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Advances in cultivation, wastewater treatment application, bioactive components of *Caulerpa lentillifera* and their biotechnological applications

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The edible seaweed *Caulerpa lentillifera*, a powerful natural food source that is rich in protein, minerals, dietary fibers, vitamins, saturated fatty acids and unsaturated fatty acids, has been mass cultured in some Asian countries and has been the focus of researchers in recent years. Here, the operational conditions of its culture, application in wastewater treatment, and bioactive components are summarized and comparatively analyzed. Based on previous studies, salinity, nutrient concentrations, irradiance and temperature are stress factors for algal growth. Moreover, dried *Caulerpa lentillifera* seaweed is efficient in the biosorption of heavy metals and cationic dyes in wastewater, and fresh seaweed can be introduced as a biofilter in aquaculture system treatment. In addition, among the rich bioactive compounds in *Caulerpa lentillifera*, the phenolic compounds show the potential ability for regulating glucose metabolism in vivo. Polysaccharides and oligosaccharides exhibit anticoagulant, immunomodulatory effects and cancer-preventing activity. Siphonaxanthin is a compound with attractive novel functions in cancer-preventing activity and lipogenesis-inhibiting effects. Furthermore, the antioxidant activity of siphonaxanthin extracted from *Caulerpa lentillifera* could be stronger than that of astaxanthin. This review offers an overview of studies of *Caulerpa lentillifera* addressing various aspects including cultivation, wastewater treatment and biological active components which may provide valuable information for the cultivation and utilization of this green alga.

Advances in cultivation, wastewater treatment application，bioactive components of *Caulerpa lentillifera* and their biotechnological applications

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Abstract:

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The edible seaweed *Caulerpa lentillifera*, a powerful natural food source that is rich in protein, minerals, dietary fibers, vitamins, saturated fatty acids and unsaturated fatty acids, has been mass cultured in some Asian countries and has been the focus of researchers in recent years. Here, the operational conditions of its culture, application in wastewater treatment, and bioactive components are summarized and comparatively analyzed. Based on previous studies, salinity, nutrient concentrations, irradiance and temperature are stress factors for algal growth. Moreover, dried *Caulerpa lentillifera* seaweed is efficient in the biosorption of heavy metals and cationic dyes in wastewater, and fresh seaweed can be introduced as a biofilter in aquaculture system treatment. In addition, among the rich bioactive compounds in *Caulerpa lentillifera*, the phenolic compounds show the potential ability for regulating glucose metabolism in vivo. Polysaccharides and oligosaccharides exhibit anticoagulant, immunomodulatory effects and cancer-preventing activity. Siphonaxanthin is a compound with attractive novel functions in cancer-preventing activity and lipogenesis-inhibiting effects. Furthermore, the antioxidant activity of siphonaxanthin extracted from *Caulerpa lentillifera* could be stronger than that of astaxanthin. This review offers an overview of studies of *Caulerpa lentillifera* addressing various aspects including cultivation, wastewater treatment and biological active components which may provide valuable information for the cultivation and utilization of this green alga.

1. Introduction

Fig. 1 *Caulerpa lentillifera* grown in Huang Hai, China（photographed by XiaolinChen）

