harvesting research area. This study employed an integrated continuous cultivation and harvesting strategy for the biorefinery of *Spirulina* microalgae. The effect of nutrient-deficient, harvesting ratio and $NaNO_3$ addition on biomass concentration, biomass productivity and phycocyanin accumulation of *Spirulina* were investigated in this study. The lowest biomass productivity of 0.015 g/L/day was observed in *Spirulina* cultivated in $NaNO_3$ deficient medium. 10% harvesting ratio showed a consistent range of harvested dry biomass weight (0.20 g to 0.22 g). By adding 2.50 g/L of NaNO3 resulted in a significant increase in C-phycocyanin (C-PC) and allophycocyanin (APC) concentration, from 34.37 to 68.35 mg/g and 27.08 to 33.23 mg/g, respectively. Biomass productivity of 1-L and 10-L batch culture was found to be 0.23 g/L/d and 0.21 g/L/d, respectively. Both 1-L and 10-L batch cultures showed a significant increase in phycocyanin accumulation due to the addition of 2.50 g/L of NaNO3. This research demonstrates a comparison of 1-L and 10-L continuous cultivation and harvesting strategy for biomass and phycocyanin production in the biorefinery of *Spirulina* microalgae.

***Keywords:*** harvesting ratio, *Spirulina*, phycocyanin, scale-up, harvesting strategy

## 5.2 INTRODUCTION

To-date, the global demand for the production of microalgae biomass as an alternative resource for various application (e.g. biofuels, cosmetics, animal feed, carbon capture, pharmaceuticals, food and nutritional supplements) is expected to achieve at least 30,000 tons per year by year 2030 (Amorim et al., 2021). *Spirulina* biomass accounts 30% of the current 10,000 tons of total algae biomass production globally (Lim et al., 2021). To realize this production target by year 2030, researchers around the world have proposed many ideas and resolutions in improving the biomass productivity in the upstream processing of microalgae. The current industrial microalgae biomass production faces five challenges which are contamination, cost, harvesting, nutrient availability and strain selection (Venkata Subhash et al., 2022; Ummalyma et al., 2022).

Extensive research over the years explored alternative solutions to overcome the issues in regards to selection, optimization and formulation of cultivation medium in the microalgae biorefinery (Fábregas et al., 2000; Salunke et al., 2016). dos Santos et al. (2016) reported that the addition of supplements (e.g. sugarcane vinasse) during the microalgae cultivation increases microalgae biomass concentration by 23%, from 0.495 to 0.609 g/L and gives higher protein content of 75%. Despite the selection and optimisation of cultivation medium or addition of supplements in culture medium, the extent of increasing biomass productivity is limited by different microalgae strains. Therefore, researcher have shifted their focuses by incorporating strategies involving genetic modification (Khoo et al., 2023). Fang et al. (2013) studied 62 mutants of *Spirulina platensis*, selected mutants have shown 78% increase in carbohydrate content and 10% increase in specific growth rate. However, genetic modification

is a relatively new field of area that requires much ongoing study and research before the mutated species can be commercially used in the industry. It is therefore crucial for researchers to carefully assess the potential risks and benefits of genetic modification in microalgae, and to develop appropriate safety measures and regulations to ensure responsible and sustainable use of this technology. Besides, closed system photobioreactors have been reported that the biomass productivity (12 $g/m^2/d$) is higher compared to open pond systems (8 $g/m^2/d$) due to its closed environment and reduced contamination (Narala et al., 2016). However, there is a problem when scaling up photobioreactor, biomass yield would decrease as it is being scale up (Benner et al., 2022). The current research has been focusing on issue related to contamination, nutrient availability and strain selection but there have been inefficient methods on harvesting research area.

Microalgae biomass harvesting methods such as coagulation and flocculation, centrifugation, electrical based processes and filtration have been studied (Singh and Patidar, 2018). Each of these harvesting methods has its own advantages and disadvantages that depends on the end-product of the process to select the suitable harvesting method. On the other hand, different cultivation strategy such as batch cultivation, semi-batch cultivation and continuous cultivation has been proposed by researchers (Peter et al., 2022). In batch cultures, the cell composition may change over time as cells get older and the bulk environment changes resulting harvested biomass is not of good quality and may contain dead cells. On the other hand, continuous cultures enable efficient control of the growing environment, leading to tailor-made biomass composition at a constant and predetermined rate. Compared to batch mode (7.3 g/L/d), continuous

cultivation systems offer higher productivity (42.6 g/L/d), ranging from 2.3 to 5 times higher, without accounting for the time required to clean and re-start a batch culture (Fernandes et al., 2015; Gao et al., 2016).

Therefore, this research work addressed the issue as mentioned above by employing an integrated continuous cultivation and harvesting strategy for the biorefinery of *Spirulina* microalgae. The objective of this research work aims to investigates the impact of different harvesting ratio (e.g. 10%, 20%, and 30%) on *Spirulina* cultivation. Additionally, this study also examines the effect of different concentration of sodium nitrate ($NaNO_3$) addition (e.g. 2.5 g/L, 3.5 g/L and 4.5 g/L) on the phycocyanin (C-phycocyanin (CPC) and Allo-phycocyanin (APC)) accumulation in *Spirulina*. Phycocyanin has been studied and reported for its various potential therapeutic properties, including antioxidant, anti-inflammatory, and immunomodulatory effects (ElFar et al., 2022). Based on the findings, $NaNO_3$ has been identified as the most important chemical for the growth of *Spirulina*. To improvise the harvesting strategy, instead of adding the entire fresh medium to replace the harvested biomass volume. We aim to investigate whether the addition of solely $NaNO_3$ is sufficient to enhance production of phycocyanin accumulation in the *Spirulina* microalgae. Thereby reducing chemical usage in the system while optimizing phycocyanin production. Finally, the tested parameters of harvesting ratio and $NaNO_3$ concentration were tested in different scale of 1-L and 10-L batch cultivation photobioreactor to evaluate and understand its effects on the biomass concentration, biomass productivity, harvested dry biomass and phycocyanin accumulation. This research work will provide insight on the impact of

continuous cultivation and harvesting strategy on biomass and phycocyanin production in the biorefinery of *Spirulina* microalgae.

## 5.3 MATERIALS AND METHODS

### 5.3.1 MICROALGAE STRAINS AND GROWTH CONDITIONS

*Spirulina platensis* microalgae was collected from Biolina Sdn. Bhd., Malaysia. Cultures were maintained in Zarrouk medium (Dineshkumar, Narendran and Sampathkumar, 2016a) with some modification (g/L). NaHCO3: 18 g/L, NaNO3: 2.5 g/L, MgSO4.7H2O: 0.2 g/L, CaCl2.2H2O: 0.04 g/L, K2HPO4: 0.5 g/L, NaCl: 1 g/L, K2SO4: 1 g/L, FeSO4.7H2O: 0.01 g/L, EDTA: 0.08 g/L, Micronutrient: 1 mL/L. The micronutrient consisted of: $H_3BO_3$: 2.86 g/L, $MnCl_2.4H_2O$: 1.81 g/L, $ZnSO_4.7H_2O$: 0.222 g/L, $Na_2MoO_4.2H_2O$: 0.3 g/L, $CuSO_4.5H_2O$: 0.07 g/L, $Co(NO_3)_2.6H_2O$: 0.04 g/L

*Spirulina* microalga were inoculated and pre-cultured in 250 mL optimized Zarrouk medium for a period of 7 days. The pre-cultured *Spirulina* microalga was then transferred to a 1-L batch cultivation. The experiments were carried out in 1-L photobioreactor. These 1-L batch cultivations were placed on a magnetic stirrer to ensure homogeneous mixing. The batch culture was cultivated under photoautotrophic growth conditions. The batch culture experiments were carried out with an illumination of about 3000 lux using LED lights. The intensity of light was measured using lux meter (UT383, UNI-T). Aeration was supplied to the batch cultivation via an air compressor (ACO-308, HAILEA, China). The air flow rate was maintained at 400 mL/min using an airflow regulator (Dwyer, Malaysia) throughout the experiment.

### 5.3.2 SCALE-UP TO 10-L AERATED BATCH CULTURES

A cylindrical closed system photobioreactor with a diameter of 12 cm and a height of 150 cm was fabricated by Donewell Resources Sdn. Bhd., Malaysia. The material used to fabricate the cultivation tank was acrylic plastic. Acrylic plastic was used because of its transparency and chemical robustness requirements (Huang et al., 2017). Transparency is crucial for *Spirulina* cultivation as *Spirulina* require light for photosynthesis. Chemical robustness is important because of its chemical resistance property where many chemicals was used to prepare the cultivation medium. The working volume of the cultivation tank is 10-L in volume.

1-L batch cultivation was prepared as the inoculum for 10-L batch cultivation. The batch culture was cultivated under photoautotrophic growth conditions. A nano-airstone (VN-132, Yek Fong Aquarium Accessories Sdn Bhd, Malaysia) were placed inside the 10-L cultivation tank to supply aeration via an air compressor for the *Spirulina* culture. The air flow rate was maintained at 20 L/min using an airflow regulator throughout the experiment. The 10-L batch culture experiments were carried out with an illumination of about 2000 lux with 4 tubes of LED lights installed surrounding the cultivation tank. The intensity of light was measured using lux meter. All scale-up cultures were supplemented with Zarrouk media.

### 5.3.3 HARVESTING RATIO STRATEGY

#### 5.3.3.1 EFFECTS OF ZARROUK MEDIUM

The effects of harvesting ratio (10%, 20% and 30%) on biomass concentration and phycocyanin accumulation were studied. Each batch of cultivation were

cultured for 10 days before harvesting. The batch cultivation was replenished with an amount of fresh culture medium equal to the harvested amount. For each batch cultivation, the absorbance of the culture was analysed daily. The harvested fresh culture was centrifuged to remove the supernatant, and the resulting biomass was freeze-dried. After the optimum harvesting ratio was determine, it was tested in 10-L scale-up cultivation tank.

#### 5.3.3.2 EFFECTS OF SODIUM NITRATE ADDITION

Instead of adding culture medium, $NaNO_3$ was added only. Previous study has demonstrated increased biomass growth, phycocyanin, and allophycocyanin accumulation in nitrate fed-batch phototrophic cultivation of *A. platensis* FACHB-314 (Manirafasha et al., 2018). However, the specific nitrate source used in the study was not specified. To address this gap, this study focused on the effects of $NaNO_3$, the primary source of nitrate in Zarrouk medium, on phycocyanin accumulation in *Spirulina*.

The harvesting ratio was fixed to be 10% based on previous optimized harvesting ratio conducted in 1-L *Spirulina* cultivation. The effect of concentration of $NaNO_3$ (2.5 g/L, 3.5 g/L and 4.5 g/L) on biomass concentration and phycocyanin accumulation were studied. Each batch of cultivation were cultured for 10 days before harvesting. The batch cultivation was replenished with fresh $NaNO_3$ solution after harvesting. After the optimum $NaNO_3$ addition was determine, it was tested in 10-L scale-up cultivation tank. **Table 5.1** shows an overview of the operating parameters investigated for the production of phycocyanin from *Spirulina* microalgae.

**Table 5.1** Operating parameters of *Spirulina* cultivation for the production of phycocyanin

| No. | Operating parameter | Variables |
|---|---|---|
| **1** | Effect of nutrients deficient media | $NaHCO_3$, $NaNO_3$, NaCl, $K_2SO_4$, $K_2HPO_4$, $MgSO_4.7H_2O$, $FeSO_4.7H_2O$ & EDTA, $CaCl_2.2H_2O$, Micronutrient |
| | Harvesting ratio (1-L) | 10%, 20%, 30% |
| **3** | Effect of $NaNO_3$ addition (1-L) | 2.5 g/L, 3.5 g/L, 4.5 g/L |
| **4** | Scale-up 10-L photobioreactor | Comparison of 1-L and 10-L<br>+ Harvesting ratio (10%)<br>+ Effect of $NaNO_3$ addition (2.5 g/L) |

### 5.3.4 BIOMASS SAMPLING

The progress of the cultivation was monitored by collecting biomass samples. The absorbance of the biomass concentration of the culture was measured with a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan) at a wavelength of 688 nm (Chen et al., 2016). However, the UV-Vis Spectrophotometer has a limitation of reading absorbance values up to 4, thus the samples had to be diluted 10 times and the average taken to ensure accurate readings. Fresh samples were used to determine the biomass concentration, biomass

productivity. Samples for phycocyanin extraction were stored at -20℃ until further analysis.

### 5.3.5 GROWTH AND BIOMASS PRODUCTIVITY DETERMINATION

#### 5.3.5.1 BIOMASS DETERMINATION WITH STANDARD CALIBRATION CURVE

A sample of mature *Spirulina* medium was subjected to dilutions of 20%, 40%, 60%, 80%, and 100% with deionized water in 15 ml centrifuge tubes. The absorbance of each diluted sample was measured and recorded using a UV-Vis spectrophotometer at a specific wavelength of 688 nm. Subsequently, the samples were centrifuged to remove the supernatant and rinse with deionised water to remove excess salt, and the resulting biomass was freeze-dried. The dry biomass weight was then measured and recorded. A standard calibration graph was generated by plotting the dry biomass weight against absorbance. The equation obtained from this graph was used to convert all measured absorbance data into dry biomass weight (g/L). The standard calibration graph allows us to estimate the dry biomass weight of *Spirulina* from the absorbance data. Finally, the remaining cultivated *Spirulina* biomass was poured into 50 mL centrifuge tubes and centrifuged at 6500 rpm for 5 min (Koyande et al., 2019). The supernatant was then discarded, and the wet biomass was freeze-dried and stored at -20℃ for further analysis. The freeze-dried biomass was used for proximate analysis to determine the phycocyanin content.

### 5.3.5.2 BIOMASS PRODUCTIVITY AND SPECIFIC GROWTH RATE

The biomass productivity ($P_b$) and specific growth rate ($\mu$) were calculated based on the following **Equation (5.1)** and **Equation (5.2)** (Chew et al., 2018a), respectively:

$$P_b \text{ (mg/L/day)} = (N_2-N_1)/(t_2-t_1) \quad (5.1)$$

$$\mu \text{ } (d^{-1}) = \ln (N_2/N_1)/(t_2-t_1) \quad (5.2)$$

where, $N_1$ and $N_2$ are biomass concentration (g/L) at time $t_1$ and $t_2$, respectively.

### 5.3.5.3 PHYCOCYANIN QUANTIFICATION

Two types of phycocyanin were analyzed: C-phycocyanin (CPC) and Allo-phycocyanin (APC). Phosphate buffer was added to a measured quantity of freeze-dried *Spirulina* biomass in a 0.009% (m/v) biomass-to-solvent ratio. The use of phosphate buffer as the solvent was chosen in this study as it has been demonstrated to improve the structural stability of protein molecules during the extraction process, which may be subject to various forces that could lead to degradation or denaturation (G-Bioscience, 2019). The mixture was homogenized using a vortex mixer and then subjected to ultrasonic sonication at an frequency of 35 kHz for 5 min (Chew et al., 2019). This process disrupted the cell walls of the *Spirulina* cells and release the phycocyanin composition. The sonicated sample was then centrifuged at 6500 rpm for 5 min, and the supernatant was collected for further analysis. The CPC and APC concentrations in the supernatant were quantified using UV-Vis spectrophotometer at various wavelengths.

CPC and APC concentration were calculated based on **Equation (5.3)** and **Equation (5.4) (**Bennett and Bogorad 1973**)**.

$$CPC\ (mg/ml) = \frac{OD_{615} - 0.474 \times OD_{652}}{5.34} \quad (5.3)$$

$$APC\ (mg/ml) = \frac{OD_{652} - 0.208 \times OD_{615}}{5.09} \quad (5.4)$$

Where, $OD_{615}$ and $OD_{652}$ is the optical density wavelength at 615 nm and 652 nm, respectively.

The purity of CPC and APC were calculated based on **Equation (5.5)** and **Equation (5.6) (**Abalde et al., 1998**)**.

$$Purity_{CPC} = \frac{OD_{620}}{OD_{280}} \quad (5.5)$$

$$Purity_{APC} = \frac{OD_{652}}{OD_{280}} \quad (5.6)$$

Where, $OD_{280}$, $OD_{620}$, and $OD_{652}$ is the optical density wavelength at 280 nm, 625 nm and 652 nm, respectively.

### 5.3.6 STATISTICAL ANALYSIS

All cultures were performed in duplicate and average values were reported. The values were then expressed in terms of mean and standard deviation. Subsequently, the data was subjected to one-way analysis of variance (ANOVA) using Microsoft Excel software to assess any significant differences between the groups with a p-value of less than or equal to 0.05.

## 5.4 RESULTS AND DISCUSSION

### 5.4.1 EFFECTS OF NUTRIENTS DEFICIENT MEDIA ON 1-L *SPIRULINA* CULTIVATION

*Spirulina* cultivation in $NaNO_3$ deficient medium has the lowest biomass productivity of 0.015 ± 0.002 g/L/day shown in **Figure 5.1**. The *Spirulina* cultivation in $NaNO_3$ deficient medium did not survive the 10 days of cultivation. This is because $NaNO_3$ is an essential nutrient for *Spirulina* cultivation as it contributes the nitrogen source in the cultivation medium. Nitrogen source is important for the synthesis of proteins, nucleic acids and chlorophyll content in microalgae (Wu and Miao, 2014). Nitrogen limitation will result in decrease in photosynthesis, protein synthesis and increase in lipid and carbohydrate synthesis (Li, Fei and Deng, 2012). Therefore, $NaNO_3$ deficient medium shows the lowest biomass productivity among others. Moreover, phycocyanin is a type of pigment-protein complex, since $NaNO_3$ is responsible for protein synthesis, this study also investigated the effect of concentration of $NaNO_3$ on phycocyanin accumulation in *Spirulina*.

