ASM Sc. J., 16, 2021

https://doi.org/10.32802/asmscj.2021.848

Strategies of Utilising Agriculture Wastewater for Microalgae Cultivation and Its Possible Applications: A Review

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Microalgae have been found to have high prospects in wastewater treatment, particularly from agriculture. However, the uneconomical algal medium growth has become the major disadvantaged in algal industry. Multiple attempts includes the development of microalgae phycoremediation technology has been integrated into wastewater treatment to reduce the cost of expensive wastewater remediation. Utilising wastewater as a low-cost nutrient medium offers a synergistic effect of wastewater nutrient removal and co-production of valuable biomass simultaneously. This paper is mainly focused on potential, ability, strategy, application (i.e., palm oil wastewater), limitation and challenges of microalgae in agricultural wastewater treatment using phycoremediation. The understanding of cultivating microalgae using agriculture wastewater shall promote the utilisation of wastewater more sustainably in the future. The possible solutions in the application of microalgae for aquaculture and agriculture sector is also discussed in this review. Overall, the utilisation of wastewater in media cultivation for microalgae is restricted due to the expensive treatment and safety concern. However, this pitfall can be reduced in the future together with a further intensive scientific study, advanced technology, better management system and applying better standard protocol.

Keywords: pre-treatment; agri-aquaculture integrated system; phycoremediation; metabolites

I. INTRODUCTION

The global threat of depleted natural resources for fuels, foods and energy has made human desperately seek for sustainable and renewable resources. Nowadays, microalgae which offer exciting industrial potential in economic activities are being explored globally. Generally, microalgae utilised sunlight as an energy source, inorganic nutrients and inorganic carbon to generate biomass through photosynthesis. The biomass could potentially be used as feedstock for bioenergy, biofertilisers, pharmaceutical, animal, protein alternative for fish and poultry feed, and other value-added products (Priyadarshani & Rath, 2012; Ansari *et al.,* 2017). The wide range of compounds derived from microalgae metabolic pathways offers in-demand compounds like fatty acids, steroids, carotenoids, polysaccharides, lectins, mycosporine-like amino acids,

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*Corresponding author's e-mail: jannatul24@gmail.com halogenated compounds, polyketides and toxins (Sathasivam *et al.,* 2017).

Approximately, 50,000 of microalgae are living on earth and capable of producing novel compounds for many purposes, and only a few were used practically for the commercial purpose (Bharathiraja *et al.*, 2015). The desirable attributes of microalgae as listed in Table 1 make microalgae a superior microorganism for multitude applications. The on-going research on utilising microalgae as a new renewable resource is crucial in this era. Producing commercial microalgae in an abundant quantity is a major problem due to its high operational cost, approximately US70 for every litre of biomass produced (Mehmod *et al.,* 2014). In order to reduce the production cost, this process can be coupled with wastewater effluent treatment for high-value products and biomass production. Agro-based industries (i.e. POME, piggery wastewater, dairy wastewater, paddy-soaked rice mill wastewater) are well established in Malaysia. Therefore, the wastewater generated from these agro-based industries could be utilised as microalgae cultivation media.

However, the microalgae biomass cultivated in wastewater treatment system has safety concern due to the high content of microorganism, COD, BOD, turbidity and suspended solids. Therefore, this paper discussed the appropriate pre-treatments process through mechanical, chemical or biological means (i.e., electrochemical oxidation, coagulation, fungal pre-treatment, autoclave, ultraviolet ray, filtration and dilution) before being processed for human and animal consumption. Microalgae are important bioremediation agent in agricultural wastewater. Agricultural wastewaters have an abundance supply of N, P, K originated from fertiliser application (Vassilev & Vassileva 2016). Microalgae can assimilate a large amount of nitrogen, phosphate and carbon for their growth and turn into biomass containing lipids, carbohydrates and proteins. It is a win-win situation where microalgae can utilise the nutrients from wastewater that is always a limiting factor for microalgae growth. Studies have been reported, some microalgae species (e.g., *Botryococcus, Chlamydomonas, Phormidium, Spirulina*) has promising efficacy in wastewater treatment (Chinnasamy *et. al.,* 2010; Stephens *et al.,* 2010).

This review focus on the recent trends of using tropical agriculture wastewater to culture microalgae and the co-production of valuable biomass is utilised into multiple applications, especially in agriculture and aquaculture applications. The approaches in wastewater pre-treatment and ways to increase the biomass yield are presented in this paper to make the wastewater as a possible source of nutrients for microalgae cultivation. In addition, the integration of applying microalgae into aquaculture and agriculture system with improved knowledge and technology will benefit many, especially the rural livelihood. Overall, the understanding of cultivating microalgae using agriculture wastewater shall promote the utilisation of wastewater more sustainably in the future.

A. The Characteristic of Microalgae

Microalgae are simple organisms with a cellular structure which can be found in almost all parts of the world, varied in sizes and structures and are classified mainly based on their pigmentations (Demirbas, 2010; Takriff *et al.,* 2016). Microalgae size are < 400 µm and generally of 1–30 µm in diameter (Demirbas, 2010; Ullah *et al.,* 2015). According to Rathod (2015), the basic elements of microalgae are carbon, hydrogen, oxygen, nitrogen and phosphorus with the stoichiometric formula of $C_{106}H_{181}O_{45}N_{16}P$. Their morphological features are round, oval, cylindrical, fusiform cells, projection-like thorn and cilia (Drew *et al.,* 2013).

The classification of algae is complex and controversial yet can be classified into prokaryotic or eukaryotic. The organelles (nucleus, chloroplast, mitochondria, Golgi body, flagella and plasmalemma) of eukaryotic microalgae are membrane-bounded while the organelles in the prokaryotic microalgae are lack of membrane-bounded (Achyuthan *et al.,* 2017). The major taxonomical classification of algae includes Rhodophyta (red algae), Chlorophyta (green algae), Phaeophyta (brown algae), Bacillariophyta (diatoms), Chrysophyta (golden algae), Haptopyta, Stramenopiles and Dynophyta (Heimann & Huerlimann 2015; Udaiyappan *et al.,* 2017). The largest group of microalgae on earth is believed from the group of diatom (Demirbas, 2011).

Genetically distinct physiological and biochemical characteristics assisted in manufacturing a variety of unique bioactive compounds (Priyadarshani & Rath 2012). It contributed 50% to the primary productivity of the aquatic ecosystem, which assimilates sunlight, water and carbon dioxide into biomass (Field, 1998; Mehmood *et al.,* 2014) The growth of algae depends very much on its physical parameter; light intensity, pH, turbulence, salinity, temperature, quality and quantity of nutrients.

