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Perspective of *Spirulina* culture with wastewater into a sustainable circular bioeconomy☆

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ABSTRACT

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. Further study is needed in modifying or exploring alternative culture media utilising waste, wastewater, or by-products from industrial processes to ensure the sustainability of environment and nutrients source for cultivation in the long term. Moreover, the current development of utilising wastewater medium only support the growth of *Spirulina* however it cannot eliminate the negative impacts of wastewater. In fact, the recent developments in coupling with wastewater treatment technology can eradicate the negative impacts of wastewater while supporting the growth of *Spirulina*. The application of *Spirulina* cultivation in wastewater able to resolve the global environmental pollution issues, produce value added product and even generate green electricity. This would benefit the society, business, and environment in achieving a sustainable circular bioeconomy.

Credit author statement

Hooi Ren Lim, Kuan Shiong Khoo: Conceptualization, Hooi Ren Lim: Writing – original draft, Hooi Ren Lim, Kuan Shiong Khoo, Kit Wayne Chew, Chih-Kai Chang, Heli Siti Halimatul Munawaroh, P. Senthil Kumar, Nguyen Duc Huy, Pau Loke Show: Writing – review & editing, Hooi Ren Lim: Visualization, Kuan Shiong Khoo and Pau Loke Show: Supervision, Pau Loke Show: Funding acquisition.

1. Introduction

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 et al., 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

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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., 2020). *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; Vu 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 et al., 2015). 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 et al., 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 in 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 review paper 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.

2. 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 et al., 2016), Conway (Dineshkumar et al., 2016), Kosaric (Rizal et al., 2017) and Synthetic Human Urine (SHU) (Marrez et al., 2013) mediums that has been formulated 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 (Dineshkumar et al., 2016; Michael et al., 2019).

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 et al., 2019; Soni et al., 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 et al., 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 source 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., 2021). The cost of culture medium accounts for about 35% of total cost of the algal biomass production (Yu et al., 2019) Therefore, research include 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 1 shows a comprehensive evaluation of different type of chemical-based culture medium.

2.1. 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 et al. (2019) revealed that the modified medium with

Table 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 Light intensity 4.5 klux 12/12 h light/dark cycle	D = 6.9 g/L P = 0.235 g/L/d	Protein: 65% Lipid: 6.84% Carbohydrate: 13.62% Fiber: 11.37% Moisture: 9.92% Ash: 9.93%	Michael et al. (2019)
	30 days, 20 mL Light intensity 4.5 klux 12/12 h light/dark cycle	D = 1.86 g/L	Not provided	Dineshkumar et al. (2016)
	30 days, 250 mL Light intensity 4.5 klux	D = 4.87 g/L		Marrez et al. (2013)
	Open pond (18.63 L) Closed reactor (17.36 L) Light intensity 1.5–4.5 klux	P: 8.568 g/L/d P: 10.231 g/L/d		Soni et al. (2019)
	10 days, 1 L	W: 16.6 g/L	Protein: 64.42% β-carotene: 1817 μg/g	Sukanya et al. (2020)
JPJM CFTRI DMSD	15 days, 200 mL 12/12 h light/dark cycle	W: 4.32 g/L W: 11.33 g/L W: 3.33 g/L	Not provided	Salunke et al. (2016)
BG11 F/2 Conway	30 days, 20 mL Light intensity 4.5 klux 12/12 h light/dark cycle	D = 4.87 g/L D = 0.46 g/L D = 0.52 g/L		(Dineshkumar et al., 2016)
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 μg/mL Allo-Phycocyanin: 51.5 μg/mL Phycoerythrin: 44.13 μg/mL	Marrez et al. (2013)
Schlösser medium	8 days, 100 mL	D = 1143 mg/L P = 136 mg/L/d	Total Protein: 18.77 U/mg	Barros et al. (2020)
Aiba & Ogawa 1977 liquid medium	15 days, 10 L Light intensity 3.0–5.0 klux 12/12 h light/dark cycle	D = 0.53 g/L	Chlorophyll *a*: 47 μg/mL Protein: 61.04% Fiber: 13.77% Lipid: 17.66%	Reham et al. (2019)

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. (2019) 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 presents a comprehensive evaluation on the different type of modified culture medium.

2.2. Alternative low-cost culture medium

2.2.1. 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. Reham et al. (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

Table 2
Evaluation on the different types of Modified Culture Medium.