As shown in Fig. 1, *Caulerpa lentillifera*, a green seaweed with high economic value, is naturally distributed in tropical and subtropical regions, such as the south China Sea, Southeast Asia, Japan, Okinawa, Taiwan and Oceania (*Paul et al., 2014*). As reported in the literature, this green seaweed was documented for the first time on the Red Sea coast (*Agardh, 1837*), and then it was observed at many other locations, especially in the Indo-Pacific region (*Hackett, 1977; Taylor, 1977; Menez and Calumpong, 1982; Coppejans and Beeckman, 1989; Phillips et al., 1999; Schils and Coppejans, 2003; Titlyanov et al., 2012*). Because its upright branches resemble grapes, *C. lentillifera* is also called "sea grapes" (*Guo et al., 2015a*), and it can grow on sand and rock bottoms in the upper sublittoral zone of tropical coral reefs (*Horstmann, 1983; Mao et al., 2011*). Because of its good texture, *C. lentillifera* is often cooked as a salad in some Asian countries. In addition, *C. lentillifera* is rich in polyunsaturated fatty acids (PUFAs) (*Saito et al., 2010*), multiple essential amino acids, minerals, dietary fibers, vitamin A and Vitamin C (*Matanju et al., 2009*) and has low levels of lipids (*Niwano et al., 2009*). Therefore, there has been increasing demand and rising market prices for *C. lentillifera* in some Asian countries in recent years. However, although this alga is widely cultivated in the Philippines (*Zemkewhite and Ohno, 1999*), Okinawa (*Kurashima et al., 2003*), Taiwan Island (*Shi, 2008*), Fujian and Hainan provinces in China (*Wang, 2011*), the commercial-scale production of *C. lentillifera* is still not sufficient, and its productivity does not meet the demand. This might be due to lack optimum cultivation conditions of the alga. Therefore, the mass culturing of *C. lentillifera* with high productivity is quite necessary.

Currently, the main research focuses on the treatment of wastewater and the use of bioactive

components. *C. lentillifera* has shown the potential ability to remove basic dyes from waste streams (*Marungrueng and Pavasant, 2006*), heavy metals from industrial wastewater (*Pavasant et al., 2006; Apiratikul and Pavasant, 2008*), and nutrients from aquaculture effluents (*Paul and De Nys, 2008*), especially NO_3-N (*Guo et al., 2015b*).

In recent years, some bioactive components of *C. lentillifera*, such as polysaccharides and pigments, and their biological potentials, including antioxidant, anti-diabetic and anticancer activities, have been documented. Therefore, in order to further understand and make better use of this seaweed, we summarized studies related to some of its aspects: its cultivation conditions, wastewater treatment abilities, and bioactive components along with their biological activity.

2. Methods

2.1 Survey Methodology

Two main databases were used to identify available literature including Web of Science and Google Scholar from the time 1900-2018. The references of selected papers were listed after the acknowledgement. From the previous researches, the results were reviewed.

2.2 Cultivation conditions for *C. lentillifera*

Table 1 The effect of cultivation conditions on *C. lentillifera*

According to the previous literature（Shown in Table 1）, we concluded that salinity, the nutrient concentration, irradiance and temperature were all stress factors for growth during all periods，when these factors changed and they will affect the physiology of the alga. Therefore,

it is important to study the optimum factors for the massive culture of the alga. Deraxbudsarakom *et al*. (*2003*) suggested that a salinity range of 25-30‰ is suitable for the normal growth of *C. lentillifera* when the alga was cultured by shrimp farm effluent. Wang (*2011*) showed that the maximum growth of *C. lentillifera* supplied by Fujian China occurs at a salinity of approximately 36‰ cultured filtered seawater with addition of salt; later, a study by Guo *et al*. (*2015b*) confirmed this result. C. *lentillifera* transported from Okinawa Japan did not survive at salinities of 15 and 55 cultured by sterile seawater. This study indicated that the specific growth rate (SGR) for *C. lentillifera* was different among the groups. The maximum SGR occurs at a salinity of 35‰, and this result was consistent with the maximum chlorophyll content and the ratio of fluorescence (Fv/Fm). At salinities of 20‰ and 45‰, only stolons regenerated from branches. However, new braches grew from stolons at salinities 30‰-40‰.

