Overview: High-values molecules from microalgae

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Abstract: Energy depletion and fluctuation of fossil-fuel price have made a great interest among researchers to find alternative sources for energy. For the third generation of biodiesel feedstock, microalgae a photosynthetic primitive plant has been used as raw material for transesterification process on biodiesel production. As a photosynthetic microorganism, microalgae needs carbon dioxide for its cultivations thus acts as a carbon dioxide bio-mitigation for controlling the Greenhouse Gases (GHG) emission from industrial flue gas. Due to its capacity to assimilate nutrients (nitrate and phosphate) and heavy metals, various researches have been made using microalgae to treat industrial, municipal and agricultural wastewater and to avoid eutrophication. Recent biotechnology advancement has revealed the potential of the microalgae was not only limited to carbon dioxide fixation, treatment of wastewater or to produced biofuel but it also has the potential to produced high-values molecules. In this study, the potential of microalgae biomass to produced high-end products that contained high-values molecules and rich in protein has been reviewed. The diversified microalgae products will surge its feasibility and become the new cutting edge for microalgae mass cultivations.

Keywords: microalgae, high-values molecules, carotenoid, polyunsaturated fatty acid, overview

1. Introduction

Algae is define as heterogeneous assemblage of organisms that range in size from tiny cells to giant seaweeds, thus algae mostly are photosynthetic species and live in aquatic habitats which this includes eukaryotes and prokaryotic cyanobacteria (blue-green algae). Algae can be differentiate by it bodies either microalgae, an algal bodies that need microscope to observe it or macroalgae which is large enough to be seen with unaided eye\cite{1}. Algae culturing has started since in the late of 1800s and early 1900s, while microalgae mass culturing begin to developed since the achievement of Allen and Nelson in 1910 by using Chlorella in Berlin, Germany\cite{2}. It is found that microalgae are the only source that can be sustainably developed in the future\cite{3}. Microalgae did not have to compete with edible oil furthermore it can be cultivated in barren soil. Less land area is needed to cultivate microalgae when compared to the oil crops. The oil productivity of many microalgae greatly exceeds the oil productivity of the best producing oil crops\cite{4}. As comparison the biodiesel source from microalgae beats bioethanol. The bioethanol has only 64\% of the energy content of biodiesel \cite{5}. Hence, indicates that biodiesel from microalgae foreseen to have a vibrant future for replacing fossil fuel.

Many study shows that there is high possibility using microalgae to treated on various wastewater either it from municipal, agriculture, aquaculture, textile industry or oil refinery industry\cite{6-14}. It was found that microalgae helps to remove heavy metal in the wastewater induced progressive reduction COD and BOD to below the discharge limit. \cite{15}. To date most researcher has move to work on extensive studies by incorporated CO\textsubscript{2} to improve microalgae culturing and in the same time use it as tool for CO\textsubscript{2} fixation to curb Green House Gases \cite{16-21}. As photosynthetic species, microalgae needs carbon dioxide (CO\textsubscript{2}) if were grown in autotrophic or mixotrophic condition. Thus, helps produced bulky microalgae biomass which have multipurpose usage or easily be sold as biofertilizer or animal feeds. \cite{22-24}. 
Abide by microalgae as an answer to growing energy demand, wastewater treatment and carbon sequestration, looking to the other side of its potentials; high-values molecules will help to boost the mass cultivation of microalgae industry. The carotenoid market has reach over US$1,000 million by the end of the decade. The nutraceutical boom has also integrated carotenoid mainly on the claim of their proven antioxidant properties, nevertheless carotenoid is important for industrial use in food products and cosmetics as vitamin supplements and health food products and as feed additives for poultry, livestock, fish, and crustaceans[25]. As such the intention of this paper was to focus on the potential of high-values molecules derived from microalgae that can be industrialized; the carotenoids (Astaxanthin, Lutein and β-carotene), natural pigment, polyunsaturated acid, pharmaceutical and nutraceutical compound.