$NaHCO_3$ deficient medium also exhibit low biomass productivity of 0.143 ± 0.012 g/L/day. The *Spirulina* cultivation in $NaHCO_3$ deficient medium survive the 10 days of cultivation. $NaHCO_3$ is an essential nutrient for *Spirulina* cultivation because it contributes carbon source in the cultivation medium. Only *Spirulina* is able to utilize carbonate or bicarbonate, supplied in the form of salts (Becker, 1994). The mechanism of $NaHCO_3$ utilization as an inorganic carbon source for microalgae differs from $CO_2$ uptake. The bicarbonate transporters are embedded in the chloroplast envelope and plasma membrane in microalgal cells. Bicarbonate ions ($HCO^{3-}$) are converted to $CO_2$ by carbonic anhydrase in the

periplasmic space, which then absorbed and utilized by microalgal cells (Mondal et al., 2017). Moreover, when $NaHCO_3$ dissociated in the water, it produces hydroxyl ions in the medium that help to sustain a high pH 9-10 throughout the cultivation condition (Michael, Kyewalyanga and Lugomela, 2019). Study have reported that *Chlorella vulgaris* under nutrient sufficient condition with $NaHCO_3$ as carbon source, the maximum cell density was able to reach 1.92 g/L almost twice as the biomass (1.04 g/L) that cultivated under aeration alone (Abedini Najafabadi et al., 2015). *Spirulina* were able to grow in $NaHCO_3$ deficient medium but resulted in low biomass productivity. $NaHCO_3$ exhibits a dual role which act as carbon source and maintaining high pH condition suitable for *Spirulina* cultivation.

Overall, the deficiency of each nutrient has shown to have lower biomass productivity as compared to the control ($0.632 \pm 0.039$ g/L/day) that contains the complete Zarrouk medium. $NaNO_3$ and $NaHCO_3$ have been identified as the most important nutrient source for *Spirulina* cultivation. Although Zarrouk medium is an established medium for cultivating cyanobacteria since 1966, not much information was released regarding the formulation of Zarrouk medium. Therefore, the current finding is important in understanding the nutrients of Zarrouk medium on the biomass productivity of *Spirulina*. Further research can be done in optimising the concentration of $NaNO_3$, $NaHCO_3$ and other nutrients to achieve a cost-effective cultivation medium for *Spirulina*. Statistical ANOVA analysis shown that the effect of nutrients deficient have significant effect ($p < 0.05$) on the biomass productivity of *Spirulina*.

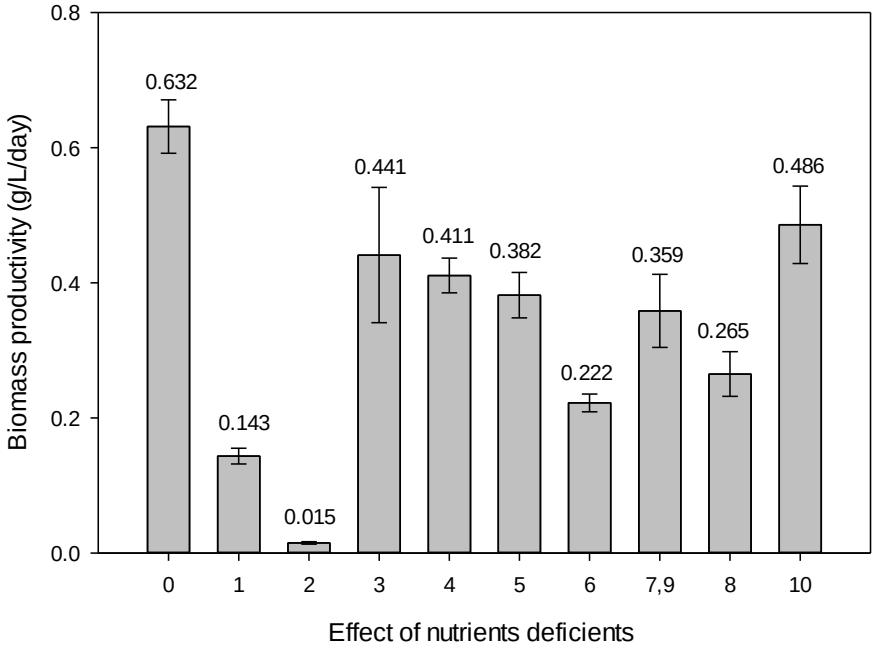



**Figure 5.1** Effect of nutrients deficient media on biomass productivity of *Spirulina* cultivation [0] Zarrouk medium as control [1] $NaHCO_3$, [2] $NaNO_3$, [3] NaCl, [4] $K_2SO_4$, [5] $K_2HPO_4$, [6] $MgSO_4.7H_2O$, [7,9], $FeSO_4.7H_2O$ and EDTA, [8] $CaCl_2.2H_2O$ and [10] Micronutrient

### 5.4.2. EFFECTS OF HARVESTING RATIO ON 1-L *SPIRULINA* CULTIVATION

**Figure 5.2(a)** shows the biomass concentration of *Spirulina* cultivated for 10 days before subjected to different harvesting ratio (e.g., 10%, 20%, and 30%). **Figure 5.2(b)** shows the dry biomass weights of *Spirulina* harvested from Day 10 to Day 13 consecutively at different harvesting ratio (10%, 20%, and 30%). Only 10% harvesting ratio shows a more consistent harvested dry biomass weights ranged from 0.2016 g to 0.2222 g. While 20% and 30% harvesting ratio, the harvested dry biomass weights decrease from 0.4566 g to 0.3344 g and 0.5671 g to 0.3635 g, respectively. A lower harvesting ratio resulted in longer

hydraulic retention time in which higher biomass density would be accumulated, thus, more nutrients would be assimilated, and the biomass concentration would be able to recover in a day. However, when the harvesting ratio increases, there is lesser biomass density accumulated, thus, lesser nutrients would be assimilated resulting decreases of harvested dry biomass weights over the four consecutive days. Min et al. (2011) reported that 25% and 33% harvesting ratio showed better nutrient removal than half of its harvesting rate.

The current study investigated on the harvesting ratio but further study can be done on investigating the harvesting frequency such as harvesting interval of 1 day, 3 days and 5 days on *Spirulina* cultivation. Cai et al. (2013) reported that the biomass productivity of *Nannochloropsis salina* increased from 87.4 to 132.1 mg/L/d when the harvesting interval reduced from 3 days to 1 day. This is because long harvesting interval used to increase the number of larger clumps which would raise the self-shading of cells and interfere with light absorption efficiency as well as photosynthetic efficiency of the microalgae, as a result the biomass productivity reduced (Ishika et al., 2021). To conclude, a low harvesting ratio and frequent harvesting would be a good harvesting strategy to produce high amount of biomass.

The reported CPC and APC concentration range from 139.413 to 172.317 mg/g and 46.667 to 59.286 mg/g, respectively shown in **Figure 5.2(c) and Figure 5.2(d), respectively**. While the reported CPC and APC purity range from 0.457 to 0.486 and 0.409 to 0.501, respectively. Statistical ANOVA analysis show that there was no significant effect ($p > 0.05$) on phycocyanin concentration or purity. While the effect of harvesting ratio has significant effect ($p < 0.05$) on the harvested dry biomass weight of *Spirulina*. Overall, the findings provided

valuable insights on the impact of harvesting ratio on harvested dry biomass and phycocyanin accumulation in *Spirulina*.

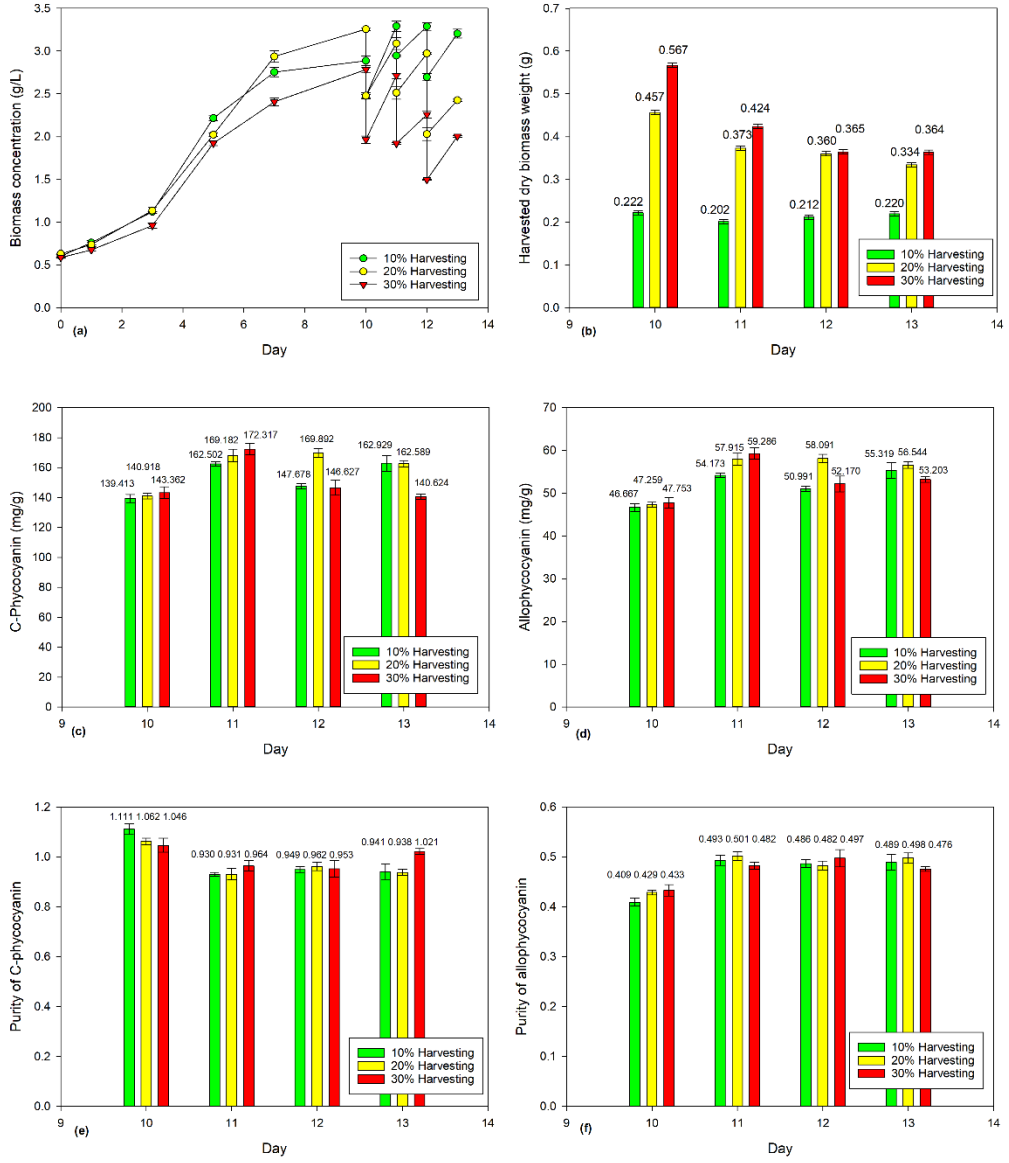



**Figure 5.2** Effect of harvesting ratio (10%, 20%, 30%) on *Spirulina* cultivation (a) biomass concentration, (b) harvested dry *Spirulina* biomass weight, (c) C-phycocyanin concentration, (d) Allo-phycocyanin concentration (e) C-phycocyanin purity and (f) Allo-phycocyanin purity

### 5.4.3. EFFECTS OF $NaNO_3$ ADDITION ON PHYCOCYANIN CONCENTRATION IN 1-L *SPIRULINA* CULTIVATION

**Figure 5.3(a)** shows the biomass concentration of *Spirulina* cultivated for 10 days before subjected to harvesting ratio of 10%. Equal volume of $NaNO_3$ (2.5 g/L, 3.5 g/L, 4.5 g/L) was added back and cultivated for another 3 days to study its effect on biomass concentration, harvested dry biomass weight and phycocyanin accumulation. After addition of $NaNO_3$, all 4-batch cultivation shows a similar trend where the biomass concentration continues to increase from Day 10 to Day 12 but decreases on Day 13. Therefore, the batch cultivations were stop and harvested. **Figure 5.3(b) s**hows that the harvested dry biomass weight increases slightly. On the other hand, the addition of 2.5 g/L of $NaNO_3$ have shown a significant increase in C-PC and APC concentration from 34.368 to 68.346 mg/g and 27.075 to 33.232 mg/g, respectively shown in **Figure 5.3(c)**. The control also shown significant increase in C-PC and APC concentration from 43.594 to 66.008 mg/g and 25.623 to 30.224 mg/g, respectively. However, the addition of 3.5 g/L of $NaNO_3$ have shown a significant decrease in C-PC concentration from 30.750 to 6.035 mg/g. Moreover, the addition of 4.5 g/L of $NaNO_3$ have shown a slight increase in C-PC and APC concentration from 26.638 to 28.613 mg/g and 26.637 to 28.903 mg/g, respectively.

The current finding has shown that both control and the addition of 2.5 g/L of $NaNO_3$ increases phycocyanin accumulation. The control uses Zarrouk medium to replace the 10% harvested *Spirulina* biomass. Instead of using Zarrouk medium that consist of many chemicals, the current study shown that 2.5 g/L of $NaNO_3$ only is capable to increase phycocyanin accumulation. This is because

phycocyanin is a type of pigment-protein complex, $NaNO_3$ that contain nitrogen source is responsible for the synthesis of proteins, nucleic acids and chlorophyll in microalgae (Wu and Miao, 2014). However, the concentration of 3.5 g/L $NaNO_3$ onwards does not shown any significant increase in phycocyanin accumulation. Sakawduan et al. (2019) reported a similar trend, the CPC concentration increase from 25.5 to 44.59 mg/L/d, when $NaNO_3$ concentration increases from 1.5 to 3.5 g/L. Conversely, the CPC concentration decreases to 8.79 mg/L/d at 4.5 g/L of $NaNO_3$. Moreover, Vieira Costa et al. (2001) reported when $NaNO_3$ increases from 0.85 g/L to 2.55 g/L, the final biomass concentration increases from 1.559 g/L to 1.992 g/L but the final biomass concentration decreases to 1.628 g/L when the $NaNO_3$ is 4.25 g/L. It can be concluded that, the tolerance level of $NaNO_3$ concentration is around 3.5 g/L. Manirafasha et al. (2018) suggested that the nitrate concentration in culture should be kept in the range between 1.2 to 1.6 g/L for high content of phycocyanin accumulation. It also worth noting that Sakawduan et al. (2019) and Vieira Costa et al. (2001) study the effect of $NaNO_3$ on the cultivation cycle but the current study investigated the effect of $NaNO_3$ on post-cultivation cycle.

The current finding has proven that the addition of 2.5 g/L of $NaNO_3$ after post-cultivation has the ability to enhance phycocyanin accumulation in *Spirulina* microalgae. This strategy offers a cost-effective solution for the microalgae industry by reducing the usage of chemicals during the cultivation process. Further research is needed to explore alternative nitrogen source (i.e., urea, ammonium nitrate and ammonium sulphate) and optimize the conditions to achieve the maximum phycocyanin accumulation in *Spirulina* cultures. Statistical ANOVA analysis show that there was no significant effect ($p > 0.05$)

on phycocyanin purity. While the effect of harvesting ratio has significant effect ($p < 0.05$) on the harvested dry biomass weight of *Spirulina*.