Nitrogen (N) and phosphorus (P) are two essential nutrients, and they are the most important nutrients for the biomass of *C. lentillifera*. Deraxbudsarakom *et al*. (*2003*) concluded that a 0.6 mmol/L NO_3-N concentration and N:P ratio of 8:1 were optimal for the rapid growth of *C. lentillifera* with salinity 25-30‰. However, Guo *et al*.(*2015b*) reported that the SGR of *C. lentillifera* transported from Okinawa Japan was the highest at a PO_4-P concentration of 0.1 mmol/L and a NO_3-N concentration of 0.5 mmol/L (an approximate N:P ratio of 5:1, water temperature 25℃ and light of 40umol photons/(cm^2·s)), which was slightly different from the results of Deraxbudsarakom *et al*. (*2003*). In addition to the nitrogen concentration, different oxidation states of N also had effects on the biomass production of *C. lentillifera.* For example, Wang *et al*. (*2017*) used four different nutrient salts ($NaNO_3$, NH_4NO_3, $CO(NH_2)_2$ and

105 NH_4HCO_3) to cultivate *C. lentillifera* supplied by Ocean University of China with temperature
106 27℃, light of 145.45umol photons/(cm^2·s) and salinity 30‰. The results showed that nitrate
107 ($NaNO_3$ and NH_4NO_3) can significantly promote the growth of the alga. Under a concentration
108 of 20 mg/L NH_4NO_3, the relative growth rate of the alga was the highest. In addition, Liu *et al*.
109 (*2016*) indicated that NH_4-N:NO_3-N ratios of 1:1 and 1:5 were the most favorable ratios for the
110 growth of the alga.

111 Different phytohormones, such as gibberellin (GA), 6-benzyl aminopurine (6-BA) and
112 indoleacetic acid (IAA), have also been shown to be efficient for the growth of *C. lentillifera*
113 (Tao *et al*., 2017). The results revealed that 0.8 and 1.4 mg/L 6-BA could induce a relatively high
114 weight gain rate and SGR of *C. lentillifera* and that 11 mg/L GA was the optimal concentration
115 for rapid growth, while IAA showed no obvious effect on the biomass of *C. lentillifera*. In
116 addition, compared to GA, which had no significant effect on the production of crude
117 polysaccharides in *C. lentillifera*, IAA obviously increased the intracellular crude polysaccharide
118 content.

119 Temperature has a major effect on the kinetics of cellular enzymes, and irradiance is an
120 essential source of photosynthetic activity in algae. Hence, the growth of *C. lentillifera* is also
121 induced by optimal temperatures and irradiances at certain degrees. A previous study showed
122 that *C. lentillifera* started to become soft and decay and the productivity decreased sharply when
123 the temperature decreased to 18℃. Moreover, Guo *et al*. (*2015a*) found that the alga reached a
124 maximum of 6.932±0.396% day^{-1} at 27.5℃ and 40 μmol photons m^{-2} s^{-1}. In addition, the
125 authors also found that higher irradiances(40-100umol photons/(m^2·s) could decrease the

chlorophyll content and rbcL expression. An experiment by Wu *et al*. (*2017*) further confirmed that different levels of light quality showed different effects on the growth and photosynthetic pigment contents of *C. lentillifera*. The concrete results showed that the light treatment of a blue/red ratio of 5/1 had significant beneficial effects on the fresh weight/length ratio, the fresh weight of regenerated vertical branches and the diameter of regenerated spherical ramuli. However, the contents of total chlorophyll, chlorophyll a, chlorophyll b and carotenoids significantly increased under full blue light. A comprehensive analysis suggested that 5/1 blue/red ratio and full white treatments were suitable for the indoor culture of *C. lentillifera*.

Besides the above cultivation parameters, the origin of the alga such as different area might lead to the different growth results. However, there was no reference to introduce the research.

With the development of culture research, different applications of *C. lentillifera* have been studied. And wastewater treatment was early studied.