Table 1. Major advantages and disadvantages of microalgae using microalgae as feedstock (Plaza *et. al.,* 2010; Vassilev & Vassileva, 2016)

Advantages	**Disadvantages**
Sustainable and renewable resources	May encounters fluctuation in algae feedstock supply due regional and seasonal availability.
Do not compete with human and animal feeds and foods	Requires high production costs for pre-treatment, cultivation, harvesting, transportation and storage.
Able to sequester CO_2	Causing other environmental problems
Able to improve standard of living in rural communities	Disturb the balance of natural ecosystems
High productivity	Neurotoxic properties of certain algae can cause serious health problem.
Highly biodegradable & suitable for bioremediation	Genetically modified algae used in the cultivation can disturb the well-being of natural algae.
Harvestable within short period of time	

II. APPLICATION OF MICROALGAE FOR WASTEWATER REMEDIATION

Microalgae are widely used in wastewater treatment due to its ability to removes contaminants and the biomass produced is considered sustainable, although some disadvantages and advantages were found (Table 1). Nitrogen, phosphorus and carbon are the three main pollutants found in wastewater (Delrue *et al.,* 2010). Primary and secondary treatment for wastewater often causes eutrophication and other environmental problems when discharged into the environment (Rathod, 2015). To some extends, it only removes organic materials but fails to eliminate inorganic materials like nitrogen, phosphorus and several heavy metals (Rathod, 2015).

The wastewater is better choice due to containing some nutrition such as N and P, which were essential elements for microalgal cell growth. Nitrogen is a major nutrient for microalgae production, but it is normally supplied as nitrate in concentration of 50 mgN/L (Fernandez *et al.,* 2018). Phosphorous is the other major nutrient required for microalgae production. In effluents, phosphorous is normally found as phosphate or in organic compounds. The wastewater usually has high chemical oxygen demand (COD) and BOD due to the presence of organic components (sugars, soluble starch, ethanol, volatile fatty acids).

The efficiency and ability of microalgae in treating mineral pollution (i.e., ammonium, nitrate, phosphate) have been well documented (Delrue *et al.,* 2016). A study reported, treatment with *Chlorella sp.* has reduced chemical oxygen demand (COD) by 70%, total nitrogen by 61% and total phosphorus by 61% (Min *et al.,* 2011). Choi and Lee (2012) found that the increasing abundance of *Chorella vulgaris* from 1 – 10 g/L during wastewater treatment increase the removal of biochemical oxygen demand (BOD) (80% - 83%), COD (78% - 82%), total nitrogen (81% - 85%) and total phosphorus (32% - 36%). Metabolically, microalgae grow in the presence of carbon dioxide (CO_2) and light through photosynthesis process thus in wastewater, it can consume bicarbonate ions or CO_2 for carbon source and obtaining inorganic nutrients (e.g., nitrogen and phosphorus) for growth.

Microalgae are able to capture carbon dioxide from the air 50 times higher than terrestrial plants and produce very high biomass (Min *et al.,* 2011). It can reduce the carbon content in the air by capturing 1.6 to 2.0 tonnes of CO_2 for every 1 ton of algal biomass produced, thus reducing the GHG emission (Abinandan & Shanthakumar, 2015; Vassilev & Vassileva, 2016). Microalgae are among superior organism in wastewater treatments process. However, their efficiencies are depending on the composition of the wastewater, and the result may vary due to its species-dependent nature, as shown in Table 2. Their capabilities are depending on the form of the wastewater and vary according to the microalgae species (i.e., *Botryococcus*, *Chlorella* and *Scenedesmus* can assimilate a large amount of CO_2) (Andreotti *et. al.,* 2017; Apandi *et al.,* 2018).

A. Challenges of Using Wastewater as Media for Biomass Production

The utilisation of wastewater as a media for microalgae culture is desirable as it can provide nutrients and reducing the operation cost at least 50% (Lardon *et. al.,* 2009; Xin *et. al.,* 2010; Dalrymple *et al.,* 2013). However, different microalgae species have different efficiencies towards wastewater treatment and can be intoxicating if not reduced to the acceptable level (Kumar & Goyal, 2010; Abinandan & Shanthakumar, 2015).

Wastewater contain a significant amount of suspended solids that may interfere with the growth process of the microalgae. The internal shading increased turbidity and limits the photosynthetic activity of microalgae (Larsdotter, 2006). Other challenges of using wastewater as media for microalgae cultivation are summarised in Figure 1.

Environmental threat and health problems: High nutrient content in wastewater media such as nitrate, phosphate and some macronutrients can limit the light penetration for mass-cultivation (Cho *et al.,* 2011). It also possesses various environmental threat (i.e., water contamination, offensive smell and potentially harmful emission to the environment) (Plaza *et al.,* 2010). Besides, wastewater contains numerous toxic substances that can impede the growth of microalgae and bioaccumulate in human's body if being consumed (Kumar & Royal, 2010).

Nutrient safety: At commercial scale, the end products need to be produced in a massive scale operation. Thus, it is vital to make sure the products are safe to be consumed when utilising wastewater as a nutrient to cultivate microalgae. It is essential to ensure their organic contents as they can be toxic if consumed (Safafar *et al.,* 2016). This is due to the possibility of microalgae to absorb either low essential nutrients or unwanted and alarming substance that lead to low yield and lack quality of biomass (Khatoon *et. al.,* 2016; Ravindran *et al.,* 2016). The best way is always to dilute the wastewater before being used for microalgae cultivation (Chiu *et. al.,* 2014; Ravindran *et al.,* 2016). Wastewater should be diluted to a tolerable limit where the selected strain could grow efficiently in wastewater.

Table 2. Summary of research done by the various researchers utilising tropical agriculture wastewater using various microalgae species for wastewater treatment

Microorganism	**Tropical agriculture Wastewater**	**Finding and remarks**	**References**
Scenedesmus sp. and *Chlorella* sp.	Palm oil mill effluent (POME)	Nutrient removal of 86% Total Nitrogen (TN), 85% Reactive Phosphate (PO_4^{-3}), 77% Total Organic Carbon (TOC) and 48% Chemical Oxygen Demand (COD)	Hariz *et al.* (2019)
C. vulgaris, C. pyrenoidosa, Haematococus pluvialis, S. obliquus, S. platensis and *Porphyridium cruentum*	Piggery wastewater	Nutrient removal of 89.5% TN and 85.3% TP	Wang *et al.* (2016a)
Mixed microalgae	Dairy Wastewater	Removal of 90% organic carbon, biochemical composition of 38% carbohydrates, 15% proteins and 22% lipids	Hemalatha *et al.* (2019)
Scenedesmus obliquus	Paddy-soaked rice mill wastewater	Removal of 96% ammonical nitrogen, 97.58 % phosphates, biochemical composition of lipids 12%, protein 40%, and carbohydrates 20%	Umamaheswari & Shanthakumar (2019)