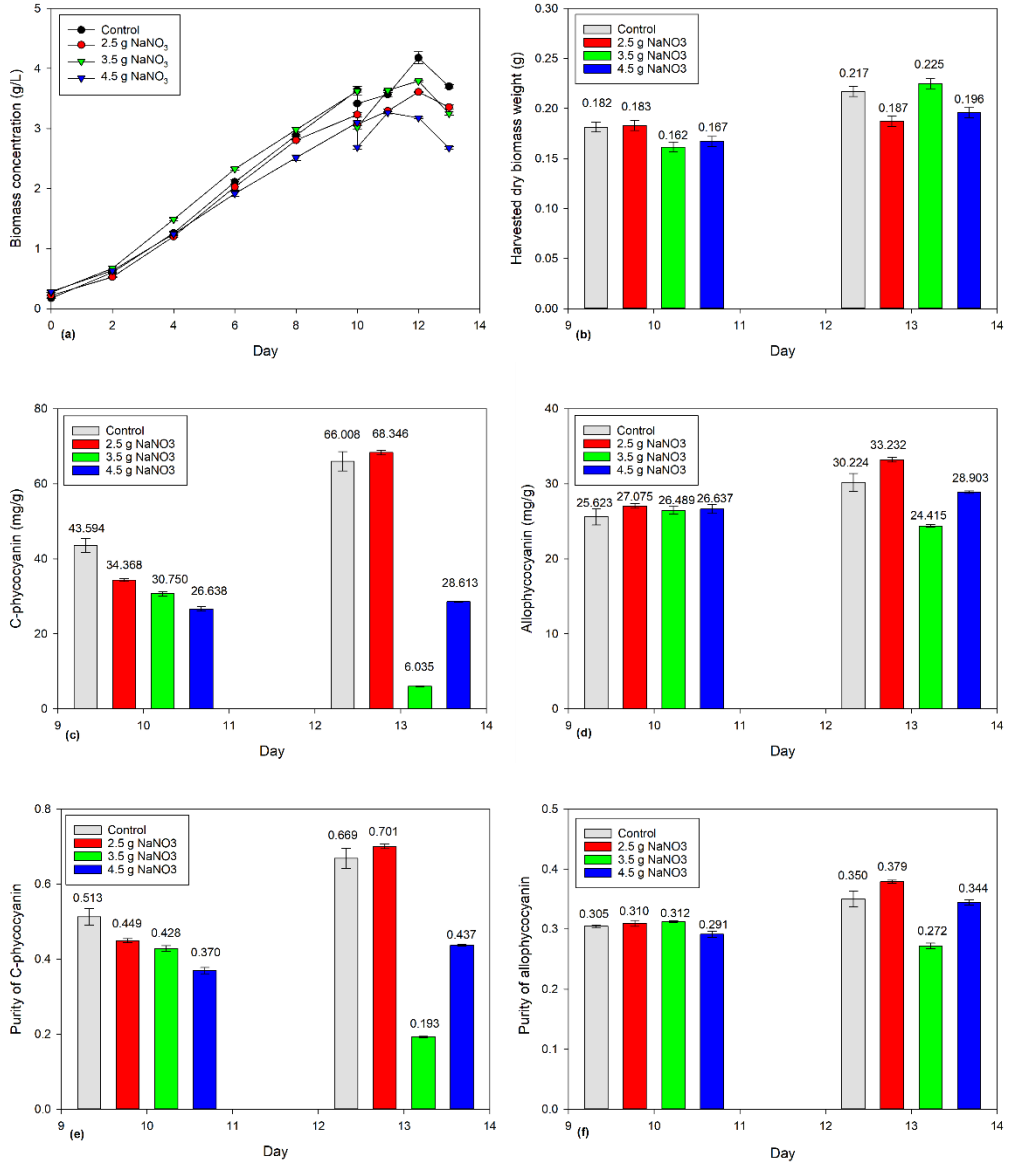



**Figure 5.3** Effect of $NaNO_3$ addition (2.5 g/L, 3.5 g/L, 4.5 g/L) on *Spirulina* cultivation (a) biomass concentration, (b) harvested dry *Spirulina* biomass weight, (c) C-phycocyanin concentration, (d) Allo-phycocyanin concentration (e) C-phycocyanin purity and (f) Allo-phycocyanin purity

### 5.4.4 COMPARISON OF 1-L AND 10-L *SPIRULINA* CULTIVATION VOLUME

#### 5.4.4.1 BIOMASS PRODUCTIVITY COMPARISON

Over the course of a 10-day cultivation period, the biomass concentration of *Spirulina* in 1-L and 10-L batch cultivation was determined to be 2.887 g/L and 2.437 g/L, respectively shown in **Figure 5.4(a)**. The biomass productivity of 1-L and 10-L batch cultivation was found to be 0.2284 g/L/d and 0.2122 g/L/d, respectively shown in **Figure 5.4(b)**. These findings suggest that the smaller-scale 1-L system is more productive than the larger 10-L system, despite the latter having a larger volume for cultivation. When cultivating microalgae on a large scale, challenges arise such as ensuring equal light distribution and mixing, providing sufficient gas exchange, and addressing the accumulation of soluble algal products in the culture medium (Jerney and Spilling, 2020).

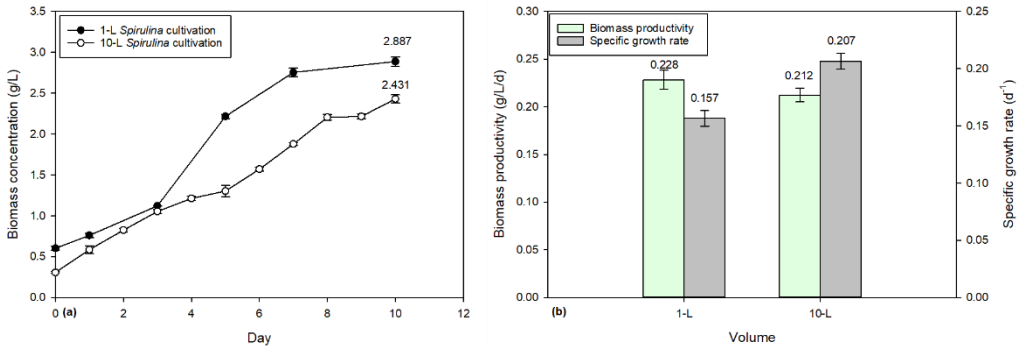



**Figure 5.4** Comparison of 1-L and 10-L *Spirulina* cultivation (a) biomass concentration and (b) biomass productivity

### 5.4.4.2 EFFECTS OF 10% HARVESTING RATIO IN 10-L SCALE-UP CULTIVATION TANKE

**Figure 5.5(a)** shows the biomass concentration of 1-L and 10-L *Spirulina* batch cultivation. The 1-L batch cultivated for 10 days while the 10-L batch cultivated for 11 days before subjected to harvesting for three consecutive days. **Figure 5.5(b)** demonstrate the results of 10% harvesting from 10-L batch culture on Day 11, 12, 13 and 14 with a harvested dry biomass weight of 1.354 g, 1.088 g, 1.200 g and 1.130 g, respectively. While 10% of the 1-L batch culture was harvested on Day 10, 11, 12 and 13 with a harvested dry biomass weight of 0.2222 g, 0.2016 g, 0.2120 and 0.2200 g. 10% from 10-L is 1-L in volume and 10% of 1-L is 0.1-L in volume. Thus, when the results of the 1-L harvested dry biomass weight were multiply by 10, it is higher compared to the harvested dry biomass weight from 10-L culture. This has also shown that the biomass productivity in 1-L batch culture is higher as compared to 10-L batch culture.

**Figure 5.5(c) and Figure 5.5(d)** shows a comparison of CPC and APC concentration between 1-L and 10-L *Spirulina* batch culture. In the 10-L batch culture, the CPC content range between 166.9 to 238.4 mg/g, APC content range between 58.43 to 77.74 mg/g. On the other hand, in the 1-L batch culture, the CPC content range between 139.4 to 162.9 mg/g, APC content range between 46.67 to 55.32 mg/g. The 10-L batch culture had higher CPC and APC content than the 1-L batch culture. However, the purity of both CPC and APC has no significant effect over the 4 days of 10% harvesting ratio.

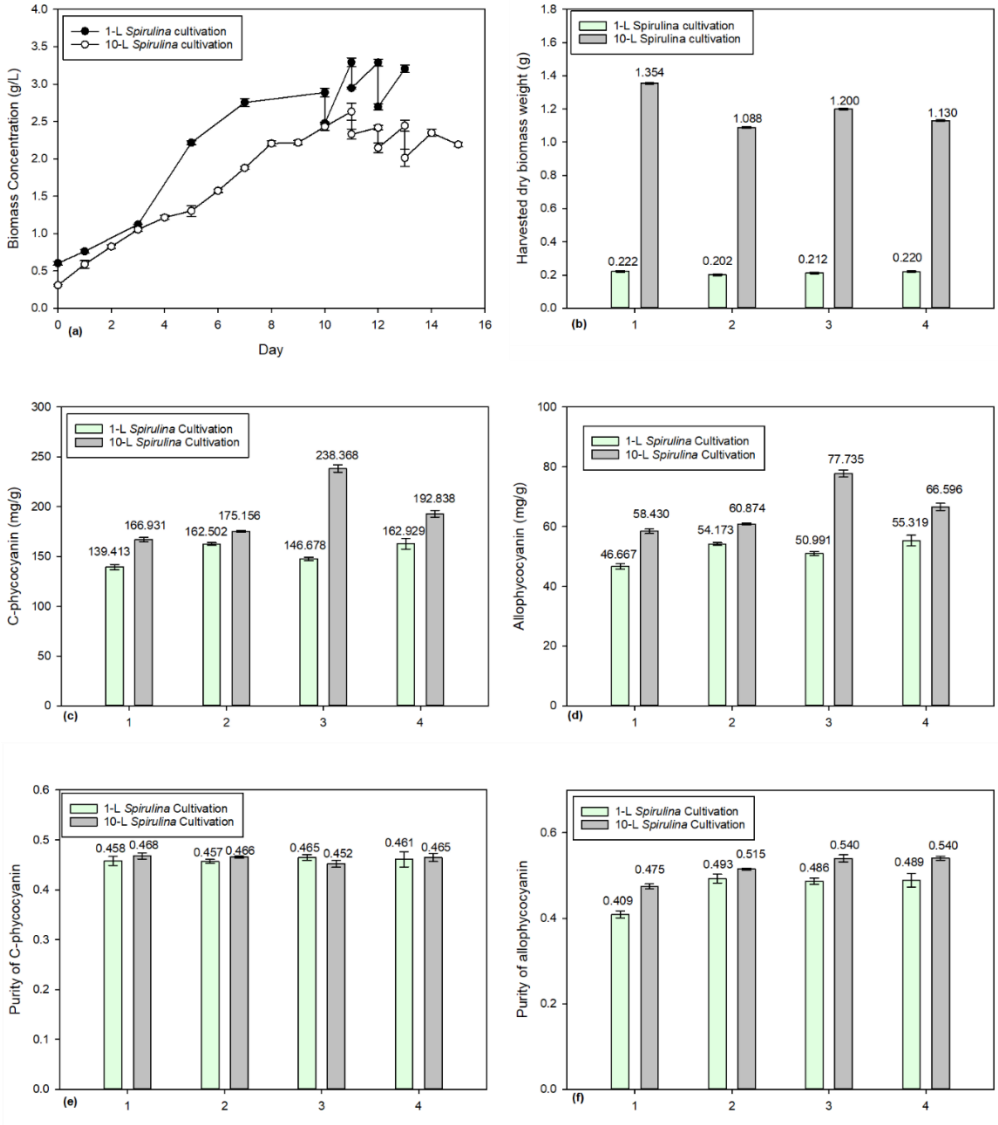



**Figure 5.5** Comparison of 1-L and 10-L *Spirulina* batch culture with 10% harvesting ratio (a) biomass concentration, (b) harvested dry *Spirulina* biomass weight, (c) C-phycocyanin concentration, (d) Allo-phycocyanin concentration (e) C-phycocyanin purity and (f) Allo-phycocyanin purity

### 5.4.4.3 EFFECTS OF $NANO_3$ ADDITION ON PHYCOCYANIN ACCUMULATION IN 10-L SCALE-UP CULTIVATION TANK

**Figure 5.6** compared the effect of 2.5 g/L of $NaNO_3$ addition between 1-L and 10-L *Spirulina* batch cultures. **Figure 5.6(a) and Figure 5.6(b)** shows CPC and APC concentration of before and after the addition of 2.5 g/L of $NaNO_3$. The initial concentration of CPC and APC in the 10-L batch culture were 218.76 mg/g and 69.38 mg/g, respectively, which increased to 232.08 mg/g and 72.79 mg/g. Meanwhile, the initial concentration of CPC and APC in the 1-L batch culture were 34.37 mg/g and 27.07 mg/g, respectively, which significantly increased to 68.35 mg/g and 33.23 mg/g. Both 1-L and 10-L batch culture have shown significant increase in phycocyanin accumulation due to the addition of 2.5 g/L of $NaNO_3$. Surprisingly, 10-L batch culture has higher phycocyanin accumulation as compared to a 1-L batch culture.

Although, the addition of $NaNO_3$ resulted an increase in both CPC and APC concentration and APC purity but it had caused the purity of CPC to decrease in both 1-L and 10-L batch culture as shown in **Figure 5.6(c)**. Therefore, this would depend on the end product of the company whether quantity or quality is favour. The current finding has demonstrated the addition of $NaNO_3$ can enhance the accumulation of phycocyanin in *Spirulina* microalgae, concurrently minimizing chemical usage. However, this approach may not be a sustainable long-term solution since it relies on raw materials extracted from the earth. Further research could be done to explore alternative nitrogen source that favour the increase in both phycocyanin concentration and purity.

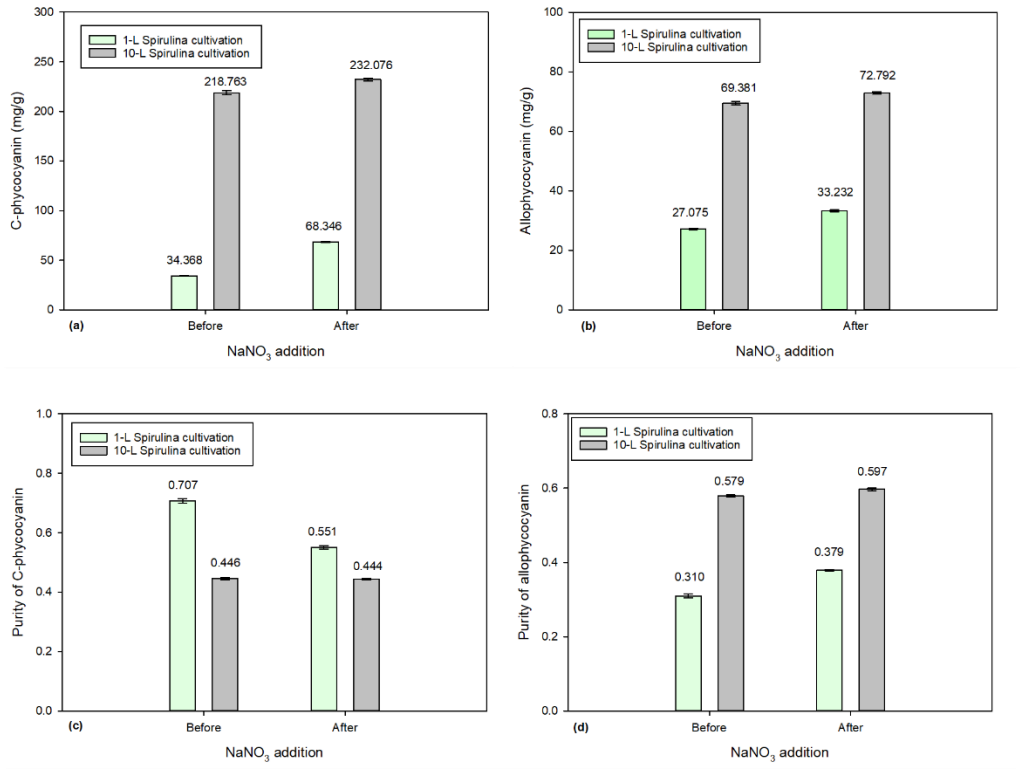



**Figure 5.6** Comparison of 1-L and 10-L *Spirulina* cultivation with the addition of 2.5 g/L of $NaNO_3$ (a) C-phycocyanin concentration, (b) Allo-phycocyanin concentration (c) C-phycocyanin purity and (d) Allo-phycocyanin purity

### 5.4.5 OPERATING COST ANALYSIS

**Table 5.2** shows the operating costs analysis of *Spirulina* cultivation in 1-L and 10-L. The operating cost consists of electricity, water and chemicals. The cost of electricity is assumed to be the same, using 4 units of LED lights with a power 18 W each and 1 unit of air pump with a power of 30 W, for both 10 bottle of 1-L Schott bottle and 1 unit of 10-L cultivation tank. The cost of water was referred based on statistics provided from Air Selangor Sdn. Bhd., Malaysia (Air Selangor, 2022). Chemicals costs were based on lab-grade chemical price as shown in **Table 5.3**. The operating costs for both 1-L and 10-L batch culture is

RM15.59. The largest expense is electricity, which cost RM 9.79 follow by chemicals which cost RM5.80. The biomass concentration of *Spirulina* microalgae is higher in the 1-L batch culture with 2.887 g/L compared to 2.431 g/L in the 10-L batch culture. As a result, the cost of producing 1 kg of *Spirulina* biomass is RM540.08 in the 1-L batch culture and RM641.39 in the 10-L batch culture. These results suggest that producing *Spirulina* microalgae in smaller volumes may be cheaper, as it produces a higher yield of dry biomass per litre of culture medium. However, the limitation of this brief calculation has yet to consider the capital cost. The capital cost of 10 units of 1-L vessels could be higher as compared to fabricating a 10-L vessel. Overall, the current findings provide a brief insight into the economic feasibility of producing *Spirulina* biomass in 1-L and 10-L batch culture. A more detailed economic feasibility of scaling-up *Spirulina* production for commercial scale should be assessed in future studies.