2.3 Wastewater treatment by *C. lentillifera*

Table 2 Different wastewater treatment process by *C. lentillifera*

As mentioned in documents, *C. lentillifera* has been studied as a biosorption material to treat wastewater, such as heavy metal wastewater, toxic dye-contaminated wastewater and aquaculture wastewater(shown in Table 2). There are several advantages to applying seaweeds as a biosorbent, including their wide availability, low cost, high metal sorption capacity, reasonably regular quality, and relatively simple application. Pavasant *et al*. (*2006*) investigated and proved

147 the ability of dried *C. lentillifera* to biosorb Cu^{2+}, Cd^{2+}, Pb^{2+} and Zn^{2+}. Moreover, the removal
148 efficiency of the alga rose with an increased pH of between 2 and 8(temperature 21±2℃), and
149 the sorption process of all metal ions only took 20 min which was much faster than that of
150 alginate/Mauritanian clay(*Ely et al., 2011*). The sorption of heavy metals on the biosorbents
151 mainly included two steps:
152 1) The metal ions were initially taken up onto the surface of the cells;
153 2) They were bioaccumulated within the cells due to the metal uptake metabolism.
154 Step 1 involved passive transport, and it took place quite rapidly, i.e., within 20-30 min, while
155 Step 2 took much longer to complete. In this case, the alga was dried and no longer active, so the
156 sorption could only take place on the surface of the cell, which controlled the whole sorption
157 process. In addition, it took place only within 20 min. Furthermore, the sorption process followed
158 the Langmuir isotherm, and the maximum sorption capacities were $Pb^{2+}>Cu^{2+}>Cd^{2+}>Zn^{2+}$.
159 In another study, the authors (*Apiratikul et al., 2008*) continued to use dried *C. lentillifera* to
160 study the biosorption process of Cu^{2+}, Cd^{2+} and Pb^{2+}, and the sorption kinetics best followed the
161 pseudo second-order kinetic model:
162
163 $$q = \frac{q_e^2 kt}{1+q_e kt} \qquad (1)$$
164

165 In Equation (1), q (mg/g) is the amount of the metal adsorbed at time t (min), q_e ($mmolKg^{-1}$) is
166 the amount of the metal adsorbed at the time of equilibrium, and k is the equilibrium rate
167 constant. The values for q_e, k and R^2 were listed in Table 3.
168 **Table 3 The values for q_e, k and R^2 of Cu^{2+}, Cd^{2+} and Pb^{2+} in pseudo second-order kinetic**

mode

In addition, the sorption isotherm data fit the Langmuir isotherm model:

$$q_e = \frac{q_{maxCe}}{1+bc_e} \quad (2)$$

In Equation (2), q_e represents the amount of metal ion taken up per unit mass of the biomass at equilibrium (mol/kg), q_{max} is the maximum amount of metal ion taken up per unit mass of the biomass (mol/kg), b is the Langmuir affinity constant (m^3/mol), and Ce is the equilibrium concentration of the heavy metal ion in solution (mol/m^3). In addition, according to the Dubinin-Radushkevich model, the sorption energies are 4-6 kJ/mol, as the process involves a physical electrostatic force. Ion exchange is believed to be a principal mechanism of the sorption, and metal ions such as Ca^{2+}, Mg^{2+} and Mn^{2+} are the main ions released from the algal biomass. In addition, the binary component systems composed of Cu^{2+}, Cd^{2+} and Pb^{2+} were also studied in dried *C. lentillifera*. The experimental data could effectively be described by the partial competitive binary isotherm model. In addition, the secondary metal ion always reduced the total sorption capacity of the previous metal ions, which implies that the concomitant metal ions competed for the same pooled binding sites during the algal biomass sorption process, and Pb^{2+} was the most adsorbed metal ion according to the study. The batch scale experiments by fixed bed column also showed that sorption capacities for various metals could also be prioritized with the same order: Pb^{2+}>Cu^{2+}>Cd^{2+}. These results were beneficial for the further design and scaling up of the system (*Apiratikul et al., 2008; Apiratikul and Pavasant, 2006*).

