Astaxanthin Production by Microalgae *Haematococcus pluvialis* Through Wastewater Treatment: Waste to Resource

Md Mahfuzur Rahman Shah

1 Introduction

Astaxanthin, known as "super antioxidant," can be obtained from synthetic and natural sources. Natural astaxanthin *can be found in fishes (*salmon), crustaceans (shrimp), *Phaffia* yeast and *Paracoccus* bacteria, zooplankton (krill), and some microalgae (e.g., *Haematococcus pluvialis*) (Higuera-Ciapara et al. 2006; Ranga Rao et al. 2014). *H. pluvialis is produced commercially as the richest source of natural* astaxanthin which has 20 times stronger antioxidant capacity than the synthetic astaxanthin (Lorenz 1999; Ranga Rao et al. 2010). Astaxanthin can be extensively applied in human nutrition, animal and aquaculture feed, and cosmetics industry.

Astaxanthin has high market value ($2500–7000/kg), and its market potentiality is estimated to increase from 280 metric tons, $447 million (in 2014), to 670 metric tons, $1.1 billion, by 2020 (Koller et al. 2014; Pérez-López et al. 2014; Industry Experts 2015). Presently, only <1% of the commercialized quantity is produced from *H. pluvialis* (Koller et al. 2014), and the interest of producing astaxanthin from *H. pluvialis* is increasing. Different approaches of production system have been reported such as photoautotrophic, heterotrophic, mixotrophic, indoor, outdoor, open raceway, photobioreactors, batch, fed-batch, two-stage mixotrophic, and attached biofilm-based system (Kang et al. 2005, 2010; Kaewpintong et al. 2007; Ranjbar et al. 2008; García-Malea et al. 2009; Issarapayup et al. 2009; Li et al. 2011; Han et al. 2013; Wang et al. 2013a, b; Park et al. 2014; Zhang et al. 2014).

The astaxanthin accumulation is controlled by various physicochemical factors such as temperature (Yoo et al. 2012), pH (Hata et al. 2001), light (Saha et al. 2013; Park et al. 2014), salinity (Kobayashi et al. 1993), plant hormones (Yu et al. 2015), and nutrient stress (Boussiba et al. 1999; Chekanov et al. 2014). Since wastewater

M. M. R. Shah (✉)
Excel Career College, Courtenay, BC, Canada

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contains (in)organic compounds, it can be a potential asset for different living creatures (Rogers et al. 2014). Microalgae have the ability to metabolize and eradicate pollutants, and also they predominate in breaking and separating resistant organic molecules from wastewater (Matamoros et al. 2016 a, b). Various bioproducts have been produced from microalgal biomass harvested during wastewater treatment (Woertz et al. 2014). Different investigations have verified the viability of utilization of microalgae in the treatment of wastewaters (municipal, agricultural, and industrial) (Chinnasamy et al. 2012; Fenton and hUallachain 2012; Dickinson et al. 2013; Neveux et al. 2016). Among them, municipal wastewater has the best potentiality for microalgae cultivation. Commonly, various culture media (BM, BG11, and M1B5) are used for cultivation of *H. pluvialis*, and for transformation of vegetative cells into cyst cells, different chemical additives such as ferric or acetate anions are used (Kobayashi et al. 1997; Ruen-ngam et al. 2010; Solovchenko 2013). Numerous experiments have been reported on the development of best synthetic medium (e.g., Gong and Feng 1997; Fábregas et al. 2000), but, as far as we are aware, a limited number of studies are accessible on the likelihood to use wastewaters for *H. pluvialis* cultivation and astaxanthin accumulation (Kang et al. 2006; Wu et al. 2013; Wang 2014; Sato et al. 2015; Ledda et al. 2015; Haque et al. 2016a; Liu 2018).

Recently, phyco-valorization (nutrient removal from wastewater and simultaneous by-product generation by microalgae) has gained great attention (Querques et al. 2015). *H. pluvialis* was explored for cultivation in diluted primary-treated sewage and primary-treated piggery wastewater which demonstrated better growth and successful uptake of nitrate and phosphorus (Kang et al. 2006). There are a lot of advantages of using wastewater such as reduction of costs and natural resource inputs and simultaneously obtainment of high-value bioproducts (Farooq et al. 2013), but there are a number of challenges involved too. The main challenges include the following:

Harvesting of the algae.
The control of biomass composition is complicated by the selection of the desired species.
Bacterial contamination.
Micro-pollutant removal.
The conceivable requirement for external CO_2.

In this chapter, *H. pluvialis*-derived astaxanthin, its application and market potential, and culture conditions and nutritional requirements of *H. pluvialis* cell growth and astaxanthin formation have been discussed. The potentiality of microalgae cultivation using various wastewater streams and integration of *H. pluvialis* culture in different wastewater streams and nutrient removal and biomass production efficiency are also discussed. Furthermore, the challenges associated with coupling *H. pluvialis* cultivation in wastewaters and possible ways to overcome such challenges have been highlighted.

2 The Green Microalga *H. pluvialis*-Derived Astaxanthin

In *H. pluvialis*, maximum accumulation of astaxanthin can reach up to 5% DW (Wayama et al. 2013). Health food supplements consisting astaxanthin from microalgae considered as safe and broadly utilized as a nutraceutical supplement (Capelli and Cysewski 2013; Yang et al. 2013). *H. pluvialis*-derived astaxanthin can be used for health benefit in dosages from 3.8 to 7.6 mg per day (Yang et al. 2013). Due to structure, function, application, and security, *H. pluvialis* astaxanthin appears to be more effective than the synthetic one (Capelli and Cysewski 2013; Pérez-López et al. 2014; Shah et al. 2016).

2.1 Applications of Astaxanthin

Astaxanthin in Medical and Nutraceutical

Many published reports are available on human health and nutraceutical applications of astaxanthin (Guerin et al. 2003; Chew et al. 2004; Higuera Ciapara et al. 2006; Palozza et al. 2009; Yuan et al. 2011). It works as an antioxidant (Hussein et al. 2006; Liu and Osawa 2007; Ranga Rao et al. 2010), protects peroxidation of membrane lipids (Naguib 2000), terminates the induction of inflammation, helps in ulcer disease (Liu and Lee 2003), enhances human digestive health (Nishikawa et al. 2005; Kamath et al. 2008), and deals with treatment of gastrointestinal pain (Andersen et al. 2007; Kupcinskas et al. 2008).

Astaxanthin can be helpful for reduction of risk for heart attacks (Iwamoto et al. 2000), increment of basal arterial blood flow (Miyawaki et al. 2008), and reduction of blood plasma level (Karppi et al. 2007). It can also reduce the effects of Alzheimer's and neurological disorders; hinder fibrosarcoma growth, cancer cells (breast and prostate), and embryonic fibroblasts (Palozza et al. 2009); and improve respiratory and sympathetic nervous system (Nagata et al. 2006) and mammary tumor (Nakao et al. 2010).

Astaxanthin also helps to protect the skin from photooxidation by UV induction and has antiaging effects (Seki et al. 2001; Yamashita 2002; Tominaga et al. 2012; Ranga Rao et al. 2013). In the case of human, astaxanthin can improve semen quality, pregnancy rate, and sperm velocity (Elgarem et al. 2002; Comhaire et al. 2005) and decrease unexplained infertility (Andrisani et al. 2015).

Astaxanthin in Aquatic Animal and Poultry Diet

Haematococcus-derived astaxanthin can provide essential nutrient for body weight increment and breeding of economically important fishes such as salmonid, red sea bream, rainbow trouts, and shellfish (shrimp). It has been proved as important compound for improvement of pigment in the fish flesh (Torrissen and Naevdal 1984; Tolasa et al. 2005). Use of *H. pluvialis* biomass has shown to enhance egg quality, growth, and rate of survival of fish (salmonid, sea bream, and rainbow trout, ornamental fish), fry (Arai et al. 1987; Ako and Tamaru 1999; Sommer et al. 1991;

Choubert and Heinrich 1993; Sheikhzadeh et al. 2012a, b), and shrimp (Arai et al. 1987; Parisenti et al. 2011). It has been demonstrated that the diet containing *H. pluvialis* improved the growth of adult yellow croaker fish (Li et al. 2014). *H. pluvialis* appeared to be effective in egg yolk coloration, improving egg-laying capacity in hen (Elwinger et al. 1997), muscle in meat-producing chicken (Inborr and Lignell 1997; Inbbor 1998), and fertility and decreasing mortality of chicken (Lignell and Inborr 1999, 2000).

2.2 Market Potential of Astaxanthin

Recently, there has been increasing pattern toward utilizing organic in food, feed, and cosmetic products. The interest for *H. pluvialis* astaxanthin in the international market worldwide has been "emerging" as a result of expanding customer attention to its medical advantages. Worldwide market for astaxanthin (synthetic and natural) is assessed in 2014 at 280 metric tons which is anticipated to achieve by 2020 at 670 metric tons (Industry Experts 2015; Panis 2015). The market value of astaxanthin is about $2500–7000/kg, and in some cases for *H. pluvialis* astaxanthin, it goes up to $15,000/kg (Borowitzka 2013; Koller et al. 2014; Pérez-López et al. 2014; Industry Experts 2015). Natural astaxanthin is 3–4 times more expensive than the synthetic one (Han et al. 2013). Considering the increasing market potentiality for natural astaxanthin for industrial utilization, large-scale production of *H. pluvialis* has great prospects and appealing commercial possibility. However, contemporary market requirement for astaxanthin from *H. pluvialis* is not fulfilled. Once the production technology is optimized, the production costs *H. pluvialis* astaxanthin would be comparable to the artificial astaxanthin (Pérez-Lópezetal et al. 2014).

