Effects of dissolved organic matter on the growth and pigments synthesis of *Spirulina platensis* (*Arthrospira*)*

MA Zengling[1], GAO Kunshan[1,2**] and WATANABE Teruo[3]

(1. Marine Biology Institute, Shantou University, Shantou 515063, China; 2. Institute of Hydrobiology, The Chinese Academy of Sciences, Wuhan 430072, China; 3. Hainan DIC Microalgae Co. Ltd., Haikou International Commercial Centre 38, Haikou 570102, China)

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Abstract Excessive accumulation of dissolved organic matter (DOM) in the culture ponds of *Spirulina platensis* is usually considered to be one of the potential factors affecting the production of *S. platensis*, however, we are not quite aware of effects of DOM on the growth and pigments synthesis of *S. platensis*. In the present study, *S. platensis* was grown in batch or semi-continuous cultures using the filtrate in the culture ponds that had not been renewed for years. It was found that disssolved organic carbon up to 60 mg/L did not bring about an inhibitory effect on the growth of *S. platensis*, but increased the contents of chlorophyll *a* and phycocyanin instead. However, further accumulation of dissolved organic matter could decrease the content of chlorophyll *a*.

Keywords: Chl *a*, dissolved organic matter, PC, specific growth rate, *Spirulina platensis* (*Arthrospira*).

Commercial mass cultivation of *S. platensis* (*Arthrospira*) has been performed since the late 1970s because of its high protein content and other unique chemical compositions[1]. Continuous recycling of medium in the production has been accepted by producers as a cost-effective measure, which, however, results in excessive accumulation of dissolved organic matter (DOM) because of fragmentation of the trichomes in harvesting systems and metabolic excretion of organic compounds[2—4]. Cell death and subsequent lysis also contribute to the accumulation. Such DOM may be toxins or taste-odor compounds[5—7]. The effects of these compounds are of general concern in terms of resulting in contaminations by other organisms and possible growth autoinhibition[8]. However, we are not aware of the possibly direct effects of such organic compounds in culture on the growth of *S. platensis*. The aim of this study is to investigate the influence of dissolved organic matter on the growth and photosynthetic pigments of *A. platensis*.

1 Materials and methods

1.1 Organism and culture

Spirulina (*Arthrospira*) *platensis* D-0083, obtained from Hainan DIC Microalgae Co. Ltd., Hainan, China, was pre-cultured in Zarrouk's medium with continued aeration at 30 ℃ under cool-white fluorescent light of 100 μmol·m^{-2}·s^{-1} (12 L:12 D).

The medium that had not been renewed for years from the production base of Hainan DIC Microalgae Co. Ltd., Hainan, China was filtrated with a filter (pore size of 0.45 μm) and frozen at −20 ℃ before being used for experiment. For preparing the media, the filtrate was treated with activated carbon (10 g/L) to reduce the DOM. Then, the main nutrient components in the Zarrouk's medium, filtrate and filtrate treated with the activated carbon were tested. Concentrations of inorganic N and P were determined with a segmented flow analyzer (SKALAR, SA2000/4000, Netherlands), and total carbon, organic carbon and inorganic carbon were determined with a total organic carbon analyzer (TOC-5000A, Shimadzu, Japan) (Table 1). To compare the effects of different kinds of DOM, the concentration of inorganic N was adjusted to be equal to the value in the filtrate, while concentrations of inorganic P and C were adjusted according to the values in the Zarrouk's medium (Table 1) by adding $NaNO_3$, K_2HPO_4 or $NaHCO_3$. Therefore, three sets of culture media were prepared: (1) modified Zarrouk's medium (MZ, Zarrouk's medium in which N was adjusted to the level equal to

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** To whom correspondence should be addressed. E-mail: ksgao@stu.edu.cn

that in the filtrate); (2) modified filtrate (MF, Zarrouk 's medium prepared with filtrate) and (3) modified filtrate treated with activated carbon (MF-C, Zarrouk 's medium prepared with the activated-carbon-treated filtrate, all the inorganic N, P and C were adjusted as mentioned above).