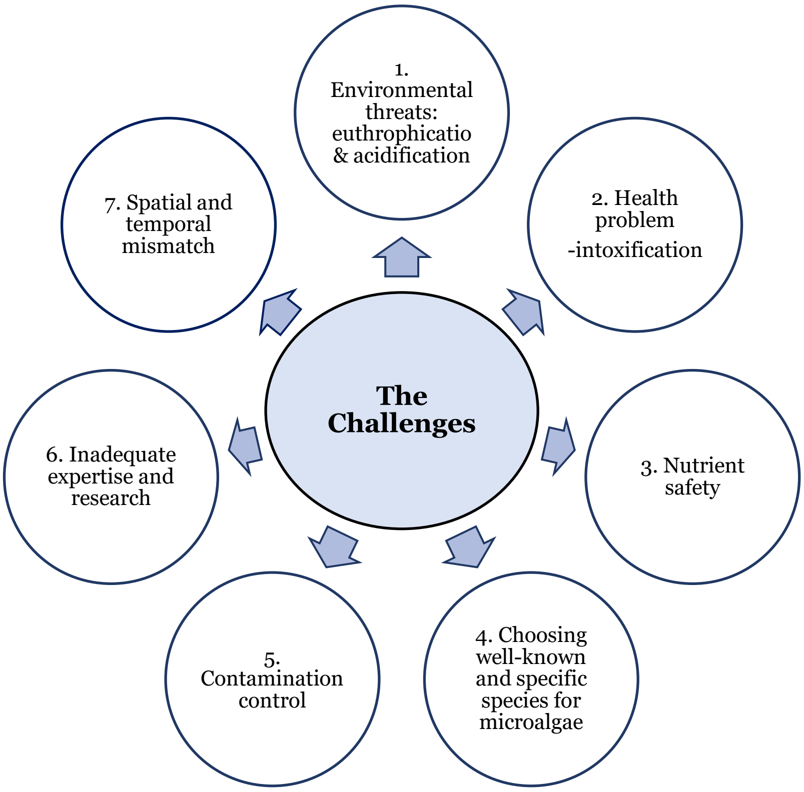

Figure 1. Challenges of using wastewater as media for microalgae (Plaza *et. al.,* 2010; Ndukwe *et. al.,* 2012; Wu *et. al.*, 2014; Muylaert *et. al.,* 2015; Delrue *et. al.,* 2016; Safafar *et. al.,* 2016; Maizatul *et al.,* 2017).

Choosing well-known and specific species of microalgae: The potential use of microalgae in wastewater treatment has been evaluated through the ability of species in treating specific pollutants (i.e., ammoniacal nitrogen, phosphorus) and wastewater (i.e., industrial, agriculture) (Delrue *et al.,* 2016). Not all microalgae species meet a specific nutritional requirement to serve their purpose. Excellent compatibility between microalgae species with the specific properties of wastewater is required to ensure the successfulness of the treatment. Thus, a preliminary screening technique is crucial to choose the suitable target species depending on their growth tolerance on the wastewater environment. Prior to that matter, there must be proper knowledge about the specific characteristics and biochemical composition of microalgae (Brown, 2002). For a commercial purpose, it is advisable to choose native microalgae species that well adapted to their environment as each product from microalgae usually distinct and species dependent (Maizatul *et al.,* 2017).

Contamination control: The occurrence of bio-contamination in microalgae cultivation tank is high when using wastewater (Wu *et al.,* 2014). For a small scale, the sterilisation method is proven useful to prevent contamination. However, the sterilisation method is not suitable to be used in large-scale cultivation. The best options are to screen and choose the strongest resistance microalgae and apply control technology to manipulate the genetic of microalgae against contamination (Chinnasamy *et. al.,* 2010; Wu *et. al.,* 2014; Gani *et al.,* 2016).

Inadequacy expertise and research: Although there are many scientific studies that were conducted to utilise wastewater for microalgae cultivation; there is still a gap for some relevant information. There have been minimal detailed investigations on other applications that include the efficacy and safety of consuming it. In recent years, there have been experiments and trials but only on a small scale (Ndukwe *et. al.,* 2012; Maizatul *et al.,* 2017). Researchers have not treated this subject in much detail, which contributes to a limited number of relevant expertise in this area. The knowledge from expertise is very critical to ensure the success in commercial applications.

Wastewater and microalgae availability: Microalgae farm often located far from the wastewater sources and it is not an economical wise to transport over a long distance. It is recommended to operate the microalgae farm near wastewater sources as the cost of transportation can be a major burden to the manufacturer. Another possible problem that may arise in an urban area is the limitation of space and high land rental cost. The spatial and temporal mismatch of microalgae between microalgae and wastewater availability makes the microalgae business look unattractive according to Muylaert *et al.* (2015). Despite all the above mention challenges of using wastewater to grow microalgae, some strategies can be applied to make it feasible as culture media (i.e., pretreatment).

III. WASTEWATER TREATMENT

Wastewater must undergo several pretreatment processes before being feasibly used as culturing media for microalgae, including various methods of mechanical, chemical or biological. Majority of microalgae species can accumulate heavy metals concentration level about 8% of their dry mass (Mehmood *et al.,* 2014). Basic wastewater treatment of raw sewage includes pretreatment, primary treatment, secondary treatment and tertiary treatment before being released into receiving bodies. As stated by regulatory agencies, industrial wastewaters must be pretreated before being discharged to the municipal sewer system to remove materials (i.e., silver ions which are toxic to bacteria) that inhibit the biological processes in secondary treatment. Pretreatment also important to reduce operational problems.

The pretreatment processes aim to lower microorganism, COD, BOD, turbidity and suspended solids in the wastewater treatment system. However, the efficacy of these processes is hindered with the occurrence of numerous inhibitory substances such as complex organics, heavy metal substances and extracellular polymeric substances (EPS) (Carballa *et. al.,* 2011; Abelleira-Pereira *et al.,* 2015). Examples of pretreatment methods used to treat wastewater are tabulated in Table 3. Pretreatment has proven to remove high unwanted nutrients (i.e., COD, BOD, TN and TP) and improved productivity.

Pre-treatment can improve microalgae cultivation process, but some method like Fenton and visible-photocatalysis pre-treatment method requires high energy demand, thus increasing the operational cost and uneconomical for field application. Therefore, it is advisable to choose the right wastewater pre-treatment to minimise the shortcoming effects before the cultivation and slight modification can reduce the energy demand. Overall, any pre-treatment process of wastewater is highly recommended to enhance microalgal growth and production of various products (i.e., cosmetic, pharmaceutical).