3 Culture Parameters for *H. pluvialis* Growth and Astaxanthin Production

Improvement of culture conditions is important to accomplish greater yield and astaxanthin generation. These conditions have diverse optimum level for cell growth and pigment production. Different kinds of media such as BG-11, BBM, OHM, and KM1-basal medium (Bischoff and Bold 1963; Rippka et al. 1979; Kobayashi et al. 1993; Fábregas et al. 2000) are used for cultivation. At nutrient-deficient conditions, astaxanthin accumulates inside the cells (Saha et al. 2013). In nitrogen-deficient condition, the production rate of astaxanthin is twice than the limitation of phosphorus. Micronutrients (selenium and chromium) play important role to increase yield and astaxanthin formation (Tripathi et al. 1999; Fábregas et al. 2000; Domínguez-Bocanegra et al. 2004). Astaxanthin generation can also be accelerated by incorporating 0.25–0.5% w/v of NaCl or combining 2.2 mM sodium acetate to the media (Sarada et al. 2002b).

The appropriate temperature for *H. pluvialis* ranges from 20 to 28 °C (Fan et al. 1994; Hata et al. 2001; Lababpour et al. 2005; Kang et al. 2010; Yoo et al. 2012; Wan et al. 2014a). However, >30 °C temperature triggers a transition from green to red stage (Tjahjono et al. 1994). pH also can significantly have an effect on the growth and synthesis of carotenoids. The optimum pH ranges from 7.00 to 7.85 (Hata et al. 2001; Sarada et al. 2002a). The optimal light irradiation ranges from 40 to 50 μmol photons m^{-2} s^{-1} (Hata et al. 2001; Chekanov et al. 2014; Park et al. 2014). Optimum light intensity to accomplish better growth rates inclines to be greater such as 70 (Zhang et al. 2014), 80 (Saha et al. 2013), 90 (Fan et al. 1994), or 177 μmol photons m^{-2} s^{-1} (Domínguez-Bocanegra et al. 2004). During green stage, the regular photoperiod (12:12 or 16: 8 h) is frequently maintained (Saha et al. 2013; Park et al. 2014) but higher growth obtained with continuous light (Domínguez-Bocanegra et al. 2004).

Culture Systems *H. pluvialis* can be grown indoor and outdoor and in open or closed system; batch, fed-batch, semicontinuous, or continuous system; and photoautotrophic, heterotrophic, or mixotrophic modes.

Photoautotrophic Culture This type of culture is generally performed in ponds/raceways or photobioreactors. Typically tubular, bubble column and airlift photobioreactors are used for cultivation. Since circumstances for maximum cell yield and astaxanthin concentration are usually incompatible, a double-step production policy is frequently followed for the industrial cultivation. The step one is to maximize vegetative growth in optimum conditions (e.g., less light intensity and with nitrogen) (Boussiba 2000; Aflalo et al. 2007; Del Rio et al. 2007). Once maximum growth is achieved, in the second step, the cells moved to stress situation (e.g., strong light and nitrogen limited, pH or salt manipulation, phosphate depletion, etc.). These stress conditions either individually or in combination with others can stimulate astaxanthin formation (Fábregas et al. 2001; Torzillo et al. 2003; Orosa et al. 2005; He et al. 2007; Hu et al. 2008; Li et al. 2010; Choi et al. 2011). The biomass production in vegetative and red stage varied from 0.01 to 0.5 g L^{-1} d^{-1} and 0.01 to 4.8 g L^{-1} d^{-1}, respectively. In terms of astaxanthin production and content, it varied from 0.44 to 21 mg L^{-1} d^{-1} and 0.8 to 4.8% of DW, respectively (Table 1). Attached cultivation strategy is utilized in the initiation of astaxanthin formation in *H. pluvialis*. In this system, the biomass and astaxanthin productivities were 2.8- and 2.4-fold greater than those of the suspended cultivation system, respectively (Wan et al. 2014b). Additional researches that used the same techniques have shown increased astaxanthin production: 124 mg m^{-2} d^{-1} (Yin et al. 2015) and 164.5 mg m^{-2} d^{-1} (Zhang et al. 2014). Attached induction system can be a potential way to enhance commercial profit and significantly lower cultivation cost (Zhang et al. 2014; Wan et al. 2014b). Park et al. (2014) invented "perfusion culture" system coupling it with stepwise increase associated with light intensity. This culture can offer greater cell growth of 0.18 g L^{-1} d^{-1}. Under stepwise improved light irradiance (150–450 μE/m2/s), cell growth of 12.3 g L^{-1} can be achieved. This cell growth is usually 3.09 and 1.67 times greater than batch and fed-batch processes, respectively (Park et al. 2014).

Table 1 Biomass production and astaxanthin content of *H. pluvialis* grown in various studies

PBRs type	Outdoor/ indoor	Mode	Biomass productivity in green stage (g L^{-1} d^{-1})	Biomass productivity in red stage (g L^{-1} d^{-1})	Astaxanthin content (%, DW)	Astaxanthin productivity (mg L^{-1} d^{-1})	Reference
Airlift column (30 L)	Indoor	Batch	0.03	0.01	2.7	0.44[b]	Harker et al. (1996)
Tubular/open pond (25,000 L)	Outdoor		0.036–0.052	N/A	2.8–3.0	N/A	Olaizola (2000)
Tubular (50 L)	Indoor	Semicontinuous	N/A	0.05	3.6	7.2 [c,d]	Torzillo et al. (2003)
Bubbling column (1.8)	Indoor	Batch	N/A	0.6	0.8	5.6 [a]	Del Rio et al. (2005)
Airlift tubular (55 L),			N/A	0.41	1.1	4.4[a]	Lopez et al. (2006)
Bubbling column (0.5 L)	Indoor	Batch	0.5	0.21	4	11.5 [b]	Aflalo et al. (2007)
Tubular (200 L)	Outdoor	Batch	0.37	0.21	3.8	10.1[b,c]	Aflalo et al. (2007)
Bubbling column (1.8 L)	Indoor	Batch	N/A	1.9	1.1	21 [a]	Del Rio et al. (2007)
Bubbling column (1 L)	Indoor	Batch	0.36	0.14	3.6	12 [b]	Ranjbar et al. (2008)
Tubular (1.8 L), outdoor	Outdoor	Continuous	N/A	0.7	1	8 [a]	Garcia-Malea et al. (2009)
Open pond	Indoor	Batch	N/A	0.15	2.79	4.3[a]	Zhang et al. (2009)
Flat type (1 L)	Indoor	Fed-batch	0.33	0.44	4.8	14[b]	Kang et al. (2010)
Airlift column	Indoor	Batch	N/A	0.14	N/A	3.3[a]	Choi et al. (2011)
Bubbling column V-shaped bottom (6 L)	Indoor	Batch	N/A	0.047	N/A	1.4[a]	Yoo et al. (2012)
Bubbling column (0.6 L)	Outdoor	Batch	N/A	0.58	2.7	17.1[b,d]	Wang et al. (2013a)

[a]One-step culture
[b]Two-step culture. Productivity value calculated based on total time required by the "green" and "red" stage
[c]Induction of astaxanthin performed outdoors
[d]A two-step process where astaxanthin productivity was calculated based on time spent on the "red stage" only

Heterotrophic and Mixotrophic Culture In heterotrophic growth, organic compounds act as carbon and energy for cell propagation and secondary metabolite construction with the absence of light. Different sources of organic carbon have been utilized in this culture. It was shown that acetate helped effectively to cyst formation and astaxanthin formation (Kobayashi et al. 1991; Kakizono et al. 1992; Orosa et al. 2000; Hata et al. 2001; Kang et al. 2005). These microalgae can be also cultured mixotrophically utilizing acetate or carbohydrates (Kobayashi et al. 1993). It is proved that biomass and astaxanthin accumulation can be improved following this culture system. For example, cell density of 0.9–2.65 g L^{-1} and astaxanthin content of 1–2% DW were achieved (Chen et al. 1997; Wang et al. 2003). A sequential, heterotrophic-photoautotrophic culture strategy was also investigated. Biomass was produced by utilizing heterotrophic culture, but for astaxanthin production, photoautotrophic culture was applied under nitrogen depletion, with bicarbonate or CO2 as carbon sources. In this system, a superior astaxanthin content (7% DW) was obtained which is 3.4-fold higher than heterotrophic induction (Kang et al. 2005).

4 Wastewater as a Resource for Microalgae Cultivation

Microalgae cultivation and biomass production require huge quantities (for 1 gram dry biomass >1 kg water) of water (Burlew 1953; Shen 2014). Wastewater (cheap and readily available) provides appropriate atmosphere (pH, dissolved CO_2, and HCO_3^-) and macronutrients (nitrate, ammonia, phosphate) and micronutrients that support for microalgal growth (Abdel-Raouf et al. 2012; Ji et al. 2013; Ajayan et al. 2015; Ding et al. 2015). Wastewater-grown microalgae biomass can be used to extract the accumulated nutrients (Mehta et al. 2015; Gouveia et al. 2016). Three nutrients (carbon, nitrogen, and phosphorus) are of most interest during evaluating a wastewater for microalgae growth enhancement (Kabra et al. 2014).

4.1 Macroelements and Microelements

The cell growth and biochemistry of microalgae require the receptiveness of 15–20 essential elements. The macronutrients consist of C, N, P, H, O, S Mg, K, Na, and Ca, and the micronutrients include Fe, Cu, Mn, Zn, Cl, V, Mo, B, Co, and Si (Eyster 1964). The macroelements are typically utilized as development materials, and the microelements are involved in biological reactions (Arnon 1961). Five microelements (Mn, Zn, Cu, Ca, and Fe) are directly associated with microalgal photosynthesis. Microelements (Cl and Mn) play an important role in O_2 evolvement. The supplementation of macroelements (C, N, and P) with essential microelements (Si, Mg, Ca, Fe, P, S, Mn, Zn, Cu, and Co) is needed for continuous microalgae growth. In case of application of wastewater for microalgae cultivation, the supply of essential microelements such as Si, Mg, Ca, Fe, P, S, Mn, Zn, Cu, and Co rarely limits microalgal growth.