Table 1. Concentration of inorganic C, N and P in the Zarrouk 's medium, filtrate and active-carbon-treated filtrate (Mean ± SD)

		Zarrouk 's medium	Filtrate	Filtrate treated with activated carbon ($10\,gL^{-1}$)
$NH4^{+}$	(mmol/L)	1.61 ± 0.07	1.13 ± 0.11	1.36 ± 0.35
$NO3^{-}$	(mmol/L)	20.31 ± 2.21	26.56 ± 3.47	17.41 ± 1.26
$NO2^{-}$	(mmol/L)	0	1.96 ± 0.06	1.40 ± 0.01
Total N	(mmol/L)	21.92	29.09	20.17
$PO4^{3-}$	(mmol/L)	2.36 ± 0.03	2.07 ± 0.04	1.61 ± 0.04
IC	(mmol/L)	180.00 ± 0.32	77.83 ± 0.20	77.16 ± 0.09
DOC	(mmol/L)	2.98 ± 0.24	4.90 ± 1.04	1.14 ± 0.40

$n = 4$

Cells of *S. platensis* in logarithmic growth phase were harvested and grown in these three kinds of media in acrylic vessels (12 cm × 20 cm × 2 cm) with transmittance of visible light (400—700 nm) being over 95%. Both batch and semi-continuous cultures were used, and were aerated (1 L/min) continuously with ambient air (360 μL/L CO_2). In the semi-continuous cultures, the media were partially renewed to maintain a constant level of cell density every other day.

1.2 Measurement of the dry mass and pigments

Cell density of the cultures was measured as dry mass by filtering 20 mL of the cultures through a pre-dried filter, drying in an oven at 80℃ for 24 h, weighing on an electronic balance and subtracting the known weight of the dried filter. Specific growth rate (μ) was calculated as $\mu = (\ln x_2 - \ln x_1)/(t_2 - t_1)$, where x_1 and x_2 represent the dry mass on day1 and day2 respectively, and units of μ are the reciprocal of time (d^{-1}).

Chl *a* was extracted by filtering 10 mL of the culture, all the sample collected was resuspended in 10 mL absolute methanol, and incubated in a water bath at 70℃ for 2 min. The supernatants obtained after centrifugation were used for the measurement with a spectrophotometer (Shimadzu UV-1206, Japan). Chl *a* content was determined according to Vonshak 's formula[9], and was normalized to the dry mass of the equal volume of culture used for the extraction. Phycocyanin (PC) was extracted by filtering 10 mL of the culture (Whatman GF/C, 25 mm). All the collected materials were resuspended in 10 mL 0.1 mol/L phosphate buffer containing 0.2 mol/L NaCl, and then frozen at −20℃ and thawed in cool water in the dark. This step was repeated until the cells disintegrated and the PC dissolved out. The extracts were centrifuged and the clear supernatant was used for the determination of PC content according to Siegelman 's method[10].

1.3 Detection of the organic matter

To detect and characterize the accumulated organic matter in the culture ponds, a small drop of filtrate was dotted on a transparent slice of KBr. Then the slice was scanned with an FIIR spectrometer (AVATAR 360, USA).

2 Results

In the batch culture, the biomass density of *S. platensis* increased with time (Fig. 1 (a)), while in the semi-continuous culture, the biomass in every two days became stable from the 4th day (Fig. 1 (b)). Specific growth rate declined with time in the batch culture (Fig. 2 (a)), whereas increased at the initial phase and then remained at about 0.4 (d^{-1}) in the semi-continuous culture (Fig. 2 (b)). However, both the biomass density and specific growth rate of

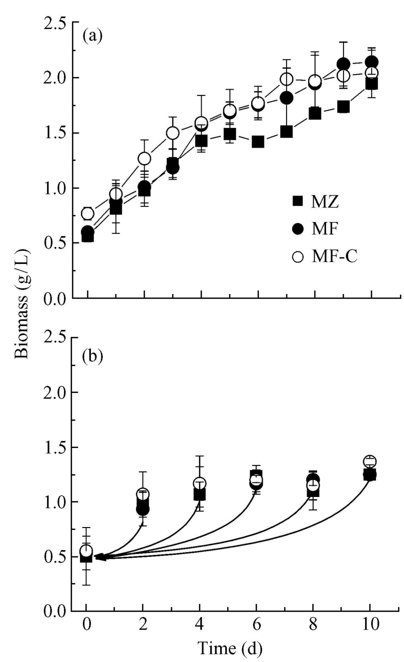


Fig. 1. The biomass density of *S. plaensis* in batch (a) and semi-continuous culture (b) at 100 μmol·m^{-2}·s^{-1} and 30℃. Mean ± SD ($n = 3$).

S. platensis did not show any significant difference ($p>0.05$) among the DOM treatments regardless of the culture conditions, though the concentration of DOC was nearly 60 mg/L in filtrate (i.e. 1.7 and 4.5 times higher than that in modified Zarrouk's medium and filtrate treated with activated carbon, respectively) (Table 2). On the other hand, the specific growth rate in the semi-continuous culture was much higher than that in the batch culture, with 2 and 10 times higher on the 4th and 10th day, respectively.

