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MICROALGAE AND THE FACTORS INVOLVED IN SUCCESSFUL PROPAGATION FOR MASS PRODUCTION

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Abstract: Recently, microalgae have been regarded as useful organisms worldwide due to their potential for extensive application in renewable energy, aquaculture, biofuel and pharmaceuticals. Different species of microalgae have drawn significant interest because of their biological and chemical composition, which could potentially be useful in developing new applications in the aquaculture, biofuel and pharmaceutical industries. Furthermore, various culture techniques have been developed based on the species and environmental condition to ensure its mass production. Although microalgae are feasible sources for a successful biological product, limitations and challenges remain, which need to be solved with the innovation of new alternative technology in culturing and producing successful mass cultures. In this review, several current microalgae species production methods will be discussed based on their applications, and biological and chemical compositions, which are influenced by their growth parameters.

Keywords: Microalgae, aquaculture, biological, chemical composition, growth parameter.

Introduction

Microalgae are defined as microscopic single cells, which may be prokaryotic, such as cyanobacteria (chloroxybacteria), or eukaryotic. They are similar to green algae (Chlorophyta). Known as a class of photosynthetic organisms, microalgae are typically found in freshwater aquatic and marine habitats, including rivers, lakes, wastewater, oceans and estuaries. The growth of algae is possible in various conditions, temperature levels, salinities, pH values and light intensities. Alga can grow on its own or symbiotically with other aquatic organisms (Barsanti *et al.,* 2008).

Some microalgae have high carbon compound content, which may be utilized for new organic-based products, including biofuels, health supplements, pharmaceuticals, and cosmetics (Das *et al.,* 2011). Furthermore, large-scale microalgae cultivation can contribute to the development of a sustainable industry for biomass production and the development of cost-effective high-value products. Many species of microalgae, such as *Chlorella vulgaris, Tetraselmis suecica* and *Isochrysis galbana* possess the potential for large-scale cultivation despite the insufficient information to run commercial trials (Xu *et al.,* 2009). A high amount of microalgae biomass is required to be on a par with the feedstock for sustainable production. According to Cheng *et al.* (2019), the growth and biomass production of microalgae is significantly dependent on cultivation conditions and concentration of microalgae, which can be manipulated based on their optimum culture parameter.

Moreover, the concentration of microalgae in a solution is an important element which quantifies the productivity for biomass production. Meanwhile, cell density is generally defined as the concentration of microalgae in a medium in terms of the number or mass of cells per unit of volume, which is essential in determining microalgae growth (Pahija *et al.,* 2019). Therefore, the attraction of microalgae as a sustainable and renewable bio-product has encouraged a new focus on biomass cultivation. Improvements in growth, culture techniques and genetic engineering can be utilized to enhance their potential as a future source of bio-products.

Microalgae as a Bioproduct

Different green algae species have been utilized as food for decades (Jensen *et al.,* 2001). The cultivation of microalgae started when it was realized there could be a lack of sources for protein-rich foods for a rapidly growing world population (Borowitzka *et al.,* 1988). Based on previous research, the first large-scale culture microalgae were the *Chlorella* species, which were reported to be used for commercial purposes in Japan in the 1960s (Iwamoto, 2004). Over the last few decades, algae culturing has expanded to new products, such as food and feed, biofuels, and biopharmaceuticals, along with the use of natural products in algal extracts in cosmetics and medicines (Luiten *et al.,* 2003). Microalgae produce a wide range of other commercially valuable products, including essential vitamins for people and animals, and aquaculture purposes (Cuellar *et al.,* 2011). In addition, microalgae contain important types of medicinally essential polysaccharide pigments, such as chlorophyll, β-carotene and other carotenoids, phycobiliproteins and astaxanthin (Guil *et al.,* 2004). Previous work from Liang *et al.* (2004) found that microalgae had been used as nutrients, colouring agents and additives in a variety of food products. Microalgae can, therefore, be a promising source of bioproducts that can be applied to new production through mass cultivation. Microalgae must be modified and enhanced in order to produce a new type of algae-based product that can be used as a food for both humans and animals.

Current Algae Production

Despite the wide range of potential applications of microalgae for aquaculture and other useful products, the production of microalgae has not been fully commercialised due to several issues, including failure to overcome physiological stresses, nutrient deficiencies, high cost of mass production and failure to identify the suitable conditions mass microalgae cultivation (Mallick *et al.,* 2016). Furthermore, some microalgae either do not produce important metabolites or produce them in small amounts under normal conditions. Some microalgae also do not fully adapt to environmental changes like pH, temperature, light, carbon dioxide concentration, salinity and nutrients (Molina *et al.,* 2003).