**Table 5.2** Comparison of operating cost between 1-L and 10-L *Spirulina* microalgae cultivation

| **Utilities** | **1-L** | **10-L** | **Justification** |
|---|---|---|---|
| **Electricity** | LED lights: 18 W, 10 days<br>$0.018\ kW\ \times\ 24\ h \times 10\ days\ \times \$\ 0.08\ per\ kWh = \$\ 1.45$<br>Air compressor: 30 W, 10 days<br>$0.030\ kW\ \times\ 24\ h \times 10\ days\ \times \$\ 0.08\ per\ kWh = \$\ 0.60$<br>Total: \$ 1.45 + \$ 0.60 = \$ 2.05 | | (Gao et al., 2016) |
| **Water** | 0.01 $m^3$ × \$0.48 per $m^3$<br>= \$0.0048 | | (Air Selangor, 2022) |
| **Chemicals** | See **Table 5.3 for detailed chemicals cost breakdown**<br>\$ 0.12 per litre × 10 L = \$ 1.20 | | Chemical price was source from local supplier |

| | | |
|---|---|---|
| **Total Operating Costs** | $ 2.05 (Electricity) + $ 0.0048 (Water) + $ 1.20 (Chemicals)<br><br>= $ 3.25 | |
| **Dry Biomass weight (g/L)** | 2.887 | 2.431 |
| **Cost of 1kg of *Spirulina* Biomass** | $ 75.59 | $ 89.77 |

*RM 1.00 = $ 0.21; currency rate based on October 2023

**Table 5.3** Detailed Zarrouk chemicals cost breakdown.

| **Zarrouk** | **g/L** | **$/kg** | **$** |
|---|---|---|---|
| **$NaNO_3$** | 2.5 | 6.30 | 0.01575 |
| **$K_2HPO_4$** | 0.5 | 12.60 | 0.00630 |
| **$MgSO_4.7H_2O$** | 0.2 | 7.35 | 0.00147 |
| **NaCl** | 1 | 6.09 | 0.00609 |
| **$K_2SO_4$** | 1 | 10.50 | 0.01050 |
| **$FeSO_4.7H_2O$** | 0.01 | 7.35 | 0.00007 |
| **$NaHCO_3$** | 18 | 4.41 | 0.07938 |
| **$CaCl_2.2H_2O$** | 0.04 | 8.82 | 0.00035 |
| **$Na_2EDTA$** | 0.08 | 16.80 | 0.00134 |
| **$H_3BO_3$** | 0.00286 | | 0.00002 |
| **$MnCl_2.4H_2O$** | 0.00181 | | 0.00003 |
| **$ZnSO_4.4H_2O$** | 0.000222 | 7.35 | 0.00000 |
| **$Na_2MoO_4$** | 0.0003 | 14.70 | 0.00001 |
| **$CuSO_4.5H_2O$** | 0.00007 | 11.34 | 0.00000 |
| **$Co(NO_3)_2.6H_2O$** | 0.00004 | 37.80 | 0.00000 |
| | | **Total** | **$ 0.12 per litre** |

## 5.6 CONCLUSION

This study demonstrates a continuous cultivation and harvesting strategy for biomass and phycocyanin production in the biorefinery of *Spirulina* microalgae. The results show that a 10% harvesting ratio provided consistent weights of harvested dry biomass for three consecutive days. Furthermore, $NaNO_3$ was found to be the most important nutrient for *Spirulina* cultivation, with the addition of 2.5 g/L significantly improving phycocyanin accumulation. The optimized parameters of a 10% harvesting ratio and 2.5 g/L of $NaNO_3$ addition were tested in a 10-L batch culture, which showed decreased biomass productivity compared to the 1-L batch culture, resulting in higher production costs. However, future studies should assess the economic feasibility of scaling up *Spirulina* production for commercial use. Additionally, further research could optimize the concentration of $NaNO_3$, $NaHCO_3$, and other nutrients for a cost-effective cultivation medium, investigate harvesting frequency, and explore alternative nitrogen sources that increase phycocyanin concentration and purity.

# CHAPTER 6

# PHYCOCYANIN PRODUCTION FROM *SPIRULINA* WITH THE NUTRIENT REPLACEMENT OF LOCAL SPENT COFFEE GROUND WASTE



## 6.1 ABSTRACT

The cultivation of *Spirulina* in various types of wastewaters has been studied, but it is crucial to select wastewater with low toxicity, such as tapioca and tofu wastewater, to avoid the production of harmful microcystins, which can promote tumor growth in humans; therefore, the current study proposes using spent coffee grounds as a medium for *Spirulina* cultivation. *Spirulina* microalgae were culture in spent coffee ground (SCG) extracts supplemented with different concentration (25%, 50%, 75%, 100%) of Zarrouk (Z) culture medium. Biomass productivity, phycocyanin accumulation and total protein of the harvested *Spirulina* biomass were analysed. The spent coffee ground used in the current study had a CHNS composition of C (21.44%), H (4.05%), N (5.95%) and S (3.22%). SCG extract supplement with 100% Z gives the highest biomass productivity of 0.0984 g/L/d. On the other hand, 25% Z show the highest TN, TP and COD percentage of removal efficiencies of 81%, 53% and 40%, respectively. Besides, the nutrients uptake pattern of the *Spirulina* microalgae in 10 days of cultivation were 67-272 mg/L of TN, 36-51 mg/L of TP and 354-418 mg/L of COD. Moreover 50% Z showed the highest phycocaynin accumulation and protein concentration of 160.778 mg/g and 427.640 mg/g, respectively. The current study has demonstrated the utilization of spent coffee ground to produce valuable phycocyanin bioactive compound and utilize the spent biomass for animal feed in achieving a sustainable circular bioeconomy.

***Keywords:*** *Spirulina*; spent coffee grounds; protein; phycocyanin; food waste

## 6.2 INTRODUCTION

*Spirulina* is widely known for its application in both food and pharmacological industry. Since the 70s, *Spirulina* has been considered a rich food source due to its high content of macro- and micronutrients (Fais et al., 2022). *Spirulina* is an excellent source of fatty acids, minerals, photosynthetic pigments, proteins, vitamins, and several secondary metabolites. *Spirulina* has a large amount of protein, with a dry weight of 60-70% (Vo, Ngo and Kim, 2015). Approximately 60% of the total protein content consists of phycobilisome, composed of C-phycocyanin (CPC) and allophycocyanin (APC) (ElFar et al., 2022). It is the presence of CPC and APC that gives *Spirulina* to possess multiple therapeutic effects including immunomodulation and booster, cholesterol depletion, anti-viral, anti-oxidant, anti-cancer, anti-allergic, anti-inflammatory, reducing blood glucose levels and anti-microbial activities (Braune et al., 2021; Liu and Blankenship, 2019; Wu et al., 2016). However, the large production of *Spirulina* biomass on an industrial scale is still hindered by the high cost of cultivation. Therefore, recent research has been exploiting the use of wastewater as a nutrient replacement for *Spirulina* cultivation. Nevertheless, it should be noted that wastewater contains toxic elements such as heavy metals, making *Spirulina* grown in such conditions unsuitable for human consumption. Moreover, from a psychological standpoint, it is unlikely that consumers would be willing to consume *Spirulina* if consumers were aware that it was cultivated in wastewater.

Recent studies have reported on the cultivation of *Spirulina* in many types of wastewater such as dye wastewater (Ismail et al., 2021), aquaculture wastewater (Cardoso et al., 2021), sewage sludge wastewater (Zheng and Zhang, 2021) and rubber wastewater (Hadiyanto et al., 2020). However, microalgae cultivate in a

body of water that are polluted with heavy metals, bacteria or harmful particles may produce toxins called microcystins (SaVanna et al., 2021). Microcystins are produced as a defence mechanism against predators. However, microsystins exposure to human may promote the growth of tumours in the liver and other organs (Schmidt, Wilhelm and Boyer, 2014). *Spirulina* grown in controlled environments exhibit lower level of microcystins (Scoglio, 2018; Roy-Lachapelle et al., 2017). Therefore, the selection of the type of wastewater medium for *Spirulina* cultivation is very important. Food-type wastewater such as tapioca wastewater and tofu wastewater are recommended for the cultivation of *Spirulina* as there are less toxic and the cultivation environment is less robust (Nur et al., 2023; Hadiyanto, Christwardana and da Costa, 2023). Thus, the current study suggests the use of spent coffee ground for the cultivation of *Spirulina*.

Each day, a staggering 2.25 billion cups of coffee are consumed globally, with an average utilization of 11 grams per cup, resulting in an annual accumulation of 18 million tonnes of discarded coffee grounds. (Mauro, 2022). Researcher have been utilizing spent coffee grounds to improve soil quality and nutritional value in agricultural products such as lettuce, wheat-soybeans, basil and tomato potting plants (Cervera-Mata et al., 2019; Hirooka et al., 2022; Ronga et al., 2016). Thus, the idea of utilizing spent coffee grounds for the cultivation of *Spirulina* came about. Spent coffee grounds have been categorized as agricultural waste with high carbon content and entrapped nutrients such as fatty acids, amino acids and polysaccharides (Rosmahadi et al., 2022). To the best of the author's knowledge, there are limited studies reported on the utilization of spent coffee grounds for the cultivation of *Spirulina*.

This study proposes the novel idea of using spent coffee grounds, an abundant agricultural waste, as an alternative nutrient source for the cultivation medium of *Spirulina*. To the best of the author's knowledge, there are limited studies on the use of spent coffee grounds specifically for *Spirulina* cultivation. The spent coffee grounds were subjected to CHNS (Carbon-Hydrogen-Nitrogen-Sulphur) analysis to determine its composition. The experiments were performed in closed-system photobioreactors (1-L) using 100% spent coffee ground extract supplemented with varying proportions (25%, 50%, 75% and 100%) of Zarrouk medium. The biomass productivity and phycocyanin accumulation were evaluated. Additionally, the nutrient removal of the cultivation medium, including total nitrogen (TN), total phosphate (TP) and chemical oxygen demand (COD) was assessed. Finally, the spent biomass obtained after the extraction of phycocyanin were subjected to protein analysis to determine the protein concentration. This study contributes to the research field of microalgae by exploring the potential utilization of spent coffee grounds as a sustainable and cost-effective medium for cultivating *Spirulina,* while also evaluating its nutrient removal capacity and biomass productivity.

## 6.3 MATERIALS AND METHODS

### 6.3.1 MICROALGAL CULTIVATION

The microalgal species, *Spirulina platensis*, was obtained from Biolina Corporation Sdn. Bhd (Malaysia). The culture medium used to cultivate *Spirulina* in this research was Zarrouk medium. A 100 mL of *Spirulina* culture starters was added into the medium in a 1-L Schott bottle as the photobioreactor.

Aeration was supplied by an air compressor with a flow rate of 400 cc/min continuously under an ambient temperature of 25 ℃ ± 1 ℃. The *Spirulina* culture was illuminated continuously with a cool-white, LED light at light intensity about 3000 lux. All *Spirulina* culture were cultivated until Day 10 to ensure consistency, where they had achieved the late-log growth phase before being harvested.

### 6.3.2 EXPERIMENTAL SETUPS FOR *SPIRULINA* CULTIVATION WITH SPENT COFFEE GROUNDS

Spent coffee grounds (SCG) was collected from local Starbucks, Malaysia. The SCG were washed thoroughly to remove impurities and dried in the oven at 70 ℃ till constant weight. 8 g/L of SCG extract was prepared by mixing the SCG in distilled water and subject to autoclave at 121℃. This help to break down some of the organic compounds present in the SCG, potentially making them more accessible for microalgae assimilation. The SCG extract was left overnight before utilizing for experimental studies. The experiments were performed using 100% SCG extract with different Zarrouk supplementations: 0%, 25%, 50%, 75% and 100%. The culture time was 10 days for every batch culture to ensure consistency.

### 6.3.3 BIOMASS PRODUCTIVITY

At the end of exponential growth phase (Day 10), the biomass was harvested by centrifugation. *Spirulina* biomass were separated from the supernatant by centrifugation at 6500 rpm for 5 minutes (Koyande et al., 2019). The supernatant was then discarded, and the wet biomass was freeze-dried and stored at -20℃

for further analysis. The freeze-dried biomass was used for proximate analysis to determine the phycocyanin and protein content.

The biomass productivity ($P_b$) were calculated based on **Equation (6.1)** (Chew et al., 2018a):

$$P_b \text{ (mg/L/day)} = (N_2\text{-}N_1)/(t_2\text{-}t_1) \quad (6.1)$$

where, $N_1$ and $N_2$ are biomass concentration (g/L) at time $t_1$ and $t_2$, respectively.

### 6.3.4 PHYCOCYANIN QUANTIFICATION

The pellet was mixed with 50 ml of 0.1 M phosphate buffer (pH 6.9) containing 0.1 M of $K_2HPO_4$ and 0.1 M $KH_2PO_4$. The phycocyanin was extracted by ultrasonic sonication at an amplitude of 35 kHz for 5 min. The cell debris was discarded by centrifugation at 6500 rpm for 5 min and the extract therefore obtained was termed as crude phycocyanin extract.

The C-phycocyanin (CPC) and allophycocyanin (APC) concentration were calculated based on **Equation (6.2)** and **Equation (6.3)** (Chia et al., 2020; Chew et al., 2019).

$$CPC\ (mg/ml) = \frac{OD_{615} - 0.474 \times OD_{652}}{5.34} \quad (6.2)$$

$$APC\ (mg/ml) = \frac{OD_{652} - 0.208 \times OD_{615}}{5.09} \quad (6.3)$$

Where, $OD_{615}$ and $OD_{652}$ is the optical density wavelength at 615 nm and 652 nm, respectively.

The purity of CPC and APC were calculated based on **Equation (4)** and **Equation (5)** (Chia et al., 2020; Chew et al., 2019).

$$Purity_{CPC} = \frac{OD_{620}}{OD_{280}} \quad (6.4)$$

$$Purity_{APC} = \frac{OD_{652}}{OD_{280}} \quad (6.5)$$

Where, $OD_{280}$, $OD_{620}$, and $OD_{652}$ is the optical density wavelength at 280 nm, 625 nm and 652 nm, respectively.

### 6.3.5 NUTRIENTS REMOVAL ANALYSIS

The SCG extract characteristics were determined following American Public Health Association on Standard Methods on the examination of water and wastewater (Cheah et al., 2018). The examination on chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) of SCG extract were conducted using Hach standard reagent and DR 2800 spectrophotometer wastewater (Cheah et al., 2018).

### 6.3.6 CHNS ANALYSIS

The carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) were measured using CHNS elemental analyser (CHNS/O 2400 Series II, Perkin Elmer, Malaysia). The 2,5-bis(5-tert-2-benzo-oxazol-2-yl) thiophene (BBOT) standard was used as the calibration materials for CHNS content determination (Hu et al., 2022). Weighed samples are combusted completely in the presence of excess oxygen and reduced to the elemental gases of $CO_2$, $H_2O$, $N_2$ and $SO_2$ in the reactor chamber of the equipment. These gases are measured by a thermal conductivity detector of the analyser (Tursunov, Dobrowolski and Nowak, 2015).

### 6.3.7 PROTEIN ANALYSIS

Proteins were extracted using the method of Rausch (1981) and total proteins were measured using method of Bradford (1976). Spectrophotometric

measurements were done at 595 nm using a UV-Vis spectrophotometer (UV-1800, Shimadzu, Japan). The percent of total proteins was calculated from a calibration curve made in the range of 0.03125 to 1.0 mg/ml standard grade bovine serum albumin (BSA). Protein concentration was calculated using **Equation (6.6) (**Bataller and Capareda, 2018**):**

$$Protein\ (mg/g) = \frac{(Absorbance - Intercept) \times Total\ extract\ (mL)}{Sample\ weight\ (g) \times Slope} \quad (6.6)$$

## 6.4. RESULTS AND DISCUSSION

### 6.4.1 CHARACTERISATION OF SPENT COFFEE GROUND

The spent coffee ground used in the current study had a CHNS composition of C (21.44%), H (4.05%), N (5.95%) and S (3.22%) as shown in **Table 6.1**. It shows a lower carbon and hydrogen content but exhibit higher nitrogen and sulphur content as compared to other research studies. The results of lower carbon content can vary depending on factors such as the type of coffee bean and the roasting process. Nonetheless, these shows a good nutrient source for the cultivation of microalgae. Nitrogen source is important for the synthesis of proteins, nucleic acids and chlorophyll content in microalgae (Wu and Miao, 2014). Sulphur are required for photosynthesis, respiration, nitrogen assimilation carbohydrate metabolism and response to abiotic and biotic stresses of microalgae (Zhang et al., 2020).