Dried *C. lentillifera* has also been utilized to treat cationic dyes, which are widely used in the textile industry, because dried *C. lentillifera* contains many functional groups (O-H, COOH, NH_2 and S=O) that exhibit chemical binding affinity toward several positively charged ions, these characteristics might also be showed by other algae. Overall, dried *C. lentillifera* was proved to effectively absorb Astrazon Blue FGRL (AB), Astrazon Red GTLN (AR), and methylene blue (MB), and its capacity was greater than that of active carbon (*Marungrueng and Pavasant, 2007*). Some parameters, including the initial dye concentration, pH, temperature, salinity, alga size and dosage, have important effects on the sorption process. In concrete, the adsorption rate constants increased with a decrease in the initial dye concentration. At low dye concentrations (20-80 mg/L), the application of an increasing amount of the alga resulted in a higher percentage of the dye(more than 95%) removed but a lower amount of the dye adsorbed per unit mass (*Marungrueng and Pavasant, 2006*). For methylene blue adsorption, a pH of 7-11 might be appropriate because this pH range can supply advantageous surface binding sites in the alga for the ionization of the dye molecule (*Ncibi et al., 2007*). Marungrueng and Pavasant (*2006*) reported that high temperatures, such as 70℃,could reduce the adsorption of FGRL, while the maximum adsorption capacity was obtained at 50℃(q_m for langmuir was 49.26mg·g^{-1}). In terms of alga size, a small size of 0.1-0.84 mm resulted in the highest adsorption capacity, followed by intermediate (0.84-2.0 mm) and larger sizes (larger than 2.0 mm) because the small size provided the most surface area and total pore volume for the adsorption of the dye. Additionally, salinity was another stress factor in the system, and high salinity caused a decrease in adsorption capacity due to the competition between Na^+ and the dye cations for the binding sites on the algal surface

and electrical repulsion (*Punjongharn et al., 2008*). Furthermore, the kinetic data for the dye adsorption matched the pseudo second-order kinetic model well, and the adsorption isotherms also followed the Langmuir model (*Punjongharn et al., 2008; Marungrueng and Pavasant, 2006; Cengiz et al., 2008*). The sorption process is controlled by both film and pore diffusion (*Marungrueng and Pavasant, 2007*).

Dried *C. lentillifera* can adsorb heavy metals and dyes, and fresh *C. lentillifera* can be used as a biofilter in aquaculture systems because it has a significant capacity for nutrient absorption, especially that of NO_3-N (*Paul and De Nys, 2008; Liu et al., 2016*). *C. lentillifera* was successfully applied at a hatchery scale to a recycling aquaculture system for juvenile spotted babylons (*Babylonia areolata*), and the results revealed that it had a positive effect on the survival rate of spotted babylons, seawater quality and the biomass of *C. lentillifera* (*Chaitanawisuti et al. 2011*). In addition, it has often been cultured in shrimp ponds using water treatment methods (*Chokwiwattanawanit, 2000*).

Besides the application in wastewater treatment, like other algae research, bioactive components of *C. lentillifera* and their bioactive activities development have also been studied in recent years.

2.4 Bioactive components of *C. lentillifera* and their biological potentials

Table 4 Studies on bioactive components of *C. lentillifera*

C. lentillifera contains abundant proteins (10.41% DW（dry weight）), PUFAs

(polyunsaturated fatty acids, 16.76% total fatty acids), and total dietary fiber (32.99% DW) (*Matanjun et al. 2009; Nagappan and Vairappan, 2014*), and the alga is also rich in some bioactive components(shown in Table 4).