To achieve the highest microalgae productivity in a cost-effective manner, the selection of cultivation method is crucial. Environmental factors play an important role in controlling the growth phases of microalgae, but they can be cultured through different methods under various conditions. (Campbell *et al.,* 2011). Environmental source of nutrients and light are needed to convert the absorbed water and CO_2 into biomass through photosynthesis (Ozkurt, 2009), leading to the creation of various products including cell components or storage materials, and vary from 20% to 50% of total biomass (Chisti, 2007). Nitrogen and phosphorus are the major nutrients required by the algae. Nitrogen is a basic component for the development of proteins and nucleic acids, while phosphate is a vital component of DNA and RNA, which are essential macromolecules for all living cells. (Juneja *et al.,* 2013).

Algae growth also requires macronutrients, including Na, Mg, Ca, and K; micronutrients, including Mo, Mn, B, Co, Fe, and Zn; and other trace elements. Additionally, wastewater from aquaculture and agriculture is a good source of nutrients for microalgae cultivation. Although different species of microalgae will go through different growth phases based on their needs for growth media in biomass production, the primary requirements are the same for almost all species (Campbell *et al.,* 2011).

Algae Culturing

Generally, the growth of microalgae may be separated into four phases – lag, log or exponential phase, stationary phase, and finally death (Moazami *et al.,* 2012). The initial lag phase is when the microalgae adapts to their surroundings, including the medium, pH, temperature, and lighting. Subsequently, the microalgae begin to undergo active cell division, which is followed by an exponential increase in the biomass of the culture (Chisti, 2007). Following that, the stationary phase takes place,

which halts the increase in the biomass due to the equal rate of the cell division and death (Jusoh *et al*., 2020). This phase mainly occurs as a result of the depletion of nutrients in the medium (Paes *et al.,* 2016). Consequently, the microalgae death rate would be higher compared to the rate of the cell division. Microalgae can be cultured through different methods under various conditions and the most important parameters in algae culturing is the type of growth system (Khan *et al.,* 2018), which should be designed according to the species and the purpose of culture.

On a large scale, although algae can be cultured in low-cost open ponds, which are ideal for commercial production, this method is easily contaminated by surrounding organisms (Borowitzka *et al.,* 2013). Bioreactors consist of continuous or batch culture facilities but are relatively high cost. The requirements for constant growth restricts this method to indoor facilities and is only feasible for relatively small-scale production (Cragg, 2000). Some algae species undergo significant growth in heterotrophic culture (Morales *et al.,* 2017), while in commercial cultivation, the culturing of microalgae in wastewater is ideal for water treatment and biomass production. Instead of freshwater, using seawater for microalgae culturing is ideal for algae growth as it reduces production cost. Marine water is a potential media for microalgae culture as it reduces nutrient preparation cost and increases the production of lipids and other useful byproducts in microalgae biomass (Park *et al.,* 2018). Most recently, ocean cultivation systems for commercial-scale production of algae have gained considerable attention due to its advantages, such as the mixing of the culture by ocean waves, utilisation of dissolved nutrients, and large area availability, which could reduce culturing and maintenance cost (Kim *et al.,* 2016).

Factors in Microalgae Cultivation

Light

Light intensity is one of the major factors in microalgae cultivation. The photosynthesis of microalgae is influenced by light radiance and intensity, which affect the biochemical composition of microalgae and biomass yield (Krzemińska *et al.,* 2014). Growth rate and biomass productivity are predicted as the function of light in the microalgae culture system (Huesemann *et al.,* 2013). Furthermore, algae species vary in terms of light requirements for optimum growth and biomass production, and the rapid growth of microalgae would not take place under extremely low and high light intensities (Mata *et al.,* 2010). Therefore, optimal light intensity needs to be observed in each species of alga to maximise CO_2 absorption with a minimum rate of photorespiration and photoinhibition (Ye *et al.,* 2012). A specific duration of light and dark periods needs to be set for algal photosynthesis. Light is also required for the synthesis of ATP (adenosine triphosphate) and NADPH (nicotinamide adenine dinucleotide phosphate), which stimulates the dark reactions of photosynthesis that produce carbon (Cheirsilp & Torpee 2012).

Previous research by Khoeyi *et al.* (2011) illustrated the differences between growth rate and biomass production, which were observed through the growth of the same algae species under different light intensities and for specific durations. Research by Jacob *et al.* (2009) found that decreased light intensities and duration would reduce microalgae growth rate and biomass yield. Most previous research has illustrated that the suitable duration of light and dark periods for algae growth are 16 and 8 hours respectively (Gunawan *et al.,* 2018; Asfouri *et al.,* 2019). Furthermore, appropriate light intensity, penetration, fixed distribution, and duration are essential in the cultivation of microalgae to avoid photo oxidation and growth inhibition (Carvalho, 2010). LED lights and fluorescent tubes could provide adequate light in algae cultivation (Wu, 2016). It was also observed by Mata *et al.* (2010) that microalgae cultivation under 12000 lx for 12 hours of daylight, produced a higher biomass yield, while the biomass decreased with the reduction of light intensity. Apart from that, a study by Khan *et al.* (2016) demonstrated that *Microcystis*

aeruginosa contributed to maximum biomass and carbohydrates productivity with red LED light at approximately 5000 lx. Daliry *et al.* (2017) reported that the *Chlorella vulgaris* produced maximum lipid production and highest growth rate at 5000-7000 lx. Therefore, photo inhibition could be prevented by increasing the light intensity through continuous illumination and mixing the light source of the culture to influence the growth and lipid production of microalgae.