**Table 6.1** Comparative analysis of CHNS content in spent coffee grounds

| C (%) | H (%) | N (%) | S (%) | References |
|---|---|---|---|---|
| **21.44%** | 4.05% | 5.95% | 3.22% | Current study |
| **49.23%** | 6.53% | 2.63% | 0.03% | (Kaya, 2020) |
| **54.9%** | 7.9% | 3.51% | 0.00% | (Ktori, Kamaterou and Zabaniotou, 2018) |
| **52.98%** | 7.59% | 3.78% | 0.31% | (Lee et al., 2022) |
| **52.35%** | 7.04% | 2.27% | 0.09% | (Semaan et al., 2023) |

### 6.4.2 BIOMASS PRODUCTIVITY OF *SPIRULINA* CULTURE IN SCG EXTRACT SUPPLEMENTED WITH DIFFERENT ZARROUK CONCENTRATION

*Spirulina* were cultivated in SCG extract supplemented with different concentration (25%, 50%, 75%, 100%) of Zarrouk medium. These were compared against a control that is cultivated in 100% Zarrouk medium prepared with distilled water. The biomass productivity of 25% Z, 50% Z, 75% Z and 100% Z were 0.0952 ± 0.0040 g/L/d, 0.0846 ± 0.0041 g/L/d, 0.0900 ± 0.0090 g/L/d and 0.0984 ± 0.0099 g/L/d, respectively as shown in **Figure 6.1**. All the biomass productivity of *Spirulina* culture in SCG extracted were lower as compared to the control which had a biomass productivity of 0.1623 ± 0.0100 g/L/d. This could have been due to the dark brown colour of SCG extracts resulting in low penetration light that causes shading effects on the *Spirulina*, ineffective photosynthesis will affect biomass productivity (Ummalyma et al., 2022). Mezzomo et al. (2010a) reported that swine wastewater was toxic for the

microalgae when used without dilution, because the coloration of the wastewater causes low penetration light in the culture medium, decreasing the growth. In the current study, 8 g/L of SCG was used to prepare the SCG extract. Therefore, future studies can investigate the growth of *Spirulina* culture in lower concentration of SCG extract. Although biomass productivities were lower as compared to control, the current findings have demonstrated another important finding which shows that the biomass productivity of 25% Z is comparable with 100% Z. This suggests that 25% Zarrouk medium supplement is sufficient to achieve a comparable biomass productivity while reducing the overall chemical input. This finding highlights the potential for optimizing resource utilization and minimizing environmental impact in biomass production processes.

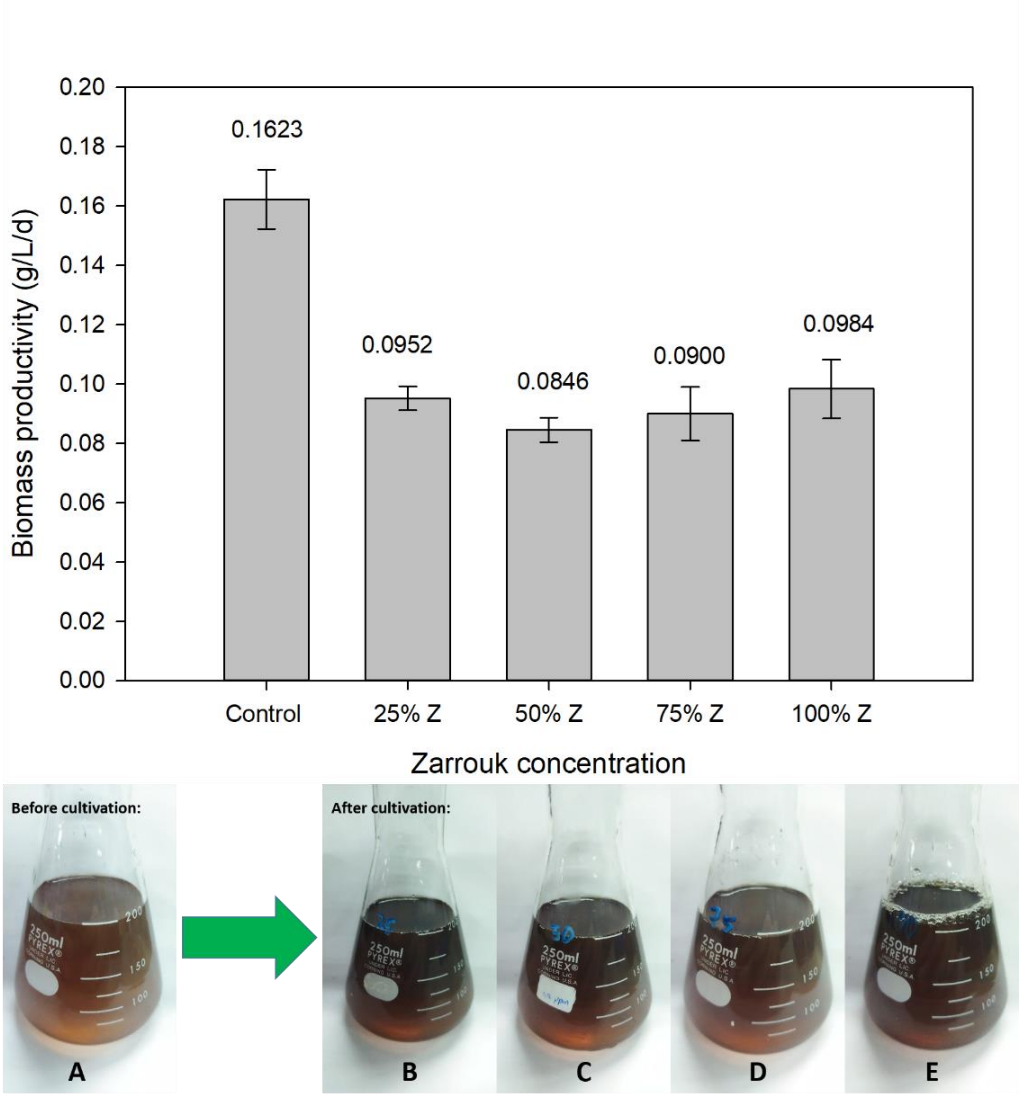



**Figure 6.1** Biomass productivity of *Spirulina* cultures in SCG extract supplemented with different concentrations of Zarrouk culture medium. (A) SCG extract (B) Recovered SCG extract (25% Z) (C) Recovered SCG extract (50% Z) (D) Recovered SCG extract (75% Z) (E) Recovered SCG extract (100% Z)

### 6.4.3 NUTRIENTS REMOVAL OF TN, TP AND COD

The parameters analysed in the study include total nitrogen (TN), total phosphate (TP) and chemical oxygen demand (COD). **Figure 6.2** shown the

initial and final concentration of TN, TP and COD of the different Zarrouk concentration supplemented in SCG extract and its percentage of removal efficiencies. Nutrients were measured on the 1$^{st}$ day and 10$^{th}$ day of the cultivation cycle. During the initial pre-trial experiments, *Spirulina* culture was cultivated in SCG extract without any supplement, but it cannot survive in the medium. The pH of the SCG extract was found to be pH 5.38. Although pH is considered one factor that might affect the growth of *Spirulina* culture but after the inoculation of *Spirulina* culture in SCG extract, the pH of the SCG extract increases to pH 10. *Spirulina* is an alkaliphile microalgae where it tends to grow better in a high pH culture medium (Zhang et al., 2018). **Figure 6.2(D)** shows that the final pH of all the batch culture at Day 10 were all above pH 10. On the other hand, this could have been due to low nutrients content present in the SCG extract that causes *Spirulina* unable to grow in the medium. The TN and TP concentration of SCG extract without any supplement were found to be between 10.00-22.67 mg/L and 67.50-78.83 mg/L, respectively. Therefore, SCG extract was decided to be supplemented with Zarrouk culture medium to ensure the growth of *Spirulina* culture. Among the different Zarrouk concentration, 25% Z show the highest TN, TP and COD percentage of removal efficiencies of 81%, 53% and 40%, respectively. The current findings have also demonstrated an important finding on the nutrient's uptake pattern of the *Spirulina* microalgae strains being studied. In 10 days of cultivation, the nutrients uptake ranges of TN, TP and COD are 67-272 mg/L, 36-51 mg/L and 354-418 mg/L, respectively. This finding is important to optimise the chemical composition of culture medium supplemented in SCG extract for the cultivation of *Spirulina*.

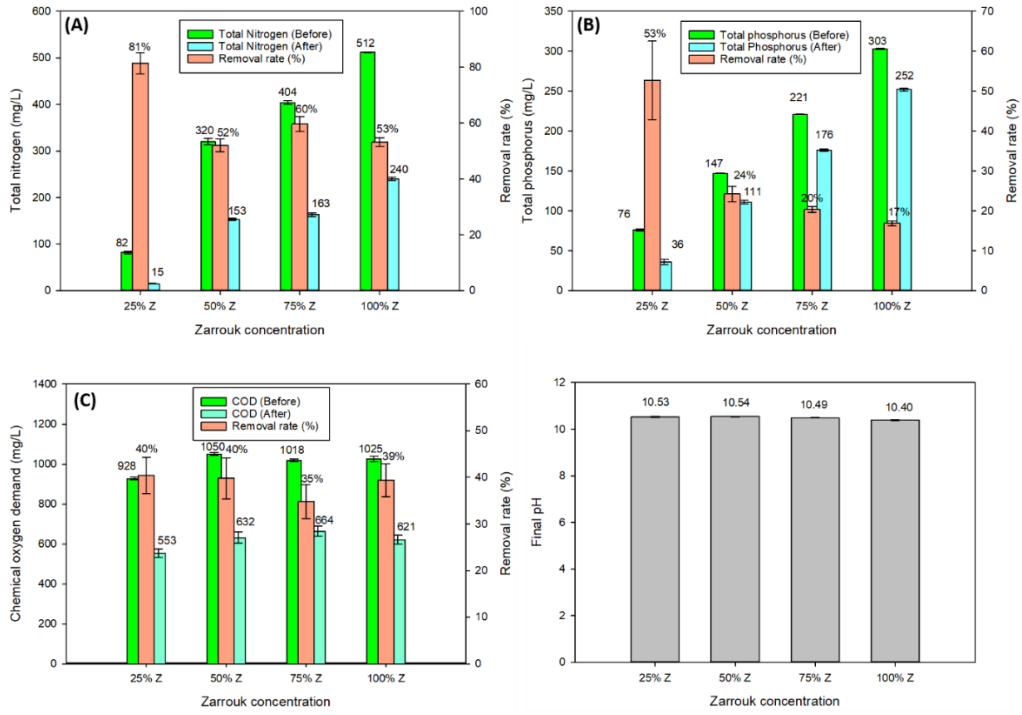



**Figure 6.2** Nutrient removal analysis of *Spirulina* culture in SCG extract supplemented with different concentrations of Zarrouk culture medium. (A) total nitrogen (B) total phosphate (C) chemical oxygen demand (D) Final pH

### 6.4.4 PHYCOCYANIN PRODUCTIVITIES OF *SPIRULINA* BIOMASS CULTIVATED WITH SPENT COFFEE GROUNDS

**Figure 6.3** shows the effect of SCG extract supplemented with different concentration of Zarrouk culture medium on phycocyanin accumulation in *Spirulina* biomass. Approximately 60% of the total protein content in *Spirulina* biomass consists of phycobilisome, composed of C-phycocyanin (CPC) and allophycocyanin (APC) (ElFar et al., 2022). Therefore, it is an important bio-compound to analyse. The CPC concentration of 25% Z, 50% Z, 75% Z and 100% Z were 137.815, 160.778, 151.511 and 140.066 mg/g respectively while the APC concentration were 38.200, 42.988, 41.842 and 38.102 mg/g respectively.

The phycocyanin accumulation of *Spirulina* culture in SCG extract supplement with Zarrouk culture medium were all lower as compared to the control (CPC: 166.515 mg/g; APC: 42.674 mg/g). The control was set to cultivate *Spirulina* culture in distilled water supplement with Zarrouk culture medium. The main factor that causes low phycocyanin accumulation is because of the dark brown colour of the SCG extract that reduces light penetration. At low light intensity, photo-limitation phenomenon occurred where the microalgal cells would encounter light scarcity, restricting its photosynthetic activity, resulting in lower phycocyanin accumulation (Wong et al., 2022). On the other hand, the phycocyanin accumulation increased from 25% Z to 50% Z and then started to decrease until 100% Z with 50% Z showing the highest phycocyanin accumulation of 160.778 mg/g. This trend can be related with the concentration of TN. As mentioned earlier, nitrogen sources are crucial for the synthesis of proteins, nucleic acids and chlorophyll content in microalgae (Wu and Miao, 2014). The TN concentration uptake for the culture conditions of 25% Z, 50% Z, 75% Z and 100% Z were 67 mg/L. 166 mg/L, 241 mg/L and 272 mg/L, respectively. In the Zarrouk culture medium, the only nitrogen source is derived from 2.5 g of $NaNO_3$, with 50% Z representing a concentration of 1.25 g of $NaNO_3$. Manirafasha et al. (2018) also suggested that the nitrate concentration in culture should be kept in the range between 1.2 to 1.6 g/L for high content of phycocyanin accumulation. The current findings demonstrate a positive correlation between the concentration of total nitrogen in the culture medium and the accumulation of phycocyanin, indicating the importance of nitrogen as a key factor in *Spirulina* phycocyanin synthesis.

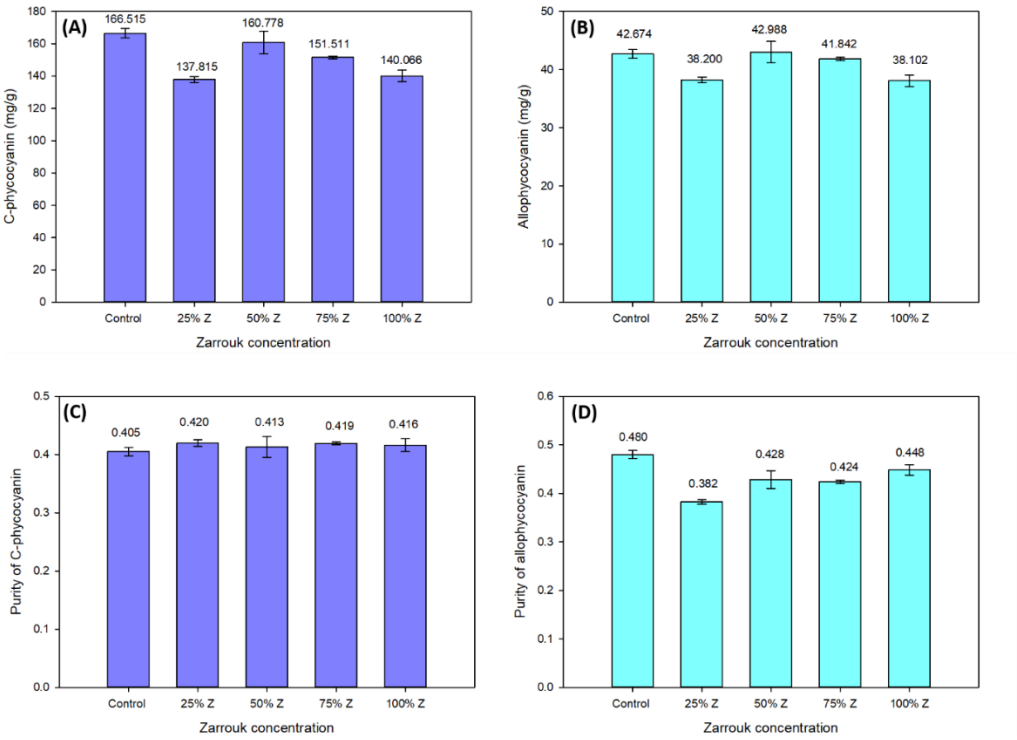



**Figure 6.3** Phycocyanin accumulation of *Spirulina* cultures in SCG extract supplemented with different concentrations of Zarrouk culture medium (A) C-phycocyanin concentration, (B) Allo-phycocyanin concentration (C) C-phycocyanin purity and (D) Allo-phycocyanin purity

### 6.4.5 PROTEIN ANALYSIS OF SPENT *SPIRULINA* BIOMASS AFTER EXTRACTION OF PHYCOCYANIN

The spent *Spirulina* biomass recovered from the extraction of phycocyanin process were oven dried overnight and subject for protein analysis. The protein concentration of 25% Z, 50% Z, 75% Z and 100% Z are 355.334 mg/g, 427.640 mg/g, 400.902 mg/g and 400.776 mg/g, respectively as shown in **Figure 6.4**. These samples were compared against a control where the *Spirulina* was cultured in distilled water medium and subjected to similar phycocyanin

extraction process. The protein concentration in all the *Spirulina* culture supplemented with Zarrouk culture medium (SCG extract) were all lower compared to the control (479.646 mg/g). Bataller and Capareda (2018) reported a total protein of 55.52% for *Arthrospira platensis* before extraction, which decreased to 33% after phycocyanin extraction. The report uses *Spirulina* powder directly and does not provide information on the cultivation duration since the study focuses on optimising extraction methods. In contrast, the current study reported a higher protein content of 47% and even *Spirulina* culture in SCG extract, the protein concentration range is between 35-42%. Moreover, the protein concentration shows a similar trend with phycocyanin accumulation where 50% Z gives the highest protein concentration as discussed in previous section. While the primary value of *Spirulina* biomass lies in its phycocyanin active biocompound, the spent biomass can still be utilized and converted into animal feed due to its remaining high protein content available.

