Biotechnological applications of microalgae

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Abstract: Microalgae are important biological resources that have a wide range of biotechnological applications. Due to their high nutritional value, microalgae such as *Spirulina* and *Chlorella* are being mass cultured for health food. A variety of high-value products including polyunsaturated fatty acids (PUFA), pigments such as carotenoids and phycobiliproteins, and bioactive compounds are useful as nutraceuticals and pharmaceuticals, as well as for industrial applications. In terms of environmental biotechnology, microalgae are useful for bioremediation of agro-industrial wastewater, and as a biological tool for assessment and monitoring of environmental toxicants such as heavy metals, pesticides and pharmaceuticals. In recent years, microalgae have attracted much interest due to their potential use as feedstock for biodiesel production. In Malaysia, there has been active research on microalgal biotechnology for the past 30 years, tapping into the potential of our rich microalgal resources for high-value products and applications in wastewater treatment and assessment of environmental toxicants. A culture collection of microalgae has been established, and this serves as an important resource for microalgal biotechnology research. Microalgal biotechnology should continue to be regarded as a priority area of research in this country.

IeJSME 2012: 6 (Suppl 1): S24-S37

Keywords: Bioactive compounds; bioremediation; biotechnology; Chlorella; Dunaliella; microalgae; Spriulina

Introduction

Algae are lower plants without structures such as leaves, roots and stems. In modern classification schemes based on molecular phylogenetic systematics, algae are not classified under the Kingdom Plantae although they resemble very much of higher plants[1]. The eukaryotic algae are grouped under the Kingdom Protista while the prokaryotic algae cyanobacteria or blue-green algae are classified as bacteria (Kingdom Monera). Algae include the microscopic (microalgae) and macroscopic forms (macroalgae), the latter consist of mainly seaweeds. In terms of the diversity in size, algae range from picoplankton of only 0.2 – 2.0 mm in diameter to giant kelps with fronds up to 60 m in length[2]. The number of species of algae has been estimated to be one to ten million, most of which are microalgae. Cyanobacteria are distinguished from other bacteria as they carry out oxygenic photosynthesis. Cyanobacteria were the first colonizers of Earth, and through photosynthesis, they released oxygen which enabled other life forms to live on this planet. The algae are further divided into several major groups, namely green, brown and red algae based on their pigmentation.

While algae are widely found in aquatic habitats, they also inhabit terrestrial habitats, including extreme environments such as snow and glaciers in the Arctic and Antarctic.[3,4] Algae are also present in the air as they may be dislodged from the soil and splashed up by the rain.[5] There have been very few studies on airborne algae although they can exert unfavorable impact on human health. Some airborne algae have been shown to produce allergic reactions in humans. A recent study conducted at Bukit Jalil, Kuala Lumpur found that cyanobacteria were the major airborne algae, with the dominance of *Phormidium tenue*.[6]

Algae represent an important group of organisms for biotechnological exploitation, especially for valuable products, processes and services, with important impact in food and pharmaceutical industries as well as in public health. A diverse range of metabolites with various bioactivities are produced in algae that are yet to be fully exploited.[7] Microalgae such as *Spirulina* and *Chlorella* have been consumed as food supplements (nutraceutical) by humans and also used as animal feeds. In addition, microalgae have been exploited for wastewater treatment and used as a biological tool for assessment of environmental toxicants. Malaysia is endowed with rich microalgal resources that are yet to be fully exploited for biotechnological applications. The aim of this paper is to give an overview of the various

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biotechnological applications of microalgae, as well as to trace the developments of microalgal biotechnology research in Malaysia.

Microalgae as food and dietary supplements

Some of the microalgae have been consumed as food or health food due to their high nutritional value. For instance, *Spirulina* has been harvested for food by the natives in Mexico and Chad (Africa) since ancient times.[2] In Mexico, *Spirulina* is collected from Lake Texcoco and used for making dry cake called tecuitlatl. In Chad, *Spirulina* is harvested from the alkaline lake Kossorom for the preparation of dry cake known as 'dihe'. In the present day, *Spirulina* still contributes significantly to the economy of Chad as the local trading value of 'dihe' is worth more than US$ 100,000.[8]

Presently, *Spirulina* is being cultured on a large scale using open ponds for commercial production of the biomass as dietary supplement in countries such as Thailand, China, United States and India. It is estimated that the annual worldwide production of *Spirulina* ranges from 3000 to 4000 metric tons.[9] *Spriulina* is regarded as a nutritious food due to its high content of proteins, γ-linolenic acid, vitamins and minerals. In addition to being consumed as a food product, *Spirulina* is known to have therapeutic implications, including for health problems such as diabetes, arthritis, anaemia, cardiovascular diseases and cancer.[10] *Spirulina* is also useful as a functional ingredient as it is incorporated into various food products to enhance their nutritional qualities and for therapeutic management of chronic disorders such as diabetes, hypertension and heart disease.[11] *Spirulina* is also well-known for its antioxidant compounds such as phycocyanin and vitamin E.[12,13]