Other than that, a study by Hubble and Harper (2001) found that microalgae cultures could have a self-shading effect. Self-shading happens when a high density of microalgae cells absorb the light, denying illumination to cells deeper in the culture (González *et al.,* 2019). According to Sorejo *et al.* (2020) the negative effect of self-shading could be overcome by giving the culture a good mixing or aeration. Other than that, Mata *et al.* (2010) also reported that an aerated culture of microalgae under uniform light intensities for 12 hours produced a higher biomass yield and prevented self-shading of the cells.

Maynardo *et al.* (2015) found that heat generated by high light intensity will increase the temperature and can cause a decline in growth rate. The optimum growth of common green microalgae such as *Chlorella* sp, *Tetraselmis* sp and *Nannochloropsis* sp will be at light intensity of 15-150 µmol of photons, which is between 1,000 and 10,000 lx (Simionato *et al.,* 2013). Therefore, to avoid high light intensity, flashing lights or adjusting the phototropic period for the 12-hour light and dark periods may obliquely control the light intensity which indirectly affects temperature (Suh *et al.,* 2003).

Temperature

Temperature plays a major role in the growth of microalgae by influencing photosynthesis. Essentially, the optimal temperature for exponential growth varies according to species, while deviating more or less from this optimal point could affect growth and activity (Bechet *et al.,* 2017). The optimum temperature range for most algal species is from 20 °C to 30 °C (Singh *et al.,* 2015). Thermophilic algae, including *Anacystis nidulans* and *Chaetoceros* sp, could endure temperatures of up to 40 °C (Covarrubias *et al.,* 2016). Non-optimal temperature could result in high biomass loss in microalgae, particularly in outdoor culture systems (Alabi *et al.,* 2009; Hu *et al.,* 2006).

Temperature is an important factor in large-scale cultivation as the algae experiences significant temperature changes over time (Bechet *et al.,* 2010). Low temperature affects photosynthesis through reduced carbon assimilation, while higher temperatures inhibit cell size and respiration (Khan *et al.,* 2018). As the decline in photosynthesis results in decreased growth rate, temperature is a major factor of algal growth and biomass production through its influence on the assimilation of CO_2. Temperature could also be used as a stress treatment to induce the production of valuable metabolites (Moller *et al.,* 2000). Temperatures from 27 °C to 31 °C are optimum for several microalgae species. It was previously shown that a culture of *Chlorella vulgaris* led to further production of carbohydrates and lipids under 25 °C instead of 30 °C (Converti *et al.,* 2009).

Nutrients

Nutrient requirements for microalgae vary between species. However, the basic nutrients for growth are the same – nitrogen, phosphorus, and carbon (Juneja *et al.,* 2013). Notably, some marine microalgae species also require silicate as a macronutrient.The presence of macronutrient during cultivation significantly affects growth rate and oil content of the algal biomass (Lardon *et al.,* 2009; Solovchenko *et al.,* 2008). Research by Aslan *et al.* (2006) found that the growth of *Chlorella sp* decreased as concentrations of nitrogen and phosphorus are reduced from 31.5 mg/l and 10.5 mg/l respectively and another study from Raehtz *et al.* (2009) found lipid production increased under conditions insufficient of phosphorus, which is attributed to the breakdown of cell membrane phospholipids into neutral lipids in order to obtain phosphorus.

The other major nutrients to be supplied were carbon, which is important for photosynthesis in microalgae growth and also contributes to a shift in microalgae nutrient composition. According to Juneja *et al.* (2013), carbon can be utilized in the form of CO_2 in the water, depending on pH, temperature and nutrient content. Previous research from Riebesell *et al.* (2000) said polyunsaturated fatty acid (PUFA) in *Emiliania huxleyi* was increased in lower CO_2 concentration, whereas fatty acid was increased in higher CO_2 levels. The high amount of fatty acid in *Dunaliella salina* was also observed to impact the culture condition due to the increased CO_2 (Muradyan *et al.,* 2004). Si (silicate) is also required as a macronutrient for diatom algae (Flynn, 2020). According to Martin *et al.* (2000) silicate incorporated for development of outer walls of algae cell, which acts as pressure vessels to prevent the enlargement of cells when water enters. Research by Hemalatha *et al.* (2014) found that growth and biochemical composition of *Chaetoceros simplex* were significantly modified when grown on the media added with silicate (Si). Other than that, the diatom *Nitzschia perspicua* can accumulated additional carbohydrate and lipids without significantly affecting the growth rate by changing the concentration of Si in the media culture (Jiang *et al.,* 2015). Although Mo, K, Co, Fe, Mg, Mn, B, and Zn are only required in trace amounts in algae cultivation, these micronutrients have a significant impact on microalgae growth due to their influence on many enzymatic activities (Hu *et al.,* 2006; Gardner *et al.,* 2017).