4.2 Composition of Wastewater

Wastewater is a compound mixture of organic, inorganic, and artificial elements. Three quarters of organic carbon in sewage are proteins, carbohydrates, fats, amino acids, and volatile acids. The inorganic parts include large amount of calcium, potassium, sodium, magnesium, chlorine, sulfur, phosphate, bicarbonate, ammonium, and heavy metals (Lim et al. 2010). Wastewaters from various sources (municipal, agricultural, and industrial) can be treated efficiently by microalgae. The typical N:P features of different wastewaters and the feasibility of cultivation of microalgae are shown in Table 2.

In the wastewater influent, nitrogen is present in the form of ammonia (NH_4^+), nitrite (NO_2^-), or nitrate (NO_3^-). Phosphorus is present as phosphates ($PO4_3^-$) in wastewater. Municipal wastewater contains several heavy metal pollutants such as arsenic, cadmium, chromium, copper, lead, mercury, and zinc (European Commission on Environment 2002). It contains comparatively lower amounts of total N and P (10–100 mg L^{-1}) (Dela Noue et al. 1992). Once the secondary treatment is done, total N and P decrease to 20–40 mg L^{-1} and 1–10 mg L^{-1}, respectively (McGinn et al. 2011), which is very suitable for microalgae growth. The N and P ration in municipal wastewater is about 11 to 13 (Christenson and Sims 2011). The widely accepted N:P ratio for microalgae growth is 16 (Larsdotter 2006; Christenson and Sims 2011; Park et al. 2011; Cai et al. 2013), on the basis of empirical formula $C_{106}H_{181}O_{45}N_{16}P$ (Stumm and Morgan 1970). The typical microalgae cell biomass contains 6.6% N and 1.3% P in dry weight (Chisti 2013) with a molar N:P ratio of 11.2, which is similar to that found in wastewater.

Agricultural wastewater derived from animal manure contains N and P concentrations of >1000 mg L^{-1} (Dela Noue et al. 1992). Agricultural runoff consists of herbicides, fungicides, and insecticides.

Industrial wastewater contains less N and P compared to agricultural and municipal wastewater. It has high levels of heavy metal pollutants such as Cr, Zn, and Cd and organic chemical toxins such as hydrocarbons, biocides, and surfactants (Chinnasamy et al. 2010). Textile, tanning, leather, and electroplating and related metal processing industry effluent possess considerable amounts of toxic metal ions (Salama et al. 2017).

4.3 Treatment of Wastewaters

Municipal Wastewater Treatment

Increasing urbanization and population expansion have resulted in large quantities of municipal wastewaters produced every day. Physical and chemical treatment methods are commonly used for removing buoyant, non-buoyant, and dissolved organic materials from wastewaters (Ruiz-Marin et al. 2010). Microalgae cultivation into the municipal wastewater treatment systems for nutrient removal has been widely studied. For example, Pittman et al. (2011) reported that *Chlorella* sp. and

Table 2 Comparison between the physicochemical characteristics of wastewaters and a common synthetic medium

Properties	Unit	Municipal wastewater	Concentrated municipal wastewater	Anaerobic digestion wastewater	Piggery wastewater	Bold's basal medium
pH	–	8.10	7.28	7.30–7.50	7.97	6.80
Alkalinity (total CO3)	mg CO_3/L	272	–	–	–	–
Salinity	g/L	1.03	–	–	–	–
TSS	mg/L	50	–	59.35–85.26	-	-
Conductivity	mS/cm	2.29	–	–	–	–
COD	mg/L	31	–	1572.45–2265.37	37,643	–
TOC	mg/L	9	180.6	–	–	–
TIC	mg/L	–	80.9	–	–	–
TN	mg/L	27	56	537.26–702.73	2055	41.01
TP	mg/L	5.04	15.8	72.62–111.58	620	53
Microbes						
E. coli	cfu/100 mL	5.4×10^6	–	–	–	E–
P. aeruginosa	cfu/100 mL	0.2×10^6	–	–	–	–
Fecal coliforms	cfu/100 mL	6.2×10^6	–	–	–	–
Total coliforms	cfu/100 mL	75.0×10^6	–	–	–	–
Metals						
Magnesium	mg/L	0.088	16.5	23.83–58.26	213	7
Manganese	mg/L	0.09	0.4	0.96–1.91	4.1	0.23
Zinc	mg/L	0.009	–	–	28.9	3.93
Copper	mg/L	–	–	0.31–0.92	10.6	0.63
Calcium	mg/L	29	65.6	–	437	7
Cobalt	mg/L	–	–	0.02–0.06	3.8	
Iron	mg/L	0.12	0.05	6.83–15.35	169.2	4.2
Aluminum	mg/L	0.04	0.02	–	–	–
Sulfate	mg/L	–	40.4	–	–	43.2
Sodium	mg/L	–	39.5	–	772	68
Potassium	mg/L	20	45.7	22.38–68.15	2524	34
Chloride	mg/L	–	–	–	–	12
Barium	mg/L	–	–	0.74–1.67	–	2.0

Adapted from Salama et al. (2017)

Scenedesmus sp. performed with very high efficiency on nutrient removal in sewage wastewater after secondary treated, particularly ammonia, nitrate, and total P, ranging from 80% to 100% removal rates in many cases. Another study indicated that *C. vulgaris* could remove more than 90% of N and 80% of P from primary-treated

municipal wastewater (Lau et al. (1995). *Dunaliella salina* showed the capacity for removing nitrate, ammonia, and phosphorous in the range of 45–88% from municipal wastewater after a 6-day cultivation (Liu and Yildiz 2018).

Agricultural Wastewater Treatment
Agriculture is another major wastewater-producing sector. Agricultural wastewater is frequently derived from livestock production and contains high levels of N and P (Wilkie and Mulbry 2002). Generally, livestock manure is often treated and used as fertilizer. However, nutrients may not be completely consumed due to the various ratios of N:P requiring by the crops. As a result, excess nutrients find their ways to the surrounding aquatic systems and cause eutrophication significantly reducing water quality (Cai et al. 2013). Piggery wastewater is typically treated with anaerobic bacteria for reduction of nutrient. However, the nutrient removal capacity of anaerobic bacteria is comparatively lower than microalgae and some cyanobacteria (Markou and Georgakakis 2011). As in the case of municipal wastewaters, previous researches have also demonstrated that microalgae can significantly assimilate N and P from manure-based wastewaters. For example, An et al. (2003) reported that 80% of nitrate content was effectively removed from piggery wastewater by *Botryococcus braunii*. Moreover, compared with microalgae that were cultivated in municipal wastewaters, Wilkie and Mulbry (2002) indicated that higher microalgae growth rates and equivalent nutrient removal efficiencies were observed in manure-added recycling wastewater.

Industrial Wastewater Treatment
The traditional methods of industrial wastewater treatments include electrowinning, precipitation, and ion exchange. Since industrial wastewaters contain lower N and P contents and greater levels of toxic elements, most microalgae cannot grow well. It is necessary to select specific strains that have high metal absorption capacities to handle industrial wastewater remediation. So far, only a few strains have been explored for metal removal capacity research. One study using carpet mill wastewater, which has relatively lower toxins and higher N and P contents, reported that *Botryococcus braunii*, *Chlorella saccharophila*, and *Pleurochrysis carterae* grew well in untreated wastewater with large amounts of biomass generated (Chinnasamy et al. 2010).

5 Integrating *H. pluvialis* Cultivation in Wastewater Treatment and Nutrient Removal

The growth rate of *H. pluvialis* is slow, and its cultivation is a highly sensitive process due to its susceptibility to contamination by other algae and microbes (Orasa et al. 2000). Generally, BM, BG11, and M1B5 media used for cultivation of *H. pluvialis* and chemical additives such as ferric or acetate anions are added to stress the cells (Kobayashi et al. 1997; Ruen-ngam et al. 2010; Solovchenko 2013).

Table 3 Research highlights on integration of different wastewaters with *H. pluvialis*

Wastewater type	Removal efficiency of TN (%)	Removal efficiency of TP (%)	Biomass production g/L	Astaxanthin production mg/L	Culture volume (L)	Culture days	Reference
Primary-treated sewage	100%	100%	0.78	39.7	130 ml	18	Kang et al. (2006)
Primary-treated piggery wastewater	100%	100%	1.43	83.9	130 ml	18	Kang et al. (2006)
Domestic secondary effluent	(93.8%	97.8%)	0.20	–	200 ml	20	Wu et al. (2013)
Coagulated wastewater	90% ± 8%	99% ± 1%		3.26	200 ml	25	Sato et al. (2015)
Piggery wastewater	99%	98%	1.31	–	300 ml	20	Ledda et al. (2015)
Bioethanol plant wastewater	91.7%	100%	4.37	–	2.2 L	16	Haque et al. (2016b)
Minkery wastewater	100%	100%	0.90	67.95	2.25 L	6	Liu (2018)

Although various researches have been performed on the development of optimal synthetic growth medium (e.g., Gong and Feng 1997; Fábregas et al. 2000), only few studies focused on the possibility to use wastewaters for *H. pluvialis* and succeeding astaxanthin production (Table 3). For instance, Kang et al. (2006) reported *H. pluvialis* cultivation in primary-treated wastewater and piggery wastewater. They showed that the cell growth rate in primary-treated wastewater was 0.24 day^{-1}, which was comparable to 0.23 day^{-1} in artificial medium; the cells were composed of 5.1 and 5.9% astaxanthin content using the two-step process; and the cells yielded 43 mg L^{-1} nitrogen and 2.6 mg L^{-1} phosphorus.

