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Pharmaceutical applications and consequent environmental impacts of *Spirulina* (*Arthrospira*): An overview

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SUMMARY: Recently, microalgae cultivation for different applications, including the production of nutritional and pharmaceutical active compounds has received increasing attention. Among the different genera, *Spirulina* (*Arthrospira* sp.) is one of the most promising blue-green microalgae (Cyanophyta) because it is rich in antioxidants, essential amino acids (EAAs), minerals, proteins, polyunsaturated fatty acids and vitamins. It has a high protein content (60-70% of the dry weight), which is a complete protein, i.e. containing all EAAs. Therefore, *Spirulina* is currently a commercial product with high nutritional value and also a significant source of complementary and alternative medicine. The objective of the present work was to review the pharmaceutical and therapeutic applications of *Spirulina,* especially its antioxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetic, anti-obesity and anti-toxicity properties. The results were obtained from experiments in the literature performed *in vitro* and *in vivo* using experimental animals. The main reported active ingredients in *Spirulina* include phycocyanin, tocopherol, β-carotene, caffeic acids and chlorogenic acid, which showed individual or synergetic effects. In addition, the present review discusses the future perspectives of genetically modified *Spirulina* as a source for industrial products while producing valuable biomass photoautotrophically. Furthermore, the consequent environmental impacts of large-scale cultivation of *Spirulina* are discussed.

***KEYWORDS*:** Arthrospira *sp.; Cultivation; Environmental impacts; GMO; Pharmaceuticals;* Spirulina

RESUMEN: ***Aplicaciones farmacéuticas e impactos ambientales de la* Spirulina *(*Arthrospira*)*. *Una visión general.*** Recientemente, el cultivo de microalgas para diferentes aplicaciones, incluida la producción de compuestos activos nutricionales y farmacéuticos, está recibiendo una atención cada vez mayor. Entre los diferentes géneros, *Spirulina* (*Arthrospira* sp.) es una de las microalgas azul-verde más prometedoras (Cyanophyta) porque es rica en antioxidantes, aminoácidos esenciales (EAAs), minerales, proteínas, ácidos grasos poliinsaturados y vitaminas. Tiene un alto contenido de proteína (60-70% del peso seco) es una proteína completa, es decir, contiene todos los EAAs. Por lo tanto, la *Spirulina* es actualmente un producto comercial con alto valor nutricional y también una fuente importante parala medicina complementaria y alternativa. El objetivo del presente trabajo es revisar las aplicaciones farmacéuticas y terapéuticas de *Spirulina*, especialmente propiedades antioxidantes, antiinflamatorias, anticancerígenas, antimicrobianas, antidiabéticas, antiobesidad y antitóxicas. Los resultados se obtienen a partir de trabajos experimentales realizados *in vitro* e *in vivo* utilizando animales de experimentación. Los principales ingredientes activos reportados en *Spirulina* incluyen ficocianina, tocoferol, β-caroteno, ácidos caféicos y clorogénico que mostraron efectos individuales o sinérgicos. Además, en la presente revisión se discute las perspectivas futuras de la *Spirulina* genéticamente modificada como fuente de productos industriales, al mismo tiempo que se produce una valiosa biomasa fotoautotrófica. Además, se discutieron los impactos ambientales consiguientes del cultivo a gran escala de la *Spirulina*.

PALABRAS CLAVE: Arthrospira *sp.; Cultivo; Impactos ambientales; OGM; Productos farmacéuticos;* Spirulina

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1. INTRODUCTION

Microorganisms have unique characteristics and therefore, they are considered good pillars for the large-scale production of many useful metabolites. Microbial cells have a high area/volume ratio, which results in high efficiency of nutrient uptake for the biosynthesis of different metabolites through different metabolic pathways. Microalgae are a wide group of photosynthetic microorganisms that can be isolated from various habitats and can grow efficiently in commercial large-scale raceway ponds or photobioreactors (Figure 1), where cells can grow on inexpensive nutrient sources to produce valuable compounds. Cyanophytes are a group of microalgae commonly grown in rice paddies and have a positive impact on growth, yield and nitrogen levels of the crop plants (Whitton, 2000). This group of microorganisms also known as cyanobacteria, as they resemble bacteria in that they are prokaryotes and lack the internal structures such as nucleus and other organelles found in eukaryotic microalgae. From the practical and economical point of view, microalgae offer numerous advantages, such as high photosynthetic efficiency which results in high growth rates, high CO_2 mitigation rate, possibility for cultivation on arid lands using marine-, fresh- or wastewater (Abomohra *et al*., 2018).

Although many previous studies focused on different cyanophytes as nitrogen-fixing organisms, *Spirulina* is gaining attention due to its high nutritional value and extensive pharmaceutical applications (Table 1). The correct identification of a certain species is a fundamental requisite for research study and/or developmental applications. The genus *Spirulina* has been incorrectly used to describe two different genera, *Spirulina* and *Arthrospira*. However, Stizenberger (1854) and Gomont (1892–1893) classified the forms with visible septa within the genus *Arthrospira* Stizenberger 1852; while coiled filaments with invisible septa were classified as belonging to the genus *Spirulina* Turpin 1829 (Tomaselli, 1997). The view of *Arthrospira* and *Spirulina* as two separate genera has been officially accepted by Bergey's Manual of Systematic Bacteriology (Castenholz, 1989). The separation between these two genera has been repeatedly affirmed on the basis of many other characteristics such as cell wall structure, helicity, trichome size, motility, gas vesicles, thylakoid pattern, GC analysis and phylogenetically using 16S rRNA. Depending on the later, 16S rRNA of two *Arthrospira* strains (PCC 7345 and PCC 8005) and one *Spirulina* strain (PCC 6313) confirmed that the two *Arthrospira* strains form a tight cluster not closely related to the *Spirulina* strain, which supports the separation of these two genera (Tomaselli, 1997). Therefore, a molecular approach is not only useful for the precise identification of a certain species, but it also helps to understand the evolutionary relationships occurring among various microorganisms.

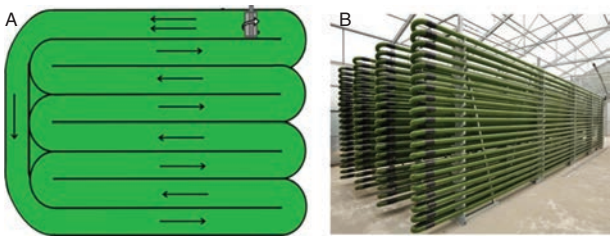

FIGURE 1. Surface view of a raceway ponds (A) and partial view of tubular photobioreactor (B) used for microalgae cultivation (Modified from Abomohra *et al.,* 2016).

Spirulina is a photosynthetic autotrophic organism containing the blue pigment phycocyanin as the main photosynthetic pigment in addition to the green pigment chlorophyll *a,* which results in the blue-green color of the cells. *Spirulina* is one of multicellular unbranched non-heterocystous filamentous microalgae which are recognizable by the unique open left-handed helix along the entire length of the filament (Figure 2). The filaments are 50-500 μm in length and 2-4 μm wide, composed of only vegetative cells containing gas vacuoles. The long size filaments and strong cells allow successful filtration comparing to unicellular microalgae which results in the cost-effective harvest of *Spirulina* compared to other microalgal species. Currently, microalgae cultivation takes place in two major cultivation systems, namely closed photobioreactors (PBR) and open ponds. Both approaches have their own advantages and disadvantages which were discussed in details by Soni *et al*., (2017). However, *Spirulina* is mainly reported to be grown commercially in open ponds. Recently, new hybrid cost-effective techniques such as poly bags can also be used to increase algal biomass productivity (Soni *et al*., 2017).

Many microorganisms are used as a massive sustainable resource for bio-pharmaceuticals which can be produced by direct extraction or after biomass

TABLE 1. Some reported applications of *Spirulina*

Applications/Activity	References
Healthy food, forage and additives	Jiménez *et al.,* (2003)
Antioxidant	Manoj *et al.,* (1992); Miranda *et al.,* (1998); Abomohra *et al.,* (2016).
Anti-inflammatory	Romay *et al.,* (1998); Vázquez-Velasco *et al.,* (2014).
Anti-cancer	Schwartz and Shklar (1987); Schwartz *et al.,* (1988); Liu *et al.,* (2000); Subhashini *et al.,* (2004).
Anti-microbial	Kokou *et al.,* (2012); Özdemir *et al.,* (2004); Santoyo *et al.,* (2006).
Anti-diabetes and anti-obesity	Schwartz *et al.,* (1988); Han *et al.,* (2006).
Anti-toxicity	Shastri *et al.,* (1999); Kim *et al.,* (2011); Abdel-Daim *et al.,* (2016); Ebaid *et al.,* (2017).