Another cyanobacterium that has been consumed as food since 2,000 years ago by the Chinese is *Nostoc*.[14] The alga is regarded as a healthy food due to its high protein and pigment contents, as well as low fat content. The main species of *Nostoc* with economic value is *Nostoc flagelliforme* or known as "fa cai" by the Chinese. *Nostoc flagelliforme* contains high amounts of pigments such as echinenone and myxoxanthophyll, as well as allophycocyanin, phycocyanin and chlorophyll.[15] It also contains 19 amino acids, of which eight are essential to human nutrition, making up 35.8-38.0% of the total amino acids.[14] The alga has also been used in traditional Chinese medicine since 400 years ago, for the treatment of diarrhea, hypertension and hepatitis. Another species, *Nostoc sphaeroides* or locally known as Ge-Xian-Mi is collected from paddy fields in certain regions of China, and used as food and herbal ingredients.[2]

Chlorella is another microalga that has been mass cultured for commercial production of health food in the form of pills and powder. The first pilot plant for mass culture of *Chlorella* was tested in Boston, USA, and this was followed by other cultivation plants in Israel, Japan and Czechoslavakia.[16] The first commercial production plant of *Chlorella* was established in Japan in 1961 by Nihon Chlorella Inc. Following this, *Chlorella* factories were constructed in countries such as Taiwan, Malaysia and Indonesia. By 1980, some 46 large-scale plants were established in Asia producing more than 1000 kg of *Chlorella* biomass per month.[17] There are more than 70 companies producing *Chlorella* in Taiwan, and the world annual sales of *Chlorella* are more than US$ 38 billion. However, the plant in Malaysia was discontinued in the early 70's. Presently, *Chlorella* products marketed in Malaysia are mainly imported from Japan and Taiwan.

The nutritional value of *Chlorella* is due to their high contents of proteins (51-58% dry weight) and carotenoids, with a wide range of vitamins.[18] In addition, the alga contains β-glucan, which is an active immunostimulator, and has other beneficial effects in scavenging free radicals and reducing blood lipids.[16] An additional product termed 'Chlorella Growth Factor' has been distributed as an agent to improve the growth of lactic bacteria.

High-value products from microalgae

Microalgae are a potential source of a wide range of

high-value products for biotechnological exploitation, which include polyunsaturated fatty acids (PUFA), carotenoids, phycobiliproteins, polysaccharides and phycotoxins (Table 1). A multitude of compounds are produced by microalgae as defense against stress conditions. An advantage of exploiting microalgae for bioactive molecules is that they can be cultured on a large scale for production of the desired chemicals. The advent of molecular biology has led to a better understanding of the biosynthesis and physiological functions of the bioactive molecules in microalgae. Genomic projects have been embarked on microalgae such as Alexandrium, Chlamydomonas, Nostoc and Synechococcus.[19] Efforts have also been invested to develop transgenic microalgae as 'green cell factories' to produce new pharmaceuticals using genetic transformation techniques.[20]

Table 1: Biotechnological applications of products from microalgae.

Product	Applications	Microalgal Producers
Polyunsaturated fatty acids (PUFA)		
Eicosapentaenoic acid (EPA)	Nutritional supplements, aquaculture feed	*Pavlova, Nannochloropsis, Monodus & Phaeodactylum*
Docohexaenoic acid (DHA)	Infant formula, nutritional supplements, aquaculture feed	*Crypthecodiuimu & Schizochytrium*
γ-linolenic acid (GLA)	Nutritional supplements	*Spirulina*
Arachidonic acid (AA)	Nutritional supplements	*Porphyridium*
Phycobiliproteins		
Phycocyanin	Natural dye for health food and cosmetics (lipsticks and eyeliners) antioxidant	*Spirulina platensis*
Phycoerythrin	Fluorescent agent, tool for biomedical research, diagnostic tool	Red algae *(e.g. Porphyridium cruentum)*
Carotenoids		
β-carotene	Food colourant; antioxidant; cancer-preventive properties	*Dunaliella salina*
Astaxanthin	Pigmenter for salmon, antioxidant	*Haematococcus pluvialis*
Mycosporine-like amino acids (MAA)	UV-screening agent ; sunscreen	*Aphanizomenon flos-aquae*
Polysaccharides	Viscosifiers, lubricants and flocculants for industrial applications; antiviral agent	*Porphyridium cruentum*
Phycotoxins – okadaic acid, gonyautoxins, & yessotoxins	Experimental tools for investigations on neurodegenerative diseases	Dinoflagellates *(e.g. Amphidinium, Prorocentrum & Dinophysis)*
Lipids – triglycerides and hydrocarbons	Biofuels	*Chlorella protothecoides Botryococcus braunii*

