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EXPERIMENTAL ARTICLES

Growth Relationships of a Lipid-Producing *Chlorella*-Alga with Common Microalgae in Laboratory Co-Cultures[1]

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Abstract—Co-existence growth relationships were studied in communities consisting of a lipid-producing alga *Chlorella* sp. HQ, another green alga and one cyanobacterium: I *Scenedesmus obliquus* and *Microcystis aeruginosa*; II *Chlamydomonas reinhardtii* and *Anabaena flos-aquae*; III *Selenastrum capricornutum* and *Microcystis wesenbergii*. The cyanobacteria and green algae except for *Chlorella* sp. HQ were commonly detected in Chinese reservoir and wastewater. The rate of increase of apparent cell number difference with other algae (k_{app}), inhibition/stimulation ratio (ISR) and the parameters of logistic model and co-existence model were determined for *Chlorella*. *Chlorella* strains were the most competitive in Combination I, and were stimulated during 75% of the cultivation time for all three combinations. *Anabaena* growth exceeded those of *Chlorella* and *Chlamydomonas* on the 5th cultivation day under 1 : 1 : 1 inoculum ratio. *Scenedesmus* colonies consisted of fewer cells, whose average length significantly shortened after the 5th cultivation day under 1 : 1 : 1 inoculum ratio. The developed co-existence model can identify the concrete growth inhibitor or stimulator among three species compared with the single method of cell number monitoring. Good correlation was found between transformed and non-transformed co-existence model through a_{mn} and b_{mn} values. Allelopathy and nutrient competition are both possible mechanisms in the above growth relationships.

Keywords: algal co-existence model, co-cultures, microalgae, growth relationship, inoculum ratio, lipid-producing alga

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Growth relationships among algal species affect their biomass in co-cultures. The mechanisms responsible for the relationship involve physical factors, allelopathic interaction and nutrient competition in aquatic ecosystems [1–3]. Allelopathy is one of the factors promoting and maintaining algal blooms in freshwater systems, and allelochemicals may affect the algal co-existence growth relationships [4]. On the other hand, changes of nutrient concentration may accelerate algal growth in co-cultures. In the south central coastal waters of Viet Nam higher ammonium concentration allowed the haptophyte *Phaeocystis globosa* to grow faster than the diatoms [5]. Allelopathy and nutrient competition are both possible mechanisms in development of algal growth relationships.

Toxin-producing cyanobacteria (*Microcystis, Anabaena*, etc.) readily form massive blooms in eutrophic natural lakes and reservoirs due to nitrogen (N) and phosphorus (P) inflow from point and diffuse sources [6]. Cultivation of microalgae offers a novel approach for N and P removal, providing biotreatment coupled with production of valuable biomass, which can be used for various purposes (lipid production, animal food, etc.) [7]. Open pond systems for mass cultivation of lipid-producing algae provide a cost-effective option since they utilize natural light, and therefore require less energy to construct and operate than closed photobioreactors [8]. However, these systems are more susceptible to contamination with unwanted microalgae, by which the growth of lipid-producing algae tends to be affected [9]. Up to now, few studies have been conducted on the growth relationships of a lipid-producing alga with other common microalgae under conditions of co-existence. The growth relationship involving the lipid-producing alga may determine their anti-contamination ability against other algae and cyanobacteria in open pond systems, and also affect its lipid production and wastewater purification efficiency. Our previous study revealed that a lipid-producing alga *Chlorella* sp. HQ grew to higher yield than *Scenedesmus obliquus* and *Chlamydomonas reinhardtii* under 1 : 1 inoculum ratio in binary co-cultures, and when co-existing with *C. reinhardtii* or *Selenastrum capricornutum* under 1 : 1 inoculum ratio, it had higher lipid contents than the same strain in monoculture [10].