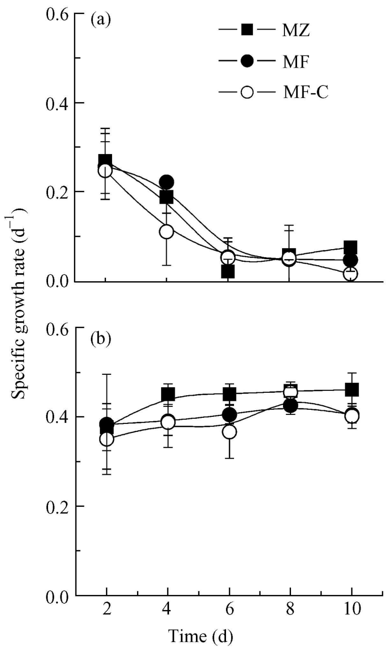


Fig. 2. The specific growth rate of *S. platensis* in batch (a) and semi-continuous (b) cultures at 100 μmol·m^{-2}·s^{-1} and 30 ℃.

Chl *a* content of *S. platensis* increased with time in the batch culture (Fig. 3 (a)) but decreased in the semi-continuous culture (Fig. 3 (b)) regardless of DOM treatments. In addition, the Chl *a* content was the lowest in the modified Zarrouk's medium (MZ) either in batch culture or in semi-continuous culture. In the batch culture, Chl *a* content was significantly higher ($p<0.05$) in the culture with the lowest DOC (MF-C, medium prepared by the filtrate that had been treated with activated carbon) as compared to that in the other DOM treatments. In contrast, in semi-continuous culture, the Chl *a* content was significantly higher ($p<0.05$) in the modified filtrate medium (MF) than that in MF-C and MZ treatments. The content of PC decreased in the initial phase in semi-continuous culture irrespective of DOM treatments, but increased from the 6th day in MF and MF-C treatments, which was much higher ($p<0.05$) in the filtrate-based media (MF) as compared to that in the modified Zarrouk's medium (MZ) (Fig. 3 (c)).

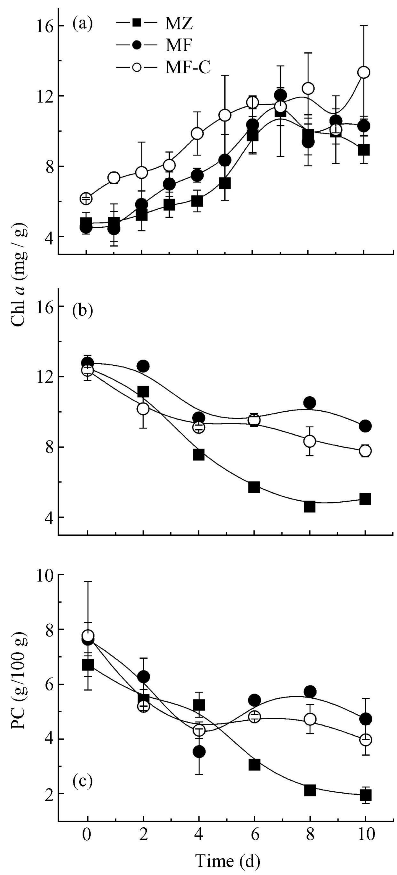


Fig. 3. Chlorophyll *a* content of *S. platensi* in batch (a) and semi-continuous (b) culture and the phycocyanin content in the semi-continuous culture (c). Mean ± SD ($n=3$).

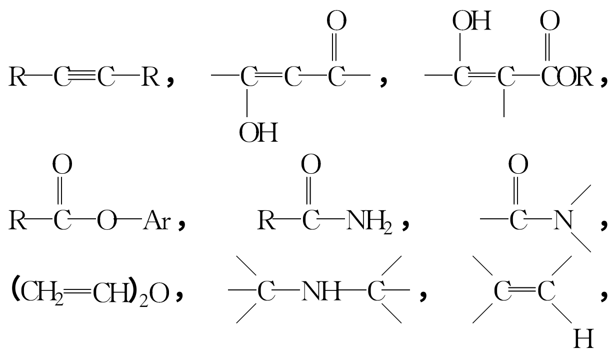
Table 2. Dissolved inorganic (IC) and organic carbon (DOC) at the beginning and end of the experiment (Mean ± SD)

Culture		MZ		MF		MF-C	
		Initial	End	Initial	End	Initial	End
Batch	IC(mg/L)	2260 ± 13	1011 ± 12	2226 ± 34	1338 ± 26	2171 ± 4	1145 ± 33
	DOC(mg/L)	35 ± 3	58 ± 7	58 ± 12	71 ± 6	13 ± 4	22 ± 8
Semi-continuous	IC(mg/L)	2245 ± 20	1492 ± 34	2202 ± 2	1767 ± 36	2158 ± 30	1896 ± 54
	DOC(mg/L)	35 ± 3	33 ± 2	58 ± 12	45 ± 4	13 ± 4	15 ± 2

$n=3$

The concentration of dissolved inorganic carbon (DIC) in all media decreased with time (Table 2). There was 50%—60% DIC left at the end of the experiment in the batch culture, while 70%—80% remained in semi-continuous culture. However, the concentration of DOC increased in batch culture, but decreased in semi-continuous culture (Table 2).

