



Effects of light and temperature on open cultivation of desert cyanobacterium *Microcoleus vaginatus*



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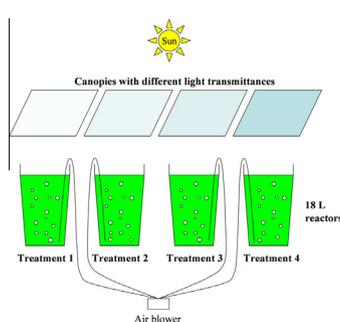
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HIGHLIGHTS

- *Chlorella* sp., *Scenedesmus* sp. and *Navicula* sp. were the main contaminating microalgae.
- High light intensity was responsible for the green algae contamination.
- Low temperature was responsible for the diatom contamination.
- High temperature was beneficial to cyanobacterial growth rather than green algae.
- All microalgal growth was demand for at least average daily light intensity $>5 \mu\text{E m}^{-2} \text{s}^{-1}$.

GRAPHICAL ABSTRACT



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ABSTRACT

Microalgae cultivation has recently been recognized as an important issue to deal with the increasingly prominent resource and environmental problems. In this study, desert cyanobacterium *Microcoleus vaginatus* was open cultivated in 4 different cultivation conditions in Qubqi Desert, and it was found *Chlorella* sp., *Scenedesmus* sp. and *Navicula* sp. were the main contaminating microalgal species during the cultivation. High light intensity alone was responsible for the green algae contamination, but the accompanied high temperature was beneficial to cyanobacterial growth, and the maximum biomass productivity acquired was $41.3 \text{ mg L}^{-1} \text{ d}^{-1}$. Low temperature was more suitable for contaminating diatoms' growth, although all the microalgae (including the target and contaminating) are still demand for a degree of light intensity, at least average daily light intensity $>5 \mu\text{E m}^{-2} \text{ s}^{-1}$. As a whole, cultivation time, conditions and their interaction had a significant impact on microalgal photosynthetic activity (Fv/Fm), biomass and exopolysaccharides content ($P < 0.001$).

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1. Introduction

With the increasingly prominent resource and environmental issues, microalgae cultivation has recently received a great deal of attention. It is proposed that microalgae are the light-dependent carbon-fixing organisms that convert CO_2 to potential biofuels,

feeds, foods and high-value bioactive substances (Chisti, 2007; Brennan and Owende, 2010). Therefore, microalgae cultivation would not only provide much more resource supplies for human beings (Mascarelli, 2009; Xia et al., 2013), but also make significant contribution to greenhouse gas CO_2 consumption (Wang et al., 2008; Brennan and Owende, 2010), as well as the utilization and purification of waste water, because microalgae can use high nutritional wastewater (containing nitrogen, phosphorus, etc.) as the medium for growth (Cantrell et al., 2008). In addition, these

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photosynthetic microorganisms are also useful in environmental improvement in desert regions, because microalgae can bind and cement sandy particles together via their growth and secretion of exopolysaccharides, so that the loose sandy particles are fixed and biological soil crusts form (Hu et al., 2012; Lan et al., 2014). Those crusts play an important pioneer role in environmental governance and ecological restoration of desert regions, promoting the development of soil microsystem and the following succession of higher vegetation communities (Bowker, 2007; Lan et al., 2014).

For microalgae cultivation, site selection is not only directly related to cultivation costs, but also fulfills the requirement of rational use of land resource. Desert environment is generally characterized by a series of harsh conditions, such as poor soils, extreme drought, high salinity, pH and radiation, large temperature variation and wind speed (Xie et al., 2007; Powell et al., 2015). In such environment, many types of vegetations are restricted, which on the one hand brings great difficulties to the local economic development, on the other hand poses a greater challenge for desertification control. However, in such environment conditions, many microalgal species can survive well due to the special physiological and ecological characteristics, such as rapid growth mechanism after obtaining water (Lan et al., 2010; Wu et al., 2013), migration in the environment to better acquire resources and avoid excessive radiation (Garcia-Pichel and Pringault, 2001), adaptation to the salinity, drought, high pH and temperature (Xie et al., 2007; Lan et al., 2010; Hu et al., 2012). Therefore, choosing desert regions as the sites of microalgae cultivation, not only can effectively use desert land resource, but also improve the environmental conditions and promote the local economic development. Meanwhile, isolating and screening microalgae resources in desert regions would provide more high-quality microalgae for the large-scale cultivation. Because in the current large-scale cultivation of microalgae, most of the algal strains screened in the lab are not able to adapt well to field changeable environmental conditions, leading to some characteristic change (such as the decrease of lipid content in lipid-producing microalgae; Zhou et al., 2013), or being contaminated and even death (Del Campo et al., 2007).