Polyunsaturated Fatty Acids (PUFA)

Some of the marine microalgae are a potential source of long chain polyunsaturated fatty acids (LC-PUFA), especially eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which have been implicated to be beneficial in the prevention of cardiovascular disease. The conventional source of such LC-PUFA is fish oil; however, fish do not synthesize these fatty acids but acquire them through their diet, which consists of marine microalgae. Microalgae-derived DHA is already commercially available in the market. A study showed that consumption of DHA-rich algal oil to be equivalent to cooked salmon in providing DHA to plasma and red blood cells in 32 healthy men and women.[21]

In humans, DHA is important for the proper development of brain and eye in infants and has been shown to support cardiovascular health in adults.[22,23] One of the major producers of DHA from algae is Martek Biosciences Corporation in USA. The oil extracted from *Crypthecodinium cohnii* contains 40-50% DHA but no EPA or other LC-PUFA.[24] The main application of the DHA oil is as a supplement in infant formula. Another company, OmegaTech (USA) cultures *Schizochytrium* to produce algal oil known as DHA Gold for use as an adult dietary supplement in food and beverages as well as animal feeds. Other applications include food for pregnant and nursing women and for cardiovascular health.[23]

Carotenoids

Carotenoids in algae mainly serve as accessory pigments in photosynthesis, and they are derived from 5-carbon isoprene units that are polymerised enzymatically to form highly-conjugated 40-carbon structures.[7] The compounds which consist of only hydrocarbons are carotenes while those with oxo, hydroxyl or expoxy groups are called xanthophylls. Of the 400 known carotenoids, the main types that have been used commercially include β-carotene and astaxanthin, and to a lesser extent, lutein, zeaxanthin and lycopene.[17] Carotenoids are used as natural food colorants and additive for animal feeds, as well as in cosmetics. In terms of nutritional relevance, certain carotenoids, especially β-carotene act as provitamin A. Carotenoids are also known to have therapeutic value, including anti-inflammatory and anti-cancer activities, which are largely attributed to their antioxidant properties.[12]

Amongst microalgae, the green alga *Dunaliella salina* has been mass cultured using open ponds for the production of β-carotene. The alga grows in water with high salt content and is able to produce β-carotene up to 14% of its dry weight.[25] Industrial plants growing this microalga have been set up in Australia, Israel, USA and China.[26,27] ß-carotene from *Dunaliella* has higher antioxidant activity than synthetic β-carotene as the latter consists of all- *trans*-isomer while the algal product has both *cis*- and *trans*-isomers.[28] The algal products in the market are in the form of β-carotene extracts, *Dunaliella* powder for human use and dried *Dunaliella* for feed use. Administration of algal β-carotene to mouse and humans has been shown that it has protective effect against atherosclerosis. For instance, administration of *Dunaliella* rich in β-carotene inhibits low density lipoprotein (LDL) oxidation and influences plasma triglycerides, cholesterol and high density lipoprotein (HDL) levels in mouse and humans.[29] Supplementation of β-carotene from *Dunaliella bardawil* has been shown to inhibit oxidation of LDL from diabetic patients, and may be important in delaying the development of atherosclerosis.[3] Besides β-carotene, other carotenoids from *Dunaliella* such as phytoene and phytofluene are also known to have health benefits, especially in protection against UV and oxidative damage leading to premature ageing and other disorders.[31]

Another carotenoid from microalgae that has commercial applications is astaxanthin. The pigment is a keto-carotenoid produced mainly by the green alga *Haematococcus pluvialis*. When under unfavourable growth conditions, the alga changes from thin-wall flagellated stage to red thick-wall resting stage due to

the accumulation of astaxanthin, which can reach up to 4-5% dry weight.[32] Astaxanthin is mainly used a food-colouring agent, natural feed additive for the poultry industry and for aquaculture, especially as a feed supplement for salmon, trout and shrimp.[33] The annual worldwide aquaculture market of this carotenoid is worth US$200 million with an average price of US$2,500/kg .[34]