2. Carotenoid

2.1 Astaxanthin

Astaxanthin, a carotenoid which the molecule has two asymmetric carbon located at the 3 and 3’ position of the benzenoid rings on either end of the molecule has been proposed as super vitamin E due to its antioxidant activities have been shown to be ten times greater than other carotenoids such as zeaxanthin, lutein, canthaxanthin, β-carotene, over 500 times greater than α-tocopherol and surpasses the antioxidant benefits of vitamin C. As a natural pigment, the major market for astaxanthin is as pigmentation source in aquaculture. Natural Astaxanthin can be produced by Haematococcus microalgae which contained 1.5% - 3% by dry weight. Astaxanthin sells for approximately US$2500 kg⁻¹ with annual worldwide aquaculture market estimated at US$200 million. The increasing growth of salmonid farming has created an enormous demand for natural astaxanthin as pigment. Salmonid are unable to synthesized astaxanthin de novo. Therefore carotenoid pigments must be supplied in their artificial aquaculture diet. Natural astaxanthin from Haematococcus has been proven in numerous feed applications. In aquaculture feed it has been used to salmon, shrimp culture, ornamental fish and sea bream. Haematococcus was found not only to provide pigmentation but also improve health and fertility in salmonid feed. In poultry feed, astaxanthin has been shown to be useful in coloration of both egg yolks and muscle tissue. Animal studies has also shown that astaxanthin can protect skin from damaging effect of UV radiation, ameliorate age-related macular degeneration, protect against chemically induced cancers, increase high density lipoprotein and enhance immune system. Epidemiological studies have demonstrated a correlation between increased carotenoid intake and reduced incidence of coronary heart disease and certain cancer, macular degeneration, and increased resistance to viral, bacterial, fungal and parasitic infection[26].

2.2 Lutein

Lutein is another important carotenoid which can be found naturally in algae. It is prominent in human serum and foods. Lutein has been used for the pigmentation of animal tissues and products, as well as important natural for the coloration of foods, drugs and cosmetics. Recent reports have shown that it plays an active role in delaying chronic disease stimulating the immune response, hampering the development of cataracts and the progression of early atherosclerosis, and preventing blindness or decrease in vision caused by age-related macular degeneration[27-29]. Global lutein market in 2004 accounted for US$139 million and is expected to increase by 6.1% per year (the fastest projected growth in individual carotenoid sales), to reach US$187 million in 2009[25]. French marigold (Tagetes patula) is currently the most widely applied source for lutein production. However, mass plantation of marigold occupies a large land area and it is easily influenced by season and climate[29]. The microalgae biomass from chlorophycean is the major source of lutein, especially Chlorella, Scenedesmus and Muriellopsis[25, 29]. Laboratory studies showed that heterotrophically
cultivated Chlorella contained considerable amount of lutein. The ability of heterotrophic growth in fermentors makes Chlorella a potential alternative resource for commercial production of lutein\[29]. Studies show that more lutein will be produced by Chlorella using Basal medium\[28]. Lutein production by heterotrophic Chlorella in batch and fed-batch cultures also has been studied\[29]. Although an established commercial system for the production of lutein from microalgae does not exist yet, the basis for outdoor production of lutein-rich cells of strains of Muriellopsis and Scenedesmus at a pilot scale have been set up\[25].

2.3 β-carotene

β-carotene is a pigment of increasing demand and a wide variety of market applications: as food coloring agent, as pro-vitamin A (retinol) in food and animal feed, as an additive to cosmetics and multivitamin preparations, and as a health food product under the antioxidant. The market value of β-carotene has been projected to reach US$253 million for 2009\[25]. β-carotene, a provitamin A is the only carotenoid, which has the potential to form two molecules of vitamin A (retinol)\[30]. Natural production of β-carotene can be produced among the microalgae genus Dunaliella, its accumulate large amount of cellular β-carotene. Dunaliella, the most halotolerant eukaryotic photosynthetic occurs in oceans, brine lakes, salt marshes, salt lagoon and salt water ditches near sea, predominantly in water bodies containing more than 2 M salt and high level of magnesium \[31]. Under such stressful environmental conditions more than 12% of Dunaliella dry weight is β-carotene, usually associated with a sharp decline in cell protein and chloroplast chlorophyll. The price for natural β-carotene is depends for it demand which can be varies from $300 to $3000. Purified β-carotene is sold in vegetable oil form range from 1% up to 20% of concentration depends on type of food product included for personal use in soft gel. Natural purified β-carotene will be accompanied by other Dunaliella carotenoid (carotenoid mix) which included; lutien, neoxanthin, zaexanthin, violaxanthin, cryptoxanthin, α-carotene. Variety of natural β-carotene can be found sold as health food and supplement under vitamin section. In powder form, natural β-carotene will be used for colorization and pro-vitamin A for animal and aquaculture feed; shrimp, fish, cattle and poultry\[31].