Table 3. Recent pretreatment methods used to treat wastewater

Wastewater type	**Pretreatment**	**Contaminant removal and productivity**	**References**
Yard waste wastewater	Electrochemical	net energy gain of 4.75 kJ/g, biogas production improved 55.4%	Panigrahi & Dubey (2019)
Sewage sludge wastewater	Microwave	OLR up to 2.80×10^{-5} kg TVS, increased the methane yield by 20%, 70% biodegradability	Gil *et al.* (2018)
Furfural wastewater	Acid	Increased 59% of methane production	Wang *et al.* (2019)
Folic acid (FA) wastewater	Three-dimensional electro-Fenton	COD removal 43.5% and TN removal 70.4% in 6 h	Zheng *et al.* (2019)
Piggery wastewater	Fermented superphosphate	Removal percentage of NH3-N reached 64%, and the COD/TN ratio increased from 0.36 to 2.28, e TN removal percentage in FSP/Pretreatment-SBR was 57%,	Luo *et al.* (2019)
Farm wastewater	Thermo oxidation and alkali	Yield methane 62% higher	Xiong *et al.* (2020)
Olive mill wastewater	Ultrasound	Increased SCOD/TCOD ratio from 0.59 to 0.79, produced approximately 20% more biogas and methane	Oz & Uzun (2015)
Palm Oil Mill Effluent (POME)	Ozone	Maximum H_2 yield (62 mL.g^{-1}), COD removed (30,000 mg COD.L^{-1})	Tanikkul *et al.* (2019)
Palm Oil Mill Effluent (POME)	Fenton oxidation	TOC reduction of 91%	Gamaralalage *et al.* (2019)

IV. CO-PRODUCTION OF VALUE-ADDED PRODUCT FROM MICROALGAE BIOMASS

Previously, microalgae grown in wastewater was meant for energy production and limited for other applications. Nowadays, microalgae are grown to accumulate their value-added products; pigment, carbohydrate, protein and lipid. Table 5 summarises the useful metabolites in different microalgae species that has been used for many applications such as for food, pharmaceutical, cosmetic and fertiliser.

Primary (i.e. carbohydrates, proteins and lipids) and secondary metabolites (i.e. antibiotics, antiviral, antitumor, antioxidant, terpenoids, phlorotannins, steroids, phenolic compounds, and halogenated ketones) in microalgae carry its own functions. Primary metabolites are for growth while secondary metabolites act as a defence mechanism for microalgae. Secondary metabolites are extracted and widely used in pharmaceutical and cosmetics (Prathana and Maruthi 2019).

These metabolites can be induced by manipulating their growth condition. For instance, when microalgae are exposed to a radical stress condition, protective systems are developed against radical stress by preventing the accumulation of free radicals (Udaiyappan *et al.,* 2017). This will help them to protect themselves from cell-damaging activities. The metabolites induced by this stress condition can be extracted and potentially be useful for human well-being.

V. THE SIGNIFICANT USE OF MICROALGAE BIOMASS CULTIVATED IN WASTEWATER FOR AQUACULTURE FEED

In a bigger picture, the whole algal biomass cultivated in wastewater is best suited applied for aquaculture feed, biohydrogen and biofertiliser purposes.

A. Aquaculture Feed

Sources for high-quality nutrition-filled food invariably one of the high demands in the aquaculture sector. Hence, the need for seafood continuously increases, which lead to the increasing value of the aquaculture market and products. Even then, at the early 1990s, most of microalgae species such as *Chlorella* sp., *Chaetoceros* sp. and *Isochrysis* sp. were already utilised as a feed for bivalves, shrimp and fish in juvenile stages (Shamsudin, 1992; Udaiyappan *et. al.,* 2017; Show *et. al.,* 2017; Cheah *et al.,* 2018). The nutritional compositions that microalgae have to offer such as high level of PUFA, crucial protein and omega-3 can potentially replace the function of fish meal and oil required in aquaculture feed (Lenihan-Geels *et. al.,* 2013; Roy *et. al.,* 2014; Yaakob *et. al.,* 2014; Khatoon *et. al.,* 2016; Younis *et al.,* 2018).

Since protein is considered the most expensive nutrient in fish feed, it may be crucial to develop sustainable alternatives to fishmeal. Compared to the other protein sources, the protein content in microalgae is higher, even higher than egg, meat and soybean (Table 4).

Table 4. The comparison of protein content from typical composition of commercially available feed Ingredients and microalgae species (Guedes *et. al.,* 2015; Koyande *et al.,* 2019).

Feed ingredients	**Protein content (% dry matter)**
Poultry meal	58.0
Corn-gluten	62.0
Soybean	44.0
Wheat meal	12.2
Soybean	44.0
Poultry meal	58.0
Whole egg	47
Fish meal	63
Chlorella sp.	50-60
Spirulina sp.	60-70

It is quite challenging in culturing microalgae in wastewater for aquaculture application because of the disease threat and food security concern. However, one research conducted by Hende *et al.* (2015) found that the harvested microalgae-bacterial flocks from aquaculture wastewater can be used as a partial inclusion in the diet of juvenile Pacific white shrimps Litopenaeus vannamei to enhance the pigmentation of the cooked shrimp tails without affecting the shrimp survival, weight gain, size distribution and food conversion rate. Another study conducted by Loo *et al.* (2012) found that bacterium Rhodovulum sulfidophilum and microalgae Nannochloropsis sp. cultured in Palm oil mill effluent can be used as a diet for marble goby larvae and has a better result if it is indirectly fed through Artemia nauplii or rotifers up to 35–55 % or 44–49 % of survival at 5 g L–1 salinity, respectively. In short, it is proven that the cultivation of microalgae using wastewater media is not impossible under controlled conditions to reduce the risk of disease and food security. It is suggested that additional pre- and post-treatment can tackle this problem quickly. Microalgae are versatile and can serve many purposes in the aquaculture sector.

1. Formulated fish pellet

The fish pellet is one of the widely used form of feed-in aquaculture, in which microalgae biomass will be incorporated into the pellet according to their specific nutritional requirements to promotes health, colour and growth of targeted aquaculture organisms (Relicardo, 2015). This method is the most preferred method by most aquaculture farmer because of its lower risk of infection by disease carrier.

Table 5. Useful metabolites in microalgae biomass

Species	**Metabolites**	**Applications**	**References**
Heterochlorella luteoviridis & *Dunaliella tertiolecta*	Luetin	Food and pharmaceutical	Diprat *et al.* (2017)
Arthrospira and *Chlorella*	Extract	Cosmetic	Patrick & Barbara (2005)
Characium terrestre, Chlorogloeopsis sp., Chlorella sorokiniana, Dunaliella tertiolecta	Sporopollenin, Scytonemin and mycosporine-like amino acid	UV protectant	Priyadarshani & Bath (2012)
Dunaliella salina	Beta-carotene	Food colorant	Priyadarshani & Bath (2012)
Chlorella vulgaris	Phenolic compounds	Immune-modulating, antitumor, antibacterial, and anti-inflammatory	Kwang *et al.* (2010) Safi *et al.* (2014)
Chlorella vulgaris & *Spirulina platensis*	Minerals and vitamin	Biofertiliser	Safi *et al.* (2014) Dineshkumar *et al.* (2017)
Tisochrysis lutea & *Nannochloropsis gaditana*	-	Inclusion in fish diet	Vizcaíno *et al.* (2018)
Cyanothece epiphytica	Exopolysaccaharides (EPS)	Bio-lubricant	Borah *et al.* (2018)
Scenedesmus sp.,	Lipid	Biofuels	Contreras-Angulo *et al.* (2019)
Chlamydomonas reinhardtii	Acetic acid	Hydrogen gas	Fakhimi & Tavakoi (2019)

2. The greenwater application

This technique involves the natural assemblages of microalgae to feed for commercialised finfish larvae directly (Neori, 2010). *Tetraselmis sp.*, *Isochrysis sp.* and *Nannochloropsis sp.* are among the suitable microalgae species for this purpose. In Korea, Japan and China, this technique is already being applied as it improved water quality, aquatic organism's survival and growth rate (Neori 2010). Further research on the understanding of the interaction between aquatic organisms and population of algae, the ways to control and manage them, the location of the farm for proper dispersal of waste and fitting technology could help in establishing this method.