Usually, inorganic nitrogen and phosphorus are absorbed as nitrates and phosphates. Urea can also be a cost-effective replacement for other inorganic nitrogen sources. For the large-scale cultivation of microalgae, environmental CO_2 must be used as a carbon source, which is not only low in cost, but has the benefit of sequestering CO_2. The lack of this nutrient highly affects the microalgae growth and results in low biomass (Ito *et al.,* 2012). Therefore, proper nutrition of microalgae is essential for rapid growth in commercial production.

Mixing

In microalgae cultivation, mixing and aerating are crucial to distribute nutrients, air and CO_2. The penetration and uniform spread of light inside the culture and the settling of biomass, which leads to aggregation, could also be promoted through aerating and mixing (Show *et al.,* 2017). Although other requirements are fulfilled without mixing, significant reduction of biomass productivity will occur. Therefore, microalgae cultures must be continuously mixed to expose all microalgae cells to light during their cultivation. Additionally, a proper mixing system in cultivation does not only enable nutrient dissolution and light penetration into the culture, but it also leads to efficient gaseous exchange (Zeng *et al.,* 2011).

Media pH

Media pH plays an important role in cell growth and biomass production for microalgae. Previous studies suggest that the optimal pH for marine algae is 7.9-8.3 and 6.0-8.0 for freshwater microalgae (Pandey *et al.,* 2010; Ying *et al.,* 2014). The optimal pH range may vary widely based on the natural habitat of microalgal (Prokop *et al.,* 2015). The maximum growth rate for the *Spirulina plantensis* was observed at pH 8.0, followed by pH 9.0 and then pH 7.0 suggesting that moderate alkalinity was necessary (Fagiri *et al.,* 2013). A study by Khalil *et al.* (2010) found that *Chlorella ellipsoidea* could grow in pH 4-10. Bartley *et al.* (2014) investigated the influence of pH on growth and lipid accumulation in *Nannochloropsis salina* and found that the highest growth rates were at pH 8.0-9.0. The changes in pH levels may occur due to the changes in the dissolved CO_2, which are generated by changing CO_2 input concentrations or adjusting CO_2 uptake by the cells due to growth rate or increased biomass (Prokop *et al.,* 2015). Besides, high CO_2 levels are also present, such as the pH levels found in gas exchange by the media pH towards acidic conditions. Furthermore, media pH usually decreases due to CO_2 dissolution, which would gradually increase with further growth of cell

(Kao *et al.,* 2014; Valdés *et al.,* 2012). Apart from that, the uptake of nutrients by the microalgae, including ammonia, nitrate, and phosphates, may also contribute to significant pH changes in the medium. As it was found that the significant pH rise occurred with the increasing biomass concentration, it was concluded that careful management is critical to avoid alteration of pH in the cell growth stages, which can influence the biomass of microalgae (Bajpai *et al.,* 2013).

Salinity

Culturing microalgae under optimum salinity may have an impact on the biomass composition of certain algae species. It was found by Renaud *et al.* (1991) and Elfituri, (2018) that the gross chemical and fatty acid compositions of *Isochrysis* sp, *Nannochloropsis oculata*, and *Nitzschia* sp were distinguishable from one another under different salinities. Furthermore, experiments conducted on marine diatoms (*Amphora* sp, *Navicula* sp, and *Cymbella* sp) and a cyanobacteria (*Oscillatoria* sp) at different salinities resulted in diatom growth, which was significantly higher at 35 ppt than at lower salinity (Khatoon *et al.,* 2010). Although the cyanobacteria displayed a higher growth at 25 ppt, the diatom growth in terms of protein and lipid composition ranged from 15 ppt to 25 ppt in low salinities, which was significantly higher (Khatoon *et al.,* 2016). Meanwhile, research by Castro Araujo and Tavano Garcia (2005) demonstrated that although carbohydrates were enhanced under high salinity, the content of lipids and protein were decreased in *Chaetoceros* sp. It was also recorded by Rao *et al.* (2007) that the growth and biochemical composition of *Botryococcus braunii*, including hydrocarbon, carbohydrate, fatty acid, and carotenoids, were influenced by salinity. However, salinity stresses could also be influenced by species and strains (Shetty *et al.,* 2019). Overall, more observations should be conducted to ensure that the optimum salinity of certain species could be identified for the optimum biomass production of microalgae.

Algae Organic-Based Byproduct

Carotenoids

Carotenoids refer to the yellow, orange and red organic pigments produced by plants, algae and several bacteria and fungi (Linnewiel *et al.,* 2016). More than 750 structurally defined carotenoids are discoverable from nature, such as land plants, algae, bacteria, including cyanobacteria and photosynthetic bacteria, archaea, fungus, and animals (Britton *et al.,* 2004). Carotenoids are the essential bio compounds which play an important role in the production of food, feed cosmetics, and biopharma around the world (Henriquez *et al.,* 2016).