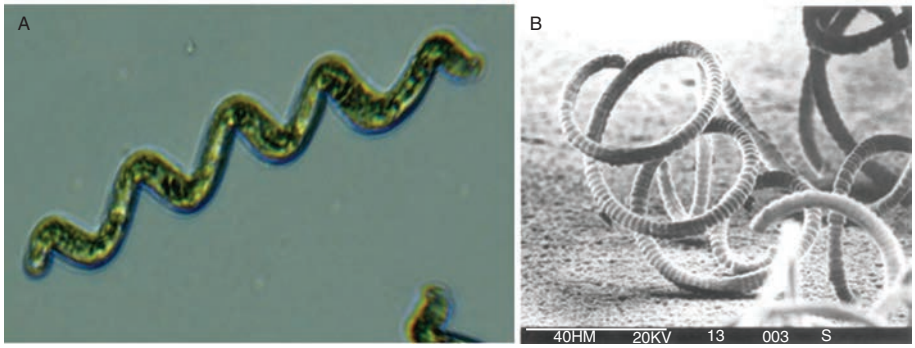

FIGURE 2. Microscopic examination of *Spirulina* under light microscope (A, Ebaid *et al.,* 2017) and scanning electron microscope (B, Ciferri 1984).

bioprocessing. During secondary metabolism, microorganisms produce special secondary metabolites which possess special chemical structures and are not critical for the growth of the producing organism. In general, such metabolites are produced under unfavorable conditions in defense against predation, herbivory and competition for space. So far, some secondary metabolites from different microorganisms were isolated and identified in pure form. For example, chlorellin is a fatty acid produced by the green microalga *Chlorella vulgaris* and was first identified as an active anti-bacterial substance in 1940s (Centeno and Ballentine, 1999). On the other hand, the limited availability of fossil fuels and their environmental issues, especially participation in global warming, enhanced the extensive worldwide search for new renewable energy sources with combined CO_2 mitigation. *Spirulina* was discussed as one of microalgae capable of producing different kinds of bioenergy with high efficiency of CO_2 fixation (Sumprasit *et al*., 2017; Shao *et al*., 2018; Rempel *et al*., 2018), which provides the advantage of an eco-friendly utilization of *Spirulina*. The main aim of the present work was to provide a critical overview of the current status of the pharmaceutical applications of *Spirulina*, including antioxidant, anti-inflammatory, anti-cancer, antimicrobial, anti- diabetes & obesity and anti-toxicity activities. In addition, future perspectives in genetic engineering technologies to enhance valuable products from *Spirulina* were discussed. The present work shed light on the environmental impacts of large-scale cultivation of *Spirulina* as one of the economically important microalgae.

2. NUTRITIONAL VALUE OF *SPIRULINA*

Compared to other foods, *Spirulina* shows extraordinary nutritional value as it provides high levels of many essential nutrients and minerals (Table 2). However, the biochemical composition of *Spirulina* is highly dependent on growth conditions and the methods used for harvest and drying. In general, Capelli and Cysewski (2010) reported that the amount of calcium in *Spirulina* is 1.8 times higher than that in whole milk, total protein is 6.7 times that of tofu, iron is 51 times greater than that of spinach and β-carotene is 31 times more abundant than in carrots. Therefore, *Spirulina* was given the label of 'super food' by The World Health Organization (WHO). In addition, it was sent to space by National Aeronautics and Space Administration (NASA) due to its high nutritional value (Khan *et al*., 2005). *Spirulina* is produced commercially in open ponds by many producers (Table 3). Earthrise Farms, located in USA, covering 437060 m^2, is the world's largest producer of *Spirulina*. The company produces dried *Spirulina* in the form of tablets and powder in over 20 countries around the world with an average annual production of 550 tons. Other large *Spirulina* production plants are Cyanotech and Hainan DIC Microalgae located in USA and China, covering 364217 and 100000 m^2 with annual production of 400 and 350 tons, respectively (Table 3). However, other companies sell *Spirulina* special products such as nutraceuticals, e.g. Myanmar *Spirulina* Factory (Yangon, Myanmar) sells tablets, chips, pasta and liquid extract.

3. PHARMACEUTICAL APPLICATIONS OF *SPIRULINA*

Spirulina is the most highly consumed microalga (Bleakley and Hayes, 2017), which has been widely used as human healthy food centuries ago; and it is currently produced commercially as healthy food and valued additives in a hugely profitable business. In addition to its high nutritional value, *Spirulina* shows many biological activities such as antioxidant, anti-inflammatory, anti-cancer, anti-microbial, anti-diabetes & obesity and anti-toxicity.

3.1. Antioxidant activities

Spirulina extracts show relatively high antioxidant activities that have attracted the attention of many researchers. Manoj *et al*., (1992) confirmed the activity of alcoholic extract of

Table 2. Typical analysis of the composition of *Spirulina* (per 3 grams of dry biomass, Moorhead *et al.,* 2012)

Items	Amount	Items	Amount
General Composition		**Phytonutrients**	
Carbohydrates	17-25%	Chlorophyll	30 mg
Protein	53-62%	Total carotenoids	15 mg
Lipids	4-6%	β-carotene	6.8 mg
Minerals	8-13%	Total phycocyanins	519 mg
Vitamins		C-phycocyanin	240 mg
Vitamin A	11,250 IU	Zeaxanthin	9 mg
Vitamin B1	3.5 µg	Superoxide dismutase	1080 units
Vitamin B2	140 µg	**Minerals**	
Vitamin B3	400 µg	Calcium	10 mg
Vitamin B6	30 µg	Magnesium	15 mg
Vitamin B12	9.0 µg	Iron	6.5 mg
Vitamin E	285 µg	Phosphorus	33 mg
Inositol	1.7 µg	Potassium	60 mg
Biotin	0.5 µg	Sodium	30 mg
Folic acid	6.2 µg	Manganese	400 µg
Pantothenic acid	4.5 µg	Zinc	90 µg
Vitamin K1	60 µg	Boron	22 µg
Vitamin K2	15 µg	Copper	20 µg
		Selenium	0.9 µg
		Iodine	15 µg

Table 3. Some current commercial producers of *Spirulina* with annual production of biomass

Company	Location	Total area (m^2)	Production (ton)
Earthrise Farms	USA	437060	550
Cyanotech	USA	364217	400
Hainan DIC Microalgae Co., Ltd	China	100000	350
Nao Pao Resins Chemical Co., Ltd	Taiwan	50000	150
Boonsom Farm	Thailand	40000	-
Siam Algae Co., Ltd.	Thailand	30000	135
Solarium Biotechnology	Chile	24000	13

Spirulina in lipid peroxidation inhibition more significantly (65%) than some chemical antioxidants like α-tocopherol (35%) and β-carotene (48%). In addition, they reported that a hot-water extract of *Spirulina* has a greater antioxidant effect (76%) than chlorogenic acid (56%) and gallic acid (54%). Miranda *et al*., (1998) studied the *in vivo* and *in vitro* antioxidant activity of *S. maxima* methanolic extract. *In vivo* antioxidant activity as lipid peroxidation was studied in the plasma, brain and liver homogenates of experimental rats using a daily dose of 5 mg of methanolic extract. The extract contained total tocopherol, β-carotene and total phenolic compounds of 18 $mg{\cdot}L^{-1}$, 27.5 $mg{\cdot}L^{-1}$ and 96.3 $mg{\cdot}L^{-1}$, respectively. Upon treatment, the antioxidant activity increased in the plasma of the *Spirulina*-treated group by 31.5% over the control group. The antioxidant effect was mainly associated with the individual or synergetic effect of tocopherol, β-carotene and phenolic compounds. In another study, the antioxidant activity was attributed to the phenolic compounds found in the methanolic alga extract including salicylic, chlorogenic, trans-cinnamic, caffeic acids, quimic and synaptic; individually or in a synergistic action (Baicus and Baicus, 2007). However, the caffeic acids and chlorogenic acid present in the *Spirulina* extract recorded more antioxidant efficiency than other acids (Henrikson, 1994); which also recorded a potential preventive action against cancer (Subhashini *et al*., 2004). Recently, Abomohra *et al*., (2016) concluded that the exposure of *A. platensis* to gamma radiation induces the cellular reactive oxygen species (ROS) by purinergic signaling,

stimulating cells to produce more antioxidants under the induced oxidative stress.