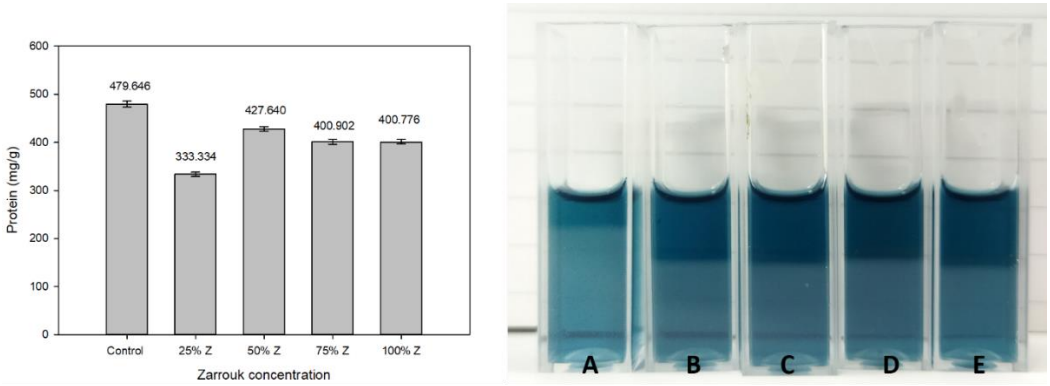



**Figure 6.4** Protein analysis of spent *Spirulina* biomass following phycocyanin extraction.

## 6.5 FUTURE PROSPECTS

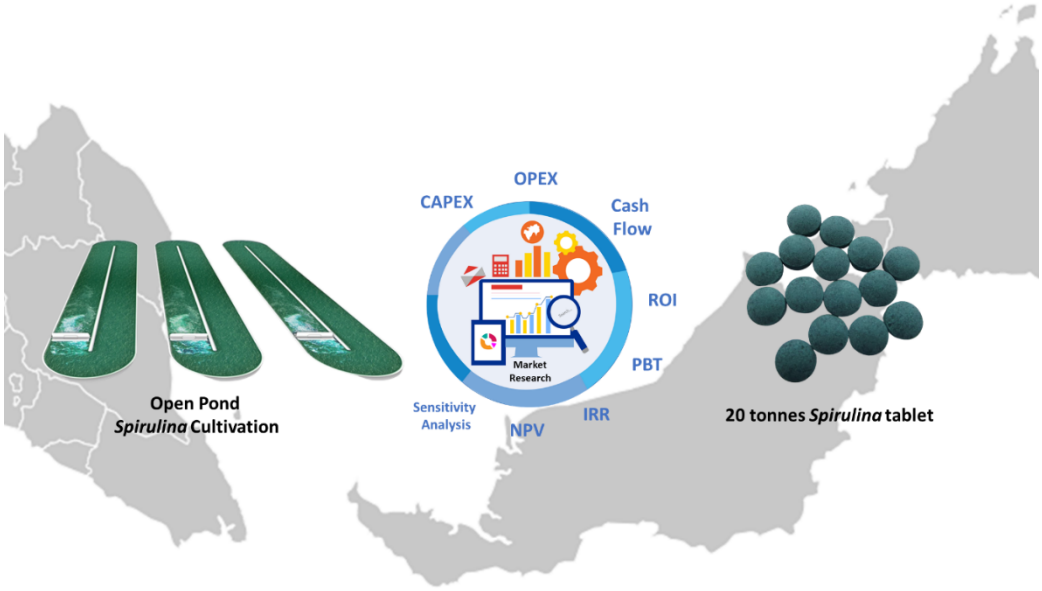
To the best of the authors knowledge, there have been limited studies reported on utilising spent coffee ground for microalgae cultivation. In a study by Rosmahadi et al. (2022), the authors investigated the use of spent coffee grounds (SCG) as a support material for the growth of *Chlorella vulgaris* microalgae. They found that an SCG dosage of 8 g/L was optimal for enhancing microalgal growth, with a density of 2.43 ± 0.04 g/g-SCG achieved under continuous illumination. Moreover, an optimised 20:4 photoperiod regime with a 4-hour dark period and 20 hours of illumination yielded a maximum microalgal density of 3.970 ± 0.8 g/g-SCG. Further research conducted by Wong et al. (2022) in this field showed that the biodiesel yielded from the optimum condition possessed high cetane number and oxidative stability due to the high saturated fatty acid methyl esters content of 63.7 % (Wong et al., 2022). The major FAME compositions ranged within C16 to C18 which were favourable for quality biodiesel production. While previous studies focused on utilising spent coffee grounds for microalgae cultivation for biofuel application, the current study uses *Spirulina* microalgae and focus on extraction of bioactive compound and utilising spent biomass for animal feed. Future research can investigate various aspects, such as exploring different dosages of spent coffee grounds, analyzing the composition of fatty acid methyl esters (FAMEs), and studying the growth of microalgae species other than *Chlorella vulgaris*. Additionally, it would be beneficial to investigate the potential applications of the extracted bioactive compounds and further explore the feasibility of utilizing spent coffee grounds for other value-added products.

## 6.6 CONCLUSION

The study had successfully utilized spent coffee grounds extract supplemented with Zarrouk culture medium for the cultivation of *Spirulina*. SCG extract supplement with 100% Z shows the highest biomass productivity of 0.0984 g/L/d. On the other hand, 25% Z show the highest TN, TP and COD percentage of removal efficiencies of 81%, 53% and 40%, respectively. Besides, the nutrients uptake pattern of the *Spirulina* microalgae in 10 days of cultivation were 67-272 mg/L of TN, 36-51 mg/L of TP and 354-418 mg/L of COD. Moreover 50% Z showed the highest phycocaynin accumulation and protein concentration of 160.778 mg/g and 427.640 mg/g, respectively. The current study has demonstrated the utilization of spent coffee ground to produce valuable phycocyanin bioactive compound and utilize the spent biomass for animal feed in achieving a sustainable circular bioeconomy. This opens up possibilities for reducing the environmental impact associated with the disposal of coffee waste while simultaneously promoting the production of valuable microalgae biomass.

# CHAPTER 7

# TECHNO-ECONOMIC ASSESSMENTS ON THE PRODUCTION OF *SPIRULINA* TABLET FROM LARGE SCALE OPEN POND CULTIVATION OF *SPIRULINA* IN MALAYSIA



## 7.1 ABSTRACT

The microalgae industry in Malaysia is currently in its infancy phase, with the government implementing various initiatives and policies to foster its growth. As part of this development, it becomes crucial to assess the feasibility and potential profitability of the production of *Spirulina* tablets in Malaysia. A techno-economic assessment study was conducted on the production of 20 tonnes of *Spirulina* tablet. The study included a comparative cost analysis of two different mediums, namely Zarrouk and fertilizer medium, used in the production process. The total fixed capital investment for the production is RM 5.3 million, and the annual operating cost is RM 3.3 million. The findings indicate that producing 20 tonnes of *Spirulina* tablets using a fertilizer medium yields higher profitability compared to the conventional Zarrouk medium. With the fertilizer medium, the return on investment (ROI) is 69%, the payback period (PBT) is 3.74 years, the net present value (NPV) is RM 5.5 million, and the internal rate of return (IRR) is 46%. On the other hand, with the Zarrouk medium, the ROI is 53%, the PBT is 4.32 years, the NPV is RM 3.6 million, and the IRR is 35%. Moreover, a sensitivity analysis identified that selling price, chemical cost, sales volume, and dry biomass weight has the most significant impact on the financial performance of the *Spirulina* plant.

***Keywords:*** *Spirulina*, economic, techno-economic, large-scale, production

## 7.2 INTRODUCTION

*Spirulina* is a type of blue-green algae that is commonly sold in supplement form and is claimed to have numerous health benefits. It is high in protein, vitamins,

and minerals, including protein, vitamins B1, B2, and B3, iron, and magnesium. Studies have found that *Spirulina* may help lower cholesterol and blood pressure levels in people with high cholesterol or hypertension, and it may also have anti-cancer properties and be effective in reducing the growth and spread of cancer cells (ElFar et al., 2022). Additionally, *Spirulina* may help improve immune function and reduce the risk of infections (ElFar et al., 2022). It may also improve muscle strength and endurance, potentially making it a useful supplement for athletes (Carvalho et al., 2018). Due to its potential health benefits, *Spirulina* is attracting significant interest in the health and wellness sector, creating a lucrative market with promising investment opportunities.

Malaysia is a strategic location for microalgae agriculture due to its consistent warm and humid climate, which allows for year-round growth of microalgae. Sarawak's five-hectare microalgae production facility was launched in February 2023, will utilize carbon dioxide ($CO_2$) waste from the Sejingkat Coal Power Station to cultivate microalgae (Nur Shazreena, 2022). This is a collaboration between Sarawak Biodiversity Centre, Sarawak Energy, Chitose Laboratory, and ENEOS Corp. This initiative not only will help reduce $CO_2$ emissions through carbon capture and utilization, but it will also create downstream opportunities across various sectors. In addition, The National Biotechnology Policy 2.0 (DBN 2.0) has recognized the potential of microalgae as a source of food and feed, as well as for the production of biofuels and other bioproducts (A. Azim, 2022). DBN 2.0 aims to encourage the development of local players in the field through the ministry's Bio-based Accelerator (BBA) program and allocate funding for research and development in the microalgae industry. This new policy will not only help Malaysia achieve high-technology nation status

by 2030, but also address issues such as food security, pandemic management, and climate change through the use of innovative biotechnology solutions.

A recent study by Pinto et al. (2022) evaluated the economics of extracting lipids and phycocyanin together from *Spirulina* and found that while none of the scenarios with lipid extraction were profitable, there is potential to be if $CO_2$ is captured and the process is optimized. They concluded that extracting phycocyanin alone would be more profitable but integrating the lipid extraction could help the microalgae access the lipid market. Taufikurahman et al. (2020) investigated using anaerobically digested dairy manure wastewater as an alternative. They tested different combinations in a laboratory-scale to find the best medium composition to produce the most phycocyanin from *Spirulina*. An economic analysis added that this method would be economically feasible on an industrial-scale, as long as there is a 10-year lifetime. The overall return on investment would be high with a gross profit margin of 12.40 and an internal rate of return of 23%, with a payback period of 3.13 years. However, further research is needed to make sure it works in industry.

Due to the good weather and implemented policy in Malaysia, as well as the ongoing microalgae start-up company in Sarawak, there is a very big potential for the microalgae industry in the country. In particular, *Spirulina* cultivation for phycocyanin production has been identified as a promising area for further study and development. Phycocyanin is a pigment found in *Spirulina* that has anti-inflammatory properties and is being explored for its potential use in various industries. As a result, this paper aims to conduct a techno-economic assessment (TEA) of *Spirulina* cultivation for *Spirulina* tablet production in order to evaluate the feasibility and potential profitability of this activity in Malaysia.

TEA is an important tool for evaluating the financial viability of a *Spirulina* production plant. It considers all cost factors, from fixed capital investment to operational cost and future cash flow. By including sensitivity analysis, TEA provides an overview to guide investors. This allows them to make informed decisions about the profitability and feasibility of their *Spirulina* venture.

## 7.3 MATERIALS AND METHODS

### 7.3.1 PROCESS DESCRIPTIONS

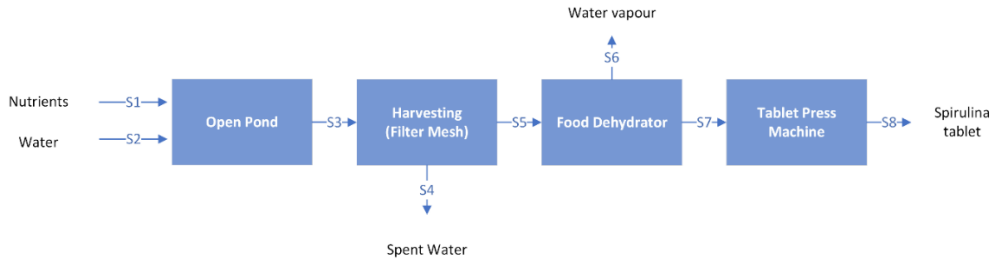



**Figure 7.1** Block flow diagram of the production of 20 tonnes of *Spirulina* tablet.

**Figure 7.1** shows the block flow diagram of the production of 20 tonnes of *Spirulina* tablet. The process description of each stage is discussed in detail in the following section.

#### 7.3.1.1 OPEN POND – CULTIVATION STAGE

A raceway pond of dimensions 25 m in length, 6 m in width, and 0.5 m in height has been designed to have a total volume of 75 $m^3$. In order to minimize water evaporation, the raceway pond will be lined with High Density Polyethylene (HDPE) (Asiedu et al., 2018). The thickness of the HDPE liner suggested by the suppliers is 0.50 mm. HDPE liners are known for their durability, with a life expectancy of 20 years, chemical resistance, ease of installation, and low maintenance requirements (Canter et al., 2014). In this techno-economic

assessment, it is assumed that 16 units of such raceway ponds will be installed inside a transparent greenhouse to make sure it is a close environment and to minimise contamination. The cost of greenhouse is RM171.60 per $m^2$ (Jovicich et al., 2005). Sunlight will be the source of illumination for the microalgae with an assumption of water loss of 1 cm per day (Benemann et al., 1982). Each raceway pond will have a paddle wheel aerators (TWC-10, THK Sales & Service (M) Sdn Bhd, Malaysia) to aerate the raceway pond. Moreover, the cost of paddle wheel aerators source from the supplier is in line with Jayanthi et al. (2021) study. In regards to *Spirulina* biomass productivity, it is assumed to be 1 g/L, although higher yields have been reported in other studies (Lim et al., 2021). The cultivation medium assume to be used is Zarrouk medium (Dineshkumar, Narendran and Sampathkumar, 2016a). Each cultivation batch is expected to last 10 days before harvesting, leading to a monthly harvest of twice. The remaining days are for harvesting and drying of mass *Spirulina* biomass.

#### 7.3.1.2 HARVEST STAGE

Mostly commercial microalgae industries use filtration equipment for harvesting. Such equipment known as inclined screens that are installed with 380-500 mesh with a filtration area of 2-4 $m^2$ per unit and are capable of harvesting 10-18 $m^3$ of *Spirulina* culture per hour (Vonshak and Richmond, 1988). The biomass removal efficiency is high up to 95% with a slurry of 8-10% of dry weight produced (Vonshak and Richmond, 1988). The electrical energy per unit of dry algae of inclined screen is 0.14 MJ/kg (Furuhashi et al., 2020).

### 7.3.1.3 DRYING STAGE

For a start-up business, it would be difficult to afford expensive equipment such as spray dryer or freeze dryer. Therefore, it was recommended to use food dehydrator in the process of biomass drying. This F-50 industrial food dehydrator (Foodehy S.L. company) is designed to efficiently and effectively dehydrate food products. Made of durable stainless steel, the dehydrator is powered by electricity, using less than 9 kW. The thermal energy needed for the process is provided by a gas boiler that can deliver up to 60 kW of power. This gas boiler can be substituted with a biomass boiler or adapted to an existing boiler in the client's facility. The dehydrator has adjustable drying air speed and can achieve a maximum drying temperature of 70ºC. The touch screen provides real-time data on temperature and humidity, and is programmable for both continuous and discontinuous drying processes. It can also be programmed for dehydration in stages, ensuring homogeneous dehydration throughout the process. The dehydrator can accommodate up to 50 $m^2$ of product and comes with stainless steel meshed trays. These trays can be further complemented with non-stick meshes. Additionally, the dehydrator has real-time product weight control and can be remotely controlled from a desktop computer, smart tablet, or smartphone.

### 7.3.1.4 TABLET PRESS MACHINE

The ZP-31 (Kimah Industrial Supplies (M) Sdn Bhd, Malaysia) is a compact and efficient tablet press machine with a 31-set die capability. It can produce tablets with a maximum pressure of 80 KN, with a maximum diameter of 20 mm and a maximum depth of 15 mm. The tablet thickness can be adjusted up to 6 mm. The turret speed is adjustable between 14-36 revolutions per minute, giving a

maximum capacity of 130,000 tablets per hour. The machine is powered by a 5.5 kw motor and has a compact dimension of 1170 mm (L) x 1150 mm (W) x 1750 mm (H). It has a total weight of 1950kg.

### 7.3.2 TECHNO-ECONOMIC ANALYSIS METHODS

In order to assess the economic viability of the production of 20 tonnes *Spirulina* tablet in Malaysia, this study conducted a comprehensive analysis of the financial aspects of the proposed system. Firstly, the total fixed capital investment required for the production of 20 tonnes of *Spirulina* tablets was estimated by gathering quotations from various local suppliers in Malaysia, and the total production cost was estimated. Major equipment such as open pond, starter pond, greenhouses, paddle wheels' aerators, harvesting filtration system, food dehydrators and tablet press machine. Whereas the operating cost focuses on three major factors which are chemical cost (medium for *Spirulina* cultivation), electricity utility cost and water utility cost. A cumulative cash flow analysis was then conducted, based on the projected lifespan of the plant (10 years), and various financial metrics, such as payback time (PBT), return on investment (ROI), net present value (NPV) and internal rate of return (IRR) were evaluated (Towler and Sinnott, 2013).

Payback time:

$$PBT = \frac{total\ investment}{average\ annual\ cash\ flow} \quad (1)$$

Return of investment:

$$ROI = \frac{net\ annual\ profit}{total\ investment} \times 100\% \quad (2)$$

Net Present Value:

$$NPV = \sum_{n=1}^{n=t} \frac{CF_n}{(1+i)^n} \quad (3)$$

Where $CF_n$ = cash flow in year n

$t$ = project life in years

$i$ = interest rate (= cost of capital, percent /100)

Internal rate of returns:

$$IRR = \sum_{n=1}^{n=t} \frac{CF_n}{(1+i')^n} = 0 \quad (4)$$

Where $CF_n$ = cash flow in year n

$t$ = project life in years

$i'$ = the discounted cash-flow rate of return (percent /100)

Sensitivity analysis was also conducted, to anticipate potential fluctuations in the system's performance, by varying seven key variables, such as selling price, yield of *Spirulina* biomass weight, sales volume, chemical cost, electricity cost, and water cost, by ±20%. Overall, the results of the economic analysis will provide insights into the financial prospects of the proposed 20 tonnes *Spirulina* tablet production in Malaysia.