Culture medium	Modification	Maximum Biomass dry (D)/wet (W) weight & Productivity (P)	Biomass composition	References
Zarrouk	without the carbon source ($NaHCO_3$)	D = 1.87 g/L P = 114 mg/L/d	Protein: 53.4% Carbohydrate: 16.6% Lipid: 12.5% Phycocyanin: 52.5 mg/g	Cardias et al. (2018)
	– $FeSO_4.7H_2O$, EDTA was completely removed – NaCl, $MgSO_4.7H_2O$ concentration were reduced – Urea was added.	Open pond P = 11.34 g/L/d Closed reactor P = 12.28 g/L/d	Chlorophyll: 0.80 mg/L/d Chlorophyll: 0.85 mg/L/d	Soni et al. (2019)
	– 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, D = 1.174 g/L, 50% Cassava flour D = 0.862 g/L, 25% Sweet potato flour 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% Carbohydrate: 11.0% Lipid: 6.4% Phycocyanin:14.2% Chlorophyll *a*: 2.04% 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% 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 D = 0.90 g/L, 50% Urea D = 0.66 g/L, 75% Urea D = 0.59 g/L, 100% Urea	Chlorophyll *a*: 7.6 mg/L Chlorophyll *a*: 9.0 mg/L Chlorophyll *a*: 7.6 mg/L 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., 2019)

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.

2.2.2. 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 compare 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

Table 3
Evaluation on the different types of Alternative Culture Medium.

Culture medium	Biomass dry/wet weight & Productivity, P	Biomass composition	References
Seawater	D = 1.42 g/L	Not Provided	Dineshkumar et al. (2016)
	D = 2.17 g/L P = 0.18 g/L/d	Protein: 8% Carbohydrate: 68.6% Lipid: 6.7%	Bezerra et al. (2020)
Fertiliser	D = 7.5 g/L P = 0.254 g/L/d	Protein: 52.85% Lipid: 6.61% Carbohydrate: 15.29% Fiber: 9.79% Moisture: 5.45% Ash: 9.55%	Michael et al. (2019)
	D = 1.82 g/L P = 177 mg/L/d	Chlorophyll: 12.84 mg/L Protein: 45.81% Lipid: 9.41%	Kumari et al. (2014)
Vermicompost	D = 0.50 g/L	Chlorophyll *a*: 32.9 μg/L Protein: 31.25% Fiber: 8.78% Lipid: 19.62%	Reham et al. (2019)
	D = 0.55 g/L	Chlorophyll *a*: 46.9 μg/L Protein: 66.31% Fiber: 14.96% Lipid: 5.21%	
Bean Sprouts Extract + Urea	D = 0.30 g/L	Phycocyanin: 257.12 mg/L	(Adharani et al., 2016.; Dianursanti et al., 2018.)
Tequesquite	D = 763.2 mg/L	Not Provided	Martínez-Jerónimo et al. (2017)
My An mineral water	P = 10 g/m^2/d	Protein: 68.32% Lipid: 7.32% Ash: 7.46% Chlorophyll *a*: 1.34%	Vu et al. (2017)

in achieving its maximum biomass dry weight for *Spirulina* cultivation. Table 3 provides a comprehensive evaluation on the different type of alternative culture medium.

3. 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 et al., 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 et al., 2015). Moreover, 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., 2020), swine wastewater (Lu et al., 2020a,b), olive oil milling wastewater (Markou et al., 2012), distillery wastewater (Sankaran and Premalatha, 2018; Krishnamoorthy et al., 2019), confectionary wastewater (El-Kassas et al., 2015), saline wastewater (Mata et al., 2020), brine wastewater (Duangsri and Satirapipathkul, 2011), tapioca wastewater (Hadiyanto et al., 2019), tofu wastewater (Hadiyanto, 2018), rubber wastewater (Hadiyanto et al., 2020), paper mill wastewater (Asthary et al., 2019) and dye wastewater (Ismail et al., 2020). Table 4 summarises the different types of wastewater for *Spirulina* cultivation.

3.1. 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 et al., 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., 2010). 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; 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.