3.2. Anti-inflammatory activities

The light-harvesting pigment "phycocyanin" is one of phycobiliproteins along with phycoerythrin and allophycocyanin, and found exclusively in cyanobacteria, cryptophytes and red algae (Hu *et al*., 2018). The presence of phycocyanin gives the characteristic blue color of cyanophytes, and hence the name of blue green algae. Eriksen (2016) concluded that *Spirulina* is the sole efficient source of natural phycocyanin production under photoautotrophic conditions. Despite the challenges facing *Spirulina* cultivation and phycocyanin extraction, there are no other particular sources of natural phycocyanin (Hu *et al*., 2018). In addition to the autotrophic growth of *Spirulina*, it is also capable of heterotrophic growth in the presence of glucose, with a phycocyanin content of 57 $mg \cdot g^{-1}$ dry weight, which represents half of that obtained in mixotrophic cultivation (Marquez *et al*., 1993). Phycocyanin can also be produced from other microalgae, e.g. from the unicellular red alga *Galdieria sulphuraria* under heterotrophic conditions. However, the phycocyanin content in *Spirulina* grown phototrophically is much higher than that of *G. sulphuraria* grown heterotrophically (Table 4), which gives the advantage of a lower cost production of phycocyanin using *Spirulina*.

In a previous study, Ebaid *et al*., (2017) estimated the phycocyanin content of 46.57 $mg \cdot g^{-1}$ dry weight in *A. platensis* grown in a Zarrouk medium for 18 days at 25 ± 3 °C and light intensity of 2500 Lux with a photoperiod of 18:6 h light: dark cycle. It is worth noting that the relative content of phycocyanin showed significant changes under different conditions of prevalent light, which was confirmed as a scavenging mechanism to prevent or reduce photoinhibitory damage (Mohanty *et al*., 1997). Romay *et al*., (1998) studied four *in vivo* experimental inflammation models (n = 7) and confirmed the activity of pure phycocyanin as an anti-inflammatory agent in all the studied models. Using 50, 100 and 200 $mg \cdot kg^{-1}$ of phycocyanin significantly reduced ear oedema induced by tetradecanoylphorbol acetate and arachidonic acid in mice by 47.6, 60.3 and 66.6%, respectively. In addition, the application of 50-200 $mg \cdot kg^{-1}$ of phycocyanin in experimental rats inhibited leukotriene B4 (LTB4), prostaglandin E2 (PGE2) levels and oedema induced by arachidonic acid (Romay *et al*., 2000). Vázquez-Velasco *et al*., (2014) concluded that *Spirulina* protects against negative proinflammatory effects induced by glucomannan in Zucker Fa/Fa rats due to the high phycocyanin content.

3.3. Anti-cancer activities

Many cancer forms result from cellular DNA damage, leading to uncontrolled cellular growth. Cellular endonucleases frequently repair the damaged DNA in order to keep the cell alive and healthy. The deactivation of these enzymes by oxidation, radiation or toxins results in errors in DNA, and eventually leads to the development of cancer. Many studies confirmed the anti-cancer activities of *Spirulina* extracts, either alone or in combination with other compounds. *In vitro* studies suggested the unique composition of *Spirulina* polysaccharides which enhance enzymatic activity and DNA repair. Decades ago, Schwartz and Shklar (1987) studied the effect of microalgae extracts on the induced squamous cell carcinoma of hamster buccal pouches in 20 animals. The results showed that *Spirulina* extract (Phycotene) resulted in total tumor regression by 30% of treated animals; while β-carotene and canthaxanthin resulted in total tumor regression by 20% and 15% of treated animals, respectively. This confirmed the higher anti-cancer activity of *Spirulina* extract than β-carotene or canthaxanthin. One year later, Schwartz *et al*., (1988) reported that the anti-cancer activity of the *Spirulina* extract was attributed to stimulation of the immune response to selectively destroy the small foci of developing malignant cells with no negative impacts on the normal cells. Liu *et al*., (2000) found that 160 $mg \cdot L^{-1}$ of c-phycocyanin from *S. platensis* significantly inhibited the *in vitro* growth of human leukemia K562 cells as colony numbers reduced from 4.8 in the control to 2.3 in the phycocyanin-treated cells after 12 days of incubation. In addition, Subhashini *et al*., (2004) reported that treatment with 50 μM of highly purified c-phycocyanin from *S. platensis* up to 48 h results in the inhibition of K562 cell proliferation by 49%. In another *in vivo* study, Simsek *et al*., (2008) suggested that *S. platensis* supplementation is useful in the treatment of anemia and leukemia induced by lead and cadmium toxicity. Moreover, Choi *et al*., (2013) investigated the anti-cancer activity of the marine cyanophyte *S. maxima* grown in deep seawater and reported effective suppression in the over-expression of the Bcl2 gene in A549 cells leading to the inhibition of various human cancer cells.

3.4. Anti-microbial activities

A large number of algal extracellular products and/or extracts have been reported as antimicrobial (anti-viral, anti-bacterial, anti-fungal, anti-algal, anti-protozoal) agents, although the detailed structure and identity of the active constituents of many of these are not yet known (Borowitzka 1995). Among microalgae, *Spirulina* is gaining more and more consideration as a natural antimicrobial agent. Kokou *et al*., (2012) confirmed the anti-bacterial activity of *Spirulina* against 6 Vibrio strains; *V. anguillarum, V. splendidus*, *V. parahaemolyticus, V. scophthalmi*, *V. lentus* and *V. alginolyticus*. The anti-microbial activity of acrylic acid, which is found in *Spirulina* in

TABLE 4. Comparison of heterotrophic cultivation of *Galdieria sulphuraria* and phototrophic cultivation of *Spirulina* for phycocyanin production (Hu *et al*., 2018).

Microalgae	Carbon source(g·L^{-1})	Growth mode	Phycocyanin content	Reference
G. sulphuraria 074G	Glucose (50)	Heterotrophic	3.6 mg/g	Schmidt *et al*. (2005)
G. sulphuraria 074G	Fructose (50)	Heterotrophic	3.4 mg/g	Schmidt *et al*. (2005)
G. sulphuraria 074G	Sucrose (50)	Heterotrophic	4.3 mg/g	Schmidt *et al*. (2005)
G. sulphuraria 074G	Glucose (500)	Heterotrophic	1.4–2.9 g/L	Graverholt and Eriksen (2007)
G. sulphuraria 074G	Glucose, fructose, glycerol (5)	Heterotrophic	2–4 mg/g	Sloth *et al*. (2006)
G. sulphuraria 074G	Glucose (5)	Heterotrophic	25–30 mg/g	Sørensen *et al*. (2013)
S. platensis UTEX 1926	Glucose (2.5)	Phototrophic	322 mg/L	Chen *et al*. (1996)
S. platensis UTEX 1926	Acetate (2)	Phototrophic	246 mg/L	Chen *et al*. (1996)

relatively high quantities, was confirmed at the end of 1970s. In addition to acrylic acid, probionic, benzoic, and mandelic organic acids were also reported as antimicrobial active compounds. Özdemir *et al*., (2004) also tested different extracts of *S. platensis* against bacteria and yeasts. They found that methanol extract was the most potent anti-microbial fraction. Santoyo *et al*., (2006) confirmed the antimicrobial activity of *S. platensis* using ethanol, hexane and petroleum ether as extracting solvents against *Escherichia coli, Staphylococcus aureus, Aspergillus niger* and *Candida albicans*. Moreover, purified c-phycocyanin extracted from *S. platensis* showed substantial inhibition in the growth of many multidrug-resistant bacteria. For example, purified c-phycocyanin showed minimum inhibitory concentrations (MIC) *in vitro* of 125, 100, 75 and 50 mg·L^{-1} against *S. aureus, E. coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* (Sarada *et al*., 2011).