Recently, astaxanthin has also been sold as a nutraceutical for humans in the form of encapsulated product. In addition, astxanthin-rich *Haematococcus* has been marketed as a dietary supplement for human consumption.[33] The health benefit of this product is mainly due to its strong antioxidant activity, which is 100 times more than α-tocopherol.[35] Studies have shown that astaxanthin has protective effects against diseases such as cancers, inflammatory diseases, metabolic syndrome, diabetes, diabetic nephropathy, neurodegenerative diseases and eye diseases.[36] The protective effects are likely to be mediated through its prevention against oxidative damage and cellular necrosis or apoptosis induced by oxidative stress. For instance, it has been shown that administration of astaxanthin in alloxan-induced diabetic rats can partially reverse oxidative stress in neutrophils.[37] In addition, feeding of astaxanthin to rats has been shown to have a protective effect against ethanol-induced gastric ulcer.[38]

Phycobiliproteins

Phycobiliproteins are water-soluble accessory photosynthetic pigments found in algae such as cyanobacteria, rhodophytes (red algae), cryptophytes and glaucophytes. There are three major types of phycobiliproteins, namely phycocyanin, allophycocyanin and phycoerythrin, which differ in their spectral properties. The contents of phycobiliproteins in microalgae vary with environmental conditions, especially intensity and spectral quality of light. For instance, phycocyanin contents in *Spirulina platensis* may vary from 0.11 to 12.7% dry weight when cultured at different light intensities.[39]

Two microalgae that have been exploited for commercial production of phycobiliproteins are *Spirulina* and *Porphyridium*. The major applications of the pigments are as natural dyes for food and cosmetic products as well as fluorescent markers in biomedical research diagnostic tools.[40] For instance, phycocyanin is marketed as a product called Lina Blue by Dainippon Ink and Chemicals (Sakura) for use in chewing gum, ice sherberts, popsicles, candies, soft drinks, dairy products and wasabi.[17] Another form of this pigment is used in natural cosmetics such as lipstick and eyeliners. As fluorescent markers, phycobiliproteins are widely used in immunology laboratories, as they can serve as labels for antibodies, receptors and other biological molecules in fluorescence-activated cell sorter, as well as in fluorescence microscopy. The prices of phycobiliprotens may range from US$ 3 to US$ 1500/mg for certain cross-linked pigments.[17]

Recent studies have shown that phycocyanin has health-promoting properties and a broad range of potential pharmaceutical applications. The beneficial effects of the pigment are mainly due to its antioxidant activities. The antioxidant potential of phycocyanin has been shown to be 16-times more efficient than trolox (vitamin E analog) and 20 times more effective than vitamin C based on its protective effect on human erythrocytes against lysis induced by peroxyl radicals.[41] The pigment has also been shown to have protective effects in human pancreatic cells[42] and against arthritis in rats[43] by attenuating oxidative stress. Administration of selenium-enriched phycocyanin from *Spirulina platensis* has been shown to protect against the development of atherosclerosis in hamster through its inhibitory effect on pro-oxidant factors.[44]

Bioactive compounds

As microalgae possess a multitude of physiological, biochemical and molecular strategies to cope with stress, they are capable of synthesising a variety of bioactive chemicals. The bioactive compounds are usually secondary metabolites, which include various types of

substances ranging from organic acids, carbohydrates, amino acids and peptides, vitamins, growth substances, antibiotics, enzymes to toxic compounds. The metabolites show a wide range of biological activities, including anticancer, antiviral, antioxidant and immunomodulatory effects. There is potential of discovering new drug leads from such metabolites. Of the various groups of microalgae, the cyanobacteria seem to feature most prominently as sources of bioactive compounds.[19]

Toxic metabolites from microalgae (phycotoxins) are a promising group of bioactive molecules for biotechnological exploitation. Such compounds are produced mainly by dinoflagellates and cyanobacteria, especially those that cause harmful algae blooms in either marine or freshwater environments. Excessive growth of dinoflagellates may cause discolouration of the sea, forming red tides while blooms of cyanobacteria in lakes due to excessive nutrients, especially nitrogen and phosphorus may cause eutrophication. While about 300 species of microalgae have been reported to form algae blooms, nearly one fourth of these species are known to produce toxins.[45] The toxins can cause health hazard to humans, domestic animals and wildlife with toxicological effects including neurotoxicity, hepatoxicity, cytotoxicity and dermatoxicity.[46] The most common freshwater algal toxins include microcystins, homo- and anatoxin-a and saxitoxins, which are produced by cyanobacteria such as *Microcystis*, *Anabaena*, *Oscillatoria* and *Nostoc* species. Human poisoning due to toxins of marine dinoflagellates include paralytic shellfish poisoning, diarrheic shellfish poisoning, neurotoxic shellfish poisoning, and ciguatera fish poisoning.[47] The common dinoflagellates that produce such toxins include *Alexandrium*, *Dinophysis*, *Karenia* and *Gymnodinium* species.