3.1.1. 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,

Table 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% P: 80.52%	Zhai et al. (2017)
Aquaculture	D = 0.22 g/L P = 0.03 g/L/d		NH_3: 19.8% NO_2^-: 100% NO_3^-: 98.7% PO_4^{3-}: 94.8%	Nogueira et al. (2018)
	D = 0.5 g/L P = 0.613 g/L/d	Lipids: 8.8% Protein: 38.3% Carbohydrate: 41.4% Ashes: 11.6%	COD: 30% NH_4^+: >99% P: 47.8%	Riãno et al. (2012)
Swine	D = 204.6 mg/L		Total P: 41.6% COD: 84.3%	Mezzomo et al. (2010)
	D = 1.28 g/L P = 74.22 mg/L/d	Chlorophyll: 0.134 mg/L/d	Total N: 85.86% Total P: 78.69% NH_4: 100% COD: 70.02%	Lu et al. (2020)
Olive oil mill	D = 1.5 g/L	Chlorophyll: 1.9 μg/L	Phenols: 80%	Mostafa et al. (2019)
Distillery	D = 8.11 g/L		TDS: 60–70% COD: 60–70%	Krishnamoorthy et al. (2019)
Desalination	D = 0.87 g/L	Phycocyanin: 2%		Matos et al. (2020)
Tapioca	P = 55.38 mg/L/d		COD: 67%	Hadiyanto et al. (2019)
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 Carbohydrate: 179.4 mg/g Chlorophyll *a*: 9.20 mg/g Carotene: 1.95 mg/g		Çelekli et al. (2016)
Real Human Urine	D = 0.81 g/L	Protein: 35.4% Lipid: 19.8%	NH_4^+: 97% TP: 96.5% Urea: 85–98%	Chang et al. (2013a, 2013b)

2006). Animal wastewater are reported to be the ideal nitrogen sources and containing essential nutrients such as sulphate, calcium, magnesium, potassium, iron and chloride (Samsudin, 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 (Chaiklahan, 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., 2010). 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. Riãno et al. (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 et al., 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.

3.1.2. *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 (1) (Larsdotter, 2006).

$$6H_2O + 6CO_2 + \text{light} \rightarrow C_6H_{12}O_6 + 6O_2 \tag{1}$$

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 and Hadiyanto (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 et al., 2013a, 2013b; Çelekli et al., 2016; Lu et al., 2020a,b). 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 et al., 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 5 shows a comprehensive evaluation on the different type of supplements in wastewater medium.

3.1.3. *Cultivation factors affecting the spirulina growth in wastewater*

Further to that, other factors such as colour of light, light intensity (Bahman et al., 2020), temperature (Subhash et al., 2014) and initial *Spirulina* biomass concentration (Sankaran and Premalatha, 2018) have some effects on the enhancement of *Spirulina* cultivation in wastewater. The optimum temperature for cultivation of *Spirulina* was 30 °C (Subhash et al., 2014). Moreover, Bahman et al. (2020) 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 et al. (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 compare 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.

3.1.4. *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

Table 5
Evaluation on the different types of supplement in wastewater medium.

Type of Wastewater	Supplementation	Biomass dry/wet weight & Productivity, P	Biomass composition	Removal efficiencies (R%)	References
Swine	8.0 g/L $NaHCO_3$, 1.5 g/L of $NaNO_3$	17.8×10^4 cells mL^{-1}	Protein: 55.88%	COD: 23% BOD: 45% NO_3^-: 49% NH_4^+: 92% PO_4^{3-}: 67%	Mezzomo et al. (2010)
	4.5 g/L $NaHCO_3$, 0.2 g/L urea	P = 12.0 g/L/d	Protein: 57.9% Total Fatty Acid: 4.18% Linoleic acid: 0.70% γ-Linolenic acid: 1.12% Phycocyanin: 19.5%	TN: 34 mg/L/d TP: 4 mg/L/d	Chaiklahan (2010)
	0.05 g/L ferrous sulphate	P = 98.67 mg/L/d	Protein: 55.15% Carbohydrate: 12.32% Lipids: 11.49% Iron: 2.03 mg/g Chlorophyll: 0.156 mg/L/d	COD: 78.15% NH_3–N: 100% TP: 83.39% TN: 91.34%	Lu et al. (2020)
	1.0 g/L $NaHCO_3$	D = 1.70 g/L P = 103.33 mg/L	Lipids: 8.52%	TN: 91.24% TP: 87.44% NH_4^+: 100%	Lu et al. (2020)
	1.0 g/L Sodium acetate	D = 2.18 g/L P = 135.33 mg/L	Lipids: 8.63%	TN: 85.72% TP: 87.02% NH_4^+: 100%	
Municipal	300 mg/L Glucose	D = 322.1 mg/L	Chlorophyll *a*: 9.93 mg/g	N: 97.58% P: >99%	Zhai et al. (2017)
Synthetic human urine	200 mg/L Sodium acetate	D = 1.75 g/L	Protein: 60.2% Lipid: 17.9%	NH_4^+: ~100%	Chang et al. (2013a, 2013b)
Olive-oil mill	1 g/L $NaNO_3$, 5 g/L $NaHCO_3$	D = 1696 mg/L	Carbohydrate: 33.64% Lipids: 16.91% Protein: 31.52% Chl: 1.02%	COD: 65.53% Carbohydrate: 88.41% Phenols: 100% P: 100%	Markou et al. (2012)
Cow Effluent	0.5 g/L $NaNO_3$	D = 1.26 g/L	Protein: 673.3 mg/g Carbohydrate: 172.4 mg/g Chlorophyll *a*: 18.8 mg/g Carotene: 4.68 mg/g Malondialdehyde: 63.60 mg/g 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 Carbohydrate: 177.5 mg/g Chlorophyll *a*: 11.33 mg/g Carotene: 5.75 mg/g Malondialdehyde: 66.51 mg/g Proline: 80.50 mg/g	Not Provided	
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 P = 0.20 g/L/d	Protein: 65.73% Phycocyanin: 16.60 mg/mL Polyunsaturated fatty acid: 38.20% γ-Linolenic: 23.29%	SO_4^{2-}: 94.01% PO_4^{3-}: 93.84% Br: 96.77% COD: 90%	Cardoso et al. (2020)
Desalination	25% Zarrouk medium	D = 1.14 g/L P = 0.05 g/L/d	Carbohydrate: 52.29% Lipids: 12.79% Ash: 2.69%	NO_3^-: 96.99% PO_4: 83.11% Zn: 96.43% TDS: 24.89% SO_4: 29.65%	Mata et al. (2020)