Algae synthesise various types of pigments, resulting in important biological activities, which have gained significant commercial interest. Among the most useful pigments produced by microalgae are the phycobiliproteins, phycocyanin, phycoerythrin, β-carotene, lutein and astaxanthin (Zhang *et al.,* 2016). According to Perez *et al.* (2011), phycobiliprotein pigments are mainly used in microscopy as fluorescent agents, while phycocyanin and other pigments from red algae exhibit antioxidant and anti-inflammatory effects, which are suitable to be used in the food and cosmetic products (Kumar *et al.,* 2014). The microalgae *Dunaliella salina* produces the carotenoid pigment β-carotene in quantities approximately 10% to 14% of its dry mass. The carotenoid pigment is important in vision and the immune system due to its relation to vitamin A (Chidambara *et al.,* 2005). Another important carotenoid pigment is astaxanthin, which is present in *Haematococcus pluvialis*. This microalgae species, which has been identified as the astaxanthin-rich source, produces 4% to 5% astaxanthin per dry biomass (Sathasivam *et al.,* 2017). Due to the strong antioxidant activity of the carotenoids, they are therapeutic in oxidative stress-related diseases and main organic pigment uses for the treatment of diabetes, ageing, cancer, obesity and stroke (Linnewiel *et al.,* 2016). Additionally, β-Carotene protects membrane lipids from peroxidation, which is linked with various

severe and lethal diseases including cancer, cardiovascular disease, Parkinson's disease, and atherosclerosis (Chidambara *et al.,* 2005).

Polyunsaturated Fatty Acids (PUFA)

Fatty acids (FAs) are the most important components of marine microalgae sources as they are structurally diverse and have gained importance due to their taxonomic specificity (Sahu *et al.,* 2013; Mathimani *et al.,* 2018). Among the fatty acids, polyunsaturated fatty acids (PUFA's) have more than one double bond in their long carbon chain, and long-chain fatty acids are broadly known for their beneficial effects on human health (Guihéneuf & Stengel, 2013). The nutritional value of microalgae is mainly related to their essential fatty acids including linoleic acid (18:2ω6; LA) and α-linolenic acid (18:3ω3; ALA) contents (Liang *et al.,* 2004). Various microalgae species including *Porphyridium cruentum*, *Arthrospira platensis*, *Odontella* sp, *Isochrysis galbana* had been explored for their ability to synthesise these valuable fatty acids (Khan *et al.,* 2018). To be specific, the previous study by Guedes (2010) revealed that large quantities of PUFA were produced by *Pavlova lutheri*. Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the most important PUFA produced by several microalgae species, which could be promising sources and the alternatives to fish oils, which are available in limited quantity and unable to fulfil the demands of EPA and DHA (Hamilton *et al.,* 2014). In the freshwater and marine food chain, DHA and EPA are incorporated by microalgae under various culture conditions (Adarme-Vega *et al.,* 2012). Various microalgae species including *Nannochloropsis* sp, *Tetraselmis* sp, *Chaetoceros* sp, *Isochrysis* sp and *Thalassiosira* sp produce sufficient amounts of DHA and EPA which are responsible for the development of various bivalve larvae (Kumar *et al.,* 2019) and previous research from Peltomaa *et al.* (2017) also mentioned that *Nannochloropsis* sp is evaluated as the most notable strain in the large volume DHA and EPA production. Other than that, recent studies also show that *Phaeodactylum tricornutum* gained attention as a potential source of EPA and DHA production (Koller *et al.,* 2014), but the commercial-scale production of microalgae for EPA and DHA products cannot be achieved because several issues lead to low product yields (Hamilton *et al.,* 2016). As a crucial fatty acid, EPA plays an important role in the regulation of biological functions as a treatment for human diseases such as heart and inflammatory diseases (Wen & Chen, 2003), while DHA acts as a protection against neuro diseases such as Alzheimer, Parkinson and multiple sclerosis (Shanab *et al.,* 2018).

Proteins and Enzymes

Some proteins, peptides and amino acids are necessary for cells and tissues to perform their normal activities. Therefore, if the human body is unable to synthesise these components, they must be obtained from an external source, which is usually food. Various species of microalgae produce a higher quantity of various essential amino acids and proteins, which could be utilised in food and used for protection against several diseases. Some species of microalgae could produce the same amount of proteins as other rich sources of proteins (Gouveia *et al.,* 2008). Bleakley *et al.* (2017) found that protein from microalgae consists of branched-chain amino acids, which are required for various functions of the body, especially in relation to muscle protein health. Furthermore, antioxidant peptides derived from hydrolysed *Pavlova lutheri* biomass and microbial hydrolysis of *Chlamydomonas* sp appear to suppress *Helicobacter pylori*-induced carcinogenesis (Hayes *et al.,* 2018). *Isochrysis galbana* produces the vital enzyme carbonic anhydrase, which plays a crucial role in converting CO_2 into carbonic acid and bicarbonate. *Microcystis aeruginosa* also produces a variety of amino acids, including proline, serine, glycine, and valine (Khan *et al.,* 2018). Microalgae can be a great source of human health bioproducts, which can be attained by mass yield cultivation. Previous research by Bleakley *et al.* (2017) show

that the green microalgae *Chlorella* sp is a rich source of different types of proteins, which have been produced in the market from 2.5 to 7.5 tons/Ha/year. Other than that, *Nannochloropsis* sp is widely used in fish-farm aqua-feed, which have highest protein production (4–15 tons/Ha/year) respectively compared to the soybean and legumes (Al Ghais & Bhardwaj, 2018).