The total contents of phenolic compounds in dried *C. lentillifera* differed due to the climate and environment in which the alga grew (*Ito and Hori, 1989*). Nguyen *et al*. (*2011*) reported that the total phenolic content of thermally dried and freeze-dried *C. lentillifera* were 1.30 mg and 2.04 mg gallic acid equivalent (GAE)/g of dry weight, respectively, which were significantly lower than the values reported by Matajun (30.86% of dry weight; *Matajun et al., 2008*). As reported in the literature, the phenolic compounds of *C. lentillifera* are often extracted using ethanol, methanol or diethyl ether and show different biological activities. The methanolic and diethyl ether extracts showed better radical-scavenging activity(2.16mM·mg^{-1} dry extract by TEAC method) and reducing power ability(362.11 uM·mg^{-1} dry extract by FRAP method) than those in other brown and red seaweeds(1.63 mM·mg^{-1} dry extract by TEAC method and 225.00 uM·mg^{-1} dry extract by FRAP method for Eucheuma cottonii; 1.66 mM·mg^{-1} dry extract by TEAC method and 268.86 uM·mg^{-1} dry extract by FRAP method) (*Matajun et al., 2008*). The ethanol extracts had strong hydrogen peroxide-scavenging activities and weak DPPH-scavenging, ferric ion-reducing and FIC activities (*Nguyen et al., 2011*). In addition, the ethanol extracts also stimulated insulin secretion in pancreatic β-cells and enhanced glucose uptake in adipocytes by decreasing dipeptidyl peptidase-Ⅳ, α-glucosidase and protein-tyrosine phosphatase 1B activities using RIN and 3T3-L1 cells as models(*Sharma and Rhyu, 2014; Sharma et al., 2017*) and regulating glucose metabolism via the PI3K/AKT signaling pathway in myocytes using L6 cells

(*Sharma et al., 2015; Abouzid et al., 2014*), which could ameliorate insulin resistance.

Polysaccharides are important components of *C. lentillifera* due to their broad spectrum of biological activity. The crude extract of *C. lentillifera* showed anticoagulant property using albino rabbits and the blood of adult dogs. And the results of the crude extract exhibited no significance with that of aspirin(*Arenajo et al., 2017*). Shevchenko *et al*. (*2009*) extracted three polysaccharide fractions, water-soluble P1, P2 and base-soluble P3. The concrete study revealed that the ranges of the molecular weights of these polysaccharides were 20-60 KDa, 20-40 KDa and more than 70 KDa, respectively. The monosaccharide contents in these three factions all contained glucose (Glc), galactose (Gal), mannose (Man) and xylose (Xyl); among these, glucose was the majority monosaccharide. Moreover, IR spectra of the polysaccharides indicated that the three fractions lacked sulfated groups. However, these results were not inconsistent with those from the study by Maeda *et al*. (*2012a*), which reported that the purified polysaccharides (SP1) contained sulfated xylogalactan with a molecular mass >100 KDa. This xylogalactan is mainly composed of galactose and xylose and small quantities of glucose and uronic acid, with 44% sulfation. Furthermore, the SP1 could enhance NO production and activate macrophage cells via NF-κB and increase the phosphorylation of p38 MAPK, which indicates that they can activate RAW 264.7 cells. In another report, β-1,3-xylooligosaccharides could inhibit the proliferation of MCF-7 human breast cancer cells and induce the condensation of chromatin, the degradation of PARP, and the activation of caspase-3/7, which indicates that oligosaccharides induce apoptosis in MCF-7 cells (*Maeda et al., 2012b*).

Valuable pigments are attracting increasing attention because of their important biological

activity. Worth mentioning is siphonaxanthin, a novel and oxidative metabolite of lutein, which is found in *C. lentillifera*. As shown in Fig. 2, its structure contains a conjugated system of 8 C=C double bonds and 1 keto group located at C-8, similar to fucoxanthin. In addition, at the C-19 position, siphonaxanthin has an extra hydroxyl group, which might make it more beneficial than other carotenoids (*Ganesan et al., 2011; Walton et al., 1973*).