To obtain microalgal biomass, open bioreactors were widely used in large-scale cultivation of microalgae because of low cost, simple structure and operating process (Blanco et al., 2007; Da Rosa et al., 2011). However, the cultivation conditions are difficult to control in the open bioreactors, thus microalgae cultivated in those reactors are often subjected to the risk of contamination (Gouveia, 2011). During the course of cultivation, the target microalgae may be contaminated by protozoa, as well as other microalgal species. Especially when the target microalgae are contaminated by other microalgal species, the contaminating microalgae not only compete with target microalgae in nutrients, light and other resources, but also may secrete some chemical substances to poison the target microalgae, ultimately restraining the growth of target microalgae (Wang et al., 2013; Mooij et al., 2015). Therefore, in microalgae cultivation, understanding the growth characteristics of different kinds of microalgae will help us better control the cultivation conditions, ultimately facilitating the growth of target microalgae, and controlling the growth of contaminating microalgae.

Desert cyanobacterium *Microcoleus vaginatus* used in this experiment was isolated from biological soil crusts in Dalate Banner region of Qubqi Desert (Inner Mongolia). This strain of microalga was the first dominant algal species in biological soil crusts, and was selected to construct artificial cyanobacterial crusts, promoting the development of biological soil crusts and restoration of local ecosystem (Lan et al., 2013, 2014). Therefore in this work, desert cyanobacterium *M. vaginatus* was open cultivated under different light and temperature conditions in the Dalate Banner region of

Qubqi Desert, in order to study the effects of light and temperature on secretion of exopolysaccharides, photosynthetic activity and growth of microalgae; and examine the contamination to target microalgae under different cultivation conditions. The results on the one hand will help us understand the cultivation conditions and metabolic characteristics of desert cyanobacteria, on the other hand will also provide an important theoretical basis for microalgal contamination control and design of cultivation processes.

2. Methods

2.1. Studying region and organisms

The experiment was conducted in Dalate Banner region of Inner Mongolia at the eastern edge of Qubqi Desert (40°21'N, 109°51'E). The region is about 15 km away from the middle reaches of Yellow River, characterized by a mass of sand dunes with average height of 5 m, with an elevation of 1040 m. The climate belongs to typical continental monsoon pattern, with windy days ($>5 \text{ m s}^{-1}$) more than 180 d y^{-1} . Mean annual sunshine time is 2525 h, and mean annual temperature is $5.8 \text{ }^\circ\text{C}$. Mean annual precipitation is 284 mm, falling predominantly between July and August. The annual potential evapotranspiration is 2288 mm, with the maximum evapotranspiration in May. Mean annual relative humidity is 50.9%, with a fluctuation between 35% and 65% (Fig. 1).

Before this experiment, to obtain sufficient microalgal biomass, *M. vaginatus* was cultivated in sterilized BG-11 medium with 1 L stoppered flasks in a incubator ($25 \pm 2 \text{ }^\circ\text{C}$), illuminated with cool white fluorescent light at $40 \mu\text{E m}^{-2} \text{ s}^{-1}$ with aeration (Xia et al., 2013; Ge et al., 2014).

2.2. Experimental setup

After obtaining adequate microalgal biomass in the incubator, *M. vaginatus* was inoculated into twelve 18 L open vertical cylindrical bioreactors, with BG-11 medium and aeration as above. The bioreactors were open, so a certain amount of water was added to the bioreactors everyday to offset the evaporation. The bioreactors were placed in four different canopied shade conditions in a transparent greenhouse, with the natural light resource. The bioreactors in different shade conditions were respectively defined as treatments 1, 2, 3 and 4. From treatment 1 to treatment 4, the light intensity (bioreactor surface) gradually decreased, and the medium temperature changed with light intensity and ambient air temperature (Fig. 2; the light intensity and temperature in the 4 treatments were monitored in a sunny day during the microalgae cultivation process).

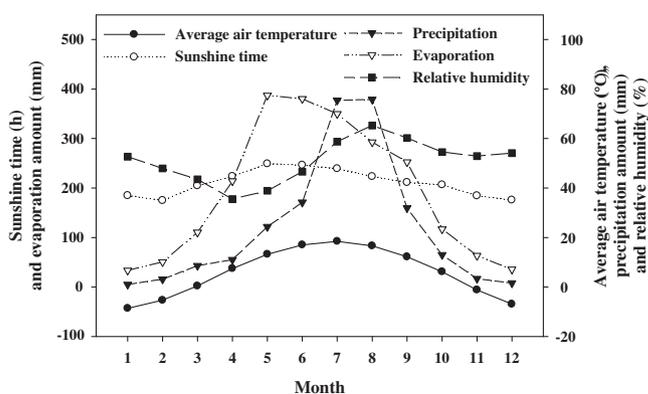


Fig. 1. Monthly average air temperature, sunshine time, precipitation, evaporation and relative humidity in Qubqi Desert based on the records from 1978 to 2005.