Algae Mass Production

The production of microalgae biomass is based on the application of selected species and the commercial value of the microalgae that could be extracted. Microalgae would be the logical source of oils for biodiesel production, which is the most ideal option for CO_2 sequestration and numerous other applications (Chisti, 2007; Grobbelaar, 2010; Tredici, 2010). The first full-scale research and production facility for the mass production of microalgae began around 1960 at Trebon in the Czech Republic (Setlik *et al.,* 1970). Furthermore, the mass culture system is applicable in the aquaculture, biofuel and pharmaceutical fields. However, the potential for mass production from algae has indicated that many investors lost a high amount of money due to high-cost maintenance (Tredici, 2010). As emphasised by Grobbelaar (2010), the objective of mass algal production is to identify the highest yields in the shortest possible time or in the high volumetric and area production rates of algal biotechnology in a cost-effective manner. To expand this novel feedstock, research and development are required in several domains, which range from the selection of suitable strains to the optimisation of the different steps required for mass operations, namely biomass production, harvesting and lipid extraction (Pawar & Gupta, 2017).

Open-pond Culture

Open ponds are the oldest and the simplest systems for algal culture. Essentially, algae are cultivated under conditions which fulfil the external environment requirement of culture species. Open-pond systems emerged in the 1950s and remain to be widely used in large-scale outdoor microalgal cultivation. The operating costs of open ponds were discussed in terms of mixing, carbon utilisation, nutrients, flocculants, salt disposal, maintenance, labour and the accumulation of photosynthetically produced oxygen (Chisti, 2007). Although various designs have emerged for open-pond systems, three successful major designs are still operating on commercial scales – race-way ponds, circular ponds, and unstirred ponds (Shen *et al.,* 2009).

Some of the key challenges in the productivity of the open-pond system are circulating and mixing the algal cells, nutrients, and CO_2 of certain species. Besides, Richmond (2000) identified four constraints for growth in the mass cultivation of algae, which include species selection, genetic manipulation, light utilisation and reactor efficiency. It was also reported that enhanced productivity in terms of biomass, and consequently lipids, could be achieved by enriching the cultures or/and extending the period of light availability. It was inferred from the present results that the incorporation of another effective variable factor was possible, namely paddlewheel speed, on productivity (Moazami *et al.,* 2012). Other than that, the selection of suitable microalgae strains for raw large-scale production by their biomass production, environmental factor, and high growth rates were equally important.

Photobioreactor (PBR)

Recently, there is prolific research into PBR for efficient and reliable culturing (Breuer *et al.,* 2015). Vargas *et al.* (2017) found that continuous microalgae production in PBR not only prevents culture contamination, it also controls important process variables. However, PBR also has disadvantages. PBR is a challenging process to reliably scale up (García *et al.,* 2011), and is expensive to construct and operate (Posten, 2009). Furthermore, although microalgae culture production in PBR is also through the batch processing of algae species (Bosma *et al.,* 2014), continuous operation would improve the culture performance as variables could be controlled,

productivity would be increased, and the risk of contamination could be reduced (Gadkar *et al.,* 2003). However, continuous PBR operation is challenging due to the requirement for large-scale units to obtain a feasible product flow. The productivity of the PBRs units also varies due to the scaling up process (García *et al.,* 2011). Meanwhile, feasible PBR design gives the opportunity to use highly reliable mathematical models, as it takes the interactions between several phenomena into account (Fernández *et al.,* 2014).

Culture Media

In a natural habitat, microalgae obtain all nutrients, minerals, and vitamins from their environment. It was observed in previous studies that the environmental factors of microalgae growth consisted pH, salinity, light, temperature, carbon and nutrients, such as nitrates, phosphates and trace metals (Mata *et al.,* 2010). In a study by Andersen *et al.* (2005), it was found that several culture media compositions, including freshwater and seawater, were commonly used to produce microalgae. To illustrate, BG-11 medium (blue and green media) or broth media has often been used to culture freshwater green algae and cyanobacteria (Grobbelaar, 2004). Furthermore, it is particularly rich in 1.5 nitrate $NaNO_3$ and exhibits a ratio of wastewater to modified media of 60:1. Similarly, the Bold Basal Medium (BBM) is also used for freshwater algae and cyanobacteria (Boggess, 2014).