In addition, Arun *et al*., (2012) confirmed the antifungal activity of *S. platensis* methanolic extract. According to Hayashi *et al*., (1993), *S. platensis* water-soluble extract also significantly inhibited the *in vitro* HSV−1 replication in HeLa cells with ED_{50} (the lowest phycocyanin concentration for reducing plaque number by 50%) at 0.173 mg·mL^{-1}. The addition of 1 mg·mL^{-1} of *S. platensis* extract 3 h before viral infection resulted in 50% inhibition of virus protein synthesis without affecting the host. Subsequently, Hayashi *et al*., (1996) isolated calcium spirulan (Ca-SP) from *S. platensis* as a novel sulfate polysaccharide. The isolated Ca-SP inhibited the *in vitro* replication of several enveloped viruses such as the human cytomegalo virus, HSV−1, influenza A virus, measles virus, HIV−1 virus and mumps virus.

3.5. Anti-diabetes and anti-obesity activities

The disease diabetes mellitus results from abnormalities in carbohydrate metabolism and is characterized by deficiencies in insulin secretion, resulting in hyperglycemia. The later was widely reported as the main reason for diabetic complications. Recently, the activity of *Spirulina* as anti-obesity and anti-diabetes was confirmed. The administration of 2 g·kg^{-1}·day^{-1} of *Spirulina* for 21 days to alloxanized-induced diabetic rats (n = 10) resulted in a significant reduction in plasma glucose levels by 13.7% below the diabetic group (El-Desouki *et al*., 2015). They attributed the antihyperglycemic action of *Spirulina* to the potentiation of the pancreatic secretion of insulin from islet β-cells, or to enhancement of blood glucose transport to the peripheral tissue. In addition, *Spirulina* supplementation promotes vasodilation and restricts vasoconstriction which decreases the levels of plasma lipids and reduces blood pressure (Schwartz *et al*., 1988). Han *et al*., (2006) reported that glycolipid H-b2 extracted from *Spirulina* was found to reduce postprandial triglyceride (TGs) levels and inhibit pancreatic lipase activity in a dose-dependent way; while phycocyanin inhibited the pancreatic lipase activity, which resulted in higher excretion of TGs in experimental animals' feces and reduced serum TGs.

3.6. Anti-toxicity activities

Shastri *et al*., (1999) investigated the protective effect of *S. fusiformis* against lead toxicity in albino mice and recorded a pronounced increase in survival rate by administration of *Spirulina*. Recently, Ebaid *et al*., (2017) studied the protective effect of *A. platensis* against liver toxicity produced by treatment with copper nanoparticles. They confirmed the beneficial role of *A. platensis* as antioxidant markers which enhanced the hepatic functional parameters. Numerous mechanisms of cellular toxicity induction under stress conditions were suggested. Lipid peroxidation is one of the main mechanisms to induce cell damage due to the reaction of free radicals with lipids (Rajesh and Kala 2015). It results in malondialdehyde (MDA), which leads to oxidative stress by further interaction with many other molecules. Nigma *et al*., (1999) attributed ROS production under copper stress to antioxidant depletion or the direct effect of copper on the peroxidation reaction. However, Kim *et al*., (2011)

reported that ROS alter various signal-transduction pathways in addition to the oxidation of vital cellular macromolecules such as DNA and lipids. ROS also increases the cytosolic Ca^{+2} levels by activation of non-selective cation channels in cell membranes (Mukherjee *et al*., 2002), promoting cell damage. The protective effect of *Spirulina* against oxidative stress was attributed to the relatively high contents of phycocyanin and carotenoids which result in antioxidant activity (Ebaid *et al*., 2017). In addition, *S. platensis* was found to positively influence the antioxidant enzyme level and oxidative stress markers under deltamethrin toxicity (Abdel-Daim *et al*., 2016), urethane and cisplatin (Premkumar *et al*., 2004) and mitomycin C and cyclophosphamide (Premkumar *et al*., 2001). In addition to its antioxidant properties, *Spirulina* showed a strong heavy metal chelating activity which reduced its toxicity as well (Rangsayator *et al*., 2002).

4. ENVIRONMENTAL IMPACTS

The scarcity of clean environments for the safe livelihood of human beings has gained global concerns. Continuous growth of the human population has resulted in an increase in petroleum consumption and growing energy demands all over the world. Due to human population growth, the world energy consumption is predicted to grow by 8.1% between 2015 and 2040, from 97.8 quadrillion British thermal units (Btu) to 105.7 quadrillion Btu (Fig. 3A). The extensive use of fossil fuels contributes to high atmospheric CO_2 levels, which are predicted to increase by 2.2% between 2015 and 2040 (Figure 3B). Increases in atmospheric CO_2 raise the global air temperature, which is known as "global warming". If this development continues, the planet will be threatened with the catastrophic changes in the earth's climate. Global warming does not only mean the earth's getting warmer, but it is associated with massive negative impacts on economic, social, and physical health which are of great distress. The melting of polar ice, violent surges of hurricanes and collapse of wildlife are all examples of how climate change can affect the planet. Microalgae are widely discussed to diminish the greenhouse effect by CO_2 sequestration and abundant oxygen production. A stoichiometric amount of 1.8 kg $CO_2 \cdot kg^{-1}$ of microalgal biomass can be consumed which makes *Spirulina* cultivation using flue gas more economically and environmentally useful (Doucha *et al*., 2005). Based on the biomass produced by the area of Earthrise Farms (550 tons in 0.437 km^2), about 990 tons of CO_2 can be mitigated annually by Earthrise Farms. Thus, 28.9% of USA land area would be theoretically used to mitigate the global CO_2 produced in 2015, which could be increased to 29.5% by the expected increase in CO_2 emission in 2040 (Figure 3B). In addition, environmental pollution might result from the corruption of human resources by various micropollutants including pharmaceutical contaminants (Xiong *et al*., 2018). The distribution of these synthetic chemicals through the air, water and soil develop multidrug-resistant microorganisms. Therefore, the use of naturally synthesized products with the limitation of using chemical compounds will enhance the optimal utilization of pharmaceuticals.

Water and land use are two important environmental factors that need to be considered. Interestingly, *Spirulina* cultivation can be done in arid lands where no other crops can survive (Soni *et al*., 2016). In addition, it needs relatively lower amount of water, as water can be recycled after harvest. After the extraction of essential compounds from the biomass, the residual biomass can be used in many other applications in what is called refining process (Figure 4). For example, sugar-based and cellulosic biorefining results in the accumulation of protein by-products, which can be used as animal feed (Soni *et al*., 2016). In addition, after the extraction of essential compounds, the residual biomass can be used as energy feedstock for biodiesel production from lipids and/or bioethanol production from carbohydrate residues. Thus, integrated food and energy systems are designed to combine, intensify, and increase the productivity of food and energy simultaneously through sustainable land management (Soni *et al*., 2016). Using *Spirulina* as bioenergy feedstock showed promising results in many studies. Sumprasit *et al*., (2017) studied energy recovery in the form of biodiesel and biogas from ubiquitous *S. platensis* and recorded a biodiesel yield of 7.1% of dry weight. The results also confirmed that lipid-free residue can be used as feedstock for biogas production with an average methane yield of about 290 mL·g^{-1} VS. In addition, Shao *et al*., (2018) reported the efficiency of Cd^{2+}-sorbed *Arthrospira platensis* for bioethanol production due to the accumulation of carbohydrates. Moreover, Chagas *et al*., (2016) studied the catalytic pyrolysis of *Arthrospira platensis* and *Arthrospira maxima* using pyrolysis/GC–MS. They found that the high acidity of HZSM-5 catalysts could maximize the conversion of biomass to aromatic hydrocarbons and the aromatic yields increased as the catalyst/biomass ratio increased from 1:1 to 10:1.By food-energy integration, *Spirulina* products are even more economically competitive with high diversification. Consequently, large-scale cultivation of *Spirulina* will not only have healthy and economic benefits, but will also help to save our planet.