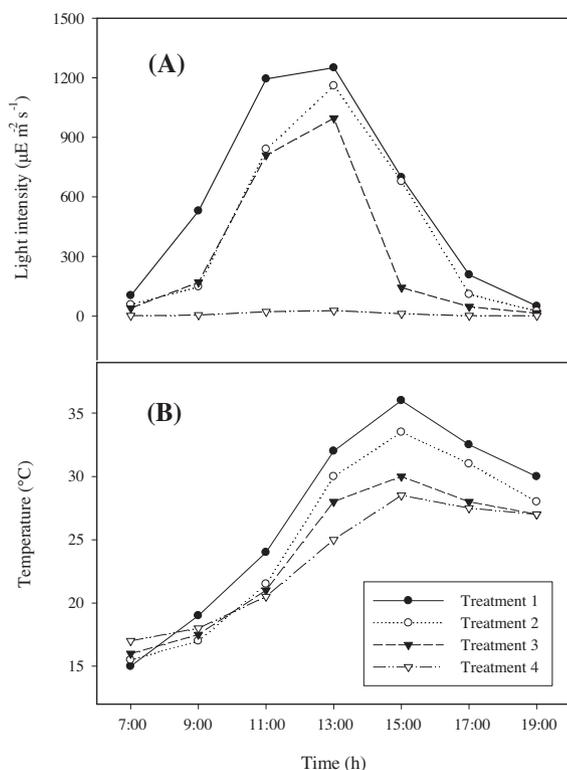


Fig. 2. Diurnal variation of the light intensity (bioreactor surface; A) and medium temperature (B) in the 4 treatments. The light intensity and temperature were monitored in a sunny day during the microalgae cultivation.

M. vaginatus in the bioreactors were cultivated for 20 d. After 10 d and 20 d of inoculation respectively, a portion of microalgae were harvested to measure microalgal biomass (dry weight) and biomass productivity. In addition, during the cultivation process, microalgae were sampled every 5 days (16:00) for determining the chlorophyll (Chl)-a, exopolysaccharides, photosynthetic activity (dark adapted Fv/Fm) and monitoring the contamination caused by other microalgae. While sampling, light intensity (bioreactor surface) and medium temperature in each treatment were measured at the same time.

2.3. Measurements

2.3.1. Dry weight

In each reactor, 0.5 L microalgae were harvested and concentrated by centrifugation, then the microalgae were placed in an oven (85 °C) and dried to constant weight, and the weight was recorded as microalgal biomass (B; mg L⁻¹). The biomass productivity (BP; mg L⁻¹ d⁻¹) was calculated according to the equation:

$$BP = (B2 - B1)/T$$

Here B2 and B1 represents microalgal biomass at the time T (days) and at the start of inoculation, respectively.

2.3.2. Chlorophyll (Chl)-a content

Before Chl-a content measurement, 25 mL microalgae were sampled and centrifuged at 900g for 10 min to remove the supernatant fluids. Then the sedimentary microalgae were ground in 10 mL 100% acetone with mortar and pestle. The extractions were placed at 4 °C overnight (for 12 h), and extracts were then centrifuged again (900g for 10 min) to remove the suspended sediments. The absorbances A_{663} , A_{490} and A_{384} of supernatant fluids were measured at 663 nm, 490 nm and 384 nm using a spectrophotometer,

and Chl-a content was calculated according to the trichromatic equation of Garcia-Pichel and Castenholz (1991).

2.3.3. Exopolysaccharides (EPS) content

For exopolysaccharides (EPS) measurement, 2 mL microalgae were sampled and centrifuged at 900g for 10 min to remove the suspended sediments, and 1 mL of each supernatant fluid was used to spectrometrically measure absorbance A_{485} at 485 nm according to the phenol-sulfuric acid method (Dubios et al., 1956). The relationship between the exopolysaccharides content (EC; µg mL⁻¹) and the A_{485} value was calculated according to the following equation:

$$EC = 120.06 \times A_{485}, R^2 = 0.995 \text{ (using sucrose as standard)}$$

2.3.4. Chl fluorescence

Before Chl fluorescence measurement, 3 mL microalgae were sampled and dark adapted for 10 min. Then the Chl fluorescence of dark adapted microalgae (liquid) was measured with a plant efficiency analyzer (PEA, Hansatech, UK). The original and maximal fluorescence, F_0 and F_m , were automatically recorded by the PEA, and variable fluorescence (F_v) was calculated by $F_v = F_m - F_0$. The ratio F_v/F_m is the maximal quantum yield of PS II photochemistry, reflecting the maximal light energy conversion efficiency of PS II, and in this experiment microalgal photosynthetic activity of was characterized by the dark adapted F_v/F_m (Baker, 2008).

2.3.5. Contamination monitoring

For contamination monitoring, the cultivated microalgae were directly observed with a microscope and the contaminating situation was recorded. Among the microalgae samples, those with the proportion of contaminating microalgal cells >10% were identified as severe contamination; those with contaminating level >1% but <10% were identified as moderate contamination, and <1% slight contamination.

2.4. Data analysis

The entire experiment was conducted in triplicate, and all the variances of Chl-a content, photosynthetic activity (F_v/F_m) and EPS content were analyzed using One-way or Two-way ANOVA at 95%. All data analyses were carried out using SPSS 13.0 software.

3. Results and discussion

3.1. Effects of light and temperature on microalgal contamination

In the 4 treated groups, light intensity (reactor surface) and medium temperature at sampling time were shown in Table 1. From treatment 1 to treatment 4, it was found the light intensity and medium temperature gradually decreased. From the diurnal variation of light intensity (Fig. 2A), it was found the maximum light intensity at noon (13:00) reached up to 1200 µE m⁻² s⁻¹ in treatments 1 and 2, but was still lower than 1000 µE m⁻² s⁻¹ in treatments 3 and 4. Especially in treatment 4, the maximum light intensity had not reached to 30 µE m⁻² s⁻¹ yet. From the diurnal variation in medium temperature (Fig. 2B), it was found a maximum temperature (15:00) was over 35 °C in treatment 1, and not more than 33 °C in treatment 2, while was lower than 30 °C in treatments 3 and 4.