Compared with most microalgal species reported in the literature, *H. pluvialis* attained highest biomass production (27.8 mg L^{-1} d^{-1}), efficient nutrient removal (both nitrogen (93.8%) and phosphorus (97.8%) were removed efficiently), and highest lipid accumulation (43%) in unsterilized domestic secondary effluent (Wu et al. 2013).

Sato et al. (2015) reported a new wastewater treatment process that involves coagulation, ozonation, and microalgae *H. pluvialis* cultivation. *H. pluvialis* grew well in the supernatant of coagulated wastewater, and the astaxanthin yield was 3.26 mg/L, and total phosphorus and nitrogen contents decreased to 99% and 90%, respectively.

In another study, wastewater treatment and astaxanthin production were conducted by a primary treatment filtering system: culture and subsequent carotenogenesis induction of *H. pluvialis* on piggery wastewater.

In this study, a drastic reduction in macro- and micronutrient concentration (up to 99% for NO_3-N and NH_4-N, 98% for TP) and astaxanthin accumulation of 1.27% on a dry weight were observed (Ledda et al. 2015). This method showed potentiality as biological wastewater treatment process since it can combine inorganic waste removal without any additives and the simultaneous production of astaxanthin.

Since *H. pluvialis* can use CO_2, CO_3, and carbohydrates as carbon sources, its production cost can be reduced by utilizing waste sources like flue gasses or waste containing carbon and nutrient compounds (Wu et al. 2013).

The required energy and nutrients in auto-, hetero-, and mixotrophic cultivation can be recycled from anaerobic digestion. Based on the culture system, carbon sources can vary. The recycled CO_2 from energy production at anaerobic digestion can be used in photoautotrophic cultivation. The required carbon (carbohydrates or acetate) can be provided from alternative source in heterotrophic cultivation. These carbon sources can be produced from waste (carbohydrate-rich food waste from food industry can be used in heterotrophic cultivation) (Wang 2014). In mixotrophic cultivation both carbon sources can be utilized. After concurrent extraction of astaxanthin and triglycerides, algal cake is used as a feedstock for biogas production through anaerobic digestion that helps in the extraction of residual energy from this integrated bioprocess (Shah et al. 2016). The biorefinery strategy is shown on Fig. 1.

In a recent study by Haque et al. (2017), high-density (4.37 g/L) *H. pluvialis* culture was obtained using the bioethanol plant waste stream as the growth media and resulted in 91.67% total nitrogen and 100% total phosphorous removal. The residual microalgal biomass, obtained after astaxanthin extraction (1.109 mg/g DW), was characterized as a potential bioenergy feedstock. This production process could be environmentally friendly and economically viable, compared to conventional astaxanthin production processes, due to integrating culture in an existing bioethanol plant and using the waste product produces in the plant. Culturing *H. pluvialis* in bioethanol wastewater streams can be a greener alternative to conventional media. The maximum vegetative growth of *H. pluvialis* was obtained in 60× diluted thin stillage, and maximum astaxanthin production was obtained in GroAst media (60% 60× thin stillage and 40% acetate-rich process condensate). The GroAst media appeared to be not only a cheaper media, compared to the chemically synthesized media, but it is also a "greener" sustainable alternative to conventional growth media (Haque et al. 2016b).

Minkery wastewater contains extremely high level of ammonia, which is a different N source from BBM. *H. pluvialis* grew well in the appropriately diluted minkery wastewater (MW) media, and a higher biomass production was realized as compared with conventional culture medium under optimal growth condition. *H. pluvialis* achieved maximum biomass at 1.5% MW cultures, yielding 906.03 ± 34.0 mg L^{-1}, with a successful removal of total nitrogen and phosphorus in a 6-day culture. The optimal initial cell density and volume ratio between microalgae and MW were

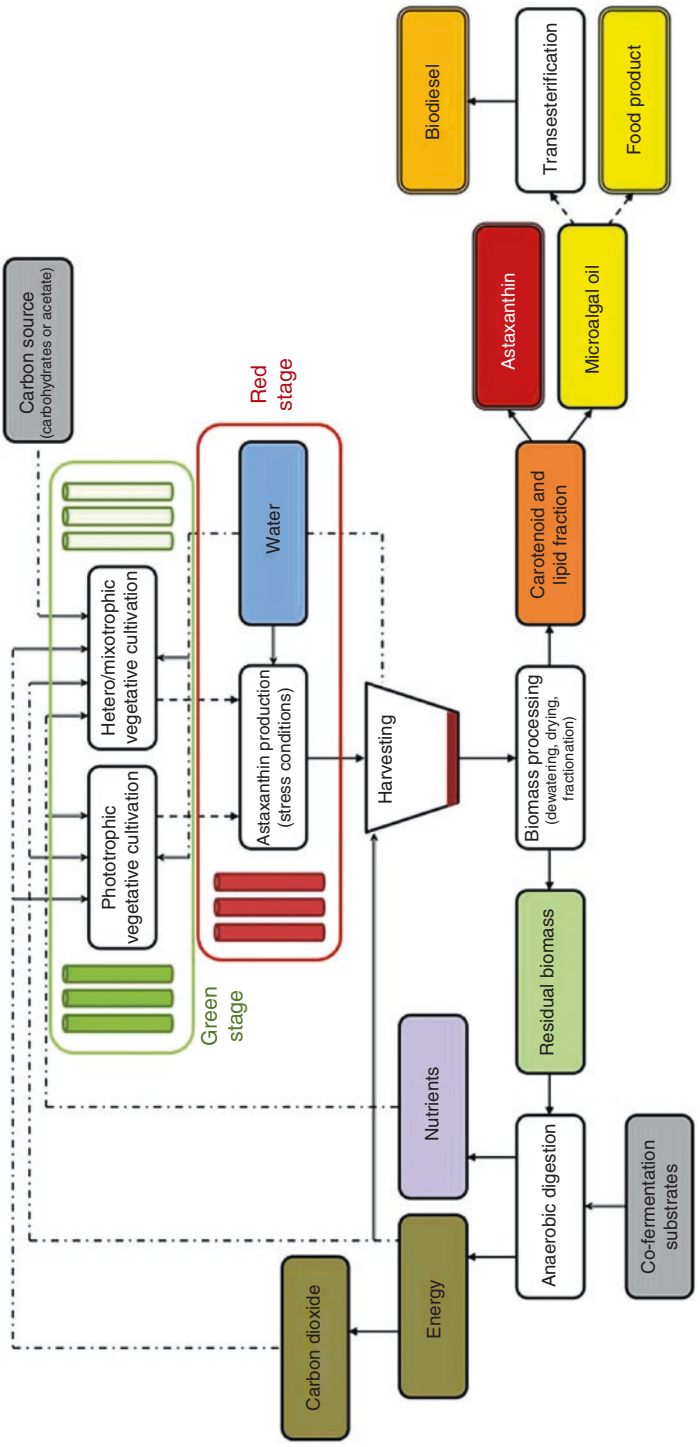

Fig. 1 Schematic diagram of two-stage cultivation *H. pluvialis* biorefinery producing astaxanthin and either edible oil or biofuel compound-biodiesel. (Adapted from Shah et al. 2016). Green stage: performed using either photoautotrophic (deep green section) or hetero−/mixotrophic (pale green section) systems. Red stage: (red section) takes place after green stage to maximize astaxanthin content. Recycling of waste is performed through an aerobic digestion process. The following annotations are used: solid arrows, subsequent steps; dashed arrows, optional steps; double lines, final products; double arrows, inputs; dotted lines, opportunities for recycling resources

also determined to have great help on maximizing the biomass yield. The findings support the claim that integration of wastewater into microalgae cultivation has the advantages of reducing cultivation costs and natural resource inputs and simultaneously obtaining high-value bioproducts (Liu 2018).

Considering the above facts, *H. pluvialis* can be considered as a promising candidate for integrated systems of wastewater treatment and microalgae cultivation while producing high-value bioproducts.

6 Challenges Associated with Growing *H. pluvialis* in Wastewater Streams

Despite the promising features of microalgae, there are huge challenges to overcome before this route can be exploited in commercially and environmentally sustainable manner. The following points are considered as the most important challenges for the cultivation of microalgae in general and *H. pluvialis* using wastewater:

- The cultivation of *H. pluvialis* in wastewater can be susceptible to contamination by fungus, zooplankton (rotifer), protozoans (e.g., amoebas, ciliates), and other microalgae due to its relatively slow growth (Han et al. 2013; Orasa et al. 2000).
- Abiotic contaminants in wastewater such as CO_2, NOx, SOx, O_2, and NH_3^+ and heavy metals can also inhibit microalgae like *H. pluvialis* growth (Kumar et al. 2010).
- In case of low concentration of trace mineral nutrients in the wastewater, it can result in poor growth, low biomass, and low lipid productivity (Christenson and Sims 2011). However, Kang et al. (2007) and Hata et al. (2001) indicated that high concentration of nutrients would also cause inhibitory effects on *H. pluvialis* growth, and thus, the suitable concentration of wastewater must be determined for *H. pluvialis* cultivation.
- Due to the lack of carbon sources in most domestic wastewater, the growth of the microalgae can be inhibited which might eventually affect the treatment of the wastewater (Craggs et al. 2011).
- High concentration of oxygen in wastewater can induce oxidative damage to microalgae cell and inhibit photosynthesis (Christenson and Sims 2011).
- The cost and energy demand of harvesting microalgae in general and *H. pluvialis* from wastewater either by flocculation or centrifugation are still very high (Razon and Tan 2011; Acién et al. 2012; Shah et al. 2016).

7 Conclusion and Prospects

This chapter provides perception regarding the recent scientific and technical improvement in different areas of *H. pluvialis*-derived astaxanthin, its application and market potential, and culture conditions and nutritional requirements of this

microalgal cell growth and astaxanthin formation. It also scans a broader image including the potentiality of microalgae cultivation using various wastewater and integration of *H. pluvialis* culture in different wastewater streams and nutrient removal and biomass production efficiency to the challenges associated with growing *H. pluvialis* in wastewater streams.