The aim of this study was to investigate the co-existing growth relationships of *Chlorella* sp. HQ with other green algae and cyanobacteria in laboratory co-cultures. The specific green algae and cyanobacteria were chosen from the algal species commonly detected

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Table 1. Inoculum cell numbers of algae and cyanobacteria in mono-cultures and co-cultures (10^4 cells mL^{-1})

Algal strain	Culture groups									
	A	B	C	D	E	F	G	H	I	J
Chlorella sp. HQ	20	100	–	–	–	–	20	100	20	20
Another green alga	–	–	20	100	–	–	20	20	100	20
Cyanobacterium	–	–	–	–	20	100	20	20	20	100

in Chinese reservoirs and wastewaters and combined to simulate co-existence growth environments in open ponds.

MATERIALS AND METHODS

Algal strains. A lipid-producing alga *Chlorella* sp. HQ unexpectedly isolated from a culture of aging *Botryococcus braunii* in our previous study was used for the present investigation. Three green algae (*Scenedesmus obliquus* FACHB-417, *Chlamydomonas reinhardtii* FACHB-479, and *Selenastrum capricornutum* FACHB-271) and three cyanobacterial strains (*Microcystis aeruginosa* FACHB-915, *Anabaena flos-aquae* FACHB-245, and *Microcystis wesenbergii* FACHB-929) were obtained from the FACHB Collection (Freshwater Algae Culture of the Institute of Hydrobiology, China). Pure cultures of green algae and cyanobacteria were maintained in sterile media SE and BG11, respectively, under (25 ± 1)°C and 60 μmol photons m^{-2} s^{-1} illumination in 14 h light/10 h dark cycles.

Algal inoculation and growth monitoring. Co-existing growth relationships of *Chlorella* sp. HQ with other microalgae and cyanobacteria were evaluated for three combinations under the same cultivation conditions. Sterilized mixture of 2/3SE + 1/3BG11 media (30 mL) was added to a series of 50 mL Erlenmeyer flasks with different densities of algal inocula (Table 1). Cells in diluted algal cultures were counted with a haemacytometer to ascertain the initial cell densities. The inocula were of known cell density and came from the exponential-phase cultures. The flasks were shaken by hand three times daily to minimize cell sedimentation. Algal cultures were aseptically sampled daily for cell number measurement using a haemacytometer. Cultivation duration was 7 days. The morphological characteristics of the cells were examined under a light microscope and cell sizes were determined with an eyepiece lens micrometer. All treatments were carried out in triplicate ($n = 3$).

Comprehensive comparison on growth relationships. The extent algal growth was affected was determined as the inhibition/stimulation ratio (ISR) defined by Eq. (1):

$$\mathrm{ISR}(\%) = \left(1 - \frac{N}{N_0}\right) \times 100, \quad (1)$$

where N_0 and N (cells mL^{-1}) are the cell numbers of one algal species in mono-cultures and co-cultures, respectively. Positive ISR values represent inhibition by other algae and cyanobacteria, and negative values represent stimulation.

The apparent density difference (ADD) was defined by Eq. (2):

$$(\mathrm{ADD})_n = (\rho_{An} - \rho_{Bn}), \quad (2)$$

where ADD (cells mL^{-1}) is the apparent density difference, n is the cultivation day, *An* and *Bn* are cell densities of *Chlorella* and another alga or cyanobacterium after n-day cultivation. An apparent rate constant (k_{app}, cells mL^{-1} day^{-1}) is used to define the rate of increase of the ADD value.

Logistic model was used to describe algal growth in Eq. (3) [11]:

$$N = \frac{N_m}{1 + \exp(a - r_m t)}, \quad (3)$$

where t is the cultivation day, N (cells mL^{-1}) is an algal cell numbers after t-day cultivation, N_m (cells mL^{-1}) is the carrying capacity, i.e., the predicted maximum cell numbers in the cultivation systems, r_m (day^{-1}) is the specific growth rate for the whole period of growth and a is a constant. Values of N_m, a and r_m could be fitted through data series of N and t by Nonlinear Curve Fit (Slogistic1) implemented in the Origin 8.6 software package.

When algal growth is in the exponential phase, the specific growth rate is not significantly affected by cell densities and is considered as a constant. Values of specific growth rate in the exponential phase can be calculated from Eq. (4) [12]:

$$\mu = \frac{\ln N_{t_2} - \ln N_{t_1}}{t_2 - t_1}, \quad (4)$$

where μ is the specific growth rate in the exponential phase (day^{-1}), t_1 and t_2 represent the starting and ending point of growth period, N_{t_1} and N_{t_2} are cell numbers of one species (cells mL^{-1}) after t_1-day and t_2-day cultivation, respectively.