On the whole, *Chlorella* sp., *Scenedesmus* sp. in green algae and *Navicula* sp. in diatoms were the main contaminating microalgal species during the cultivation of *M. vaginatus* in Dalate Banner region (Table 2). In the present experiment, severe contamination caused by *Chlorella* sp. appeared in treatments 1 and 2 after 5 days

Table 1

Light intensity (bioreactor surface) and medium temperature at sampling time (16:00) of the different treatments.

	Treatment time (d)	Treatment 1	Treatment 2	Treatment 3	Treatment 4
Light intensity ($\mu\text{E m}^{-2} \text{s}^{-1}$)	0	208	110	47	2
	5	416	168	110	6.2
	10	266	96	38	4
	15	114	6.8	5	1.2
	20	148	18.6	8.4	2.8
Temperature ($^{\circ}\text{C}$)	0	33.3	32.5	29	28
	5	28.5	28	25	23
	10	34.5	31	29.8	29
	15	24.5	22.5	21	20.5
	20	29	24	21.5	21

Table 2

The contaminated microalgae in the different treatments during the cultivation process (+++ severe; ++ moderate; + slight).

Species	Treatment 1	Treatment 2	Treatment 3	Treatment 4
<i>5 d</i>				
<i>Chlorella</i> sp.	+++	+++	++	++
<i>Scenedesmus</i> sp.	+	++	+	+
<i>Navicula</i> sp.	+	++	+++	+
<i>10 d</i>				
<i>Chlorella</i> sp.	+++	+	+	+
<i>Scenedesmus</i> sp.	+	+	+	+
<i>Navicula</i> sp.	+	++	+++	+
<i>Euglena</i> sp.		+		
<i>15 d</i>				
<i>Chlorella</i> sp.	+	+	+	+
<i>Scenedesmus</i> sp.	++	+	+	+
<i>Navicula</i> sp.	+	++	+++	+
<i>20 d</i>				
<i>Chlorella</i> sp.	+	+	+	+
<i>Scenedesmus</i> sp.	+	+	+	+
<i>Navicula</i> sp.	++	++	+++	+

of inoculation, which might be due to the high light intensity appeared at that time (the light intensity of $416 \mu\text{E m}^{-2} \text{s}^{-1}$ at sampling time was maximum during the whole experiment; Table 1), because it has been reported that green algae seem to prefer high light intensity (Lionard et al., 2005). Later, the *Chlorella* contamination was gradually lightened, which on the one hand might be due to the decrease of light intensity since the 5th day (Table 1); on the other hand, the mass growth and bloom of the cyanobacteria might suppress the growth of *Chlorella* sp.

During the whole experiment, contamination caused by *Navicula* sp. was moderate in treatment 2 and severe in treatment 3, which might be due to the temperature difference among the different treatments, because it was proposed the low temperatures were more suitable for the growth of diatoms (Tilman et al., 1986; the maximum medium temperature in treatment 3 had not reached to 30°C). However, although the medium temperature was also low in treatment 4 (the maximum medium temperature was lower than 30°C similarly), only slight *Navicula* contamination appeared. That may imply us the growth of diatoms is still demand for a degree of light intensity, because the average daily light intensity was less than $5 \mu\text{E m}^{-2} \text{s}^{-1}$ in treatment 4.

3.2. Effects of light and temperature on microalgal biomass

Although severe *Chlorella* contamination appeared in treatments 1 and 2, moderate and severe *Navicula* contaminations

appeared in treatments 2 and 3 respectively, the final microalgal dry weight in treatments 1 and 2 was not significantly affected, more than 440 mg L^{-1} after 20 days of cultivation (Table 3). In treatment 3, although microalgal dry weight reached to 300 mg L^{-1} after 10 days of cultivation, this value decreased to only 123 mg L^{-1} at the end of experiment. During the whole experiment, microalgal dry weight in treatment 4 maintained at a low level, and was less than 50 mg L^{-1} .

Chl-a is a type of photosynthetic pigment, existing in all the photosynthetic organisms, and the proportion in photosynthetic organisms is relatively stable, thus is often used to indicate photosynthetic biomass (Schumann et al., 2005). From the Fig. 3, it was found that Chl-a contents were higher in treatments 1 and 2 than those in treatments 3 and 4 after 20 days of cultivation ($P < 0.05$). From the changing curves of Chl-a (Fig. 4), it was found Chl-a contents in treatments 1 and 2 gradually increased, although a slight decrease was observed later in treatment 1. However in treatment 3, although the Chl-a content increased to some extent in the first 10 days, then it decreased rapidly. Similarly as the results of dry weight, Chl-a content in treatment 4 maintained at a low and inoculation level during the whole experiment. Two-way ANOVA showed that Chl-a content was significantly affected by cultivation time, treatment and their interaction ($P < 0.001$; Table 4).