Phycotoxins show a wide range of biological activities including cytotoxic, antitumor, antibiotic, antifungal, immunosuppressant and neurotoxic activities, which offer a great potential for biotechnological exploitation.[47] Many patents relating to such toxins have been filed. As many of the algal toxins are neurotoxic compounds, they are suitable as experimental tools used to explore how the brain works and for investigations on neurodegenerative diseases. For instance, okadaic acid from *Dinophysis* is a potent neurotoxin used in the studies on the therapeutic effects of atypical antipsychotic drugs in the treatment of cognitive impairment and schizophrenia.[48] In addition, pectentoxins from *Dinophysis* show cytotoxic activity against several human cancer cell lines.[49] The macrolide amphidinolides produced by *Amphidinium* has been shown to have potent antitumour properties against murine lymphoma L1210 and human epidermoid carcinoma KB cells.[50] The major challenge faced in the biotechnological exploitation of dinoflagellate toxins is the need to produce sufficient quantities of the bioactive materials. The main problem faced in culturing dinoflagellates is their lower growth rates compared to other typical microalgae.[47]

Amongst the toxins from cyanobacteria, those isolated from *Lyngbya majuscula* show a variety of chemical structures, including nitrogen-containing compounds and polyketides.[51] One of the compounds, curacin A is a potent inhibitor of cell growth and mitosis that inhibits microtubulin assembly. It has been shown to have cytotoxic activity against L1210 leukaemic cell line by inhibiting tubulin polymerisation.[52] A neurotoxic lipopeptide, kalkitoxin isolated from the microalga is a sodium channel block, which is a useful tool to understand neural transmission.[53] Successful chemical synthesis of this toxin and its analogues, and testing of their biological activities was recently reported by Umezawa *et al.* (2012).[54]

Several cyclic or linear peptides and depsipeptides isolated from cyanobacteria are protease inhibitors, which have potential applications in medicine for treatment of diseases such as strokes, coronary artery occlusions and pulmonary emphysema.[19,51] For instance, aeruginosins isolated from *Microcystis aeruginosa* have been shown to have inhibitory activity against thrombin, plasmin and trypsin.[55] Other depsipeptides such as micropeptin, microcystilide, cyanopeptolin, oscillapeptin and nostocyclin are inhibitors of enzymes

such as trypsin, plasmin, thrombin and chymotrypsin.[56]

Microalgae are also potential sources of antiviral compounds that are yet to be fully explored. For instance, screening of extracts from 600 cultures of cyanobacteria showed a hit rate of 10% in inhibiting cellular infection of HIV-1, HSV-2, and antirespiratory syncytia virus.[57] A novel compound from cyanobacteria, cyanovirin-N has been shown to be a potent virucidal agent against HIV, which blocks the interaction of the viral glycoprotein gp120 with CD4.[58] There is potential of developing a topical vaginal microbicide against HIV based on this compound.[59] The sulfated polysaccharide calcium spirulan derived from *Spirulina platensis* shows antiviral activity by inhibiting the entry of enveloped viruses such as Herpes simplex, human cytomegaloviruses and measles virus into the cell.[60,61] Sulfated polysaccharide from the red alga *Porphyridium* has also been shown to display antiviral activities against HSV-1, HSV-2 and Varicella zoster virus by preventing the adsorption of the virions.[62]

Bioactive molecules with anticancer activities are one major group of targeted compounds from microalgae. A large-scale screening of about 1,000 extracts of cyanobacteria isolated from various habitats for antineoplastic activity was conducted by Patterson et al. (1991).[63] Antineoplastic activity was assessed based on the inhibitory effects on the differentiation of human leukaemic cells, and a hit rate of about 7% was reported. In another screening, more than 501 extracts from marine microalgae were assessed using mechanism-based assays, including activities against protein kinase C, protein tyrosine kinase and inosine monophosphate dehydrogenase.[64] A novel chlorosulfolipid isolated from *Poteriochromonas malhamensis* was shown to inhibit protein tyrosine kinase activity. Marine cyanobacteria are potential producers of bioactive compounds that are effective in killing cancer cells by inducing apoptotic death, or affecting cell signaling through activation of signaling enzymes belonging to protein kinase C family.[65]

The polyketide-derived macrolides scytophycins isolated from *Scytonema pseudohofmanni* have been shown to inhibit a variety of mammalian cells, including the epidermoid carcinoma cells.[66] The compounds are also active against intraperitoneally implanted lymphocytic leukaemia and lung carcinoma.[67] Another metabolite isolated from *Nostoc* ATCC 53789, cryptophycin is a prominent anticancer compound[68] while scytonemin isolated from *Stigonema* is a protein serine/threonine kinase inhibitor, which may provide an excellent drug with antiproliferative and antiinflammatory activities.[69]