Co-existence model was used to describe the co-existing interaction among three algal species in Eq. (5):

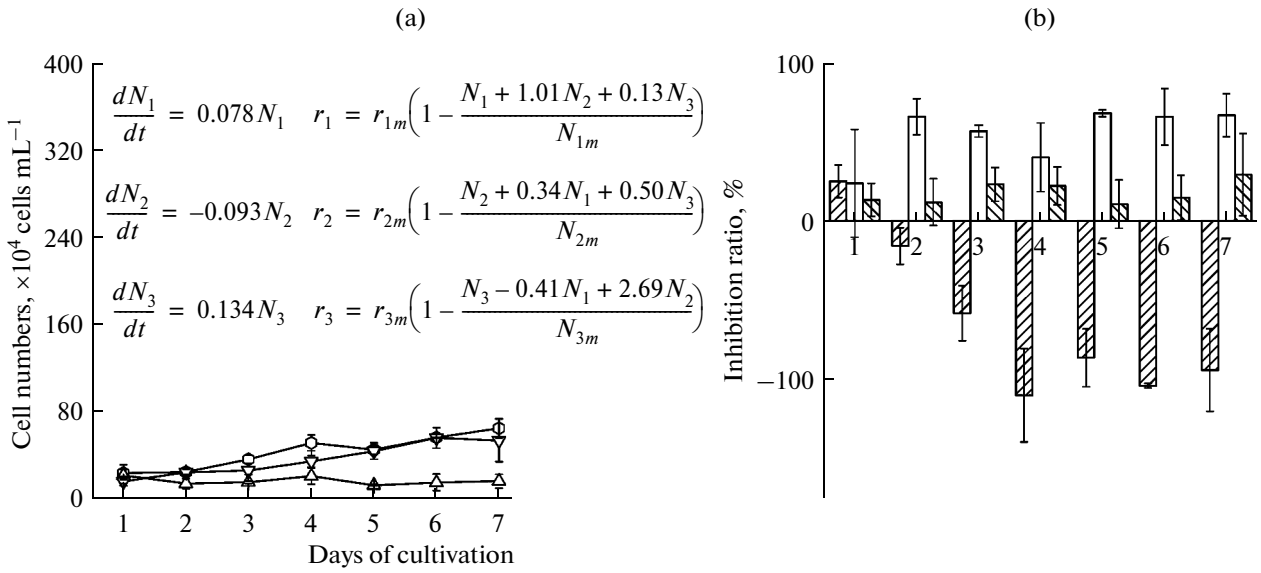

Fig. 1. Growth curves and inhibition/stimulation ratios of *Chlorella* sp. HQ (hexagon; squares with left diagonal), *Scenedesmus obliquus* (up triangle; blank squares) and *M. aeruginosa* (down triangle; squares with right diagonal) under 1 : 1 : 1 inoculum ratio. (a)—Growth curves, (b)—inhibition/stimulation ratios, means ±SD, $n = 3$.

$$\frac{dN_1}{dt} = \mu_1 N_1, \quad \mu_1 = r_{1m}\left(1 - \frac{N_1 + a_1N_2 + a_2N_3}{N_{1m}}\right),$$

$$\frac{dN_2}{dt} = \mu_2 N_2, \quad \mu_2 = r_{2m}\left(1 - \frac{N_2 + a_3N_1 + a_4N_3}{N_{2m}}\right), \quad (5)$$

$$\frac{dN_3}{dt} = \mu_3 N_3, \quad \mu_3 = r_{3m}\left(1 - \frac{N_3 + a_5N_1 + a_6N_2}{N_{3m}}\right),$$

where N_n (cells mL^{-1}) is the algal cell numbers after t-day cultivation, N_{nm} (cells mL^{-1}) is the predicted maximum algal cell numbers in co-cultures, μ_n (day^{-1}) is the specific growth rate in the exponential growth phase, r_{nm} (day^{-1}) is the specific growth rate during the whole period of growth and a_n is a fitted parameter. Negative values of a_n represent antagonism while positive values represent synergism. When synergism is observed between two species, co-existence model can be transformed into another model in Eq. (6) to verify this interaction:

$$\mu_1 = r_{1m}\left(1 - \frac{N_1}{N_{1m} + b_1N_2}\right),$$
$$\mu_2 = r_{2m}\left(1 - \frac{N_2}{N_{2m} + b_2N_1}\right), \quad (6)$$

where b_n was a fitted parameter. b_n values are negative in the case of synergism.