Either Chl-a content or dry weight discussed as above, as well as OD (optical density) value monitored in other reports (Xia et al., 2013; Zhou et al., 2013), are three common parameters used to indicate microalgal biomass. In the present experiment, the results from Chl-a content were consistent with the results of dry weight. Additionally the determination of Chl-a required less sample size but presented a more accurate indication, therefore Chl-a content could be an ideal approach to representing desert filamentous cyanobacterial biomass. In addition, although OD value is also often used to determine microalgal biomass, this value is not suitable for desert filamentous cyanobacteria, because the filamentous cyanobacteria deposit rapidly while determination, thus OD values are always difficult to be exactly determined.

Obtaining high microalgal biomass is an important purpose of microalgae cultivation. In the present experiment, the difference of microalgal biomass occurred among different treatments, which on the one hand might be because light and temperature directly affected the growth of microalgae; on the other hand microalgal contamination also had a significant impact on the biomass of target microalgae. From the view of categories of the target and main contaminating microalgae, the target microalga was prokaryotic cyanobacterium, and still able to grow well under high temperatures above 35°C . Generally, the optimal incubation temperature for most prokaryotes is about 37°C (Nan et al., 1999), while the temperature for eukaryotic fungi and algae is $25\text{--}28^{\circ}\text{C}$ (Lan et al., 2010, 2013). Therefore in treatments 1 and 2, although the high light intensity was beneficial to green algae, the high temperature accompanied with high light intensity restricted the growth of green algae. As a result, the contamination caused by green algae

Table 3
Comparison of microalgal biomass (dry weight) in the different treatments after 10 and 20 days of inoculation respectively.^a

Treatments	10 d		20 d	
	Biomass (mg L ⁻¹)	Biomass productivity (mg L ⁻¹ d ⁻¹)	Biomass (mg L ⁻¹)	Biomass productivity (mg L ⁻¹ d ⁻¹)
Treatment 1	473.9 ± 41.2	41.3 ± 3.9	444.4 ± 41.3	19.2 ± 1.9
Treatment 2	372.8 ± 24.8	31.4 ± 2.0	546.0 ± 13.1	24.3 ± 0.4
Treatment 3	300.6 ± 16.3	23.6 ± 1.1	123.3 ± 6.4	2.9 ± 0.1
Treatment 4	78.2 ± 5.2	3.0 ± 0.3	39.3 ± 6.9	–

^a Data are represented as mean ± standard deviation of triplicates.

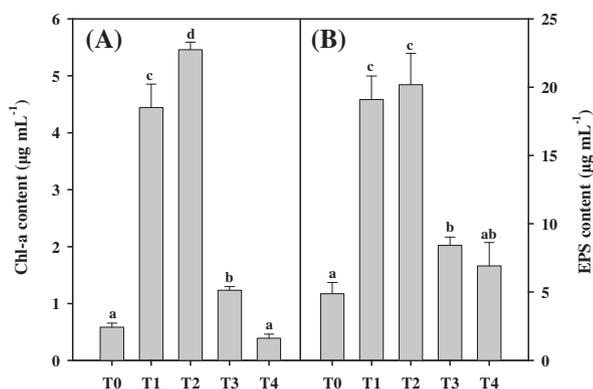


Fig. 3. Chl-a (A) and EPS content (B) of microalgae after 20 days of inoculation in the different treatments. T0 indicates the Chl-a and EPS content just after inoculation. Data are represented as mean ± standard deviation of triplicates, and the different letters indicate the differences are significant at 0.05 level ($P < 0.05$).

did not seriously affect cyanobacterial biomass. However, if the high light intensity was accompanied with relatively moderate temperatures during the cultivation process, the green algae contamination might have a serious impact on the desert cyanobacterial biomass. So in the practice of desert cyanobacterial cultivation in spring of Dalate Banner region (high light intensity was accompanied with relatively lower temperatures), at the same time of controlling light intensity, increasing temperature is another common mean to control the contamination caused by green algae.

Light and temperature are the important environmental factors affecting microalgal growth, and high light intensity and temperature may lead to photoinhibition and even death (Heber et al., 2001; Lan et al., 2012), while low light intensity and temperature are also not conducive to the most microalgal photosynthetic carbon fixation. In the treatment 4 of this experiment, low light intensity might be the main reason for the low microalgal biomass. In

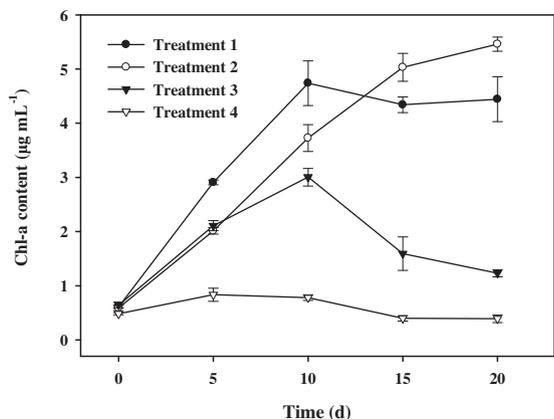


Fig. 4. Change of Chl-a content in the different treatments during the microalgal cultivation. Data are represented as mean ± standard deviation of triplicates.