Recently the demand from *H. pluvialis* is increasing. A number of developments have been obtained concerning production and processing to achieve astaxanthin during the last decade. Still its large-scale cultivation is very expensive for mass adoption of natural astaxanthin compared to the synthetic one. *H. pluvialis* has been cultured in different ways. Various studies have been focused on optimization of parameters (media, light, pH, temperature, etc.) for maximum growth. For biomass accumulation and astaxanthin production, most of these parameters found different.

There is not much can be done to solve this challenges since it is fundamentally connected with the whole life cycle of *H. pluvialis*. We believe that integration of *H. pluvialis* cultivation with wastewater treatment could be a great option to produce astaxanthin effectively in large scale. Research in the use of wastewater for cultivating *H. pluvialis* is still very limited as compared to research for growing other microalgae species. Therefore, further clarifications are needed to prove the feasibility of *H. pluvialis*-based systems in full scale. A number of wastewater types are encouraged to be investigated in *Haematococcus* cultures. Moreover, the relevant optimal production routes and advances in technologies are needed. The improvements in integration processes, harvesting, and extraction technology will contribute to accelerate the speed of the *Haematococcus*-derived astaxanthin production from laboratory scale to commercial scale. Further study in these areas can have a profound influence on the market of natural astaxanthin from *H. pluvialis*.

The challenges of wastewaters directly for *H. pluvialis* culture should be addressed since they restrict the utilization of the easily accessible and low-cost wastewater. There are a number of areas that can improve the integrated *H. pluvialis* cultivation using wastewater for nutrient removal and efficient astaxanthin production. These include the following:

Consideration of sterilized wastewater for microalgal cultivation to prevent biotic contamination.
Coupling of immobilize or attached cultivation of *H. pluvialis* in wastewater stream to maximize the biomass production.
In cases of low concentration of nutrients, there is a need to supplement these nutrients in wastewater to achieve high productivity.
Bubbling of CO_2 can improve algae growth while using domestic wastewater with lack of carbon source for *H. pluvialis* cultivation.
Technological advancement in cost-effective *H. pluvialis* biomass harvesting from large-scale wastewater culture to make it more economically attractive.

Future improvements in these fields can have a thoughtful effect on the commercial implementation of *H. pluvialis* astaxanthin products. Finally, global microalgae industry can be benefited in the near future.

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References

Abdel-Raouf N, Al-Homaidan AA, Ibraheem IBM (2012) Microalgae and wastewater treatment. Saudi J Biol Sci 19:257–275

Acien G, Fernández JM, Magán JJ, Molina E (2012) Production cost of a real microalgae production plant and strategies to reduce it. Biotechnol Adv 30:1344–1353

Aflalo C, Meshulam Y, Zarka A, Boussiba S (2007) On the relative efficiency of two- vs. one-stage production of astaxanthin by the green alga *Haematococcus pluvialis*. Biotechnol Bioeng 98:300–305

Ajayan KV, Selvaraju M, Unnikannan P, Sruthi P (2015) Phycoremediation of tannery wastewater using microalgae Scenedesmus species. Int J Phytoremediation 7:907–916

Ako H, Tamaru CS (1999) Are feeds for food fish practical for aquarium fish? Int Aqua Feeds 2:30–36

An JY, Sim SJ, Lee JS, Kim BW (2003) Hydrocarbon production from secondarily treated piggery wastewater by the green alga *Botryococcus braunii*. J Appl Phycol 15:185–191

Andersen LP, Holck S, Kupcinskas L, Kiudelis G, Jonaitis L, Janciauskas D, Permin H, Wadstrom T (2007) Gastric inflammatory markers and interleukins in patients with functional dyspepsia treated with astaxanthin. FEMS Immunol Med Microbiol 50:244–248. https://doi.org/10.1111/j.1574-695X.2007.00257.x

Andrisani A, Donà G, Tibaldi E, Brunati AM, Sabbadin C, Armanini D, Alvisi G, Gizzo S, Ambrosini G, Ragazzi E, Bordin L (2015) Astaxanthin improves human sperm capacitation by inducing lyn displacement and activation. Mar Drugs 13:5533–5551. https://doi.org/10.3390/md13095533

Arai S, Mori T, Miki W, Yamaguchi K, Konosu S, Satake M, Fujita T (1987) Pigmentation of juvenile coho salmon with carotenoid oil extracted from Antarctic krill. Aquaculture 66:255–264. https://doi.org/10.1016/0044-8486(87)90111-6

Arnon D (1961) Cell-free photosynthesis and the energy conversion process. Light Life 1961:489–566

Bischoff HW, Bold HC (1963) Psychological studies IV. In: Some soil algae from enchanted rock and related algal species. University of Texas Publications, Austin, p 95

Borowitzka MA (2013) High-value products from microalgae—their development and commercialisation. J Appl Phycol 25:743–756. https://doi.org/10.1007/s10811-013-9983-9

Boussiba S (2000) Carotenogenesis in the green alga *Haematococcus pluvialis*: cellular physiology and stress response. Physiol Plantarum 108:111–117. https://doi.org/10.1034/j.1399-3054.2000.108002111.x

Boussiba S, Bing W, Yuan JP, Zarka A, Chen F (1999) Changes in pigments profile in the green alga *Haematococcus pluvialis* exposed to environmental stresses. Biotechnol Lett 21:601–604. https://doi.org/10.1023/A:1005507514694

Burlew JS (1953) Algal culture: from laboratory to pilot plant. Carnegie Institute, Washington Publication, p 600

Cai T, Park SY, Li Y (2013) Nutrient recovery from wastewater streams by microalgae: status and prospects. Renew Sust Energy Rev 19:360–369

Capelli GC, Cysewski G (2013) The worlds' best kept health secret natural astaxanthin. Cyanotech Corporation, Kailua-Kona

Chekanov K, Lobakova E, Selyakh I, Semenova L, Sidorov R, Solovchenko A (2014) Accumulation of astaxanthin by a new *Haematococcus pluvialis* strain BM1 from the White Sea coastal rocks (Russia). Mar Drugs 12:4504–4520. https://doi.org/10.3390/md12084504

Chen F, Chen H, Gong X (1997) Mixotrophic and heterotrophic growth of *Haematococcus lacustris* and rheological behaviour of the cell suspensions. Bioresour Technol 62:19–24. https://doi.org/10.1016/S0960-8524(97)00115-6

Chew BP, Park JS, Hayek MG, Massimino S, Reinhart GA (2004) Dietary astaxanthin stimulates cell-mediated and humoral immune response in cats. FASEB J 18:533

Chinnasamy S, Bhatnagar A, Hunt RW, Das KC (2010) Microalgae cultivation in a wastewater dominated by carpet mill effluents for biofuel applications. Bioresour Technol 101:3097–3105
Chinnasamy S, Rao PH, Bhaskar S, Rengasamy R, Singh M (2012) Algae: a novel biomass feedstock for biofuels. In Arora R (Ed.) Microbial Biotechnology: Energy and Environment, pp. 224–239
Chisti Y (2013) Constraints to commercialization of algal fuels. J Biotechnol 167:201–214
Choi Y, Yun Y, Park JM, Yang J (2011) Determination of the time transferring cells for astaxanthin production considering two-stage process of Haematococcus pluvialis cultivation. Bioresour Technol 102(24):11249–11253
Choubert G, Heinrich O (1993) Carotenoid pigments of the green alga *Haematococcus pluvialis*: assay on rainbow trout, *Oncorhynchus mykiss*, pigmentation in comparison with synthetic astaxanthin and canthaxanthin. Aquaculture 112:217–226. https://doi.org/10.1016/0044-8486(93)90447-7
Christenson L, Sims R (2011) Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol Adv 29:686–702
Comhaire FH, El Garem Y, Mahmoud A, Eertmans F, Schoonjans F (2005) Combined conventional/ antioxidant "Astaxanthin" treatment for male infertility: a double blind randomized trial. Asian J Androl 7:257–262. https://doi.org/10.1111/j.1745-7262.2005.00047.x
Craggs RJ, Heubeck SM, Lundquist TJ, Benemann J (2011) Algal biofuels from wastewater treatment high rate algal ponds. Water Sci Technol 63:660–665
Dela Noue J, Laliberte G, Proulx D (1992) Algae and wastewater. J Appl Phycol 4:247–254
Del Río E, Acién FG, García-Malea MC, Rivas J, Del Rio E, Acién FG, García-Malea MC, Rivas J, Molina-Grima E, Guerrero MG (2005) Efficient one-step production of astaxanthin by the microalga *Haematococcus pluvialis* in continuous culture. Biotechnol Bioeng 91:808–815
Del Rio E, Acien FG, Garcia-Malea MC, Rivas J, Molina-Grima E, Guerrero MG (2007) Efficiency assessment of the one-step production of astaxanthin by the microalga *Haematococcus pluvialis*. Biotechnol Bioeng 100:397–402. https://doi.org/10.1002/bit.21770
Dickinson KE, Whitney CG, McGinn PJ (2013) Nutrient remediation rates in municipal wastewater and their effect on biochemical composition of the microalga *Scenedesmus* sp AMDD. Algal Res 2:127–134
Ding J, Zhao F, Cao Y, Xing L, Liu W, Mei S, Li S (2015) Cultivation of microalgae in dairy farm wastewater without sterilization. Int J Phytoremediation 17:222–227
Domínguez-Bocanegra AR, Guerrero Legarreta I, Martinez Jeronimo F, Tomasini Campocosio A (2004) Influence of environmental and nutritional factors in the production of astaxanthin from *Haematococcus pluvialis*. Bioresour Technol 92:209–214. https://doi.org/10.1016/j.biortech.2003.04.001
Elgarem Y, Lignell A, Comhaire FH (2002) Supplementation with Astaxanthin (Astacarox) improves semen quality in infertile men. In: Proceedings of the 13th international carotenoid symposium, Honolulu, pp 180–197
Elwinger K, Lignell A, Wilhelmson M (1997) Astaxanthin rich algal meal (*Haematococcus pluvialis*) as carotenoid source in feed for laying hens. In: Proceedings of the VII European symposium on the quality of eggs and egg products, Poznan, pp 52–59
European Commission on Environmnet (2002) Heavy metals in wastes. COWI A/S, Copenhagen
Eyster C (1964) Micronutrient requirements for green plants, especially algae. In: Algae and man. Springer, pp 86–119
Fábregas J, Domínguez A, Regueiro M, Maseda A, Otero A (2000) Optimization of culture medium for the continuous cultivation of the microalga *Haematococcus pluvialis*. Appl Microbiol Biotechnol 53:530–535. https://doi.org/10.1007/s002530051652
Fábregas J, Otero A, Maseda A, Domínguez A (2001) Two-stage cultures for the production of astaxanthin from *Haematococcus pluvialis*. J Biotechnol 89:65–71. https://doi.org/10.1016/S0168-1656(01)00289-9
Fan L, Vonshak A, Boussiba S (1994) Effect of temperature and irradiance on growth of *Haematococcus pluvialis*(Chlorophyceae). J Phycol 30:829–833. https://doi.org/10.1111/j.0022-3646.1994.00829.x