3 Natural Dyes (Pigment)

3.1 Chlorophyll

In practice most green leaf material contains about 0.3% chlorophyll and yields about 5% extractive, but grass and Lucerne (alfalfa) are the materials of choice being cheap, readily available, easy to dry, and low in the destructive chlorophyllase enzyme. In more esoteric extractions, leaves of nettles and elder are preferred, whilst algae and silk worm droppings have also been used commercially\[32]. However, most algae cultured under optimum condition were reported contained about 4% dry weight of chlorophyll from overall cell weight. Cyanobacteria, the blue green algae typically contain chlorophyll-a while species of green algae mostly have chlorophyll-b. Among species of microalgae, Chlorella was reported to have high amount of chlorophyll. Chlorophyll provides a chelating agent activity which can be used in ointment, treatment for pharmaceutical benefits especially liver recovery and ulcer treatment. Besides that, it repairs cells, increases haemoglobin in blood and faster the cell growth\[33]. Testing the cytotoxicity of Chlorophyll derivatives; pheophorbide and pheophytin against tumor cell revealed that the cellular uptake and inhibition of myeloma cell multiplicity was greater for pheophorbide that pheophytin. Hence it indicates that chlorophyll derivatives may play a role in cancer prevention\[30]. Chlorophyll too is used as natural pigment ingredient in processed foods and cosmetic. Because of its strong green pigment and consumers demand for natural foods, chlorophyll is gaining importance as food additive. This
in turn is encouraging food processors to switch from artificial pigments to chlorophyll-based natural colouring[33].

3.2 Phycobiliprotein
Phycobiliprotein a high-potential molecules, been utilized commercially as natural dyes and variety application in pharmaceutical. It is classified according to UV-visible absorption maxima as phycocyanin (blue pigment), phyceroerythrins (red pigment), and allophycocyanins (pale-blue pigment). *Arthrospira* also known as *Spirulina* is the major sources for phycocyanins, which up to 20% of its dry weight. Others algae that can be source for phycobiliprotein is *Aphanizomenon flos-aquae*[30, 34]. Albeit it major used as natural food pigment, phycocyanins has been utilized in medical diagnostic as phycoflours highly sensitive fluorescent reagent. This makes phycobiliprotein will be utilized for labeling antibodies applied in clinical tests such as immunofluorescence or flow cytometry due to phycobiliprotein bound to biological active molecules such as immunoglobulin, biotin or protein. It is reported that phycocyanin acted as antioxidant against radical. In vitro experiment has been conducted and shows that phycocyanin scavenges radicals. It is proven that phycocyanin is 20 times more efficient than ascorbic acid against hemolysis induced by peroxy radicals in human erythrocytes[30].

4. Polyunsaturated Fatty Acid (PUFA)
Polyunsaturated Fatty Acid (PUFA) which included; Docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA), Arachidonic acid (AA), γ-Linolenic acid (GLA) is widely recognized to have beneficial towards human health[35]. Natural PUFA can be obtained from fish or extracted from fish-oil. However there is report on the possibility of PUFA derived from fish sources accumulated toxin, furthermore the unpleasant smell, taste and poor oxidative stability has limits the application of fish oil as food additive[36].

DHA is the only algal PUFA commercially available. DHA is an omega-3 fatty acid found in tissues throughout the body. It is a major structural fatty acid in the grey matter of the brain and in the retina of the eye, and is a key component of the heart tissue. DHA is important for correct brain and eye development in infants and has been shown to support cardiovascular health in adults[36]. The inclusion of DHA in infant formula for preterm and full term infants is recommended[6]. DHA helps to fight cancer, AIDS, heart disease, lower cholesterol, boost immune system, and detoxify body. It was studied that marine microalgae having significantly more DHA contents compared to fresh water microalgae; mainly consists saturated or monounsaturated fatty acids [33].

*Cryptecodinium cohnii* is a non-photosynthetic, marine dinoflagellate in which DHA fatty acids predominate, which nearly 30–50% of its constituent fatty acids is DHA and no other PUFAs are present in excess of 1%. Therefore it is easy to separate DHA from the fatty acid mixtures, hence *Cryptecodinium cohnii* represents a promising microalgae for the commercial production of DHA[37]. *Schizochytrium* produce low-cost DHA oil. which currently used as an adult dietary supplement in food and beverages, health foods, animal feeds and maricultural products. Other markets include foods for pregnant and nursing women and applications in cardiovascular health. Finally, the Nutrinova process (Frankfurt, Germany) uses *Ulkenia sp.* which grows in 80m$^3$ fermenters. [36]. Other algae strain that have high DHA such as *Schizochytrium mangrove*, reported to have main component of DHA in a range of 33–39% of total fatty acid, *Amphidium caryerea* (17.0%) and *Throutocytrium aureum* (16.1%)[33]. *Nannochloropsis* has been proposed as the sources of PUFA, due to high content of EPA[38]. Thus, *Nannochloropsis* production been used to addresses the
rotifer requirements if 8,000,000 gilthead sea bream fingerlings produced annually [39]. The diatoms; *Phaeodactylum tricornutum* and *Nitzschia laevis* is another algae strain to be utilized as sources of EPA[36, 40]. Others, such as *C. muelleri var. subsalsum* and *Isochrysis galbana* are the algae strain that producing high percentage of DHA and EPA[39, 41].