5. GENETIC ENGINEERING OF *SPIRULINA*

Genetic engineering facilitates the duplication and manipulation of specific fragments of DNA for industrial, medical and research purposes. Recently, genetic engineering led to a revolution in molecular biology with many benefits in different fields, especially in medicine. The cloning of recombinant

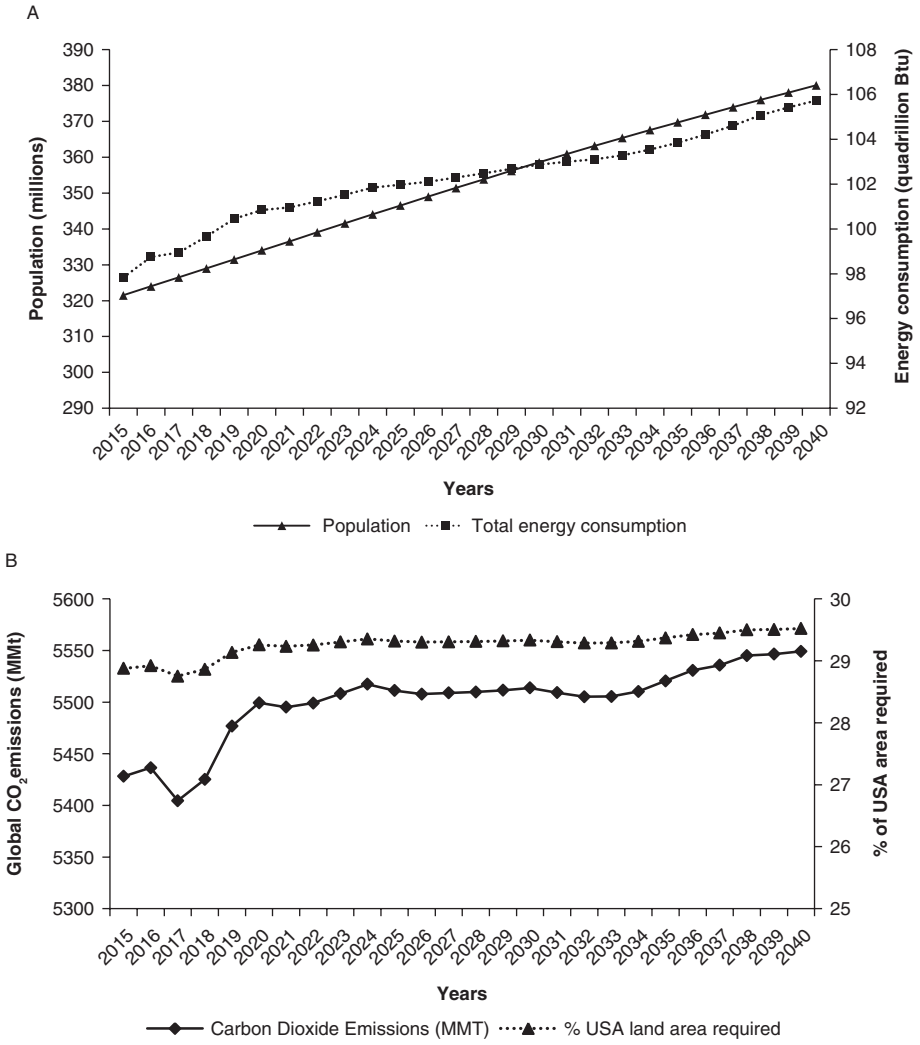

FIGURE 3. Current and predicted global population and total energy consumption (A) and global CO_2 emission as a result of fuel combustion with the estimated USA area required for global CO_2 mitigation (B). Data in part was calculated from "Global Energy Statistical Yearbook 2015".

DNA facilitates the use of bacteria to produce important proteins such as human insulin which is of great importance and now marketed throughout the world. It was revealed that biosynthetic pathways for the production of different cellular macromolecules are complex processes which include many genes (Bhunia *et al*., 2018). Many industrial compounds can be produced by several molecular techniques such as rational and non-rational mutagenesis, including site-specific mutagenesis, random mutagenesis, and site-saturation mutagenesis. In the last few decades, extensive research has been carried out to understand the biosynthesis genetic regulations of different valuable products in cyanophytes. For example, enhanced alcohol production via genetic engineering has been implemented in two independent cyanobacterial strains through the over-expression of pyruvate decarboxylase and alcohol dehydrogenase II genes (Deng and Coleman, 1999). In addition, previous studies on the regulation of desaturase gene expression in *Spirulina platensis* revealed that the mRNA level and mRNA stability of the D6D gene increased drastically by decreasing the growth temperature from 35 to 22 °C, which led to an increase in the cellular γ-linolenic acid level by approximately 30% (Deshnium *et al*., 2000; Honsthong *et al*., 2003). Further, site-specific mutation studies by Subudhi *et al*., (2008) demonstrated that the sequence TATAAT located at −33 bp was essential for the D6D promoter function and suggested a complex regulation of D6D gene expression in *Spirulina*.

Currently, most cloned genes are introduced into bacteria or fungi on heterotrophic growth media for industrial enzyme production. However, developing a protocol for using *Spirulina* instead involves an economic and procedural reason for considering it as an alternate producer of industrial enzymes. *Spirulina*

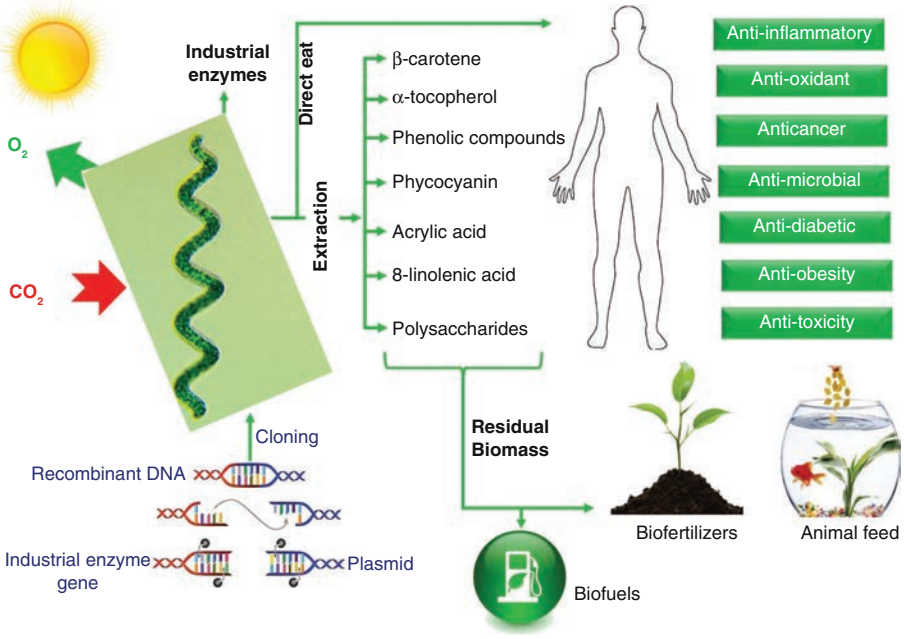

FIGURE 4. Illustration showing the refining concept and different pathways of *Spirulina* applications.