Farooq W, Lee Y, Ryu B, Kim B, Kim H, Choi Y, Yang J (2013) Two-stage cultivation of two Chlorella sp. strains by simultaneous treatment of brewery wastewater and maximizing lipid productivity. Bioresour Technol 132:230–238

Fenton O, hUallachain DO (2012) Agricultural nutrient surpluses as potential input sources to grow third generation biomass (microalgae): a review. Algal Res 1:49–56

García-Malea MC, Acién FG, Del Río E, Fernández JM, Cerón MC, Guerrero MG (2009) Production of astaxanthin by *Haematococcus pluvialis*: taking the one-step system outdoors. Biotechnol Bioeng 102:651–657. https://doi.org/10.1002/bit.22076

Gong XD, Feng C (1997) Optimization of culture medium for growth of *Haematococcus pluvialis*. J Appl Phycol 9:437–444

Gouveia L, Graça S, Sousa C, Ambrosano L, Ribeiro B, Botrel EP, Neto PC, Ferreira AF, Silva CM (2016) Microalgae biomass production using wastewater: treatment and costs: scale-up considerations. Algal Res 16:167–176

Guerin M, Huntley ME, Olaizola M (2003) Haematococcus astaxanthin: applications for human health and nutrition. Trends Biotechnol 21(5):210–216

Han D, Li Y, Hu Q (2013) Biology and commercial aspects of *Haematococcus pluvialis*. In: Richmond A, Hu Q (eds) Handbook of microalgal culture: applied phycology and biotechnology, 2nd edn. Blackwell, Hoboken, pp 388–405

Haque F, Dutta A, Thimmanagari M, Chiang YW (2016a) Integrated *Haematococcus pluvialis* biomass production and nutrient removal using bioethanol plant waste effluent. Process Saf Environ Prot 111:128–137

Haque F, Dutta A, Thimmanagari M, Chiang YW (2016b) Intensified green production of astaxanthin from *Haematococcus pluvialis*. Food Bioprod Process 99:1–11

Harker M, Tsavalos AJ, Young AJ (1996) Autotrophic growth and carotenoid production of *Haematococcus pluvialis* in a 30 liter air-lift photobioreactor. J Ferment Bioeng 82:113–118

Hata N, Ogbonna JC, Hasegawa Y, Taroda H, Tanaka H (2001) Production of astaxanthin by *Haematococcus pluvialis* in a sequential heterotrophic-photoautotrophic culture. J Appl Phycol 13:395–402. https://doi.org/10.1023/A:1011921329568

He P, Duncan J, Barber J (2007) Astaxanthin accumulation in the green alga *Haematococcus pluvialis*: effects of cultivation parameters. J Integr Plant Biol 49:447–451. https://doi.org/10.1111/j.1744-7909.2007.00468.x

Higuera-Ciapara I, Felix-Valenzuela L, Goycoolea FM (2006) Astaxanthin: a review of its chemistry and applications. *Crit Rev Food Sci Nutr* 46:185–196. https://doi.org/10.1080/10408690590957188

Hu Z, Li Y, Sommerfeld M, Chen F, Hu Q (2008) Enhanced protection against oxidative stress in an astaxanthin-overproduction *Haematococcus* mutant (Chlorophyceae). Eur J Phycol 43:365–376. https://doi.org/10.1080/09670260802227736

Hussein G, Sankawa U, Goto H, Matsumoto K, Watanabe H (2006) Astaxanthin, a carotenoid with potential in human health and nutrition. J Nat Prod 69:443–449. https://doi.org/10.1021/np050354+

Inbbor J (1998) *Haematococcus*, the poultry pigmentor. Feed Mix 6:31–34

Inborr J, Lignell Å (1997) Effect of feeding astaxanthin-rich algae meal (*Haematococcus pluvialis*) on performance and carotenoid concentration of different tissues of broiler chickens. In: Proceedings of the XIII WPSA conference on Poultry meat quality in Poznan, Poland, pp 39–43

Industry Experts (2015) Global Astaxanthin market – sources, Technologies and Applications. Available online at: http://industry-experts.com/verticals/healthcare-and-pharma/global-astaxanthin-market-sources-technologies-and-applications

Issarapayup K, Powtongsook S, Pavasant P (2009) Flat panel airlift photobioreactors for cultivation of vegetative cells of microalga *Haematococcus pluvialis*. J Biotechnol 142:227–232. https://doi.org/10.1016/j.jbiotec.2009.04.014

Iwamoto T, Hosoda K, Hirano R, Kurata H, Matsumoto A, Miki W (2000) Inhibition of low-density lipoprotein oxidation by astaxanthin. J Atheroscler Thromb 7:216–222. https://doi.org/10.5551/jat1994.7.216

Ji MK, Abou-Shanab RAI, Kim SH, Salama ES, Lee SH, Kabra AN, Lee YS, Hong S, Jeon BH (2013) Cultivation of microalgae species in tertiary municipal wastewater supplemented with CO2 for nutrient removal and biomass production. Ecol Eng 58:142–148

Kabra AN, Salama el S, Roh HS, Kim JR, Lee DS, Jeon BH (2014) Effect of mine wastewater on nutrient removal and lipid production by a green microalga *Micractinium reisseri* from concentrated municipal wastewater. Bioresour Technol 157:84–90

Kaewpintong K, Shotipruk A, Powtongsook S, Pavasant P (2007) Photoautotrophic high-density cultivation of vegetative cells of *Haematococcus pluvialis* in airlift bioreactor. Bioresour Technol 98:288–295. https://doi.org/10.1016/j.biortech.2006.01.011

Kakizono T, Kobayashi M, Nagai S (1992) Effect of carbon/nitrogen ratio on encystment accompanied with astaxanthin formation in a green alga *Haematococcus pluvialis*. J Ferment Bioeng 74:403–405

Kamath BS, Srikanta BM, Dharmesh SM, Sarada R, Ravishankar GA (2008) Ulcer preventive and antioxidative properties of astaxanthin from *Haematococcus pluvialis*. Eur J Pharmacol 590:387–395. https://doi.org/10.1016/j.ejphar.2008.06.042

Kang CD, Lee JS, Park TH, Sim SJ (2005) Comparison of heterotrophic and photoautotrophic induction on astaxanthin production by *Haematococcus pluvialis*. Appl Microbiol Biotechnol 68:237–241. https://doi.org/10.1007/s00253-005-1889-2

Kang CD, An JM, Park TH, Sim SJ (2006) Astaxanthin biosynthesis from simultaneous N and P uptake by the green alga *Haematococcus pluvialis* in primary-treated wastewater. Biochem Eng J 31:234–238

Kang CD, Lee D, Park J, Sim S (2007) Complementary limiting factors of astaxanthin synthesis during photoautotrophic induction of *Haematococcus pluvialis*: C/N ratio and light intensity. Appl Microbiol Biotechnol 74:987–994

Kang CD, Han SJ, Choi SP, Sim SJ (2010) Fed-batch culture of astaxanthin-rich *Haematococcus pluvialis* by exponential nutrient feeding and stepwise light supplementation. Bioprocess Biosyst Eng 33:133–139. https://doi.org/10.1007/s00449-009-0362-5

Karppi J, Rissanen TH, Nyyssönen K, Kaikkonen J, Olsson AG, Voutilainen S (2007) Effects of astaxanthin supplementation on lipid peroxidation. Int J Vitam Nutr Res 77:3–11. https://doi.org/10.1024/0300-9831.77.1.3

Kobayashi M, Kakizono T, Nagai S (1991) Astaxanthin production by a green alga, *Haematococcus pluvialis* accompanied with morphological changes in acetate media. J Ferment Bioeng 71:335–339. https://doi.org/10.1016/0922-338X(91)90346-I

Kobayashi M, Kakizono T, Nagai S (1993) Enhanced carotenoid biosynthesis by oxidative stress in acetateinduced cyst cells of a green unicellular alga, *Haematococcus pluvialis*. Appl Environ Microbiol 59:867–873

Kobayashi M, Kurimura Y, Tsuji Y (1997) Light-independent, astaxanthin production by the green microalga *Haematococcus pluvialis* under salt stress. Biotechnol Lett 19:507–509

Koller M, Muhr A, Braunegg G (2014) Microalgae as versatile cellular factories for valued products. Algal Res 6:52–63. https://doi.org/10.1016/j.algal.2014.09.002