Fig. 2 Structure of siphonaxanthin

Siphonaxanthin is a specific keto-carotenoid that mainly exists in green algae, such as *Codium fragile*, *C. lentillifera*, *Umbraulva japonica*, and *Caulerpa racemosa*. The content of siphonaxanthin is approximately 0.03%-0.1% of its dry weight (*Sugawara et al., 2014*). Initially, this keto-carotenoid was proved to facilitate the highly efficient energy transfer of carotenoids to chlorophylls (*Akimoto et al., 2008*). Moreover, it might have a largely light-harvesting function in the green light-rich underwater habitat to reduce light damage (*Wang et al., 2013*). In addition to its physiological functions, siphonaxanthin has been found to show many biological activities. It was involved in cancer-preventing action in human leukemia HL-60 cells by increasing in TUNEL-positive cells and increasing chromatin condensation in the cells by decreasing the expression of Bcl-2 but up-regulating the expression of DR5. Furthermore, the inhibition function of siphonaxanthin was stronger than that of fucoxanthin and siphonein, which is an esterified form of siphonaxanthin (*Ganesan et al., 2011*). In addition, siphonaxanthin can inhibit adipogenesis in 3T3-L1 preadipocytes and lipid accumulation in the white adipose tissue of KK-

Ay mice by inhibiting protein kinase B phosphorylation and regulating the expression of *CEBPA*, *PPARG*, *FABP4* and *SCD1* (*Li et al., 2015*). Zheng *et al*. (*2018*) found that siphonaxanthin can inhibit lipogenesis in hepatocytes by suppressing the excess accumulation of triacylglycerols induced by liver X receptor α agonist and down-regulating nuclear transcription factors.

3. Subheadings

Salinity, nutrients concentration, irradiance and temperature were the most important factors to influence Caulerpa lentillifera growth.

Dried seaweed could be used as biosorbent for heavy metals and cationic dyes, and fresh seaweed could be biofilter for the aquaculture system.

The phenolic compounds showed good antioxidant activity and could regulate glucose metabolism.

Polysaccharides and oligosaccharides exhibited immunodulatory effects and cancer-preventing activity.

Siphonaxanthin as a novel function compound showed cancer-preventing activity and lipogenesis inhibiting effect.

4. Conclusion

The green seaweed *C. lentillifera* is quite common and popular in Southeast Asian countries and Japan due to its delicious taste and abundant nutrients. During the past 30 years, it has been mass cultivated in some Asian countries, such as the Philippines and Malaysia, and some

cultivation conditions, such as the nutrient concentration, salinity, irradiance and temperature, have been studied in relation to the growth of *C. lentillifera*. In addition, this species has been applied to treat wastewater using heavy metal, cationic dye biosorption and aquaculture system. Recently, some bioactive components, such as phenolic compounds, polysaccharides, and siphonaxanthin, have been extracted from *C. lentillifera*, and their biological activities have also been analyzed by cells. In conclusion, these compounds showed high antioxidant, anticoagulant and immunostimulatory, hypoglycemic, cancer-prevention and lipogenesis inhibition activities, etc in vitro. It is believed that this seaweed will be a new source of health products with its cultivation at an increasing scale. In addition, maybe, *C. lentillifera* will be used as the resource of biofuel or CO_2 fixation just like other algae with further research.

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Figure 1

Caulerpa lentillifera

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Figure 2(on next page)

Structure of siphoxanthin

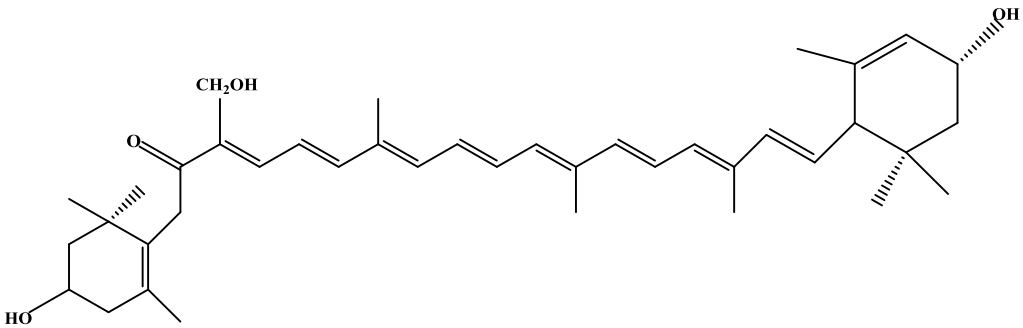

Fig.2 Structure of siphoxanthin

Table 1(on next page)