EPA plays an important role in higher animals as a precursor of a group of eicosanoids which are crucial in regulating developmental and regulatory physiology. The eicosanoids are hormone-like substances including prostaglandins (PG), thromboxanes (TX) and leukotrienes (LT). AA and EPA are precursors of eicosanoid compounds. However, the eicosanoids from these two fatty acids are different both structurally and functionally, and are sometimes even antagonistic in their effects. A balanced uptake of EPA/AA can prevent eicosanoid dysfunctions and may be effective in treating a number of illnesses and metabolic disorder[40]. *Porphyridium cruentum*, red algae has been mass cultured due to relatively high AA and EPA. Nevertheless, *Porphyridium cruentum* biomass is high in protein 34.1%, carbohydrates 32.1% and essential minerals[42]. AA is potentially used for Infant formulas either for full-term or preterm infants, AA also been used as nutritional supplement[36]. *Arthrospira* is the richest algal source of GLA. GLA is important to the infant formulas for full-term infant as well as it has shown to lower low-density lipoproteins in hypercholesteremic patients, alleviate of premenstrual syndrome and treated atopical eczema[36, 43]. GLA may attenuate the signs and symptoms of inflammatory diseases such as rheumatoid arthritis and atopic dermatitis[44, 45].

5. Pharmaceutical & Nutraceutical compounds

5.1 β-1,3-glucan

β-1,3-glucan is important to human health. It is an active immunostimulator, a free radical scavenger and helps to reduced lipids in blood. *Chlorella* is major source of β-1,3-glucan[46]. β1,3-glucan is widely used as a dietary supplement, with well-established stimulating effects on the immune defense system. Positive effects were also found in patients after cardiopulmonary bypass, and inhibition of antiviral activity has been found in HIV-infected patients. Some β1,3-glucans are routinely used in patients for tumor immunotherapy[47]. The use of β-1,3-glucan is of special interest in the cancer patient undergoing chemotherapy and/or radiation treatment. It can also stimulate recovery of the bone marrow following chemotherapy, something vital to restricting tumor growth and preventing infectious complications during treatment. Studies shows that β-1,3-glucan has potential to be used against Anthrax infection[48].

5.2 Other applications

Algae have a potential to be the source of compounds having antibiotic and anticancer activity. Studies have found that wide variety of cyanobacteria produce compounds including sulfolipids, which were active against a herpes virus, pneumonia virus and HIV. Cyanobacteria, *Tolypothrix* and *Sceytomena* have shown a producing tolytoxin, an anti fungal antibiotic, which has low toxicity to human. Nevertheless, *Lyngbya majuscula* have potent against human lung tumor and mouse neuroblastoma cell line due to producing aurilides B and C. *Laphocladia* an algae producing lophocladine alkaloids which shows anticancer effect on lung tumor and Brest cancer line. Therefore, once the algal sources of useful medicinal compound are identified, the algae can be cultivated in mass scale and the material can be extracted, purified and marketed[1]. *Arthrospira* and *Chlorella* species are established in the skin care market as well as sun protection and hair care products, this include anti-aging cream, refreshing or regenerant care products, emollient and as an anti-irritant in peelers. Microalgae are also represented in. A protein-rich extract from Arthrospira repairs the signs
of early skin aging, exerts a tightening effect and prevents stria formation and an extract from *Chlorella vulgaris* stimulates collagen synthesis in skin, thereby supporting tissue regeneration and wrinkle reduction. Recently, an ingredient from *Nannochloropsis oculata* with excellent skin-tightening properties (short and long-term effects) and an ingredient from *D. salina*, which shows the ability to markedly stimulate cell proliferation and turnover and to positively influence the energy metabolism of skin.[36].

**Conclusion**

It is hope that this review has highlighted about the microalgae potential to produced high-end product apart from being converted as biodiesel. One should noticed that as the biofuels industry is still new and just started to blooming, hence it is crucial to have various type of branch algae industry; the pharmaceutical, nutraceutical, chemical, agricultural & aquacultural industry which will support the objective to make algaefuels industry as the main renewable energy source. With combination of multiple integrated industry, this will enhanced the algae industry by having variety of product, thus strengthen the algaefuel feasibility and production.

**References**