3. Live feed

Live feed usually preferred by the aquatic larvae organisms when their feeding ability is not yet developed. Most aquatic larvae have an incomplete digestive system, small mouth size for feeding upon hatching. Proper handling technique and technology is needed to ensure the balanced nutrition is delivered to the larvae (Brown, 2002). Whole microalgae cells are sufficient to fulfil fish larvae diet. Therefore, live microalgae are often used as a feed. The criteria for suitable microalgae to be used as live feed are it must be easily digested, high growth rate and contain proper nutrition (Brown, 2002). Microalgae are used as tools to deliver important nutrients like PUFA (SC-PUFA and HUFA) and FAA to fish larvae through natural live prey (rotifer, brine shrimp, daphnia and etc.) because naturally these live preys are missing with important nutrients for fish larvae (Vu *et al.,* 2019). Microalgae can serve as a complementary diet for these natural live preys prior to feeding to fish larvae in the hatchery. It is estimated that more than 40 species of microalgae have been isolated and cultivated as pure strains in intensive systems (Becker, 2004; Shield & Lupatsch, 2012). The most common species used in commercial mariculture are diatoms *Skeletonema costatum*, *Thalassiosira pseudonana*, *Chaetoceros gracilis*, *C. calcitrans*, the *flagellates Isochrysis galbana*, *Tetraselmis suecica*, *Monochrysis lutheri*, *Nannochloropsis spp.*, and the *benthic diatoms Nitzschia palaecea* and *N. closterium* (Becker, 2004).

B. Biohydrogen Producer

Lage *et al.* (2018) pointed out that microalgae biomass grown in wastewater is not suitable to be used as food, feed, or biofertiliser instead be used as a source of energy. The increasing demand for energy and depleted natural resources for energy production has initiated the search for alternative green energy resources. Some green microalgae, cyanobacteria and photosynthetic & non-photosynthetic bacteria are capable of producing biohydrogen gas (Khetkorn *et al.,* 2017). The mechanism of hydrogen gas production in microalgae is facilitated through the action of hydrogenase enzyme that is produced under specific favourable condition. Several microalgae that may possess a hydrogenase enzyme for hydrogen production are identified from the species of Anabaena, Botryococcus, Chlamydomonas, Chlorella, Nostoc, Scenedesmus, Tetraspora and etc.

Due to high production cost and low production yield, commercial hydrogen production from microalgae biomass is still under ongoing studies. Nevertheless, with the current advancement of metabolic and genetic engineering approaches, more efficient biohydrogen production from microalgae and other microorganism is expected from the future. By studying the microalgae composition, the biochemical content of protein, carbohydrate and lipid is known to be dependent on growth conditions and microalgae species. Xiao *et al.* (2010) evaluated that glucose was able to produce 18 times more hydrogen gas in thermal treated sludge. This is because Hydrogen Producing Bacteria (HPB) can hydrolyse sugar faster during the dark fermentation process (Bai *et al.,* 2004).

C. Biofertiliser for Agriculture

The controversial issue of using chemical fertiliser can be easily tackled using microalgae grown in wastewater. The subsequent biomass produced from the wastewater treatment could be used as cheap biofertiliser in the agriculture sector. Biological material with high concentrations of sequestered plant nutrients and fertiliser from microalgae biomass could reduce the cost and dependency on conventional fertiliser. Unfortunately, there is limited research on evaluating the performance efficacy of these materials like fertilisers, specifically in horticultural applications.

Nowadays, the introduction of biofertiliser has emerged as highly sustainable agriculture due to its green resource, cheap and non-toxic properties. By having a less negative impact on the environment and greener alternative for the agriculture sector, biofertiliser is considered superior over conventional chemical fertiliser in agriculture (Chatterjee *et al.,* 2017). Banayo *et al.* (2012) simplified biofertiliser as free-living organisms associated with root surfaces. Still, they may also contain endophytes microorganisms which able to colonise the intercellular and intracellular spaces of plant tissues without causing apparent damage to the host plant. The microorganisms, which are commonly used as biofertilisers, belong to families of bacteria, blue-green algae and fungi (Mazid & Khan, 2014). Algal biofertiliser is believed to improve the quality of soil by separating the sodium salt from the soil and convert alkaline soil into fertility soil (Embrandiri *et al.,* 2012). The potential to mobilise insoluble forms of inorganic phosphates to restore soil nutrients by secreting exopolysaccharides and bioactive substances into the agricultural soil has nominated the blue-green algae (BGA) as a suitable candidate for biofertiliser (Chatterjee *et al.,* 2017).

A study conducted by Garcia-Gonzalez and Sommerfeld (2015) utilising dried green alga Acutodesmus dimorphuswere as a primer for plant seeds, foliar spray, and biofertiliser was found to enhance seed germination, plant growth and floral production in tomatoes. Another study conducted by Subramanian and Jayasingam (2017) reported potential usage of marine microalgae as alternative fertiliser in maize cultivation by enhancing high growth and yield, which require further study to produce microalgae-based fertiliser composition. It also believes that some red algae used as biofertilisers also helps to increase the growth nutritional value and yield of agriculture plants (Chatterjee *et al.,* 2017). Mazid and Khan (2014) reported that combination of algae and rock phosphate improved rice straw and grain yield production as phosphate promotes root and height development, fresh bulb weight, root colonisation and phosphate uptake of wheat plants (Mazid & Khan, 2014).

Biofertiliser can be presented in liquid or solid form. Liquid bio-fertilisers are formulations of special liquid containing desired microorganisms added with the unique chemical that act as cell protectants to the resting spores so that it becomes more tolerant to harsh condition and can last longer (Mazid & Khan, 2014). The solid form of biofertiliser is always incorporated with a suitable carrier which their expiry period is only limited to 6 months depending on the type of carrier. The prolong use of microalgae as biofertiliser for 3 to 4 years is believed to lessen and completely eradicate the need to use any chemical fertiliser in the future.

VI. INTEGRATING WASTEWATER TREATMENT WITH AQUACULTURE AND AGRICULTURE APPLICATION: OLD CONCEPT WITH NEW APPLICATION

The application of microalgae grown in wastewater (i.e., palm oil wastewater) can be integrated with the aquaculture and agriculture for more sustainable approaches by utilising waste material to sustain another activity. In Greece, the concept of integrated aquaculture for the last 30 years has been adopted to minimise the environmental impacts and simultaneously reduce the feeding cost (Vatsos *et al.,* 2015). Meanwhile, the other parts of Asia are prevalent in applying rice-fish farming as an integrated production system (Halwart & Gupta, 2004).