Use of microalgae for biodiesel production

The potential use of microalgae as feedstock for biodiesel production has been receiving increased interest in recent years. It is advantageous to use microalgae for biodiesel production compared to other crop plants because it will not compromise production of food, fodder and other feedstocks derived from those crops. In Malaysia, palm oil has been the major raw material for biodiesel production; however, the supply of palm oil is not sufficient to meet the demand. There is concern that there may not be enough palm oil left for food production if it is used for biodiesel production. Microalgae appear to be the only source of biodiesel that has great potential to replace fossil diesel.[70] The oil content of microalgae may range from 16 to 68% dry weight.[71] The oil yield from microalgae can reach up to 136,900 L/ha compared to other plant crops, which range from 172 to 5950 L/ha. Manipulation of culture conditions can further enhance the lipid content of microalgae. For instance, some microalgae can accumulate storage lipids (triglycerides) up to 70% dry weight under nitrogen-starvation.[72] Amongst the microalgae, *Chlorella* appears to be a potential feedstock for biodiesel production. For instance, *Chlorella protothecoides* produces a crude lipid content of 55.2% dry weight when grown under heterotrophic condition on glucose.[73] The biodiesel produced by this alga has been shown to be of high quality, with high heating value and viscosity.

Mass culture of microalgae not only generates biomass for biodiesel production, it is also useful for mitigating the problem of global warming due to carbon dioxide (CO_2). Microalgae have much higher photosynthetic efficiency than terrestrial plants, and are able to grow at high CO_2 level and temperature.[74] In theory, microalgae are able to use up 9% of the incoming solar energy to produce 280 tons of dry biomass per ha^{-1} yr^{-1} while sequestering about 513 tons of CO_2.[75] Species which have been shown to grow at high levels of CO_2 include *Spirulina* sp., *Scenedesmus obliquus* and *Chlorella vulgaris* which can grow up to 18% CO_2.[76] Such microalgae are useful for bioremediation of flue gas which contains 10 – 13% CO_2.[77] The CO_2 fixation rate by *Chlorella vulgaris* grown in flue gas can reach 4.4 g CO_2 L^{-1} day^{-1}. With a gas residence time of 2 s, *Chlorella vulgaris* can remove up to 74% of the CO_2 when grown in a photobioreactor.[78] Thus, an integrated system of using microalgae for CO_2 biofixation and biodiesel production will be most attractive.

Applications of microalgae in environmental biotechnology

Microalgae have many applications in environmental biotechnology, especially for bioremediation, bioassay and biomonitoring of environmental toxicants. The discharge of wastewater containing various contaminants into the aquatic ecosystems is a major concern as it can be a threat to public health. The high rate algae pond (HRAP) system has been shown to be an efficient system to treat wastewater.[79,80] The system consists of shallow pond mixed by paddle-wheels to enhance nutrient transfer and photosynthetic efficiency to optimise algal growth. The HRAP system is particularly suitable for tropical climate with the warm weather and abundance of sun light throughout the year. Apart from the efficient reduction of pollutants such as chemical oxygen demand (COD), biological oxygen demand (BOD), nitrogen and phosphorus, HRAP generates algal biomass that has potential applications as animal feed and feedstock for biodiesel. Microalgae grown in HRAP have been shown to be useful in treating various wastewaters including rubber effluent, palm oil mill effluent (POME) and municipal wastewater.[80,81] Reduction of phosphate from anaerobically digested starch factory wastewater of more than 99% was attained by *Spirulina platensis* grown in HRAP.[82] The HRAP system is useful for tertiary treatment of the wastewater after undergoing the conventional treatment before discharge.[79] For instance, *Chlorella vulgaris* grown in HRAP has been shown to be useful for final polishing of textile wastewater before discharge, especially for colour removal.[83] Besides single-species culture, a combination of several microalgae has also been evaluated in some studies for efficiency in wastewater treatment. For instance, a consortium of five microalgae grown in HRAP was successfully used to treat landfill leachate.[84]

In addition to suspended cultures, immobilised microalgae system can further enhance the efficiency in the removal of environmental toxicants.[85] For instance, *Chlorella vulgaris* immobilised in alginate was found to be effective in removing colour from textile dyes.[86] Immobilised *Chlorella vulgaris* and *Scenedesmus obliquus* have been shown to be effective in removing nitrogen and phosphorus from urban wastewater operated on a semi-continuous mode.[87] In another study, co-immobilisation of microalgae and bacteria have been shown to be more effective in removing nutrients such as nitrate, ammonium and phosphate from wastewater compared with immobilised microalgae without bacteria.[88]