that treatment, although the contamination was slight, cyanobacterial growth and biomass accumulation were the worst. The slight diatom contamination also imply us diatom growth is still demand for a degree of light intensity, although low temperature is more suitable for the growth of diatoms (Tilman et al., 1986). In the low temperature environment meeting the illumination requirements (treatment 3), the diatoms would rapidly multiply and contaminate the target cyanobacteria, ultimately resulting in the decrease of cyanobacterial biomass. So in the practice of desert cyanobacterial cultivation in spring of Dalate Banner region, at the same time of controlling green algae contamination, maintaining a certain temperature to prevent the contamination caused by diatoms is also necessary.

3.3. Effects of light and temperature on microalgal metabolism

As an important metabolic indicator of photosynthetic activity, the dark adapted Fv/Fm generally remains at a constant value under the unstressed conditions, >0.8 for higher plants, about 0.65 for eukaryotic algae, and lower in prokaryotic cyanobacteria (Lan et al., 2010). However when subjected to environmental stress, Fv/Fm will decline in photosynthetic organisms (Baker, 2008; Lan et al., 2010). In the present study, it was found microalgal photosynthetic activity (Fv/Fm) was significantly affected by cultivation time, treatment and their interaction ($P < 0.001$; Table 4).

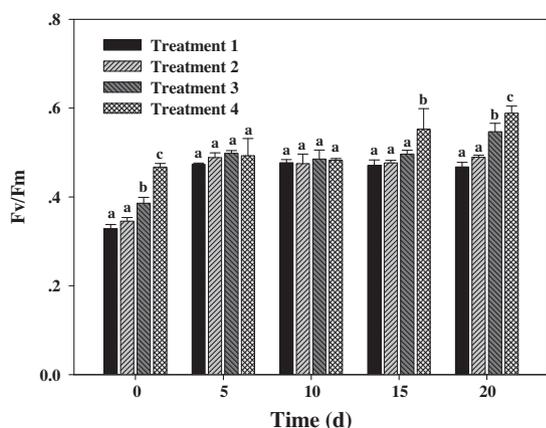
Just after inoculation, the photosynthetic activity showed an obvious increasing trend from treatment 1 to treatment 4 (Fig. 5), which might be due to the gradual decreasing light intensity. Because under low light intensity, the inoculated microalgae could recover more easily; in contrast the high light intensity would bring light stress or even death to the microalgae (Hill et al., 2005). In the treatments 1, 2 and 3 of this experiment, although the light intensity had caused some stress on microalgae immediately after inoculation, microalgal photosynthetic activity was not affected in the later cultivation process (Fig. 5). When the microalgae entered the late cultivation stage, their photosynthetic activities in treatments 3 and 4 were significantly higher than those in treatments 1 and 2, which might be due to the low cyanobacterial biomass and thus relative large proportion of contaminating eukaryotic algae in treatments 3 and 4. Because compared with prokaryotic cyanobacteria, eukaryotic algae have higher Fv/Fm, which is considered to be caused by following two aspects (Papageorgiou, 1996; Lan et al., 2012): (1) the principal light harvesting complexes (LHCs) are phycobilisomes in cyanobacteria, while Chl-a and Chl-b in eukaryotic algae; (2) cyanobacteria have higher ratio of PS I to PS II than eukaryotic algae. The Fv exclusively arises from PS II complexes, while Fm not only arises from PS II complexes, but also from phycobiliproteins, and also possibly from PS I complexes.

Microalgal EPS is a type of polysaccharide substances secreted by microalgae, playing an important role in the adaptation of microalgal cells to environments (Lan et al., 2010; Mager and Thomas, 2011). However, under different cultivation conditions (such as light and temperature in this study), or at different cultivation

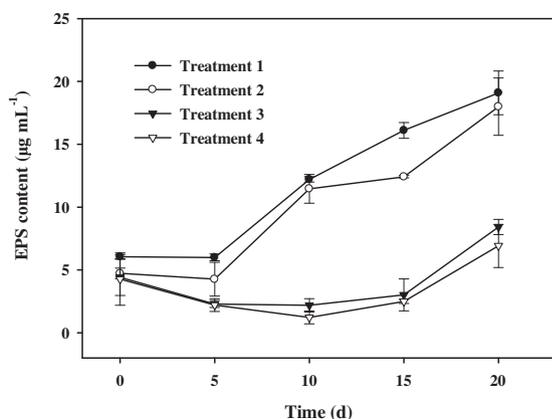
Table 4

Two-way ANOVA of effects of cultivation time, treatment and their interactions on microalgal Chl-a, EPS content and Fv/Fm ratio.