Kumar A, Ergas S, Yuan X, Sahu A, Zhang Q, Dewulf J, Xavier F, Langenhove MHV (2010) Enhanced CO2 fixation and biofuel production via microalgae: recent developments and future directions. Trends Biotechnol 28:371–380

Kupcinskas L, Lafolie P, Lignell A, Kiudelis G, Jonaitis L, Adamonis K (2008) Efficacy of the natural antioxidant astaxanthin in the treatment of functional dyspepsia in patients with or without *Helicobacter pylori* infection: a prospective, randomized, double blind, and placebo-controlled study. Phytomedicine 15:391–399. https://doi.org/10.1016/j.phymed.2008.04.004

Lababpour A, Shimahara K, Hada K, Kyoui Y, Katsuda T, Katoh S (2005) Fedbatch culture under illumination with blue light emitting diodes (LEDs) for astaxanthin production by *Haematococcus pluvialis*. J Biosci Bioeng 100:339–342. https://doi.org/10.1263/jbb.100.339

Larsdotter K (2006) Wastewater treatment with microalgae-a literature review. Vatten 62:31

Lau PS, Tam NFY, Wong YS (1995) Effect of algal density on nutrient removal from primary settled wastewater. Environ Pollut 89:59–66

Ledda C, Villegas GR, Adani F, Fernández FA, Grima EM (2015) Utilization of centrate from wastewater treatment for the outdoor production of *Nannochloropsis gaditana* biomass at pilot-scale. Algal Res 12:17–25

Li Y, Sommerfeld M, Chen F, Hu Q (2010) Effect of photon flux densities on regulation of carotenogenesis and cell viability of *Haematococcus pluvialis* (Chlorophyceae). J Appl Phycol 22:253–263. https://doi.org/10.1007/s10811-009-9453-6

Li J, Zhu DL, Niu J, Shen SD, Wang G (2011) An economic assessment of astaxanthin production by large scale cultivation of *Haematococcus pluvialis*. Biotechnol Adv 29:568–574. https://doi.org/10.1016/j.biotechadv.2011.04.001

Li M, Wu W, Zhou P, Xie F, Zhou Q, Kangsen Mai K (2014) Comparison effect of dietary astaxanthin and *Haematococcus pluvialis* on growth performance, antioxidant status and immune response of large yellow croaker *Pseudosciaena crocea*. Aquaculture 434:227–232. https://doi.org/10.1016/j.aquaculture.2014.08.022

Lignell A, Inborr J (1999) Agent for increasing the production of/in breeding and production mammals. Varmdo: European patent. EP0912106

Lignell ANGK, Inborr J (2000) Agent for increasing the production of/in breeding and production mammals. Varmdo: United States patent and trademark office granted patent. WO97/35491

Lim S, Chu W, Phang S (2010) Use of *Chlorella vulgaris* for bioremediation of textile wastewater. J Bioresour Technol 101:7314–7322

Liu Y (2018) Optimization study of biomass and astaxanthin production by *Haematococcus pluvialis* under minkery wastewater cultures. Masters Thesis, Dalhousie University, Halifax, Nova Scotia, p 139

Liu BH, Lee YK (2003) Effect of total secondary carotenoids extracts from Chlorococcum sp. on Helicobacter pylori-infected BALB/c mice. Int Immunopharmacol 3:979–986. https://doi.org/10.1016/S1567-5769(03)00096-1

Liu X, Osawa T (2007) Cis astaxanthin and especially 9-cis astaxanthin exhibits a higher antioxidant activity *in vitro* compared to the all-trans isomer. Biochem Biophys Res Commun 357:187–193. https://doi.org/10.1016/j.bbrc.2007.03.120

Liu Y, Yildiz I (2018) The effect of salinity concentration on algal biomass production and nutrient removal from municipal wastewater by *Dunaliella salina*. Int J Energy Res 42:2997

Lopez MCGM, Sanchez ED, Lopez JLC, Fernandez FGA, Sevilla JMF, Rivas J, Guerrero MG, Grima EM (2006) Comparative analysis of the outdoor culture of *Haematococcus pluvialis* in tubular and bubble column photobioreactors. J Biotechnol 123:329–342

Lorenz RT (1999) A technical review of Haematococcus algae. NatuRose™ Technical Bulletin #060. Cyanotech Corporation, Kailua-Kona

Markou G, Georgakakis D (2011) Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: a review. Appl Energy 88:3389–3401

Matamoros V, Rodriguez Y, Albaiges J (2016a) A comparative assessment of intensive and extensive wastewater treatment technologies for removing emerging contaminants in small communities. Water Res 88:777–785

Matamoros V, Uggetti E, García J, Bayona JM (2016b) Assessment of the mechanisms involved in the removal of emerging contaminants by microalgae from wastewater: a laboratory scale study. J Hazard Mater 301:197–205

McGinn PJ, Dickinson KE, Bhatti S, Frigon JC, Guiot SR, O'Leary SJ (2011) Integration of microalgae cultivation with industrial waste remediation for biofuel and bioenergy production: opportunities and limitations. Photosynth Res 109:231–247

Mehta CM, Khunjar WO, Nguyen V, Tait S, Batstone DJ (2015) Technologies to recover nutrients from waste streams: a critical review. Crit Rev Environ Sci Technol 45:385–427

Miyawaki H, Takahashi J, Tsukahara H, Takehara I (2008) Effects of astaxanthin on human blood rheology. J Clin Biochem Nutr 43:69–74. https://doi.org/10.3164/jcbn.2008048

Nagata A, Tajima T, Takahashi J (2006) Effect of astaxanthin 5 mg on anti fatigue and task performance of human. Carotenoid Sci 10:102–106

Naguib YMA (2000) Antioxidant activities of astaxanthin and related carotenoids. J Agric Food Chem 48:1150–1154. https://doi.org/10.1021/jf991106k

Nakao R, Nelson OL, Park JS, Mathison BD, Thompson PA, Chew BP (2010) Effect of astaxanthin supplementation on inflammation and cardiac function in BALB/c mice. Anticancer Res 30:2721–2725

Neveux N, Magnusson M, Mata L, Whelan A, de Nys R, Paul NA (2016) The treatment of municipal wastewater by the macroalga Oedogonium sp and its potential for the production of biocrude. Algal Res 13:284–292

Nishikawa Y, Minenaka Y, Ichimura M, Tatsumi K, Nadamoto T, Urabe K (2005) Effects of astaxanthin and vitamin C on the prevention of gastric ulcerations in stressed rats. J Nutr Sci Vitaminol (Tokyo) 51:135–141. https://doi.org/10.3177/jnsv.51.135

Olaizola M (2000) Commercial production of astaxanthin from *Haematococcus pluvialis* using 25,000-liter outdoor photobioreactors. J Appl Phycol 12:499–506

Orosa M, Torres E, Fidalgo P, Abalde J (2000) Production and analysis of secondary carotenoids in green algae. J Appl Phycol 12:553–556. https://doi.org/10.1023/A:1008173807143

Orosa M, Franqueira D, Cid A, Abalde J (2005) Analysis and enhancement of astaxanthin accumulation in *Haematococcus pluvialis*. Bioresour Technol 96:373–378

Palozza P, Torelli C, Boninsegna A, Simone R, Catalano A, Mele MC (2009) Growth-inhibitory effects of the astaxanthin-rich alga *Haematococcus pluvialis* in human colon cancer cells. Cancer Lett 283:108–117. https://doi.org/10.1016/j.canlet.2009.03.031

Panis G (2015) Commercial Astaxanthin production derived by green alga *Haematococcus pluvialis*: a microalgae process model and a techno-economic assessment all through production line. Master Thesis, Utrecht University, Netherlands

Parisenti J, Beirao LH, Maraschin M, Mourino JL, Nascimento Viera F, Do Nascimento Vieira F, Bedin LH (2011) Pigmentation and carotenoid content of shrimp fed with *Haematococcus pluvialis* and soy lecithin. Aquac Nutr 17:530–535. https://doi.org/10.1111/j.1365-2095.2010.00794.x

Park J, Craggs R, Shilton A (2011) Wastewater treatment high rate algal ponds for biofuel production. Bioresour Technol 102:35–42

Park JC, Choi SP, Hong ME, Sim SJ (2014) Enhanced astaxanthin production from microalga, *Haematococcus pluvialis* by two-stage perfusion culture with stepwise light irradiation. Bioprocess Biosyst Eng 37:2039–2047. https://doi.org/10.1007/s00449-014-1180-y

Pérez-López P, González-García S, Jeffryes C, Agathos SN, McHugh E, Walsh D (2014) Life-cycle assessment of the production of the red antioxidant carotenoid astaxanthin by microalgae: from lab to pilot scale. J Clean Prod 64:332–344

Pittman JK, Dean AP, Osundeko O (2011) The potential of sustainable algal biofuel production using wastewater resources. Bioresour Technol 102:17–25

Querques N, Cesta M, Santos RM, Chiang YW (2015) Microalgal phycocyanin productivity: strategies for phyco-valorization. J Chem Technol Biotechnol 90:1968–1982. https://doi.org/10.1002/jctb.4796

Ranga Rao A, Harshvardhan Reddy A, Aradhya SM (2010) Antibacterial properties of *Spirulina platensis, Haematococcus pluvialis, Botryococcus braunii* micro algal extracts. Curr Trends Biotechnol Pharm 4:809–819

Ranga Rao A, Sindhuja HN, Dharmesh SM, Sankar KU, Sarada R, Ravishankar GA (2013) Effective inhibition of skin cancer, tyrosinase, and antioxidative properties by astaxanthin and astaxanthin esters from the green alga *Haematococcus pluvialis*. J Agric Food Chem 61:3842–3851. https://doi.org/10.1021/jf304609j

Ranga Rao A, Siew Moi P, Ravi S, Aswathanarayana RG (2014) Astaxanthin: sources, extraction, stability, biological activities and its commercial applications—a review. Mar Drugs 12:128–152