While microalgae that are tolerant to toxicants are useful in bioremediation, sensitive species are useful tools for bioassay and biomonitoring of environmental pollutants.[89] Microalgae have been used as bioassay organisms to assess the toxicity of pollutants such as heavy metals, pesticides and pharmaceuticals. Heavy metals have been detected in the aquatic ecosystems and even in marine organisms such as fish and mussel.[89,90] Residual organochlorine and organophosphate pesticides have been detected in the Selangor River due to intense agricultural and urban activity.[91] The presence of pharmaceutically-active compounds such as antibiotics in the environment, due to their excess use in aquaculture and animal husbandry has also

been a concern in recent years.[92]

While chemical assays are used to determine the contents of the pollutants, only bioassays can really assess the biological effects on living organisms. Adverse impacts of environmental toxicants on microalgae can be far-reaching as they form the basis of the food chain. Thus, microalgae are often included in the bioassays of environmental pollutants. The common microalgae used for bioassays of toxicants include *Pseudokirchneriella subcapitata*, *Dunaiella tertiolecta*, *Isochrysis galbana*, *Chlorela* spp.[89,93] Various endpoints are used to assess the toxicity of pollutants to microalgae and these are usually based on the effects on growth, photosynthesis, movement behavior, oxidative stress markers, biochemical composition and pigmentation.[89,94]

Runoff of excess nutrients from fertilizers and industrial wastewater to the aquatic environment can trigger overgrowth of microalgae that causes eutrophication.[95] Microalgae are useful tools for the assessment of nutrient enrichment due to nitrogen and phosphorus. For instance, three tropical microalgae, namely *Chlorella vulgaris*, *Scenedesmus quadricauda* and *Ankistrodemsus convolutus* were found to be useful for the assessment of nitrogen and phosphorus enrichment in freshwater ecosystems.[96]

Development of microalgal biotechnology in Malaysia

In Malaysia, early studies on microalgae focused mainly on the taxonomy and ecological distribution of freshwater algae. The fundamental studies in phycology have resulted in the publications of several checklists and monographs that documented the diversity of microalgae in Malaysia.[97-99] The early studies in applied phycology focused mainly on the use of microalgae for the treatment of agro-industrial wastewater.[100] Efficient removal of nitrogen from palm oil mill effluent (POME) was attained using such a system. Following this, the HRAP system was shown to be useful for the treatment of agro-industrial wastewaters such as rubber effluent[80], sago starch factory wastewater[82], textile wastewater[83] and landfill leachate.[84] In another study, marine microalgae grown in POME were suggested to be useful as aquaculture feed due to their high contents of PUFA.[101]

There has been much interest in screening the indigenous microalgal resources of Malaysia for fine chemicals such as PUFA and pigments since 1990's. Microalgae were isolated from various habitats in Malaysia and screened for those valuable products.[102] The University of Malaya Algae Culture Collection (UMACC) was then established to serve as a repository for the microalgal cultures.[103] The UMACC which holds more than 150 microalgal isolates, is the largest and only microalgae culture collection in Malaysia. Of the many interesting microalgae in the collection, *Chlorella vulgaris* UMACC 001 is a very well-studied strain.[104] The alga has potential applications in wastewater treatment and removal of heavy metals, and it grows well on organic carbon and at high nitrogen levels. Although the strain was isolated from freshwater habitat, it grows well at a wide range of salinities.

Microalgal lipids, especially PUFA and carotenoids are amongst the targeted products from the algal resources of Malaysia.[105] For instance, the marine diatom *Nitzschia inconspicua* produces appreciable amounts of EPA and arachidonic acid.[106] Factors such as nitrate and silicate levels, and carbon source were found to affect the growth and fatty acid profiles of this diatom. Cultures aerated with 5% CO_2 were found to produce highest yields of biomass and EPA. The current interest in microalgal lipids is mainly focused on their potential use for biodiesel production. The priority areas include screening of microalgal strains for high lipid producers, and the use of genomic, proteomic and metabolomic approaches to understand and enhance lipid synthesis in microalgae.

The chlorophyte *Ankistrodesmus convolutus* was found to produce appreciable amounts of carotenoids, especially lutein and may have potential application as poultry feed.[107] The biomass attained by this microalga increased when grown on glucose, although the carotenoid content decreased. Further manipulation by varying the irradiance and light-dark cycle to enhance

biomass and pigment production by *Ankistrodesmus convolutus* was also conducted.[108] The cultures of the microalga were scaled up using a 6.5 L airlift fermenter to enhance biomass and pigment productivity.[109]