Variables	Factors	Sum of Squares	df	Mean Square	F	P
Chl-a content	Time	51.224	4	12.806	192.448	<0.001
	Treatment	84.963	3	28.321	425.610	<0.001
	Time × treatment	44.715	12	3.726	55.998	<0.001
EPS content	Time	723.279	4	180.820	102.855	<0.001
	Treatment	821.129	3	273.710	155.693	<0.001
	Time × treatment	330.640	12	27.553	15.673	<0.001
Fv/Fm	Time	.138	4	0.035	127.368	<0.001
	Treatment	.045	3	0.015	54.912	<0.001
	Time × treatment	.036	12	0.003	10.988	<0.001

**Fig. 5.** Change of Fv/Fm in the different treatments during the microalgal cultivation. Data are represented as mean ± standard deviation of triplicates, and the different letters indicate the differences are significant at 0.05 level ($P < 0.05$).

times, a significant difference still existed in the secreted EPS contents ($P < 0.05$; Table 4). In the present study, the EPS contents at the end of experiment were significantly different among the 4 treatments ($P < 0.05$), and higher than those values just after the inoculation, although the difference was not significant in treatment 4 ($P > 0.05$). From the accumulation of EPS, the 4 treatments could be clearly divided into two groups (Fig. 6): (1) in treatments 1 and 2, the EPS contents began to accumulate rapidly after 5 days of inoculation; (2) in treatments 3 and 4, the EPS contents first decreased and then followed by a slight increase. It was considered that the apparent difference of EPS accumulation in the two groups was mainly due to the difference of light intensity. Because it has been reported that high light intensity is more conducive to micro-

**Fig. 6.** Change of EPS content in the different treatments during the microalgal cultivation. Data are represented as mean ± standard deviation of triplicates.

algal EPS accumulation (Ge et al., 2014), while in low-light environments, the carbon fixed by microalgal photosynthesis may be not able to fully meet the requirements of cell metabolism. In this case, it is possible for microalgae to utilize the secreted EPS as a carbon source for basic metabolic activity (Mager and Thomas, 2011). That also may be the reason of decrease in EPS contents in treatments 3 and 4.

4. Conclusions

In this study, *Chlorella* sp., *Scenedesmus* sp. and *Navicula* sp. were the main contaminating microalgal species during the open cultivation of desert cyanobacterium *M. vaginatus*. Furthermore, it was found high light intensity alone was responsible for the green algae contamination, but high temperature accompanied with high light intensity was beneficial to cyanobacterial growth (final biomass achieved $>546 \text{ mg L}^{-1}$). Low light intensity and temperature inhibited cyanobacterial growth (biomass maintained $<100 \text{ mg L}^{-1}$), because low temperature was more suitable for the growth of diatoms, and all microalgal growth was demand for at least average daily light intensity $>5 \mu\text{E m}^{-2} \text{ s}^{-1}$.

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References

- Baker, N.R., 2008. Chlorophyll fluorescence. A probe of photosynthesis in vivo. *Annu. Rev. Plant Biol.* 59, 89–113.
- Blanco, A.M., Moreno, J., Campo, J.A.D., Rivas, J., Guerrero, M.G., 2007. Outdoor cultivation of lutein-rich cells of *Muriellopsis* sp. in open ponds. *Appl. Microbiol. Biotechnol.* 73, 1259–1266.
- Bowker, M.A., 2007. Biological soil crust rehabilitation in theory and practice. An underexploited opportunity. *Restor. Ecol.* 15, 13–23.
- Brennan, L., Owende, P., 2010. Biofuels from microalgae – a review of technologies for production, processing, and extractions of biofuels and co-products. *Renewable Sustainable Energy Rev.* 14, 557–577.
- Cantrell, K.B., Ducey, T., Ro, K.S., Hunt, P.G., 2008. Livestock waste-to-bioenergy generation opportunities. *Bioresour. Technol.* 99 (17), 7941–7953.
- Chisti, Y., 2007. Biodiesel from microalgae. *Biotechnol. Adv.* 25, 294–306.
- Da Rosa, A.P.C., Carvalho, L.F., Goldbeck, L., Costa, J.A.V., 2011. Carbon dioxide fixation by microalgae cultivated in open bioreactors. *Energy Convers. Manage.* 52, 3071–3073.
- Del Campo, J.A., Garcia-Gonzalez, M., Guerrero, M.G., 2007. Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. *Appl. Microbiol. Biotechnol.* 74, 1163–1174.
- Dubois, M., Gilles, K.A., Hamilton, J.K., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28, 350–356.
- Garcia-Pichel, F., Castenholz, R.W., 1991. Characterization and biological implications of scytonemin, a cyanobacterial sheath pigment. *J. Phycol.* 27, 395–409.
- Garcia-Pichel, F., Pringault, O., 2001. Cyanobacteria track water in desert soils. *Nature* 413, 380–381.