In Malaysia, approximately 3.75 tonnes of palm oil mill effluent (POME) was produced and requires a larger space of ponding system POME treatment (Ahmad *et al.,* 2003). The treated wastewater and biomass produced should not be wasted; therefore, recent technology incorporating of POME treatment with microalgae seems promising. By integrating wastewater treatment with aquaculture and agriculture application, nothing will be wasted and at the same time can generate side income for smallholder farmers. This integration concept would benefit land used efficiency by using optimum space for installation. This method maximises resources utilisation by promoting crops diversity. Livestock production, well-organised land management and preventing any further destruction to the Earth to meet human's need for the future.

The circular valorisation or operational circularity concept is useful in attaining more sustainable approaches by converting waste material into beneficial product and enhance the economic growth among farmers (Figure 2). Utilisation of microalgae to treat POME is a good example of this concept. The treated wastewater will be fully utilised to fertilised crops and biomass produced as food for aquaculture organism. Hence, this concept promotes zero-waste application while reducing the dependency on chemical fertiliser and aquaculture artificial feed.

The overall integrated process is illustrated in Figure 3. This affordable integration system can be installed at a very low-cost with the appropriate technology. The concept of this process is to grow microalgae in the fish pond so it can consume the free flows of nutrient from fish manure. After several days cultivated in the fish pond, the cultured microalgae will be used to inoculate POME in the treatment ponds. The valuable microalgae biomass harvested from the treatment ponds can be utilised as fish feed, and simultaneously, the treated wastewater can be used to irrigate crops field. This process would minimise the negative impact associated with agricultural activities by reducing the GHGs emission and degrade the organic material present in POME effectively. With low energy consumption and labour force, this treatment system can be turned into high profitability business in the market, especially for rural farmers. It has a promising impact on the economy and social improvement among poor farmers. It has more potential in promoting more sustainable income with available waste resources and hence promoting more environmental-friendly integrated agriculture-aquaculture system. As a result, it can attract more collaboration between both conventional farmers and fish farmer to share their water resources to overcome waters scarcity issue.

The concept of integrating wastewater treatment with aquaculture and agriculture application has been put forward in this paper to give a social benefit, especially for rural communities. It is a win-win situation where the industry gets to eliminate wastewater, and society gets to increase its in-house income. The main idea in this system is to reduce the impact of wastewater on the environment by converting it into useful products and to maximise the production of the crop. This concept embarks with the higher sustainability approach to improve food security for the future generation and ensure the land availability is well sustained.

VII. STRATEGIES TO INCREASE YIELD OF MICROALGAE BIOMASS

Traditional methods of microalgae cultivation for commercialisation are always associated with low biomass yield, high operational costs, harvesting problems which often lead to poor techno-economic performance. Usually, algal storage causes nutrient degradation and affecting their maximum capabilities for utilisation. For various algal applications, it is essential to find ways which can increase the yield of microalgae biomass at its best. This includes co-cultivation the microalgae with other organisms, manipulating microalgae growth conditions and improving culture system/design. Recently there has been growing interest in co-cultivation systems incorporating microalgae with fungi, yeast and bacteria to enhance biomass production, enhanced lipid production and decrease nutrient/energy inputs leading to low-cost installation and effective bio-flocculation of microalgae biomass.

A. Co-Cultivation

The previous studies have proven microalgae-fungi co-cultivation had improved the growth of microalgae, suggesting a potential for microalgae-fungal symbiosis (Simpson, 2018; Zhou *et al.,* 2018). Fungi are unique; the filamentous structure allows them to self-pelletise eases harvesting process in algae-fungi co-cultivation system. The coagulative machinery use spores for palletisation, whereas non-coagulative machinery consist of the germinated hyphae from the spores, which then will be interlinked to form pellets (Gultom & Hu, 2013). Fungi can metabolise glucose ($C_6H_{12}O_6$) and releases carbon dioxide (CO_2). Later, microalgae assimilate CO_2 through photosynthesis to release oxygen gasses (O_2). Ammonia is produced when microalgae metabolised NO^{2-} to and taken up by fungi as a nutrient (Hom and Murray 2014). Few fungal such as *Aspergillus* spp, *Basidiomycete* spp, *Phanerochaete* spp shows coagulative ability which causes the cell to aggregate into lumps (Wrede *et al.,* 2014). Microalgae-fungi cultivation is being used for many environmental applications (i.e., bio-flocculation, nutrient and CO_2 removal in wastewater treatment, wastewater pre-treatment, and in aquaculture application) (Ummalyma *et. al.,* 2017; Zhou *et al.,* 2018). It was found that bio-flocculation of microalgae cell using fungi are superior method in term of its economics, harvesting efficiency, technological possibilities (Ummalyma *et al.,* 2017). Besides, their co-production of value-added product form the symbiotic of microalgae with fungal has been industrially utilised for its astaxanthin production (Dong & Zhao 2004), biodiesel (Shu *et al.,* 2013), lipid and carotenoid (Santos *et al.,* 2013), and as a model for micro-ecosystem (Hom & Murray 2014).

Co-cultivation of microalgae with bacteria has been reported to cause both stimulation and inhibition effect to microalgae growth. Some bacteria are capable of killing microalgae by producing an enzyme that can break the algal cell wall and cause algal lysis (Morris, 1962; Cole, 1982; Fergola *et al.,* 2007). Bacteria stimulates microalgae growth by degrading the intractable compounds (i.e., ammonium, nitrogen, phosphate and carbon dioxide) for nutrient absorption and in returns, microalgae provide essential nutrients (i.e., Vitamin B12) for bacteria (Croft *et. al.,* 2005; Zhang *et al.,* 2012). However, not all microalgae-bacteria interaction stimulates microalgae growth. Fakhimi and Tavakolia (2019) had shown the effect of bacteria co-cultivation of *Chlamydomonas reinhardtii* with *Escherichia coli, Pseudomonas stutzeri* and *Pseudomonas putida* enhanced hydrogen production up to 56% but reduced the growth of *Chlamydomonas*.

Co-cultivation of microalgae with yeast has proven to improve about 40–50% of biomass and 60–70% of total lipid in 5L photobioreactor cultivation (Yen *et al.,* 2015). The mechanism involves between algae-yeast co-culture system are the result of gas utilisation and providing of trace elements to each other after the natural cells lysis (Yen *et al.,* 2015). Microalgae-yeast co-culture is also beneficial for wastewater treatment as it can remove 96% of nitrate, 100% of TAN and 93% of orthophosphate as it allows of aerobic fermentation condition (Walls *et al.,* 2019).