The ability to utilize higher inoculum density by an alga or a cyanobacterium was expressed by the Δhu value defined in Eq. (7):

$$(\Delta hu)_n = \frac{\rho_{co_n} - \rho_{uni_n}}{\rho_{uni_n}}, \quad (7)$$

where n is the cultivation day, ρ_{uni_n} and ρ_{co_n} are cell numbers in mono-culture and co-existence environments after n-day cultivation, respectively. The higher the value of $(\Delta hu)_n$ is the stronger the ability to utilize higher inoculum density.

Data analysis and graphing. Growth curves and diagrams in this work were created by the data analysis and graphing software package Origin 8.6. Paired-samples t test, independent-samples t test and one-way ANOVA were calculated using the IBM SPSS Statistics 20 program. Mean values and standard deviations (SD) were calculated from triplicates. Parameters in co-existence model and its transformed form in Eq. (6) were fitted by Datafit 9.0 Software. In these two models cell numbers (N_n) were defined as independent variables, r_n as dependent variables, a_n and b_n as fitted parameters and r_{nm} and N_{nm} as constants.

RESULTS AND DISCUSSION

Growth of *Chlorella* sp. HQ with *Scenedesmus obliquus* and *M. aeruginosa*. Figure 1a shows the growth curves of *Chlorella* sp. HQ, *Scenedesmus obliquus* and *M. aeruginosa* in co-cultures under 1 : 1 : 1 inoculum ratio. *Chlorella* cell numbers remained higher than those of *Scenedesmus obliquus* and *M. aeruginosa* throughout the whole cultivation period. ISR values (Fig. 1b) showed the stimulation interaction on *Chlorella* kept growing from day 2 to day 4, and remained at a high level during the latter 3 days. A linear fitting between ISR values and

cultivation time showed good correlations (ISR (%) = $43.17t - 69.01$, $r = 0.996$). It indicates that *Chlorella* obtained a growth advantage when co-existing with *Scenedesmus* and *Microcystis* under 1 : 1 : 1 inoculum ratio, and there might be an allelopathic interaction. Calculation and fitting results on ADD values with t showed the ADD values for *Chlorella* with *Scenedesmus* and *Microcystis* in mixed cultures inoculated with the same cell numbers of all components both remained positive and kept rising, especially fast with *Scenedesmus obliquus* (with *Microcystis*: $k_{app} = 1.90 \times 10^4$ cells mL^{-1} day^{-1}, $r = 0.186$; with *Scenedesmus*: $k_{app} = 8.19 \times 10^4$ cells mL^{-1} day^{-1}, $r = 0.928$). It indicates that *Scenedesmus* capacity for growth was the weakest in environmental setup, and much lower than that of *Chlorella*.

In mixed cultures inoculated with different cell numbers of all components, algae with higher inoculum density (1×10^6 cells mL^{-1}) tend to obtain a growth advantage. However, in the case of high-density *Scenedesmus obliquus* inoculum, its cell numbers declined during the first 5 days and approached those of *Chlorella* sp. HQ and *M. aeruginosa* on the 4th cultivation day (Fig. 2). It indicates that the comprehensive effect of antagonistic interaction with *Chlorella* and *Microcystis* on *Scenedesmus* was strong enough to override the growth advantage of high-density inoculum. Results of paired-samples t test showed *Scenedesmus* growth was significantly inhibited in co-cultures under 1 : 1 : 1 inoculum ratio ($P < 0.05$). Submerged macrophytes *Najas minor* and *Potamogeton malaianus* significantly inhibited the growth of *Scenedesmus obliquus*, exhibiting allelopathic effects [13]. Our previous study showed that water extracts of a giant reed *Arundo donax* L. inhibited the growth of *M. aeruginosa*, with its cell size decreasing with an increasing extract concentration, which was also an allelopathic effect [14]. The results of the present work showed *Scenedesmus* cells were smaller in mixed cultures inoculated with the same cell numbers of all components than in mono-cultures (Table 2). It was speculated that co-cultivation with *Chlorella* sp. HQ and *M. aeruginosa* led to an allelopathic growth inhibition on *Scenedesmus* and a decrease in its cell size.