- Ge, H.M., Zhang, J., Zhou, X.P., Xia, L., Hu, C.X., 2014. Effects of light intensity on components and topographical structures of extracellular polymeric substances from *Microcoleus vaginatus* (Cyanophyceae). *Phycologia* 53 (2), 167–173.
- Gouveia, L., 2011. Microalgae as a Feedstock for Biofuels. Springer, Heidelberg, pp 26–28.
- Heber, U., Bukhov, N.G., Shuvalov, V.A., Kobayashi, Y., Lange, O.L., 2001. Protection of the photosynthetic apparatus against damage by excessive illumination in homoiohydric leaves and poikilohydric mosses and lichens. *J. Exp. Bot.* 52, 1999–2006.
- Hill, R., Frankart, C., Ralph, P.J., 2005. Impact of bleaching conditions on the components of non-photochemical quenching in the zooxanthellae of a coral. *J. Exp. Mar. Biol. Ecol.* 322, 83–92.
- Hu, C.X., Gao, K.S., Whitton, B.A., 2012. Semi-arid regions and deserts. In: Whitton, B.A. (Ed.), *Ecology of Cyanobacteria II: Their Diversity in Space and Time*. Springer Science + Business Media, Dordrecht, pp. 345–369.
- Lan, S.B., Wu, L., Zhang, D.L., Hu, C.X., Liu, Y.D., 2010. Effects of drought and salt stresses on man-made cyanobacterial crusts. *Eur. J. Soil Biol.* 46, 381–386.
- Lan, S.B., Wu, L., Zhang, D.L., Hu, C.X., 2012. Composition of photosynthetic organisms and diurnal changes of photosynthetic efficiency in algae and moss crusts. *Plant Soil* 351, 325–336.
- Lan, S.B., Wu, L., Zhang, D.L., Hu, C.X., 2013. Assessing level of development and successional stages in biological soil crusts with biological indicators. *Microb. Ecol.* 66, 394–403.
- Lan, S.B., Zhang, Q.Y., Wu, L., Liu, Y.D., Zhang, D.L., Hu, C.X., 2014. Artificially accelerating the reversal of desertification: cyanobacterial inoculation facilitates the succession of vegetation communities. *Environ. Sci. Technol.* 48, 307–315.
- Lionard, M., Muylaert, K., Gansbeke, D.V., Vyverman, W., 2005. Influence of changes in salinity and light intensity on growth of phytoplankton communities from the Schelde river and estuary (Belgium/The Netherlands). *Hydrobiologia* 540, 105–115.
- Mager, D.M., Thomas, A.D., 2011. Extracellular polysaccharides from cyanobacterial soil crusts: a review of their role in dryland soil processes. *J. Arid Environ.* 75, 91–97.
- Mascarelli, A.L., 2009. Algae: fuel of the future? *Environ. Sci. Technol.* 43 (19), 7160–7161.
- Mooij, P.R., Stouten, G.R., Van Loosdrecht, M.C.M., Kleerebezem, R., 2015. Ecology-based selective environments as solution to contamination in microalgal cultivation. *Curr. Opin. Biotechnol.* 33, 46–51.
- Nan, Z.D., Xiang, Y., Zeng, X.C., Zhang, H.L., 1999. Investigations of optimum growth temperature of bacteria by microcalorimetric method. *J. Sichuan Univ.* 36 (6), 1147–1150.
- Papageorgiou, G.C., 1996. The photosynthesis of cyanobacteria (blue bacteria) from the perspective of signal analysis of chlorophyll a fluorescence. *J. Sci. Ind. Res.* 55, 596–617.
- Powell, J.T., Chatziefthimiou, A.D., Banack, S.A., Cox, P.A., Metcalf, J.S., 2015. Desert crust microorganisms, their environment, and human health. *J. Arid Environ.* 112, 127–133.
- Schumann, R., Häubner, N., Klausch, S., Karsten, U., 2005. Chlorophyll extraction methods for the quantification of green microalgae colonizing building facades. *Int. Biodeterior. Biodegrad.* 55, 213–222.
- Tilman, D., Kiesling, R., Sterner, R., Kilham, S., Johnson, F., 1986. Green, bluegreen and diatom algae-taxonomic differences in competitive ability for phosphorus, silicon and nitrogen. *Arch. Hydrobiol.* 106, 473–485.
- Wang, B., Li, Y., Wu, N., Lan, C.Q., 2008. CO₂ bio-mitigation using microalgae. *Appl. Microbiol. Biotechnol.* 79 (5), 707–718.
- Wang, H., Zhang, W., Chen, L., Wang, J.F., Liu, T.Z., 2013. The contamination and control of biological pollutants in mass cultivation of microalgae. *Bioresour. Technol.* 128, 745–750.
- Wu, L., Lan, S.B., Zhang, D.L., Hu, C.X., 2013. Functional reactivation of photosystem II in lichen soil crusts after long-term desiccation. *Plant Soil* 369, 177–186.
- Xia, L., Ge, H.M., Zhou, X.P., Zhang, D.L., Hu, C.X., 2013. Photoautotrophic outdoor two-stage cultivation for oleaginous microalgae *Scenedesmus obtusus* XJ-15. *Bioresour. Technol.* 144, 261–267.
- Xie, Z.M., Chen, L.Z., Li, D.H., Shen, Y.W., Hu, C.X., Liu, Y.D., 2007. The research on the function of soil filamentous cyanobacteria in desertification control. *Acta Hydrobiol. Sinica* 31 (6), 886–890.
- Zhou, X.P., Xia, L., Ge, H.M., Zhang, D.L., Hu, C.X., 2013. Feasibility of biodiesel production by microalgae *Chlorella* sp. (FACHB-1748) under outdoor conditions. *Bioresour. Technol.* 138, 131–135.