## 7.4 RESULTS AND DISCUSSION

### 7.4.1 FIXED CAPITAL INVESTMENT (FCI)

The techno-economic analysis of the production of *Spirulina* tablet from *Spirulina* cultivation in 16 units of large scale open-pond in country Malaysia is studied using several analyses. The cost of item 1-7 was source from local suppliers in Malaysia or literature studies. Whereas, warehouse, prorateable

expenses, field expenses, home office & construction and project contingency were assumed 4%, 10%, 10%, 20% and 10%, respectively of the total direct cost (TDC) (Davis et al., 2016). The working capital investment was assumed as 20% of the fixed capital investment (Taufikurahman et al., 2020). Therefore, the total fixed capital investment consists of TDC and working capital investment (WCI). The fixed capital investment for the production of 20 tonnes of *Spirulina* tablet is estimated to be RM 4.5 million. Details cost breakdown of FCI is presented in **Table 7.1** Based on **Figure 7.2**, the construction of open pond contributes the highest 41% of FCI follow by greenhouses (19% of FCI) and home office & construction (13% of FCI) excluding WCI.

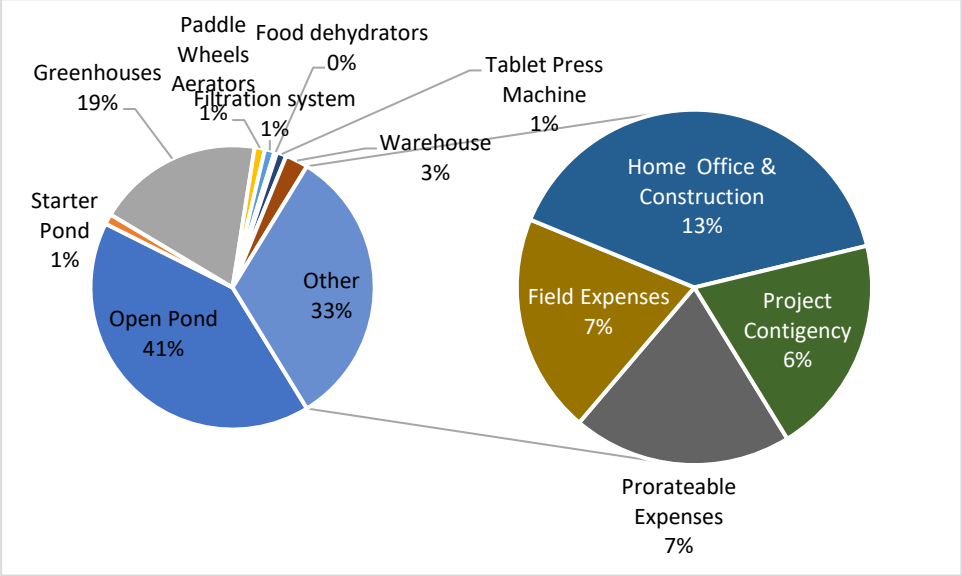



**Figure 7.2** Distribution breakdown of the fixed capital investment.

**Table 7.1** Fixed capital investment (FCI) for the production of 2 tonnes of *Spirulina* tablet

| | Item | Quantity | Unit Price (RM) | Total Price (RM) | References/ Source |
|---|---|---|---|---|---|
| 1 | **Open Pond** | 16 | RM70,000.00 | RM1,120,000.00 | GTEK Resources Sdn Bhd., Malaysia |
| 2 | **Starter Pond** | 16 | RM2,000.00 | RM32,000.00 | GTEK Resources Sdn Bhd., Malaysia |

| | | | | | |
|---|---|---|---|---|---|
| 3 | **Greenhouses** | 3000 $m^2$ | RM171.60 per $m^2$ | RM514,800.00 | (Jovicich et al., 2005) |
| 4 | **Paddle Wheels Aerators** | 16 | RM2,000.00 | RM32,000.00 | THK Sales & Service (M) Sdn Bhd, Malaysia |
| 5 | **Inclined-screen filtration** | 1 | RM30,000.00 | RM30,000.00 | JX Filtration (China) Co., Ltd |

| | | | | | |
|---|---|---|---|---|---|
| 6 | **Food dehydrators** | 1 | RM8,000.00 | RM8,000.00 | Foodehy SL, Spain |
| 7 | **Tablet Press Machine** | 1 | RM30,000.00 | RM30,000.00 | Kimah Industrial Supplies (M) Sdn Bhd, Malaysia |
| | | | TDC | RM1,766,800.00 | |
| 8 | **Warehouse** | | | RM70,672.00 | (Davis et al., 2016) |

| | | | |
|---|---|---|---|
| 9 | **Prorateable Expenses** | RM176,680.00 | (Davis et al., 2016) |
| 10 | **Field Expenses** | RM176,680.00 | (Davis et al., 2016) |
| 11 | **Home Office & Construction** | RM353,360.00 | (Davis et al., 2016) |
| 12 | **Project Contingency** | RM176,680.00 | (Davis et al., 2016) |
| 13 | **Working capital investment** | RM897,534.40 | (Taufikurahman et al., 2020) |
| | Total FCI | RM5,385,206.40 | |

### 7.4.2 ANNUAL OPERATING COST

**Table 7.2** shows an overview of the annual operating cost for the production of 20 tonnes of *Spirulina* tablet. Operating cost is controlled mainly by employee salary, supervision (1% of employee salary), equipment maintenance (1% of FCI), insurance (1% of FCI) and taxes (1% of FCI) (Taufikurahman et al., 2020). Utilities cost is consisting of the cost of electricity and water. Electricity utility cost was estimated based on major equipment such as paddle wheel aerators, food dehydrator, incline screen filtration and tablet press machine as shown in **Table 7.3**. On the other hand, water utility cost is estimated based on assuming all 16 units of open ponds will be cultivated for 2 cycle (20 days) every month. Moreover, electricity-powered paddle wheel aerators are assumed to be running 365 days without any downtime maintenance. Inclined screen machine, food dehydrator and tablet press machine are assumed to run for 4 days per month. Chemical costing was sourced from online website such as Alibaba website for their industrial market price as shown in **Table 7.4**. Overall, the total annual operating cost for the production of 20 tonnes of *Spirulina* tablet is about RM 3.4 million. Based on **Figure 7.3**, chemical cost contributes about 74% of the annual operating cost follow by employee salary (16%).

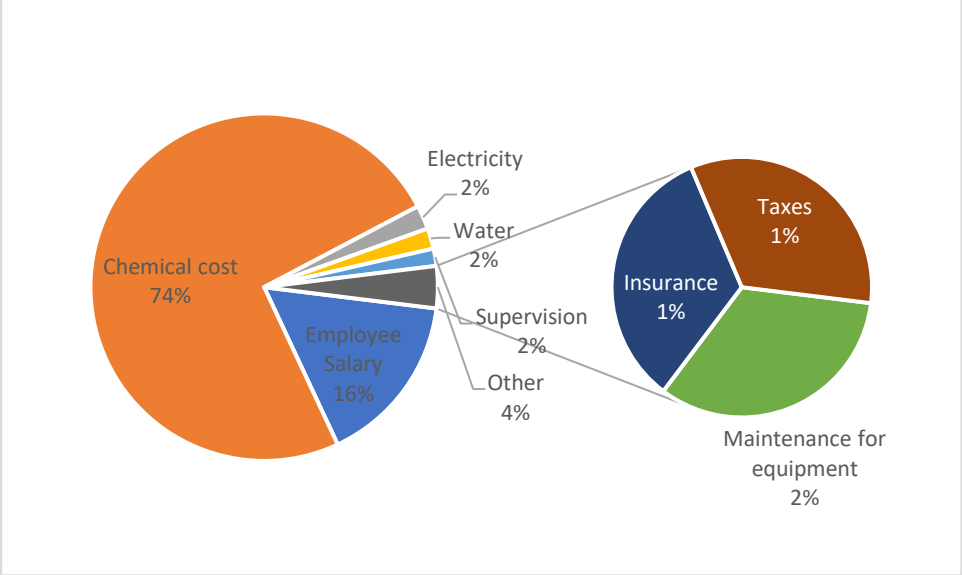



**Figure 7.3** Distribution of annual operating cost for the production of 20 tonnes of *Spirulina* tablet.

**Table 7.2** Overview of annual operating cost for the production of 20 tonnes of *Spirulina* tablet

| Item | Cost per month / RM | Annually Cost / RM | **References** |
|---|---|---|---|
| Employee Salary | RM46,000.00 | RM552,000.00 | |
| Chemical cost | RM212,426.36 | RM2,549,116.38 | Detail calculation in **Table 7.4** |
| Electricity | RM6380.18 | RM76,562.12 | Detail calculation in **Table 7.3** |
| Water | RM5,472.00 | RM65,664.00 | Detail calculation in **Table 7.3** |
| Supervision | RM4,600.00 | RM55,200.00 | (Taufikurahman et al., 2020) |
| Maintenance for equipment | RM3,739.73 | RM44,876.72 | (Taufikurahman et al., 2020) |
| Insurance | RM3,739.73 | RM44,876.72 | (Taufikurahman et al., 2020) |
| Taxes | RM3,739.73 | RM44,876.72 | (Taufikurahman et al., 2020) |
| | Total | RM3,393,978.67 | |

**Table 7.3** Detailed calculation of water and electricity utility cost for the production 20 tonnes *Spirulina* tablet

| **Utility** | **Estimated utility cost** | **References** |
|---|---|---|
| Water | Total volume 16 units open pond:<br>$25 \times 6 \times 0.5 \times 16 = 1200\ m^3$<br>Cost of water utility per month:<br>$1200\ m^3 \times$ RM 2.28 per $m^3 \times 2$ = RM 5472.00 | (Hafizi Md Lani, Yusop and Syafiuddin, 2018) |
| Electricity | Paddle wheel aerators: 1 horsepower-hour<br>0.7457 kWh × 24 h × 365 days × 16 units ÷ 12 months = 8709.77 kWh per month | Source from supplier, THK Sales & Service (M) Sdn Bhd, Malaysia |
| | Food dehydrator: 69 kWh<br>69 kWh × 24 h × 4 days = 6624.00 kWh per month | Source from Foodehy S.L. company |

| | | |
|---|---|---|
| | Incline-screen filtration: 0.14 MJ/ kg of biomass<br><br>0.14 MJ/ kg × 2280 kg × 277.78 kWh/MJ = 8709.77 kWh per month | (Furuhashi et al., 2020). |
| | Tablet press machine: 5.5 kWh<br><br>5.5 kWh × 24 h × 4 days = 88.67 kWh per month | Source from Kimah Industrial Supplies (M) Sdn Bhd, Malaysia) |
| | Total power usage: 15950.44 kWh<br><br>Cost of electricity utility per month:<br><br>15950.44 kWh × RM 0.40 per kWh = RM 6380.18 | (Foong et al., 2023) |

1 horsepower-hour = 0.7457 kWh

**Table 7.4** Detailed calculation of chemical cost as of 2023 for the production of 20 tonnes of *Spirulina* tablet

| Zarrouk chemicals | g/L | RM/kg | RM/month | Fertiliser chemicals | g/L | RM/kg | RM/month |
|---|---|---|---|---|---|---|---|
| Sodium nitrate | 2.5 | 0.86 | Sample calculation: 2.5 g/L × 1,200,000 L × 2 harvest per month × RM 0.86 = RM5,148.00 | NPK fertiliser (16:16:16) | 0.5 | 8.00 | RM9,600.00 |
| Dipotassium hydrogen phosphate | 0.5 | 4.29 | RM5,148.00 | Urea | 0.2 | 6.50 | RM3,120.00 |
| Magnesium sulfate heptahydrate | 0.2 | 2.15 | RM1,029.60 | Sodium bicarbonate | 15 | 4.29 | RM154,440.00 |
| Sodium chloride | 1 | 4.29 | RM10,296.00 | Sodium chloride | 1 | 4.29 | RM10,296.00 |
| Potassium sulfate | 1 | 1.50 | RM3,603.60 | | | | |

| | | | | | |
|---|---|---|---|---|---|
| Iron(II) sulfate heptahydrate | 0.01 | 3.43 | RM82.37 | | |
| Sodium bicarbonate | 18 | 4.29 | RM185,328.00 | | |
| Calcium chloride dihydrate | 0.04 | 0.86 | RM82.37 | | |
| Ethylenediaminetetraacetic acid | 0.08 | 8.58 | RM1,647.36 | | |
| Boric acid | 2.86 | 4.29 | RM29.45 | | |
| Manganese(II) chloride tetrahydrate | 1.81 | 5.92 | RM25.72 | | |
| Zinc sulfate heptahydrate | 0.222 | 4.29 | RM2.29 | | |
| Sodium molybdate dihydrate | 0.0177 | 27.03 | RM1.15 | | |
| Copper(II) sulfate pentahydrate | 0.08 | 12.87 | RM2.47 | | |
| | | Total | RM212,426.36 | Total | RM177,456.00 |

### 7.4.3 CUMULATIVE CASH FLOW

The sales price of *Spirulina* tablet used in this preliminary design is set at RM250 per kg. An online survey was conducted on the current market price of *Spirulina* tablet. The sales price is lower than the average current market price of *Spirulina* tablet which is between RM300 to RM600 per kg of *Spirulina* tablet. The sales price of RM250 per kg, although lower than the average market price, suggests that the product could attract potential customers due to its affordability. This pricing strategy may allow for capturing a larger market share and gaining a competitive edge over other suppliers who offer *Spirulina* tablets at higher prices.

**Figure 7.4** shows a cumulative cash flow comparison between Zarrouk medium and fertiliser medium for the production of 20 tonnes of *Spirulina* tablet. The ROI of Zarrouk medium and fertiliser medium is 53.04% and 69.66%, respectively. This indicate that both approaches yield attractive returns of investment. These figures suggest that investing in *Spirulina* tablet production using either medium has the potential to generate substantial profits. Furthermore, the payback period serves as a crucial indicator to calculate the amount of time it takes to recover the cost of an investment (Coker, 1995). The reduction in the payback time from 4.32 to 3.74 years when using fertiliser medium implies that this approach allows for a faster recovery of the investment, enhancing the overall financial feasibility of the project. This reduction in payback time indicates improved cash flow and shorter time to profitability.

The NPV values of RM 3.6 million and RM 5.5 million for Zarrouk and fertiliser medium, respectively, demonstrate the positive net value generated by the investment over the project's lifetime. These positive NPVs indicate that both production methods are financially attractive, with fertiliser medium exhibiting

a higher net value. Moreover, the IRR values of 35.8% and 46.0% for Zarrouk and fertiliser medium, respectively, further strengthen the case for the economic viability of *Spirulina* tablet production. These IRR values surpass the minimum acceptable rate of return range of 32-48% for a high-risk project, suggesting that both approaches offer favourable returns on the investment (Bidar and Shahraki, 2018).

Overall, the findings from this economic cost analysis highlight the production of *Spirulina* tablets using fertiliser medium presents a more favourable economic outlook. The availability of a lower-priced product coupled higher ROI, shorter payback period and both higher NPV and IRR. This shows that *Spirulina* tablet production project as a promising venture with significant potential for profitability and success in the market.