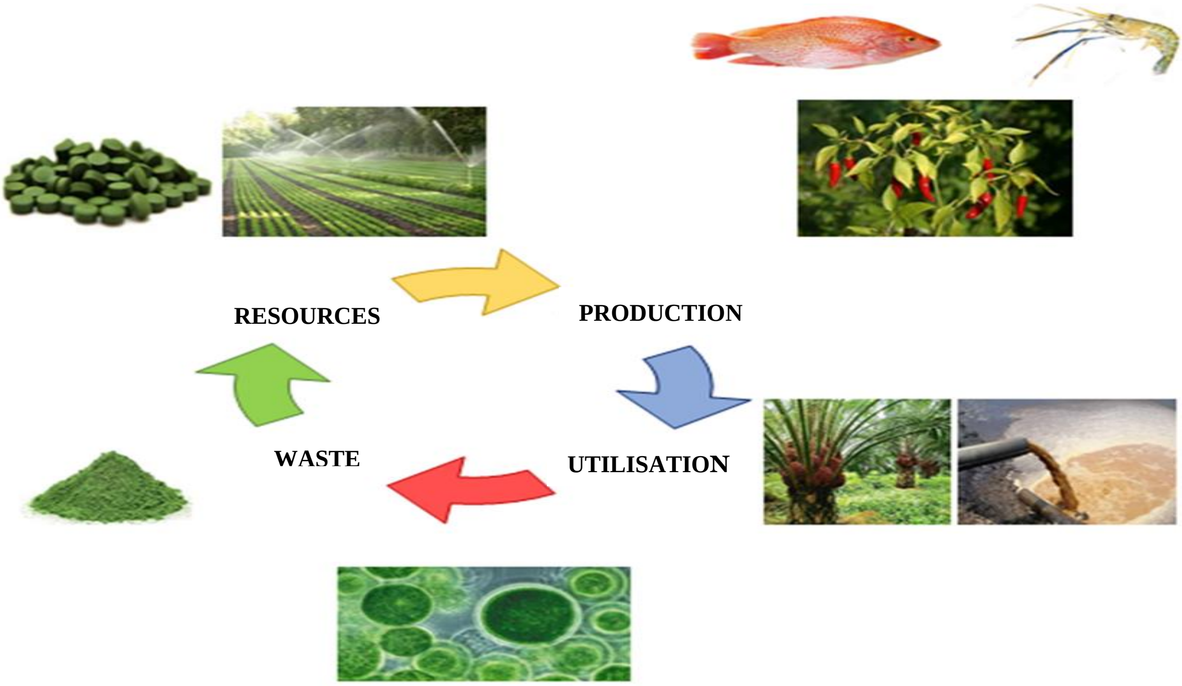

Figure 2. The operational concept of circularity

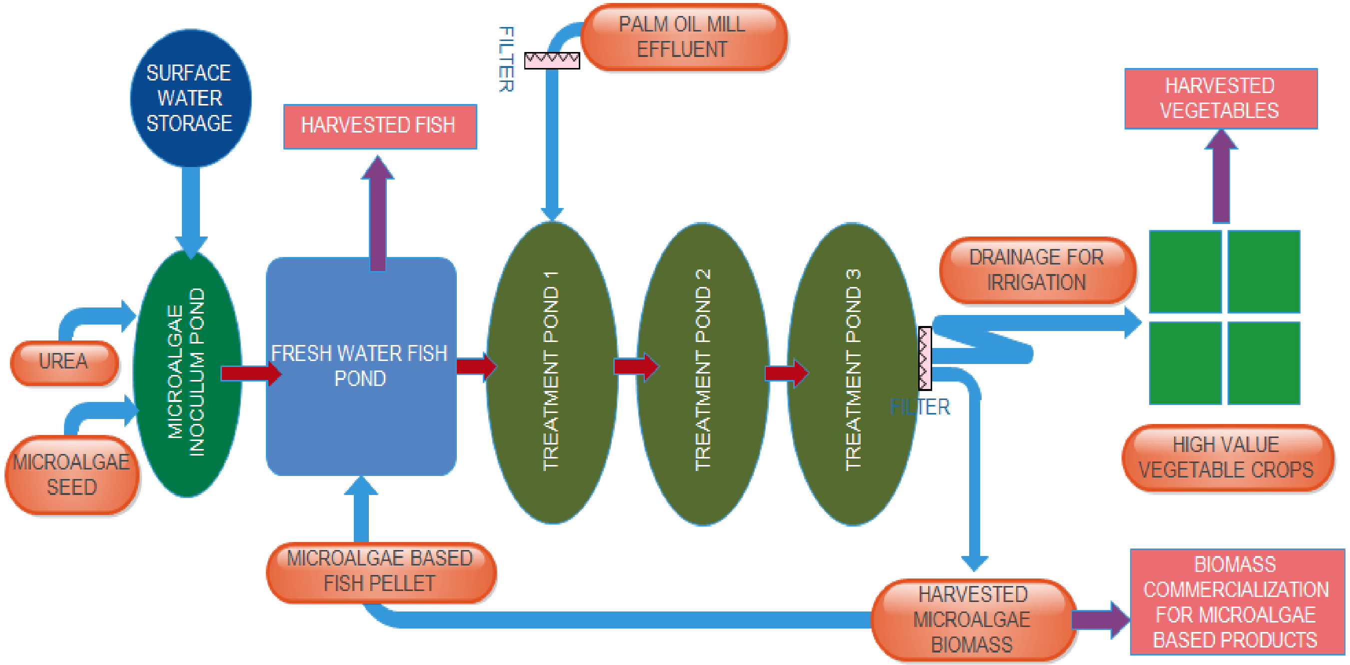

Figure 3. The overall process flow of the treatment system

B. Manipulating The Growth Conditions and Acclimatisation

Four main microalgae conditions that can be manipulated for optimum growth are photoautotrophy, heterotrophy, mixotrophy and photo-heterotrophy (Chojnacka & Noworyta, 2004). A study reported a 1.9-fold increment in biomass output was obtained by manipulating cultivation condition can maximising cost efficiency in industrial production at a large scale (Engin *et al.,* 2017). Another study conducted by Wu and Shi (2007) on heterotrophic cultivation of *Chlorella pyrenoidosa* produced microalgae cell densities as high as 104.9 g·L−1 of dry weight while Gupta and Pawar (2018) found that mixotrophic cultivation of microalgae improved the quality of lipid and acetate. Ra *et al.* (2017) reported *Isochrysis galbana, Phaeodactylum tricornutum* and *Nannochloropsis salina* produced 1.03 g DCW/L, 0.95 g DCW/L, 0.85 g DCW/L, and 0.62 g DCW/L respectively when introduced with mix LED wavelength. Therefore, it is necessary to find economical and effective ways to increase the microalgae yield for commercial scale and to minimise the environmental impact using the methods discussed above.

Under normal culture condition, the differences in the growth medium and environment could challenge microalgae performance. Therefore, an acclimation step can be applied to help microalgae for better adapt to the new environment (Hu *et al.,* 2019). The acclimatisation of microalgae has many potentials. For instance, the acclimatised *Chlorella* sp. to hypersaline condition offers a mean to reduce contamination (Anandraj *et al.,* 2020). Hu *et al.* (2019) also reported acclimated *S. obliquus*, *C. vulgaris* and *C. sorokiniana* achieved higher biomass than those without acclimation thus remove organic pollutants efficiently and the biomass produced were used for further applications. Acclimation had successfully shortened the microalgae's lag time as proven by Khalid AAH *et al.* (2018) and further improvement of the microalgae growth are proven feasible. In short, the acclimatised microalgae can reduce or eliminate the high cost of energy-intensive microalgae processes.