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 (Samsudin, 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., 2013a, 2013b; Markou et al., 2014; Chang et al., 2016; Hadiyanto et al., 2019).

Frequent studies have been reported on the coupling of adsorption technology with *Spirulina* cultivation using natural zeolite, synthetic zeolite and granular γ-alumina adsorbent. Markou et al. (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 γ-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 et al. (2013a, 2013b) 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 et al. (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.

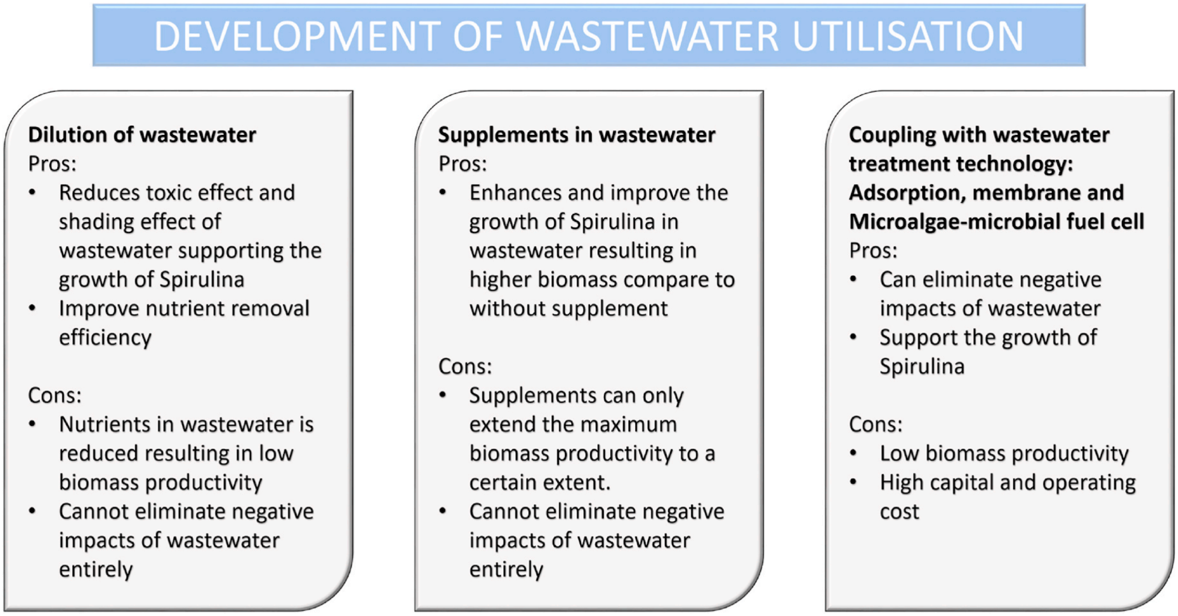
Microalgae-microbial fuel cell is a relatively new and promising technology coupling with the cultivation of *Spirulina*. Hadiyanto et al. (2019) design a MMFC system where tapioca wastewater was placed at the anode while *Spirulina* cultivation was placed at the cathode. The COD removal efficiency in 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. Fig. 1 summarise the pros and cons of the development of wastewater utilisation for the cultivation of *Spirulina*.