A study by Dayananda *et al.* (2007) compared several culture media, including BG-11 and BBM, for the culture of *Botryococcus braunii*, and it was concluded that BG-11 was the most ideal medium for biomass and hydrocarbon production. In the case of saltwater algae, the modification and formulation of media, including Conway and f/2 media are normally implemented to produce microalgae monoculture in the laboratory (Xin *et al.,* 2010). These media have been generated based on the basic nutrients needed for algae growth, which are similar to the nutrients needed in the natural habitat condition (Panahi *et al.,* 2019). Based on the research of Lananan *et al.* (2013), specific growth rate of different genera of algae including *Dunaliella* sp, *Chlorella* sp, *Chaetoceros* sp and *Tetraselmis* sp were enhanced in f/2 medium by 72.00%, 40.36%, 22.40% and 4.13% respectively, while *Pavlova* sp and *Isochrysis* sp thrived in Conway Medium by 16.39% and 4.64% respectively.

NP Ratio

Algae composition is essential in determining the nutrients for commercial uses in aquaculture industries, such as fish larvae production, live-feed culture and pellet fish feed production (Samat *et al.,* 2020). Nutrient composition, which consists of protein, amino acid, fatty acid and lipid, is highly important for the growth of the aquaculture of fish, marine zooplankton and algae (Craig *et al.,* 2017; Rasdi *et al.,* 2018; Jónasdóttir, 2019). Furthermore, algal growth is related to nitrogen and phosphorus supply in the culture medium (Zhang & Hu, 2011). Specifically, nitrogen is important in amino acid and protein synthesis, while phosphorus is an important component of phospholipids and in energy metabolism and nucleic acid synthesis for algal growth (Rasdi *et al.,* 2016). Therefore, the low amount of proteins and high carbohydrates in algae cells would be affected by the limitations of N and P (Ganf *et al.,* 1986; Reitan *et al.,* 1994).

The internal composition of marine phytoplankton has been established as 106:16:1, which is represented as a molar ratio for C:N:P known as the Redfield Ratio (Redfield, 1934). However, in the case of freshwater microalgae, the Redfield Ratio becomes an exception instead of being accounted for the N:P molar ratios, which ranged from 8:1 to 45:1 (Hecky *et al.,* 1996; Ptacnik *et al.,* 2010) through a specific species. Furthermore, freshwater microalgae possess the ability to adjust the N and P concentration in their biomass in relation to the concentration of the surrounding water (Beuckels *et al.,* 2015; Choi & Lee, 2015). Furthermore, as the biomass P accumulation was influenced by the external P and N supply,

while the accumulation of N was independent of P (Beuckels *et al.*, 2015). Microalgae growth can be considerably affected by the manipulation of N:P ratios in the media culture. Previous research by Rasdi and Qin (2014) indicated that the growth of *Nannochloropsis oculata* and *Tisochrysis lutea* increased when the N:P ratio increased from 5:1 to 20:1, while the growth was decreased from 20:1 to 120:1. Similarly, the density of *Nannochloropsis* sp was enhanced when cultured under N:P ratios of 16:1 and 32:1, compared to 64:1 and 80:1 (Mayers *et al.,* 2014). In the case of the biomass production of certain microalgae species, the manipulation of N and P ratio should be considered when designing the culture reactor based on the environmental condition. According to Whitton *et al.* (2016), the selection of species and nutrient concentrations should be considered, which include N and P in the biomass and the ability to adapt to external concentrations due to its impacts on the maximum growth and composition rate.

Microalgae and Wastewater

Microalgae was successfully used in the purification or the treatment of post-culture waters. Wastewater treatment systems, which involve microalgae, present a low-cost and environment-friendly wastewater treatment option compared to conventional processes (Liu *et al.,* 2013). The oxygen produced by microalgae during photosynthesis may reduce the biological oxygen demand in wastewater. Moreover, the eutrophication of nutrients in wastewater, including nitrogen and phosphorus, can be capably removed by microalgae (Lee *et al.,* 2002). Previous researched by Hawrot *et al.* (2020) mentioned that the concentration of total nitrogen and phosphorus in the wastewater decreased by 87.9% and 99.1% after 10 days of treatment with *Chlorella minutissima*. Similarly, Gao *et al.* (2016) stated that the total nitrogen and total phosphorus in aquaculture wastewater reduced by 86.1% and 82.7% respectively in *Chlorella vulgaris*. Otherwise, algae wastewater cultivation processes should undergo the same operation as any biological wastewater treatment systems, in which the environmental parameters are considered in the design of algal cultivation processes to ensure high production of lipids and biomass at low cost (Hwang *et al.,* 2016). In a previous study by Khatoon *et al.* (2016), it is found that the growth and biomass production of *Chaetoceros calcitrans*, *Nannochloropsis maculate* and *Tetraselmis chuii* in aquaculture wastewater was equivalent with the Conway medium. Previous research by Hawrot *et al.* (2020) also stated that lipid content of *Chlorella minutissima* growth in F/2 media compared well with wastewater, which is 51.67% and 46.37 % dry weight, respectively. Overall, it was indicated that the nutrients in aquaculture wastewater were sufficient for microalgae growth.