cultivation conditions

The effect of cultivation conditions on *C. lentillifera*

1 **Table 1：**

2 **Table 1 The effect of cultivation conditions on *C. lentillifera***

Cultivation conditions	Effect	Reference
Salinity	Suitable salinity range of 25-30‰; the maximum growth at a salinity of approximately 35-36‰	Deraxbudsarakom *et al*. (*2003*); Wang (*2011*) and Guo *et al.* (2015b)
Nitrogen and phosphorus	Optimal for the rapid growth at 0.6 mmol/L NO_3-N and N:P ratio of 8:1;	Deraxbudsarakom *et al*. (*2003*);
	Highest SGR at a 0.1 mmol/L PO_4-P and 0.5 mmol/L NO_3-N;	Guo *et al.* (2015b);
	Nitrogen types: $NaNO_3$ and NH_4NO_3) can significantly promote the growth of the alga; NH_4-N:NO_3-N ratios of 1:1 and 1:5 were the most favorable ratios for the growth of the alga	Wang *et al*. (*2017*); Liu *et al*. (*2016*);
Phytohormones	6-BA and GA could induce the growth of the alga, but IAA could increase the intracellular crude polysaccharide content	Tao *et al*.(*2017*)
Temperature	Ideal temperature range 22-28°C	Friedlander *et al*.(*2006*) and Guo *et al*. (*2015a*)

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Table 2(on next page)

Different wastewater treatment process

Different wastewater treatment process by *C. lentillifera*

1 **Table 2：**

2 **Table 2 Different wastewater treatment process by *C. lentillifera***

Alga types	Wastewater types	References
Dried alga	Cu^{2+}, Cd^{2+}, Pb^{2+} and Zn^{2+}	Pavasant *et al*. (*2006*) Apiratikul and Pavasant, *(2006*)
Dried alga	Cationic dyes: Astrazon Blue FGRL (AB), Astrazon Red GTLN (AR), and methylene blue (MB)	Marungrueng and Pavasant(2006; *2007*); Ncibi *et al.*(*2007*); Cengiz and Cavas (*2008*); Punjongharn *et al.*(*2008*)
Fresh alga	Used as a biofilter in aquaculture systems for nutrient absorption, especially NO_3-N	Paul and De Nys(*2008*); Liu *et al.*(*2016*); Chokwiwattanawanit(*2000*)

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Table 3(on next page)

The values for pseudo second-order kinetic mode

Table 3：

Table 3 The values for q_e, k and R^2 of Cu^{2+}, Cd^{2+} and Pb^{2+} in pseudo second-order kinetic mode

Parameters	Cu^{2+}	Cd^{2+}	Pb^{2+}
q_e(mmol Kg^{-1})	6.14	3.97	2.64
K(Kg mol^{-1} min^{-1})	254	621	2036
R^2	0.999	1.000	1.000

Table 4(on next page)

Bioactive components of *C. lentillifera*

Studies on bioactive components of *C. lentillifera*

1 **Table 4：**

2 **Table 4 Studies on bioactive components of *C. lentillifera***

Components	Biological activity	References
Phenolic compounds	Radical-scavenging activity and reducing power ability;	Matajun et al.(*2008*); Nguyen et al.(*2011*);
	Stimulated insulin secretion in pancreatic β-cells and enhanced glucose uptake	Sharma and Rhyu(*2014*); Sharma et al.(*2017*); Sharma et al.(*2015*); Abouzid et al.(*2014*)
Polysaccharides	Increase the phosphorylation of p38 MAPK;	Maeda *et al*. (*2012a*);
	Inhibit the proliferation of MCF-7	Maeda et al.(*2012b*)
Siphonaxanthin	cancer-preventing action;	Ganesan et al.(*2011*);
	Inhibit adipogenesis;	Li et al.(*2015*); Zheng et al. (*2018*)

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