4. 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 et al., 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 wastewater, 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



DEVELOPMENT OF WASTEWATER UTILISATION

Dilution of wastewater

Pros:

- Reduces toxic effect and shading effect of wastewater supporting the growth of Spirulina
- Improve nutrient removal efficiency

Cons:

- Nutrients in wastewater is reduced resulting in low biomass productivity
- Cannot eliminate negative impacts of wastewater entirely

Supplements in wastewater

Pros:

- Enhances and improve the growth of Spirulina in wastewater resulting in higher biomass compare to without supplement

Cons:

- Supplements can only extend the maximum biomass productivity to a certain extent.
- Cannot eliminate negative impacts of wastewater entirely

Coupling with wastewater treatment technology: Adsorption, membrane and Microalgae-microbial fuel cell

Pros:

- Can eliminate negative impacts of wastewater
- Support the growth of Spirulina

Cons:

- Low biomass productivity
- High capital and operating cost

Fig. 1. Development of wastewater utilisation for the cultivation of *Spirulina*.

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 wastewater 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 2–10 L per day per head, cattle is 25–100 L per day per head and pig is 7–113 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 serve 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, 2018; Tan and Lim, 2019). About 1 tons of fresh fruit bunch produces 0.66 tons of palm oil mill effluent (Indriyati, 2018; 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 mitigate 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., 2020). 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 et al., 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. Fig. 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.

5. 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 wastewater 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,

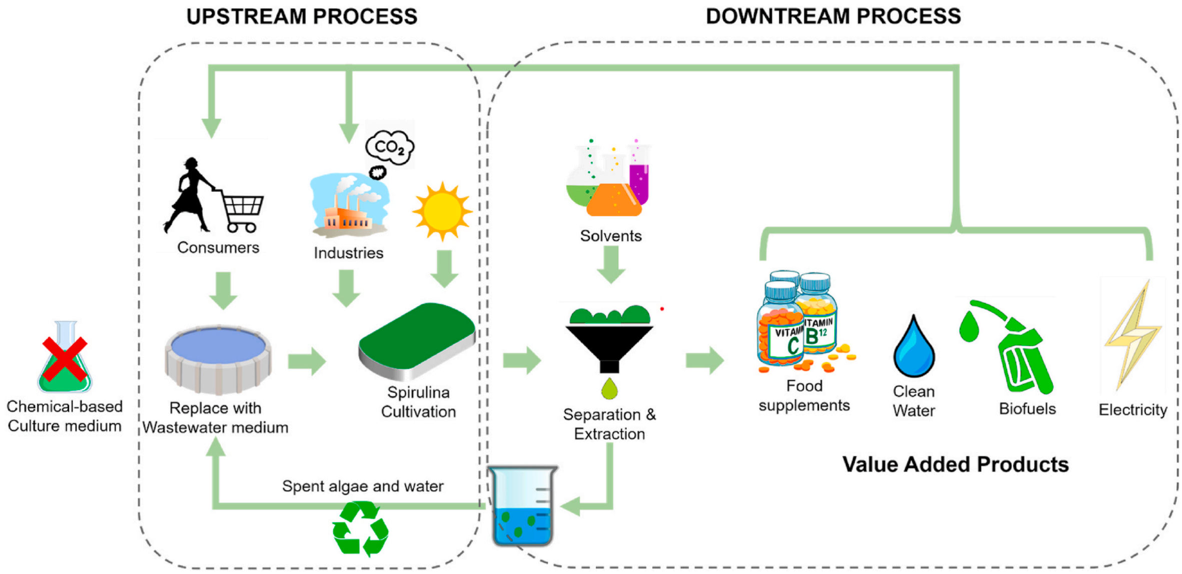


Fig. 2. The process flow of spirulina cultivation from upstream to downstream process (adopted from Pienkos et al., 2012).

the cost of membrane is around $100,000 for treating wastewater with a flow rate of 10–20 gallons per minute, while the cost of zeolites may range within $1960 to $48,000 for treating 1000 gallons of water (Albatrni et al., 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.

6. Conclusions

The current development of chemical-based culture medium has been established as there are many different types 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.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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