The Applications of Microalgae in Environmental Biotechnology

Microalgae have been used for various applications in environmental biotechnology, in particular, bioremediation, bioassays, and biomonitoring of environmental toxicants and the development of *Spirulina platensis* in the High Rates Algal Pond System (HRAP) (Phang *et al.,* 2001). Microalgae grown in HRAP have been shown to be beneficial as the treatments for various wastewater, including municipal wastewater (Garcia *et al.,* 2000). Furthermore, over 99% reduction of phosphate from anaerobically digested starch in factory wastewater occurred in *Spirulina platensis* grown in HRAP. Moreover, the immobilised microalgae system also enhanced its applicability in the removal of environmental toxicants (Bashan *et al.,* 2010). Meanwhile, *Chlorella vulgaris* grown in HRAP was found to be useful in the final polishing of textile wastewater before its discharge, especially in the color removal process (Chu *et al.,* 2009). Additionally, it was found in a previous study by Ruiz-Marin *et al.* (2010) that the immobilised *Chlorella vulgaris* and *Scenedesmus obliquus* were effective in the removal of nitrogen and phosphorus from

urban wastewater, which operated on a semi-continuous mode (Ruiz-Marin *et al.,* 2010).

Common Algae Species Produced by Industries

Algae are an important group of aquatic organisms for biotechnological exploitation, especially for valuable products, and processes and services in the food, pharmaceutical and aquaculture industries. A wide range of metabolites with various bioactivities produced in algae are yet to be fully exploited (Cardozo *et al.,* 2007). There is a diverse range of algae species, which are widely used around the world, including *Spirulina* sp and *Chlorella* sp. It was emphasised by Chu (2012) that the aforementioned algae have been consumed as food supplements (nutraceutical) by humans and as animal feed.

Currently, *Spirulina* sp is cultured in open ponds for mass and commercial production of biomass as a dietary supplement in several countries, including Thailand, China, United States and India (Soni *et al.,* 2017). It is estimated that 3,000 to 4,000 metric tons of *Spirulina* sp is produced worldwide (Belay, 2007). Besides the consumption of food product, *Spirulina* sp is known for its therapeutic effects on health problems, including diabetes, arthritis, anaemia, cardiovascular diseases and cancer (Sigamani *et al.,* 2016). Moreover, *Spirulina* sp is beneficial as a functional ingredient due to its incorporation into various food products to enhance their nutritional qualities and perform therapeutic management of chronic disorders, such as diabetes, hypertension, and heart disease (Mani, 2007).

Chlorella sp is another microalgae species which have been mass cultured for the commercial production of healthy food in the form of pills and powder (Priyadarshani & Rath, 2012). The first commercial production of this type of microalga was established in Japan in 1961 by Nihon Chlorella Inc. Following this, *Chlorella* sp factories were developed in several countries including Taiwan, China, and Indonesia, and some 46 large-scale plants had been established in Asia by 1980, producing more than 1000 kg of *Chlorella* sp biomass per month (Spolaore *et al.,* 2006). Currently, *Chlorella* sp products marketed in Malaysia are mainly imported from Japan and Taiwan. The nutritional value of *Chlorella* sp is due to their high content of protein, which consist of 51% to 58% of dry weight, and carotenoids, with a wide range of vitamins (Becker, 2004).

Another algae species produced on an industrial scale are *Nannochloropsis* sp and *Isochrysis* sp. A study by Sirakov *et al.* (2015) mentioned that *Isochrysis* sp and *Nannochloropsis* sp have been used as direct or indirect feed for cultured larval organisms. Indirect means supplying the algae as enrichment for copepod, rotifers and daphnia before fed to the target larval organisms (Rasdi & Qin, 2018a; Rasdi & Qin, 2018b; Yuslan *et al.,* 2021). Industrial production of *Nannochloropsis* sp, known as Nanno 3600 from Reed Mariculture Inc, have been widely used in aquaculture as a booster for increasing yield of rotifer, which is the main diet for shrimp culture (Román-Reyes *et al.,* 2014). Other than that, *Isochrysis* sp also have been used in aquaculture due to their high nutritional value and small 4-6 μm size, which is easily digestible by the larva (Thu *et al.,* 2015). In aquaculture, especially shrimp farming, Algaefeed© Isochrysis powder have widely been used as co-feed and showed better result than probiotics, which can prevent the dominance of *Vibrio* sp, as well as improve egg hatching and larval survival of shrimp culture (Molina *et al.,* 2014).

Conclusion

Microalgae have proven their high potential in creating new types of organic-based products, including sustainable aquaculture sources and biofuels, bioactive medicinal products and food supplements for humans. Similarly, low-cost microalgae culture may also be feasible as a treatment for wastewater based on the growth requirement of several species of these algae. The upgrade and modification of algae culture techniques and bioproduct technology, such as biofuel, is possible from small scale

to commercial level, when the challenges and limitations around them are solved. This review has discussed the potential of by-products from microalgae and their extensive applications in bioenergy, aquaculture and pharmaceuticals, which could be achieved through mass-culture production. As a result, an understanding of the growth requirement would be developed, which could be applied to enhance the feasibility and applicability of microalgae for their commercialisation.

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