The spectra obtained with FIIR spectrometry indicated that there were two higher peaks and several lower peaks in the functional molecule area ($4000\ cm^{-1}$—$1300\ cm^{-1}$) (Fig. 4). These peaks are associated with different groups of compounds, and the height of the peaks indicates relative amounts of these compounds. Though the molecular structure of DOM in pond water cannot be exactly identified, based on the FIIR spectra, these compounds were characterized by the groups of $-CH_3$,



$R-NO_2$ and so on. Therefore, they could be hydrocarbon, alkene, alkine, ketone, ester, aniline, acyl, alcoholic aldehyde or hydroxybenzene. Among the above compounds, some are biomolecules and some are toxins.

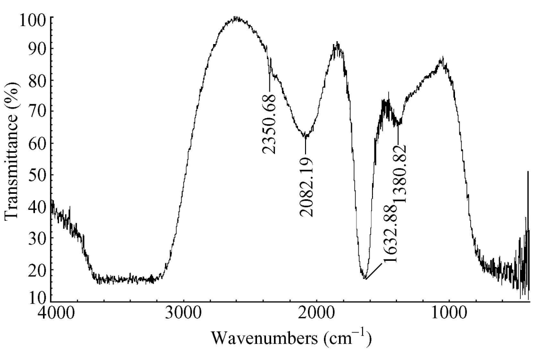


Fig. 4. Infrared spectrum of the filtrate collected from the ponds of Hainan DIC production base.

3 Discussion

It was reported that dissolved organic carbon could be accumulated with time in the culture of *S. pacifica*[4]. *S. platensis* was also found to excrete polysaccharides[2] even under optimal growth conditions, and the excretion of the organic carbon depended on the nutritive status and growth phase[11—13]. In the present study, analysis with infrared spectrum showed that dissolved organic matter was accumulated in the pond. However, dissolved organic matter up to 60 mg/L in the culture pond did not influence the growth of *S. platensis*, although some dissolved matter may be toxic[5—7]. Our explanation is that the level of the organic matter could be too low to exert any effects on the growth of *S. platensis*. And *S. platensis* has been found to be able to use many organic compounds for mixotrophic growth[14—17]. Dissolved organic matter might play dual roles, i.e. inhibiting or enhancing the metabolism of the cells, which depends on the levels of the accumulation of these compounds.

Further increase in DOM content may inhibit the growth of *S. platensis*. It was reported that treatment of filtered pond water with charcoal to remove organic matter brought about a much higher growth rate of *Spirulina* than the untreated control sample, but when culture and harvest conditions were improved to reduce fragmentation of cells, there was no difference in growth of *Spirulina* observed between charcoal treatment and untreated controls[8]. It was generally recognized that bacteria in natural waters rapidly utilize labile compounds, such as acids and monosaccharides even at low concentration (nM)[18,19]. In a laboratory study, about 70% labile compounds (glucose, glutamate) were rapidly (in less than 48 hours) utilized by natural assemblage marine bacteria and about 15% of these compounds were reverted to refractory DOM that persisting in decomposition[20]. So the variance in number of bacteria would lead to the fluctuation of DOM content and affect biomass yield of *S. platensis* ultimately.

The content of photosynthetic pigments in *S. platensis* could be influenced by many factors, such as irradiance, cell density and nutrition conditions[18]. Self-shading of the cells due to high cell density in batch cultures could lead to a higher Chl *a* content than that in semi-continuous cultures. The DOM accumulated in the filtrate may also improve the Chl *a* content in *S. platensis* as seen in semi-continuous culture. However, further accumulation of DOC (>58 mg/L) in the modified filtrate in the batch culture resulted in the decrease of the Chl *a* content. It has been reported PC formation could be strengthened by organic matter[16]. In our present study, the PC content was higher in the filtrate, suggesting that the dissolved organic matter might contribute to the increase of PC in *S. platensis*.

Previous studies have shown that excessive accumulation of organic matter often resulted in contamination by other algae[8,19] and overmuch foaming[8] in the *S. platensis* culture ponds, which would affect the production. However, as it was shown in the present study, the accumulation of organic matter itself did not bring about significant inhibitory effects on the growth of *S. platensis*, indicating that the accumulation of organic matter should be controlled to a certain level. Thus, to optimize the growth conditions of *S. platensis* should still be considered for improving the production.

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