can grow autotrophically by converting light energy into biomass rich in minerals, protein, vitamin B_{12}, essential fatty acids, amino acids and β-carotene. In addition, *Spirulina* does not require physical or chemical pre-treatment in order to become digestible due to the absence of a cellulosic cell wall (Toyomizu *et al*., 2001a). Moreover, residual biomass after the extraction of valuable molecules can be used as feed for fish, farm animals and poultry (Toyomizu *et al*., 2001b; Abomohra *et al*., 2014). Therefore, the development of a method for stable gene cloning into *Spirulina* as a biological factory for recombinant enzyme production will have many advantages. Unfortunately, the genetic engineering methods for *Spirulina* have been almost non-existent due to the absence of plasmids in *Spirulina* (Vachhani and Vonshak, 1997; Toyomizu *et al*., 2001a). Some *Spirulina platensis* genes involved in fundamental processes such as protein synthesis, carbon dioxide fixation and nitrogen metabolism were successfully cloned (e.g. Bini *et al*., 1992; Nelissen *et al*., 1994; Murata *et al*., 1996). However, the development of gene transfer systems is a big challenge. Using the plasmid pKK232–8 carrying a promoterless chloramphenicol acetyltransferase (CAT) gene, Cheevadhanarak *et al*., (1993) transformed *S. platensis* to stably express the CAT gene which maintained under selection pressure for several generations. However, the stability of the transformants was a major problem, as the transformation system developed for *Spirulina* must be efficient and allow not only a good degree of expression of the introduced gene but also to maintain itself in a fast-growing culture. Toyomizu *et al*., (2001a) studied the optimization of electroporation of plasmid pHSG399 in *S. platensis*. The plasmid contained a chloramphenicol resistance gene which produces an *E. coli* protein, Cm acetyltransferase (CAT). They reported that *Spirulina* is a suitable organism for the expression of genetically engineered proteins, but the gene transfer system has not yet been established. Later, Kawata *et al*., (2004) employed a new approach using natural transposase, mini-Tn5 transposon, and a cation liposome to transform *Spirulina platensis* as a stable recombinant DNA methodology. According to Toyomizu *et al.,* (2001a), more research is needed to investigate other requirements for a 'stable' *S. platensis* gene transfer system, which include; (1) cloning and assessment of *Spirulina* promoters using a plasmid carrying a promoterless CAT reporter gene, (2) developing a simple and rapid method for sensitive fluorometric determination of *Spirulina* transformants, and (3) subjecting *Spirulina* transformants to protoplast fusion in glycine, for cloning. Thus, genetic engineering techniques currently used in plant and microbial biotechnology are required to enable *Spirulina*, as an autotrophic factory, to produce desired molecules in order to incorporate algal biotechnology products into industrial technologies.

6. CONCLUSIONS

Spirulina is considered as a valuable pharmaceutical agent which offers many unique features with many environmental and health benefits. Despite their extensive applications for nutrition, the industrial

production of *Spirulina* is still more or less limited to relatively small areas. In addition, the molecular engineering of *Spirulina* seems as a neglected research field in spite of its importance to resolving many challenges, and any piece of work accumulated at this stage will be undoubtedly of great value. There is much more research which needs to be done, including innovative cultivation systems and development of successful genetic and molecular biology tools for *Spirulina*. The development of a reliable genetic engineering methodology will open the way, not only for better basic research, but also will enable a better program for the development of new and better strains to be used by the industry. This could integrate *Spirulina* into the industrial sector for the production of essential industrial products while producing valuable nutrient-rich biomass. In addition, nutritional awareness and increasing the acceptance level of *Spirulina* are required in order to develop such *Spirulina*-fortified products. At the same time, further scientific, clinical and toxicological research is required to evaluate the safety of large-scale production of *Spirulina* with harvest at minimum contamination rates, since contamination with harmful elements or toxic substances might potentially cause several serious human health effects.

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REFERENCES

Abdel-Daim M, El-Bialy B, Abdel Rahman H, Radi A, Hefny H, Hassan A. 2016. Antagonistic effects of *Spirulina platensis* against subacute deltamethrin toxicity in mice: Biochemical and histopathological studies. *Biomed. Pharmacother.* **77,** 79–85. https://doi.org/10.1016/j.biopha.2015.12.003

Abomohra A, El-Sheekh M, Hanelt D. 2014. Pilot cultivation of the chlorophyte microalga *Scenedesmus obliquus* as a promising feedstock for biofuel. *Biomass Bioenergy* **64**, 237–244. https://doi.org/10.1016/j.biombioe.2014.03.049

Abomohra A, El-Shouny W, Sharaf M, Abo-Eleneen M. 2016. Effect of Gamma Radiation on Growth and Metabolic Activities of *Arthrospira platensis*. *Braz. Arch. Biol. Technol.* **59**, e16150476. https://doi.org/10.1590/1678-4324-2016150476

Abomohra A, Eladel H, El-Esawi M, Wang S, Wang Q, He Z, Feng Y, Shang H, Hanelt D. 2018. Effect of lipid-free microalgal biomass and waste glycerol on growth and lipid production of *Scenedesmus obliquus*: Innovative waste recycling for extraordinary lipid production. *Bioresour. Technol.* **249**, 992–999. https://doi.org/10.1016/j.biortech.2017.10.102

Arun N, Gupta S, Singh DP. 2012. Antimicrobial and antioxidant property of commonly found microalgae *Spirulina platensis*, *Nostoc muscorum* and *Chlorella pyrenoidosa* against some pathogenic bacteria and fungi. Int. *J. Pharm. Sci. Res.* **3**, 4866–4875. https://doi.org/10.13040/IJPSR.0975-8232.3(12).4866-75

Baicus C, Baicus A. 2007. *Spirulina* did not ameliorate idiopathic chronic fatigue in four N-of-1 randomized controlled trials. *Phytother. Res.* **21**, 570–573. https://doi.org/10.1002/ptr.2114

Bhunia B, Uday U, Oinam G, Mondal A, Bandyopadhyay T, Tiwari O. 2018. Characterization, genetic regulation and production of cyanobacterial exopolysaccharides and its applicability for heavy metal removal. *Carbohydr. Polym.* **179**, 228–243. https://doi.org/10.1016/j.carbpol.2017.09.091

Bini F, De Rossi E, Barbierato L, Riccardi G. 1992. Molecular cloning and sequencing of the ß-isopropylmalate dehydrogenase gene from the cyanobacterium *Spirulina platensis*. *J. Gen. Microbiol.* **138**, 493–498. https://doi.org/10.1099/00221287-138-3-493

Bleakley S, Hayes M. 2017. Algal proteins: Extraction, application, and challenges concerning production. *Foods* **6**, 33. https://doi.org/10.3390/foods6050033

Borowitzka M. 1995. Microalgae as sources of pharmaceuticals and other biologically active compounds. *J. Appl. Phycol.* **7**, 3–15. https://doi.org/10.1007/BF00003544

Capelli B, Cysewski GR. 2010. Potential health benefits of *Spirulina* microalgae. *Nutrafoods* **9**, 19–26. https://doi.org/10.1007/BF03223332

Castenholz RW. 1989. Subsection III, Order Oscillatoriales. In Stanley JT, Bryant MP, Pfenning N, Holt JG (Eds), *Bergey's Manual of Systematic Bacteriology*, Vol. 3, Baltimore: William and Wilkins, p. 1771.

Centeno P, Ballentine DL. 1999. Effects of culture conditions on production of antibiotically active metabolites by the marine alga *Spyridia filamentosa* (Ceramiaceae, Rhodophyta), I. Light. *J. Appl. Phycol.* **11**, 217–224. https://doi.org/10.1023/A:1008038418777

Chagas B, DoradoC, Serapiglia M, Mullen C, Boateng A, Melo M, Ataíde C. 2016. Catalytic pyrolysis-GC/MS of *Spirulina*: evaluation of a highly proteinaceous biomass source for production of fuels and chemicals. *Fuel* **179**, 124–134. https://doi.org/10.1016/j.fuel.2016.03.076

Cheevadhanarak S, Kanokslip S, Chaisawadi S, Rachdawong S, Tanticharoen M. 1993. Transformation system for *Spirulina platensis*. In Masojidek J, Setlik I. (Eds) *Book of Abstracts of the 6***th** *International Conference on Applied Algology*, Czech Republic, p. 109.

Chen F, Zhang Y, Guo S. 1996. Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. *Biotechnol. Lett.* **18**, 603–608. https://doi.org/10.1007/BF00140211

Choi WY, Kang DH, Lee HY. 2013. Enhancement of immune activation activities of *Spirulina maxima* grown in deep sea water. *Int. J. Mol. Sci.* **14**, 12205–12221. https://doi.org/10.3390/ijms140612205

Ciferri O. 1984. *Spirulina*, the edible microorganism. *Microbiol. Rev.* **47**, 551–578.

Deng MD, Coleman JR. 1999. Ethanol synthesis by genetic engineering in cyanobacteria. *Appl. Environ. Microbiol.* **65**, 523–528.