A strain of marine cyanobacterium from the collection, *Oscillatoria* UMACC 216 was found to produce high amounts of phycoerythrin.[110] Cells grown at 15 parts per thousand (ppt) of salt were found to produce highest amounts of phycoerythrin. In another study, the influence of irradiance and inoculum density on the pigmentation of *Spirulina platensis* was investigated.[39] The highest yield of phycocyanin was attained by cultures grown at low irradiance, developed from low-density inoculum. Under nitrogen-starvation, three strains of *Spirulina platensis* were also shown to produce high amounts of poly(3-hydroxybutyrate), which has potential application as bioplastic.[111]

Several microalgae from the UMACC, including *Spirulina platensis*, *Ankistrodesmus convolutus* and *Synechococcus elongatus* were screened for their antiproliferative activities against nasopharyngeal carcinoma (NPC) cells using bioassay-guided fractionation approach.[112] It was found that Fraction 7' obtained from column chromatography separation of the methanol extract from *Synechococcus elongatus* showed antiproliferative activity against NPC cells. It was suggested that the mechanism of action of the fraction was through induction of apoptosis and the active compound could be a derivative product of chlorophyll a. In another study, the inhibitory activities of extracts from the microalgae *Ankistrodesmus convolutus*, *Synechococcus elongatus* and *Spirulina platensis* against the release of Epstein-Barr virus (EBV) from lymphoblastoid cells were investigated.[113] The HPLC sub-fraction SEF1' from the methanol extract of *Synechococcus elongatus* was found to be most active in reducing the cell-free EBV DNA from lymphoblastoid cells. It was postulated that the active principles could be pigment components. Several microalgae, especially *Spirulina platensis* were also shown to have high antioxidant activity based on chemical assay.[114] The aqueous extract from food-grade *Spirulina platensis* was also shown to have protective effect against apoptotic cell death due to free radicals.[13]

Another interesting study on Malaysian microalgae was their potential application as larvicidal agents against *Aedes aegyptii*. A survey was conducted to investigate the correlation between the occurrence of microalgae and mosquito larvae in various mosquito breeding habitats in Malaysia.[115] A total of 196 microalga were identified and some common microalgae found in the mosquito breeding sites include *Chlorella* sp. and *Scenedesmus* sp. The microalgae isolated were used to feed the larvae of *Aedes aegypti*.[116,117] Of the chlorophytes tested, it was found that the percentage mortality of larvae fed with *Chlorella vulgaris* was the highest. There was also delayed pupation and body size reduction of the mosquitoes fed with *Chlorella vulgaris*. Interestingly, larvae fed with another alga, *Ankistrodesmus convolutus* were larger than those fed with normal insectory feed.

The applications of the microalgae from the UMACC for environmental biotechnology were further expanded with the addition of new isolates of from the Arctic and Antarctic. This new collection was established following the venture of Malaysian researchers into polar research since the year 2000.[3,4] Diversity of polar microalgae at Ny Alesund in the Arctic[3] and Casey Station in the Antarctic[4] was documented following the expeditions to those sites. The cultures of polar microalgae established are very useful for the studies on stress response of such microalgae to global warming and increased ultraviolet radiation (UVR), in comparison with tropical and temperate species. The effects of climate change on microalgae, especially on how they respond and adapt to global warming and increased UVR has received much attention in recent years.[118,119] For instance, the response of microalgae of similar taxa from the Antarctic, tropical and temperate regimes to UVR was compared based on their growth and biochemical composition.[120] Fatty acid profile was found to be a useful biomarker to assess the response of some Antarctic microalgae to UVR stress. The influence of temperature stress on the growth, biochemical composition and fatty acid

profiles of six Antarctic microalgae was reported by Teoh *et al.* (2004).[121] The Antarctic *Chlorella* was found to be able to grow at temperatures much higher than the ambient, even up to 30°C. The differential gene expression of the Antarctic *Chlorella* in response to temperature was conducted.[122] The gene that encodes Photosystem II P680 chlorophyll a apoprotein CP47 (PSII-CP47) increased when the alga was exposed to cold temperature stress at 4°C.

Conclusion

There is indeed a wide range of applications of microalgae in biotechnology. There is great potential to further exploit the rich microalgal resources of Malaysia for various biotechnological applications. In terms of applications in medical biotechnology, microalgae are potential sources of high-value products, including nutraceuticals, and bioactive molecules that may lead to the discovery of new drugs. The use of microalgae as a biological tool for monitoring and assessment of environmental toxicants is another application that has attracted much interest. Microalgal biotechnology should continue to be regarded as a priority area of research in Malaysia.

Acknowledgements

The author would like to thank Prof Phang Siew Moi from the University of Malaya for her encouragement, support and mentorship throughout his endeavor in microalgal biotechnology research. The funding from the Ministry of Science, Technology and Innovation (MOSTI), Ministry of Higher Education (MOHE) as well as IMU internal grants in supporting research in microalgal biotechnology is gratefully acknowledged.

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