Ranjbar R, Inoue R, Shiraishi H, Katsuda T, Katoh S (2008) High efficiency production of astaxanthin by autotrophic cultivation of *Haematococcus pluvialis* in a bubble column photobioreactor. Biochem Eng J 39:575–580. https://doi.org/10.1016/j.bej.2007.11.010

Razon LF, Tan RR (2011) Net energy analysis of the production of biodiesel and biogas from the microalgae: Haematococcus pluvialis and Nannochloropsis. Appl Energy 88:3507–3514

Rippka R, Deruelles J, Waterbury M, Herdman M, Stanier R (1979) Generic assignments, strain histories and properties of pure culture of cyanobacteria. J Gen Microbiol 111:1–61. https://doi.org/10.1099/00221287-111-1-1

Rogers JN, Rosenberg JN, Guzman BJ, Oh VH, Mimbela LE, Ghassemi A, Betenbaugh MJ, Oyler GA, Donohue MD (2014) A critical analysis of paddlewheel-driven raceway ponds for algal biofuel production at commercial scales. Algal Res 4:76–88

Ruen-ngam D, Shotipruk A, Pavasant P (2010) Comparison of extraction methods for recovery of Astaxanthin from *Haematococcus pluvialis*. Sep Sci Technol 46:64–70

Ruiz-Marin A, Mendoza-Espinosa LG, Stephenson T (2010) Growth and nutrient removal in free and immobilized green algae in batch and semi-continuous cultures treating real wastewater. Bioresour Technol 101:58–64

Saha SK, McHugh E, Hayes J, Moane S, Walsh D, Murray P (2013) Effect of various stressregulatory factors on biomass and lipid production in microalga *Haematococcus pluvialis*. Bioresour Technol 128:118–124. https://doi.org/10.1016/j.biortech.2012.10.049

Salama ES, Kurade MB, Abou-Shanab RAI, El-Dalatony MM, Yang IS, Min B, Jeon BH (2017) Recent progress in microalgal biomass production coupled with wastewater treatment for biofuel generation. Renew Sust Energ Rev 79:1189–1211

Sarada R, Bhattacharya S, Ravishankar GA (2002a) Optimization of culture conditions for growth of the green alga *Haematococcus pluvialis*. World J Microbiol Biotechnol 18:517–521. https://doi.org/10.1023/A:1016349828310

Sarada R, Tripathi U, Ravishankar GA (2002b) Influence of stress on astaxanthin production in *Haematococcus pluvialis* grown under different culture conditions. Process Biochem 37:623–627. https://doi.org/10.1016/S0032-9592(01)00246-1

Sato H, Nagare H, Huynh TNC, Komatsu H (2015) Development of a new wastewater treatment process for resource recovery of carotenoids. Water Sci Technol 72:1191–1197

Seki T, Sueki H, Kono H, Suganuma K, Yamashita E (2001) Effects of astaxanthin from *Haematococcus pluvialis* on human skin-patch test; skin repeated application test; effect on wrinkle reduction. Fragrance J 12:98–103

Shah MMR, Liang Y, Cheng JJ, Daroch M (2016) Astaxanthin-producing green microalga *Haematococcus pluvialis*: from single cell to high value commercial products. Front Plant Sci 7:531

Sheikhzadeh N, Panchah IK, Asadpour R, Tayefi-Nasrabadi H, Mahmoudi H (2012a) Effects of *Haematococcus pluvialis* in maternal diet on reproductive performance and egg quality in rainbow trout (*Oncorhynchus mykiss*). Anim Reprod Sci 130:119–123

Sheikhzadeh N, Tayefi-Nasrabadi H, Oushani AK, Najafi Enferadi MH (2012b) Effects of *Haematococcus pluvialis* supplementation on antioxidant system and metabolism in rain- bow trout (*Oncorhynchus mykiss*). Fish Physiol Biochem 38:413–419

Shen Y (2014) Carbon dioxide bio-fixation and wastewater treatment via algae photochemical synthesis for biofuels production. RSC Adv 4:49672–49722

Solovchenko A (2013) Physiology and adaptive significance of secondary carotenogenesis in green microalgae. Russ J Plant Physiol 60:1–13

Sommer TR, Potts WT, Morrissy NM (1991) Utilization of microalgal astaxanthin by rainbow trout (*Oncorhynchus mykiss*). Aquaculture 94:79–88

Stumm W, Morgan JJ (1970) Aquatic chemistry; an introduction emphasizing chemical equilibria in natural waters. Willey-VCH Verlag GmbH & Co. KGaA, Weinheim

Tjahjono AE, Hayama Y, Kakizono T, Terada Y, Nishio N, Nagai S (1994) Hyper accumulation of astaxanthin in a green alga *Haematococcus pluvialis* at elevated temperatures. Biotechnol Lett 16:133–138. https://doi.org/10.1007/BF01021659

Tolasa S, Cakli S, Ostermeyer U (2005) Determination of astaxanthin and canthaxanthin in salmonid. Eur Food Res Technol 221:787–791. https://doi.org/10.1007/s00217-005-0071-5

Tominaga K, Hongo N, Karato M, Yamashita E (2012) Cosmetic benefits of astaxanthin on humans subjects. Acta Biochim Pol 59:43–47

Torrissen OJ, Naevdal G (1984) Pigmentation of salmonids — genetical variation in carotenoid deposition in rainbow trout. Aquaculture 38:59–66

Torzillo G, Goksan T, Faraloni C, Kopecky J, Masojidek J (2003) Interplay between photochemical activities and pigment composition in an outdoor culture of *Haematococcus pluvialis* during the shift from the green to red stage. J Appl Phycol 15:127–136

Tripathi U, Sarada R, Ramachandra Rao S, Ravishankar GA (1999) Production of astaxanthin in *Haematococcus pluvialis* cultured in various media. Bioresour Technol 68:197–199. https://doi.org/10.1016/S0960-8524(98)00143-6

Wan M, Zhang J, Hou D, Fan J, Li Y, Huang J (2014a) The effect of temperature on cell growth and astaxanthin accumulation of *Haematococcus pluvialis* during a light–dark cyclic cultivation. Bioresour Technol 167:276–283. https://doi.org/10.1016/j.biortech.2014.06.030

Wan M, Hou D, Li Y, Fan J, Huang J, Liang S (2014b) The effective photoinduction of *Haematococcus pluvialis* for accumulating astaxanthin with attached cultivation. Bioresour Technol 163:26–32. https://doi.org/10.1016/j.biortech.2014.04.017

Wang P (2014) Culture medium and culture method for culturing *Haematococcus pluvialis* by using brewery wastewater. Patent no CN103966103A

Wang B, Zarka A, Trebst A, Boussiba S (2003) Astaxanthin accumulation in *Haematococcus pluvialis*(Chlorophyceae) as an active photoprotective process under high irradiance. J Phycol 39:1116–1124

Wang JF, Han DX, Sommerfeld MR, Lu CM, Hu Q (2013a) Effect of initial biomass density on growth and astaxanthin production of *Haematococcus pluvialis* in an outdoor photobioreactor. J Appl Phycol 25:253–260. https://doi.org/10.1007/s10811-012-9859-4

Wang JF, Sommerfeld MR, Lu CM, Hu Q (2013b) Combined effect of initial biomass density and nitrogen concentration on growth and astaxanthin production of *Haematococcus pluvialis* (Chlorophyta) in outdoor cultivation. Algae 28:193–202

Wayama M, Ota S, Matsuura H, Nango N, Hirata A, Kawano S (2013) Three-dimensional ultrastructural study of oil and astaxanthin accumulation during encystment in the green alga *Haematococcus pluvialis*. PLoS One 8:e53618. https://doi.org/10.1371/journal.pone.0053618

Wilkie AC, Mulbry WW (2002) Recovery of dairy manure nutrients by benthic freshwater algae. Bioresour Technol 84:81–91

Woertz IC, Benemann JR, Du N, Unnasch S, Mendola D, Mitchell BG, Lundquist TJ (2014) Life cycle GHG emissions from microalgal biodiesel–a CA-GREET model. Environ Sci Technol 48:6060–6068

Wu YH, Yang J, Hu HY, Yu Y (2013) Lipid-rich microalgal biomass production and nutrient removal by *Haematococcus pluvialis* in domestic secondary effluent. Ecol Eng 60:155–159. https://doi.org/10.1016/j.ecoleng.2013.07.066

Yamashita E (2002) Cosmetic benefit of dietary supplements containing astaxanthin and tocotrienol on human skin. Food Style 21:112–117

Yang Y, Kim B, Lee JY (2013) Astaxanthin structure, metabolism, and health benefits. J Hum Nutr Food Sci 1003:1–11

Yin S, Wang J, Chen L, Liu T (2015) The water footprint of biofilm cultivation of *Haematococcus pluvialis* is greatly decreased by using sealed narrow chambers combined with slow aeration rate. Biotechnol Lett 37:1819–1827

Yoo JJ, Choi SP, Kim BW, Sim SJ (2012) Optimal design of scalable photobioreactor for phototropic culturing of *Haematococcus pluvialis*. Bioprocess Biosyst Eng 35:309–315

Yu X, Chen L, Zhang W (2015) Chemicals to enhance microalgal growth and accumulation of high-value bioproducts. Front Microbiol 6:56

Yuan JP, Peng J, Yin K, Wang JH (2011) Potential health promoting effects of astaxanthin: a high-value carotenoid mostly from microalgae. Mol Nutr Food Res 55:150–165

Zhang B, Geng Y, Li Z, Hu H, Li Y (2009) Production of astaxanthin from *Haematococcus* in open pond by two-stage growth one-step process. Aquaculture 295:275–281

Zhang W, Wang J, Wang J, Liu T (2014) Attached cultivation of *Haematococcus pluvialis* for astaxanthin production. Bioresour Technol 158:329–335