Deshnium P, Paithoonrangsarid K, Suphatrakul A, Meesapyodsuk D, Tanticharoen M, Cheevadhanarak S. 2000. Temperature-independent and -dependent expression of desaturase genes in filamentous cyanobacterium *Spirulina platensis* strain C1 (*Arthrospira* sp. PCC 9438). *FEMS. Microbiol. Lett.* **184**, 207–213. https://doi.org/10.1111/j.1574-6968.2000.tb09015.x

Doucha J, Straka F, Livansky K. 2005. Utilization of flue gas for cultivation of microalgae (*Chlorella* sp.) in an outdoor open thin layer photobioreactor. *J. Appl. Phycol.* **17**, 403–412. https://doi.org/10.1007/s10811-005-8701-7

Ebaid R, Elhussainy E, El-Shourbagy S, Ali S, Abomohra A. 2017. Protective effect of *Arthrospira platensis* against liver injury induced by copper nanoparticles. *Orient. Pharm. Exp. Med.* **17**, 203–210. https://doi.org/10.1007/s13596-017-0264-z

El-Desouki N, Tabl G, Abdel-Aziz K, Salim E, Nazeeh N. 2015. Improvement in beta-islets of Langerhans in alloxan-induced diabetic rats by erythropoietin and *Spirulina*. *J. Basic App. Zool.* **71**, 20–31. https://doi.org/10.1016/j.jobaz.2015.04.003

Eriksen NT. 2016. Research trends in the dominating microalgal pigments, β-carotene, astaxanthin, and phycocyanin used in feed, in foods, and in health applications. *J. Nutr. Food Sci.* **6**, 507. https://doi.org/10.4172/2155-9600.1000507

Global Energy Statistical Yearbook, Enerdata 2015. Available at https://yearbook.enerdata.net, Accessed on December 2017.

Gomont M. 1892–1893. Monographie des Oscillariées. *Ann. Sci. Nat. Bot.* **15**, 263–368, **16**, 91–264.

Graverholt OS, Eriksen NT. 2007. Heterotrophic high-cell-density fedbatch and continuous-flZ cultures of *Galdieria sulphuraria* and production of phycocyanin. *Appl. Microbiol. Biotechnol.* **77**, 69–75. https://doi.org/10.1007/s00253-007-1150-2

Han LK, Li DX, Xiang L, Kondo Y, Suzuki I, Okuda H.2006. Isolation of pancreatic lipase activity-inhibitory component of *Spirulina platensis* and it reduce postprandial triacylglycerolemia. *Yakugaku. Zasshi.* **126**, 43–49. https://doi.org/10.1248/yakushi.126.43

Hayashi K, Hayashi T, Morita N. 1993. An extract from *Spirulina platensis* is a selective inhibitor of herpes simplex virus type 1 penetration into HeLa cells. *Phytother. Res.* **7**, 76–80. https://doi.org/10.1002/ptr.2650070118

Hayashi T, Hayashi K, Maedaa M, Kojima I. 1996. Calcium spirulan, an inhibitor of enveloped virus replication, from a blue-green alga *Spirulina platensis*. *J. Nat. Prod.* **59**, 83–87. https://doi.org/10.1021/np960017o

Henrikson R. 1994. Microalga *Spirulina*, superalimento del futuro. Ronore Enterprises. 2nd ed. Ediciones Urano, Barcelona, Spain, pp. 222.

Honsthong A, Deshnium P, Paithoonrangsarid K, Cheevadhanarak S, Tanticharoen M. 2003. Differential responses of three acyl-lipid desaturases to immediate temperature reduction occurring in two lipid membranes of *Spirulina platensis* strain C1. *J. Biosci. Bioeng.* **96**, 519–524. https://doi.org/10.1016/S1389-1723(04)70143-7

Hu J, Nagarajan D, Zhang Q, Chang J, Lee D. 2018. Heterotrophic cultivation of microalgae for pigment production: A review. *Biotechnol. Adv.* **36**, 54–67. https://doi.org/10.1016/j.biotechadv.2017.09.009

Jimenez C, Cossio BR, Niell FX.2003. Relationship between physicochemical variables and productivity in open ponds for the production of *Spirulina*: A predictive model of algal yield. *Aquaculture* **221**, 331–345. https://doi.org/10.1016/S0044-8486(03)00123-6

Kawata Y, Yano S, Kojima H, Toyomizu M. 2004. Transformation of *Spirulina platensis* strain C1 (*Arthrospira* sp. PCC9438) with Tn5 transposase–transposon DNA–cation liposome complex. *Mar. Biotechnol.* **6**, 355–363. https://doi.org/10.1007/s10126-003-0037-1

Khan Z, Bhadouria P, Bisen P. 2005. Nutritional and therapeutic potential of *Spirulina*. *Curr. Pharm. Biotechnol.* **6**, 373–379. https://doi.org/10.2174/138920105774370607

Kim D, Kim J, Goh E, Kim W, Kim S, Seo Y, Jang C, Kang S. 2011. Antioxidant response of *Arabidopsis* plants to gamma irradiation: Genome-wide expression profiling of the ROS scavenging and signal transduction pathways. *J. Plant. Physiol.* **168**, 1960–1971. https://doi.org/10.1016/j.jplph.2011.05.008

Kokou F, Makridis P, Kentouri M, Divanach P. 2012. Anti-bacterial activity in microalgae cultures. *Aquacult. Res.* **43**, 1520–1527. https://doi.org/10.1111/j.1365-2109.2011.02955.x

Liu Y, Xu L, Cheng N, Lin L, Zhang C. 2000. Inhibitory effect of phycocyanin from *Spirulina platensis* on the growth of human leukemia K562 cells. *J. Appl. Phycol.* **12**, 125–130. https://doi.org/10.1023/A:1008132210772

Manoj G, Venkataraman LV, Srinivas L. 1992. Antioxidant properties of *Spirulina* (*Spirulina platensis*). In: Seshadri CV, Jeejibai N, editors. *ETTA National Symposium on Spirulina*. MCRC Publishers, New York, USA, pp. 148–154.

Márquez FJ, Sasaki K, Kakizono T, Nishio N, Nagai S. 1993. Growth characteristics of *Spirulina platensis* in mixotrophic and heterotrophic conditions. *J. Ferment. Bioeng.* **5**, 408–410. https://doi.org/10.1016/0922-338X(93)90034-6

Miranda MS, Cintra RG, Barros SM, Mancini-Filho J. 1998. Antioxidant activity of the microalga *Spirulina maxima*. *Braz. J. Med. Biol. Res.* **31**, 1075–1079. https://doi.org/10.1590/S0100-879X1998000800007

Mohanty P, Srivastava M, Krishna K. 1997. The Photosynthetic Apparatus of *Spirulina*: Electron Transport and Energy Transfer. In: Vonshak A, *Spirulina platensis* (*Arthrospira*) Physiology, cell-biology and biotechnology. 1997, Taylor & Francis Ltd., London.

Moorhead K, Capelli B, Cysewski G. 2012. *Spirulina: Nature's Superfood*. 3rd edition, Cyanotech Corporation, Kailua Kona, Hawaii, USA.

Mukherjee SB, Das M, Sudhandiran G, Shaha C. 2002. Increase in cytosolic Ca^{2+} levels through the activation of non-selective cation channels induced by oxidative stress causes mitochondrial depolarization leading to apoptosis-like death in *Leishmania donovani* promastigotes. *J. Biol. Chem.* **277**, 24717–24727. https://doi.org/10.1074/jbc.M201961200

Murata N, Deshnium P, Tasaka Y. 1996. Biosynthesis of gamma-linolenic acid in the cyanobacterium *Spirulina platensis*. In Huang Y, Milles DE (Eds) *Gamma-linolenic acid, metabolism and its role in nutrition and medicine*, AOC Press, Champaign, Illinois, p. 22.