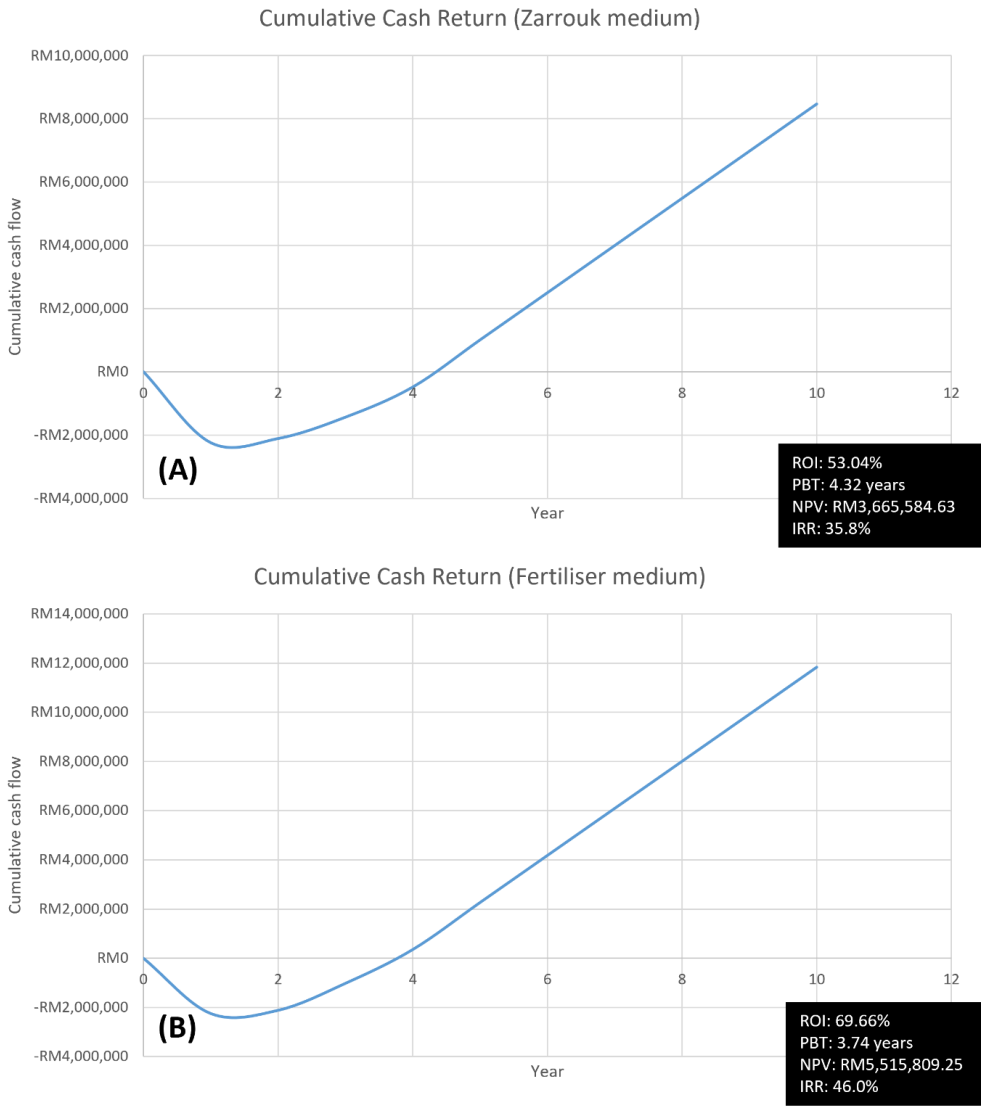



**Figure 7.4** Cumulative cash flow comparison between (A) Zarrouk medium and (B) fertilizer medium for the production of 20 tonnes of *Spirulina* tablets.

### 7.4.4 SENSITIVITY ANALYSIS

In view of the 10-year lifespan of the plant, it is important to consider potential variations in the economy and labor market during its operation, as these factors may pose a risk to the plant's financial viability. Sensitivity analysis was used to anticipate potential variations on the payback time (PBT), return of investment (ROI), net present value (NPV) and internal rate of return (IRR) of *Spirulina*

tablets using six variables which are selling price, chemical cost, sales volume, dry biomass weight, electricity cost and water cost with a ±20% range (shown in **Figure 7.5**). Among the six variables, selling price, chemical cost, sales volume and dry biomass weight has the most significant impact on the financial performance of the production of *Spirulina* tablets process. The selling price directly affects the revenue generated from sales, while the chemical cost directly impacts the production cost. The sales volume and dry biomass weight are crucial factors that determine the quantity of *Spirulina* tablets produced and sold.

By conducting sensitivity analysis on these variables, the plant operators can assess the plant's financial resilience under various economic scenarios. For example, if the selling price decreases by 20%, it would directly reduce the revenue and potentially increase the payback time and lower the ROI. Similarly, an increase in the chemical cost may reduce the profitability of the plant. The sales volume and dry biomass weight variables are interconnected. A decrease in sales volume would result in a lower quantity of *Spirulina* tablets sold, impacting the revenue and profitability. Likewise, a decrease in the dry biomass weight would directly reduce the production output and subsequently affect the financial indicators.

Considering these variables within a range of ±20% allows for a comprehensive evaluation of the plant's financial viability under different market conditions. The sensitivity analysis helps in identifying potential risks and enables the plant operators to make informed decisions regarding pricing strategies, cost optimization, and production planning. By proactively addressing these factors,

the plant can enhance its adaptability to changing economic and market conditions, ensuring its long-term sustainability and success.

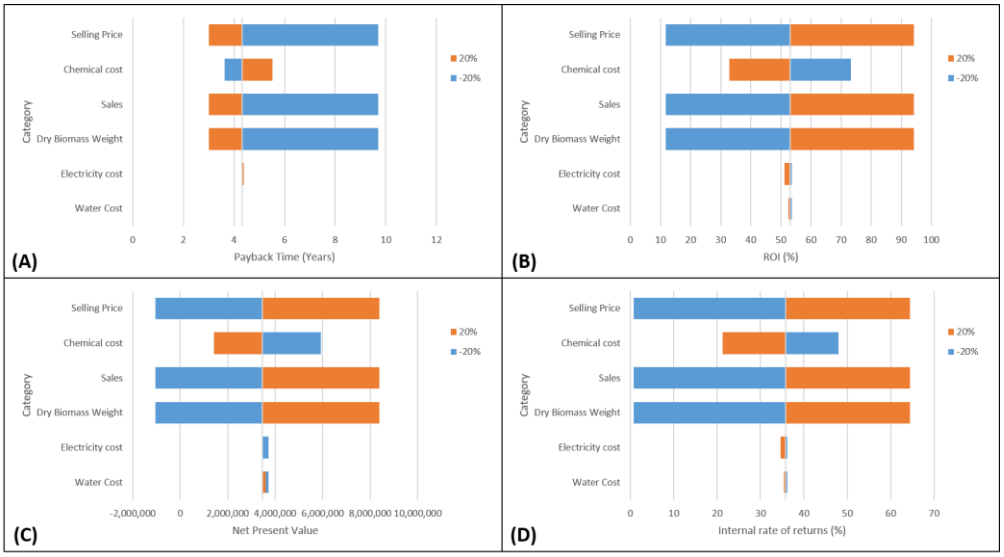



**Figure 7.5** Sensitivity analysis of the production of 20 tonnes of *Spirulina* tablet (A) payback time, (B) return of investment, (C) net present value and (D) internal rate of return.

## 7.5 CONCLUSION

This study has successfully conducted a techno-economic assessment to evaluate the feasibility and potential profitability of producing *Spirulina* tablets in Malaysia. The total fixed capital investment for the production is RM 5.3 million, and the annual operating cost is RM 3.3 million. The findings indicate that producing 20 tonnes of *Spirulina* tablets using a fertilizer medium yields higher profitability compared to the conventional Zarrouk medium. With the fertilizer medium, the return on investment (ROI) is 69%, the payback period (PBT) is 3.74 years, the net present value (NPV) is RM 5.5 million, and the

internal rate of return (IRR) is 46%. On the other hand, with the Zarrouk medium, the ROI is 53%, the PBT is 4.32 years, the NPV is RM 3.6 million, and the IRR is 35%. Moreover, a sensitivity analysis identified that selling price, chemical cost, sales volume, and dry biomass weight has the most significant impact on the financial performance of the *Spirulina* plant. The findings of this study provide valuable insights and investment opportunities within the microalgae industry in Malaysia. Further exploration and development in this sector can contribute to the growth and success of the country's microalgae industry.

# CHAPTER 8

# CONCLUSION AND FUTURE WORKS

This thesis project established a sustainable cultivation and harvesting strategy for the upstream processing of *Spirulina* biorefinery while integrating Internet of Things monitoring system in it. Within this chapter, a comprehensive overview of the research accomplishments attained during this study will be summarised. Moreover, this section also highlights the main takeaways from the project and suggests some possible future work.

## 8.1 RESEARCH ACHIEVEMENTS

This thesis presents a sustainable cultivation and harvesting strategy for the upstream processing of *Spirulina* biorefinery while integrating Internet of Things monitoring system. One of the key sustainable approaches was to utilize rainwater resources for the cultivation of *Spirulina* to reduce water footprint in the cultivation process. On the other hand, another sustainable approached was to use spent coffee ground as an alternative nutrient source for the cultivation of *Spirulina*. Moreover, one of the studies develops a continuous cultivation and harvesting approach for improving biomass and phycocyanin production. The thesis explores a diverse approach in the upstream processing from water, culture medium and harvesting. In addition, one of the studies applied IoT monitoring technology to a *Spirulina* cultivation system to monitor key parameters such as absorbance, temperature, light intensity, and water level using the ThingSpeak open-source software. Lastly, the thesis conducts a detailed techno-economic analysis of the production of *Spirulina* tablets in Malaysia, providing valuable insights and investment opportunities for the

emerging microalgae industry in Malaysia. The comprehensive description of each research achievement is stated as followed:

(A) **Upstream bioprocessing of *Spirulina* platensis microalgae using rainwater and recycle medium from post-cultivation for c-phycocyanin production:** This study evaluates the growth of *Spirulina* in recycled culture medium and rainwater medium for the preparation of its culture medium. Different methods of pre-treatment (such as microfiltration and UV-light) were applied to the recycled medium and rainwater before the transfer of *Spirulina* culture into the medium. The growth of *Spirulina* was evaluated by measuring the biomass concentration, biomass productivity and phycocyanin accumulation under different pre-treatment conditions. The recycled medium without any pre-treatments shows a reduction of biomass productivity from 0.2246 to 0.0802 g/L/d after three cultivation cycles, which could be caused by high salinity and biological contaminants that affect the growth of *Spirulina*. However, using pre-treatment methods with microfiltration and UV-light on the recycled medium increased biomass productivity from 0.1395 to 0.1861 g/L/d. Furthermore, pre-treatment methods were also used to treat the rainwater, biomass productivity improved from 0.3211 to 0.3837 g/L/d. These results suggest that the use of pre-treatment methods and alternative rainwater sources can improve the biomass productivity of *Spirulina* and potentially lower the water footprint of the microalgae cultivation process.

(B) **Evaluation of real-time monitoring on the growth of *Spirulina* microalgae: Internet-of-Things and microalgae technologies:** The

study applied IoT monitoring technology to a *Spirulina* cultivation system. It monitored key parameters such as absorbance, temperature, light intensity, and water level using the ThingSpeak open-source software. The temperature and light sensors performed well, but the absorbance (TCS3200) and water level sensors had some limitations. The TCS3200 sensor could only measure absorbance up to 1.7, while conventional UV-Vis spectrophotometers could measure values above 3.0. This limited the accuracy of measuring higher absorbance levels. The water level sensor was affected by prolonged immersion in the *Spirulina* culture, which caused *Spirulina* attachment that interfered with the measurement accuracy. Moreover, the study demonstrated the feasibility of monitoring *Spirulina* conditions using the ThingSpeak mobile application interface, which provided real-time tracking. These results are important for advancing IoT-based methods in the *Spirulina* microalgae industry. By integrating IoT, Industry 4.0 benefits can be achieved, such as automation and data-driven decisions. To sum up, this research introduces IoT to *Spirulina* cultivation and evaluates its effectiveness in parameter monitoring. The identified limitations suggest the need for sensor improvements. The potential of ThingSpeak as a monitoring interface indicates its future microalgae industry applications, in line with Industry 4.0's transformative potential.

**(C) Effect of nutrients-deficient and harvesting ratio strategy for biomass and phycocyanin production from *Spirulina* microalgae:** This study develops a continuous cultivation and harvesting approach for improving biomass and phycocyanin production in the context of

*Spirulina* microalgae biorefinery. The results show that a 10% harvesting ratio maintained a constant amount of dry biomass harvested over three consecutive days. Importantly, the key role of $NaNO_3$ in *Spirulina* cultivation was revealed, as adding 2.5 g/L significantly increased phycocyanin accumulation. The optimized parameters of a 10% harvesting ratio and 2.5 g/L $NaNO_3$ were tested in a 10-L batch culture setup. This larger scale showed lower biomass productivity than the 1-L batch culture, resulting in higher production costs, but it provided a valuable perspective on the feasibility aspect. However, further studies should focus on a comprehensive economic evaluation of scaling up *Spirulina* production for feasible commercial application. In this regard, it is essential to optimize the concentrations of $NaNO_3$, $NaHCO_3$, and other critical nutrients, to establish a cost-effective cultivation medium. Furthermore, the investigation of different harvesting frequencies and alternative nitrogen sources could enhance phycocyanin concentration and purity. In summary, this research highlights the complex interactions of continuous cultivation and harvesting approaches in improving biomass and phycocyanin yields in the *Spirulina* microalgae biorefinery framework.

**(D) Phycocyanin production from *Spirulina* with the nutrient replacement of local spent coffee ground waste:** This study explored the novel use of spent coffee ground extract supplemented with Zarrouk medium for *Spirulina* cultivation. The findings revealed that the highest biomass productivity of 0.0984 g/L/d was achieved by the supplementation of 100% Zarrouk culture medium with spent coffee

grounds extract. Meanwhile, the highest removal efficiencies of TN, TP, and COD obtained by 25% Zarrouk medium and spent coffee grounds extract were 81%, 53%, and 40%, respectively. These findings suggested that lower concentrations of Zarrouk medium could improve nutrient removal efficiency. The study also quantified the nutrient uptake of TN, TP, and COD for a period of 10 days were 67-272 mg/L, 36-51 mg/L, and 354-418 mg/L, respectively. In addition, the 50% Zarrouk medium had the highest phycocyanin and protein concentrations of 160.778 mg/g and 427.640 mg/g, respectively. These compounds are valuable bioactive substances. This finding showed the opportunities of using spent coffee grounds to produce phycocyanin and also proposed that the remaining biomass contained high amount of proteins could be utilized as animal feed. This could create a sustainable circular bioeconomy and reduce the environmental impact of coffee waste disposal. Therefore, this research presented a new opportunity for producing high-value microalgae biomass.

**(E) Techno-economic assessments on the production of *Spirulina* tablet from large scale open pond cultivation of *Spirulina* in Malaysia:** This paper presents a detailed techno-economic analysis of the production of *Spirulina* tablets in Malaysia. The production process requires a total fixed capital investment of RM 5.3 million and an annual operating cost of RM 3.3 million. The analysis shows that using a fertilizer medium to produce 20 tonnes of *Spirulina* tablets is more profitable than using the conventional Zarrouk medium. The fertilizer medium yields a return on investment (ROI) of 69%, a payback period (PBT) of 3.74 years, a net

present value (NPV) of RM 5.5 million, and an internal rate of return (IRR) of 46%. The Zarrouk medium, on the other hand, yields an ROI of 53%, a PBT of 4.32 years, an NPV of RM 3.6 million, and an IRR of 35%. The sensitivity analysis reveals that the selling price, chemical cost, sales volume, and dry biomass weight are the main factors that affect the financial performance of the *Spirulina* production facility. This paper provides valuable insights and investment opportunities for the growing microalgae industry in Malaysia. Further research and development in this field can enhance the growth and prosperity of the country's microalgae industry.

## 8.2 FUTURE WORKS

This section discusses on the challenges and future works recommended to improve the upstream processing of microalgae industry towards commercialization in a greener and sustainable approach aligning with Industrial Revolution (IR) 4.0.

The limitation of TCS3200 sensor absorbance limit and the need for dilution of collected *Spirulina* culture sample to accurately measure biomass concentration remains a drawback. Future work can focus on develop an automation machine mechanism that can extract a small amount of *Spirulina* culture sample and dilute it with distilled water. The automation machine mechanism can be set to measure 1-hour, 6-hour or daily. The continuous data collection of biomass concentration is a very important indicator that helps us to understand the impact of factors (i.e., nutrients, temperature, light, pH, salinity) on the growth curve of *Spirulina*. Sensors such as temperature, light, pH, and salinity are readily available in the industry. However, sensors to measure nutrients in water are not

yet available. Nonetheless, there are a type of sensor in the agriculture industry known as NPK sensor used for measuring the nutrients content (nitrogen, phosphorus and potassium) in the soil. The NPK sensor was developed to measure nutrients content in soil but not in water. Therefore, future studies can also focus to develop a nutrient sensor that can measure nutrients in water. Addressing the two main concern of biomass concentrations and nutrient concentrations will serve as an advancement to the development of real-time monitoring applications in the microalgae industry. With this real-time monitoring development, we can monitor microalgae biomass concentrations and nutrients availability in real-time with accuracy. With this much of data collected, this would also push forward the development of machine learning and data analytics of microalgae.

Besides, the culture medium used to cultivate *Spirulina* are chemical based. Although some of the current microalgae industry uses fertilizer as an alternative culture medium for the cultivation of microalgae due to ease of preparation and cost-effectiveness. It is not a sustainable practice in the long term. The production of chemicals or synthetic fertilizer have negative impacts on the environment (Penuelas, Coello and Sardans, 2023). Therefore, there is a need to formulate a sustainable and green culture medium for the cultivation of microalgae. Although many studies have reported the potential of wastewater as a valuable source of nutrients for microalgae growth but the selection of the type of wastewater is important. Wastewater that are robust and high toxicity may cause the microalgae to produce toxins called microcystins that are harmful to human consumption. Therefore, the current study suggests food waste or food wastewater. Future studies should be directed toward formulating a culture

medium that harnesses the potential of specific components. For instance, coffee waste could serve as a nitrogen source, eggshells as a viable carbon source, and fruit skins as a wellspring of essential micronutrients. Such a concerted focus holds promise for advancing sustainable microalgae cultivation practices while mitigating the associated environmental and health-related concern. In conclusion, forging an innovative and environmentally conscious approach to formulating microalgae culture mediums holds the potential to revolutionize the industry's sustainability while addressing prevailing ecological and health challenges.

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