C. Culture System and Design

The cultivation systems can be classified into three major systems, hybrid production systems (Medipally *et al.,* 2015), closed and open system (Wu *et al.,* 2012). Open-air systems in shallow ponds, tanks, circular ponds and raceway ponds are widely used to imitate the natural habitat of microalgae. This system is characterised by simple and inexpensive, but the biomass yield and system stability are more reduced than other systems (Borowitzka, 1999). Meanwhile, for closed culture system, the photobioreactors (PBR) are made of distinct configurations such as tubular, flat plate (Wu *et al.,* 2012), fluidised-bed bioreactors (FBR), parallel-plate bioreactors (PPR), air-lift bioreactors (ALR), hollow-fibre bioreactor (HFR) and column photobioreactors (Malik, 2002). Closed culture system allowing higher cell density at higher capital and operating cost but easier to control and manage (Wu *et al.,* 2012). Nevertheless, the efficiency of phototrophic growth of microalgae in both systems subject to the light source and intensity (Medipally *et al.,* 2015). Approximately 200–400 μM photons $m^{-2}s^{-1}$ average of light intensity, required by most of algae species to obtain the maximum photosynthesis (Medipally *et al.,* 2015). Better results could be obtained by combining both open and closed culture system (hybrid system). In a hybrid system, the required amount of contamination-free inocula obtained from photobioreactors is transferred to open ponds or raceways to get higher biomass output (Grobbelaar, 2000; Greenwell *et al.,* 2009). This system was used by Olaizola (2000) and Huntley and Redalje (2007) in the production of astaxanthin from *Haematococcus pluvialis*. However, this system is more expensive, and it is also a batch culture system rather than a continuous culture system.

D. Strain Improvement by Metabolic Engineering

Recent technology advancement in microalgae is aimed in the improvement of microalgae biomass productivity. The utilisation of numerous genomic tools and advance biotechnology methods such as synthetic gene construction and manipulation of metabolic pathways empowered growth of cell production and its metabolic activity (Jagadevan *et al.,* 2018). Improving strain development is the main focus in most of the metabolic engineering studies and necessitates reliable tools, knowledge and resources which are very limited at the moment. Maximising algal growth and its metabolic output through metabolic engineering is future feasibility as a substitution to the non-renewable fossil fuels. Further research in this technology is essential, particularly in commercial-scale on productions of biofuels from microalgae concurrently obtaining maximisation profit output (Medipally *et al.,* 2015).

E. Nutrient Supplementation And Chemical Enhancer

The growth of microalgae in wastewater media might be limited, but nutrient supplementation is always helpful in enhancing the microalgae biomass. Ansari *et al.* (2017) used sodium nitrate supplementation in aquaculture wastewater to improve the productivities of biomass, lipid, carbohydrate and protein. The result showed the biomass productivity of *Ankistrodesmus falcatus* and *Chlorella sorokiniana* were comparable with synthetic media cultivation. Research conducted by Cheah *et al.* (2018) was done to enhance the production of biomass and lipid in microalgae cultured in POME using nutrient supplementation of glucose, urea and glycerol. This study found that glycerol had higher growth performance as compared to the other supplementation. However, the lipid production was enhanced when supplemented with the mixture of urea, glucose and glycerol supplementation.

Various enhancer chemicals had proven effective and economical for large scale cultivation. The chemicals are classified into four categories: chemicals regulating biosynthetic pathways, chemicals inducing oxidative stress responses, phytohormones and analogues regulating multiple aspects of microalgae metabolism, and chemicals directly as metabolic precursors (Yu *et al.,* 2015). Herein is the summarised chemical used in enhancing the microalgae products (Table 6).

VIII. FUTURE PERSPECTIVE

Overall, the utilisation of wastewater in media cultivation for microalgae is restricted due to the expensive treatment and safety concern. However, this pitfall can be reduced in the future together with a further intensive scientific study, advanced technology, a better management system and applying better standard protocol. The utilisation of microalgae cultured in wastewater is still limited to certain microalgae species. Therefore, a new local species with improved characteristics and nutrient qualities could improvise the utilisation of microalgae grown in the targeted wastewater. Apart from this, the type of wastewater used to grow microalgae should be diversified not only limited to agriculture wastewater. The characteristic of the wastewater should be well studied. More research should be conducted on the interaction of microalgae with another organism as they can produce valuable substances from the interaction, such as the production of the certain antibiotic. At the same time, a major problem in rural agriculture with untreated agro-waste that contribute to environmental pollution can be reduced with the full utilisation of agro-waste usage.

IX. CONCLUSION

Agriculture wastewater management is the bottleneck when agriculture activity is the main economic activities. Recent trends have highlighted that microalgae have the potential to remediate wastewater and can turn into profitable biomass. It is feasible to integrate microalgal cultivation in wastewater for green energy production and waived the security and toxicity concern. Still, for other purposes like for animal feed production, additional efforts must be put to secure the security concern. Therefore, a clear picture of multiples strategies to turn microalgae biomass into multiple applications, especially in agriculture and aquaculture has been set forth in this paper. This study concludes that with a considerable effort, agriculture wastewater can be used as microalgae feedstock for co-production of value-added products from microalgae biomass provided appropriate treatment and ways to increase microalgae yield is optimised.

X. ACKNOWLEDGEMENT

We gratefully acknowledged the financial support from Geran Universiti Penyelidikan (GUP-2018-092) from Universiti Kebangsaan Malaysia.

Table 6. Chemicals used to enhance growth and products in microalgae (Yu *et. al.*, 2015; Cho *et al.*, 2019).

Species	**Products**	**Chemicals**
Chlorella vulgaris	Biomass	Brassinosteroids (BRs) Indomethacin (IM) Salicylic acid (SA) Diamines, polyamines Zeatin
Haematococcus pluvialis	Astaxanthin	Salicylic acid (SA) 2, 4-Epibrassinolide (EBR) Jasmonic acid (JA) Salicylic acid (SA) Methyl jsmonate (MJ), gibberellic acid (GA3)
Schizochytrium sp. HX-308	DHA	Ethanol, sodium acetate, malic acid
Scenedesmus obliquus	Biomass	Methanol
Chlorella sorokiniana	Biomass and lipid	2-phenylacetic acid (PAA), Indole butyric acid (IBA), 1-naphthaleneacetic acid (NAA), Gibberellic acid (GA3), Zeatin, thidiazuron, Humic acid, Kelp extract, Methanol, Fe, Putrescine, Supermidine
Spirulina platensis	Total carotenoids and α-tocopherol, glutathione (GSH), and ascorbic acid (AsA)	H_2O_2
Dunaliella salina	Biomass and glycerol	Copper
Chlorella vulgaris	Lipid	Fe
Chlamydomonas reinhardtii	Biomass and fatty acid	Indole acetic acid (IAA), gibberellic acid (GA3), kinetin, 1-triacontanol, abscisic acid
Schizochytrium sp. HX-308	DHA	Malic acid (MA) ethanol
Chlorella vulgaris	Biomass and lipid	Bacterial volatile compounds

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