Nelissen B, Wilmotte A, Neefs JM, De Wachter R. 1994. Phylogenetic relationships among filamentous helical cyanobacteria investigated on the basis of 16S ribosomal RNA gene sequence analysis. *Syst. App. Microbiol.* **17**, 206–210. https://doi.org/10.1016/S0723-2020(11)80009-3

Nigma D, Shukla G, Agarwal A. 1999. Glutathione depletion and oxidative damage in mitochondria following exposure to cadmium in rat liver and kidney. *Toxicol. Lett.* **106**, 151–157. https://doi.org/10.1016/S0378-4274(99)00059-4

Özdemir G, Karabay NU, Dalay MC, Pazarbasi B. 2004. Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. *Phytother. Res.* **18**, 754–757. https://doi.org/10.1002/ptr.1541

Premkumar K, Abraham SK, Santhiya ST, Ramesh A. 2004. Protective effect of *Spirulina fusiformis* on chemical-induced genotoxicity. *Fitoterapia* **75**, 24–31. https://doi.org/10.1016/j.fitote.2003.07.008

Premkumar K, Pachiappan A, Abraham SK, Santhiya ST, Gopinath PM, Ramesh A. 2001. Effect of *Spirulina fusiformis* on cyclophosphamide and mitomycin-C induced genotoxicity and oxidative stress in mice. *Fitoterapia* **72**, 906–911. https://doi.org/10.1016/S0367-326X(01)00340-9

Rajesh V, Kala M. 2015. Antiproliferative and chemopreventive effect of *Annona muricata* Linn. on Ehrlich ascites carcinoma and Benzo[a]pyrene induced lung carcinoma. *Orient. Pharm. Exp. Med.* **15**, 239–256. https://doi.org/10.1007/s13596-015-0199-1

Rangsayator N, Upatham ES, KruatrachureM, Pokethitiyook P, Lanza G. 2002. Phytoremediation potential of *Spirulina* (*Arthrospira*) *platensis*: Biosorption and toxicity studies of cadmium. *Environ. Pollut.* **119**, 45–53. https://doi.org/10.1016/S0269-7491(01)00324-4

Rempel A, Machado T, Treichel H, Colla E, Margarites A, Colla L. 2018. Saccharification of *Spirulina platensis* biomass using free and immobilized amylolytic enzymes. *Bioresour. Technol.* **263**, 163–171. https://doi.org/10.1016/j.biortech.2018.04.114

Romay C, Ledon N, Gonzalez R. 1998. Further studies on anti-inflammatory activity of phycocyanin in some animal models of inflammation. *Inflamm. Res.* **47**, 334–338. https://doi.org/10.1007/s000110050338

Romay C, Ledon N, Gonzalez R. 2000. Effects of phycocyanin extract on prostaglandin E2 levels in mouse ear inflammation test. *Arzneim -Forsch.* **50**, 1106–1109.

Santoyo S, Herrero S, Señorans M, Cifuentes F, Ibáñez A. 2006. Functional characterization of pressurized liquid extracts of *Spirulina platensis*. *Eur. Food Res. Technol.* **224**, 75–81. https://doi.org/10.1007/s00217-006-0291-3

Sarada DL, Kumar CS, Rengasamy R. 2011. Purified C-phycocyanin from *Spirulina platensis* (Nordstedt) Geitler: A novel and potent agent against drug resistant bacteria. *World J. Microb. Biot.* **27**, 779–783. https://doi.org/10.1007/s11274-010-0516-2

Schmidt RA, Wiebe MG, Eriksen NT. 2005. Heterotrophic high cell density fed-batch cultures of the phycocyanin producing red alga *Galdieria sulphuraria*. *Biotechnol. Bioeng.* **90**, 77–84. https://doi.org/10.1002/bit.20417

Schwartz J, Shklar G. 1987. Regression of experimental hamster cancer by beta carotene and algae extracts. *J. Oral. Maxillofacial Surg.* **5**, 510–515. https://doi.org/10.1016/S0278-2391(87)80011-3

Schwartz J, Shklar G, Reid S, Trickler D. 1988. Prevention of experimental oral cancer by extracts of *Spirulina-Dunaliella* algae. *Nutrition Cancer* **11**, 127–134. https://doi.org/10.1080/01635588809513979

Shao W, Ebaid R, Abomohra A, Shahen M. 2018. Enhancement of *Spirulina* biomass production and cadmium biosorption using combined static magnetic field. *Bioresour. Technol.* **265**, 163–169. https://doi.org/10.1016/j.biortech.2018.06.009

Shastri D, Kumar MM, Kumar A. 1999. Modulation of lead toxicity by *Spirulina fusiformis*. *Phytother.* Res. **13**, 258–260. https://doi.org/10.1002/(SICI)1099-1573(199905)13:3<258::AID-PTR438>3.0.CO;2-2

Simsek N, Karadeniz A, Kalkanc Y, Keles ON, Unal B. 2008. *Spirulina platensis* feeding inhibited the anemia- and leucopenia-induced lead and cadmium in rats. *J. Hazard. Mater.* **164**, 1304–1309. https://doi.org/10.1016/j.jhazmat.2008.09.041

Sloth JK, Wiebe MG, Eriksen NT. 2006. Accumulation of Phycocyanin in heterotrophic and mixotrophic cultures of the acidophilic red alga *Galdieria sulphuraria*. *Enzym. Microb. Technol.* **38**, 168–175. https://doi.org/10.1016/j.enzmictec.2005.05.010

Soni R, Sudhakar K, Rana R. 2016. Sustainable biomass production from microalgae for food, feed and biofuels: An integrated approach. *Biosci. Biotech. Res. Comm.* **9,** 729–736.

Soni R, Sudhakar K, Rana R. 2017. *Spirulina* – From growth to nutritional product: A review. *Trends Food Sci. Technol.***69**, 157–171. https://doi.org/10.1016/j.tifs.2017.09.010

Sørensen L, Hantke A, Eriksen NT. 2013. Purification of the photosynthetic pigment C-phycocyanin from heterotrophic *Galdieria sulphuraria*. *J. Sci. Food Agric.* **93**, 2933–2938. https://doi.org/10.1002/jsfa.6116

Stizenberger E. 1854. *Spirulina* und *Arthrospira* (nov. gen.). *Hedwigia* **1**, 32–41.

Subhashini J, Mahipal SK, Reddy MC, Reddy MM, Rachamallu A, Reddanna P. 2004. Molecular mechanisms in C-Phycocyanin induced apoptosis in human chronic myeloid leukemia cell line- K562. *Biochem. Pharmacol.* **68**, 453–462. https://doi.org/10.1016/j.bcp.2004.02.025

Subudhi S, Kurdrid P, Hongsthong A, Sirijuntarut M, Cheevadhanarak S, Tanticharoen M. 2008. Isolation and functional characterization of *Spirulina* D6D gene promoter: Role of a putative GntR transcription factor in transcriptional regulation of D6D gene expression. *Biochem. Biophys. Res. Commun.* **365**, 643–649. https://doi.org/10.1016/j.bbrc.2007.11.018

Sumprasit N, Wagle N, Glanpracha N, Annachhatre A. 2017. Biodiesel and biogas recovery from *Spirulina platensis*. *Int. Biodeterior. Biodegrad.* **119**, 196–204. https://doi.org/10.1016/j.ibiod.2016.11.006

Tomaselli L. 1997. Morphology, Ultrastructure and Taxonomy of *Arthrospira* (*Spirulina*) *maxima* and *Arthrospira (Spirulina) platensis*. In: Vonshak A, *Spirulina platensis (Arthrospira) Physiology, cell-biology and biotechnology*. Taylor & Francis Ltd., London.

Toyomizu M, Suzuki K, Kawata Y, Kojima H, Akiba Y. 2001a. Effective transformation of the cyanobacterium *Spirulina platensis* using electroporation. *J. App. Phycol.***13**, 209–214. https://doi.org/10.1023/A:1011182613761

Toyomizu M, Sato K, Taroda H, Kato T, Akiba Y. 2001b. Effects of dietary *Spirulina* on meat colour in muscle of broiler chickens. *Bri. Poult. Sci.* **42**, 197–202. https://doi.org/10.1080/00071660120048447

Vachhani A, Vonshak A. 1997. Genetics of *Spirulina*. In: Vonshak A, *Spirulina platensis (Arthrospira) Physiology, cell-biology and biotechnology*. 1997, Taylor & Francis Ltd., London.

Vázquez-Velasco M, González-Torres L, López-Gasco P, Bastida S, Benedí J, Sánchez-Reus M, González-Muñoz M, Sánchez-Muniz F. 2014. Liver oxidation and inflammation in Fa/Fa rats fed glucomannan/*Spirulina*-surimi. *Food Chem.* **159**, 215–221. https://doi.org/10.1016/j.foodchem.2014.03.015

Whitton BA. 2000. Soils and rice-fields. In: Whitton BA, Potts M, editor. *The ecology of cyanobacteria*. Kluwer Academic, Dordrecht, Netherlands, pp. 233–255.

Xiong J, Kurade M, Jeon B. 2018. Can Microalgae Remove Pharmaceutical Contaminants from Water?. *Trends Biotechnol.* **36**, 30–44. https://doi.org/10.1016/j.tibtech.2017.09.003