M. aeruginosa FACHB-915 is an international standardized toxic cyanobacterium, and our previous study showed its ability to release microcystins [15]. *Scenedesmus obliquus* CPCC5 exhibited higher sensitivity to microcystin standards (variants MC-LF, LR, RR, YR) than *Chlorella vulgaris* CPCC111 [16]. Unlike other *Chlorophyta*, *Chlorella kesslerii* only responded to microcystins-LR and -RR when at the highest concentration used in the work, and the effects of microcystins-LR were weaker, apparent only after 11-day exposure [17]. *Scenedesmus obliquus*, *Chlorella vulgaris* and *M. aeruginosa* could each resist the grazing by a rotifer *Brachionus calyciflorus* applying different ecological strategies, such as enhancing colony formation, accelerating growth and enhancing toxin formation to keep their population at a certain level [18]. In this study, fewer *Scenedesmus* cells were found to form colonies in co-cultures than in mono-cultures. It is speculated that *Chlorella* sp. HQ might be more resistant to microcystin than *Scenedesmus obliquus*, whose possible mechanisms for the resistance might be a release of allelochemicals and resource competition.

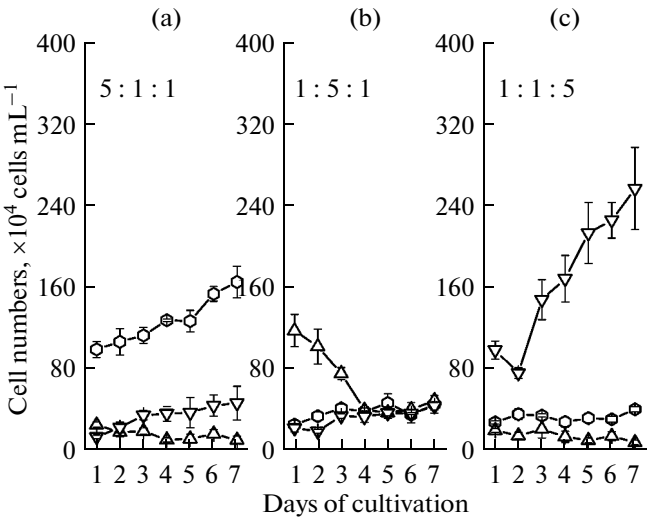

Fig. 2. Growth curves of *Chlorella* sp. HQ (hegaxon), *Scenedesmus obliquus* (up triangle) and *M. aeruginosa* (down triangle) under 5 : 1 : 1, 1 : 5 : 1 and 1 : 1 : 5 inoculum ratio (*Chlorella* to *Scenedesmus* and *Microcystis*), means ±SD, $n = 3$.

Growth of *Chlorella* sp. HQ with *Chlamydomonas reinhardtii* and *A. flos-aquae*. Figure 3 shows the growth curves of *Chlorella* sp. HQ, *Chlamydomonas reinhardtii* and *A. flos-aquae* under different inoculum ratios (1 : 1 : 1 and 5 : 1 : 1). During the first four days

Table 2. The decrease on average *Scenedesmus* cell size co-existing under 1 : 1 : 1 inoculum ratio (μm)

Size category	Cultivation days		
	5th day	6th day	7th day
Mono-cultured	10.22 ± 0.66	11.27 ± 0.43	10.48 ± 0.26
Co-existing	7.94 ± 0.10	7.55 ± 0.20	7.01 ± 0.31
Value of decrease	2.28 ± 0.58	3.72 ± 0.62	3.47 ± 0.30

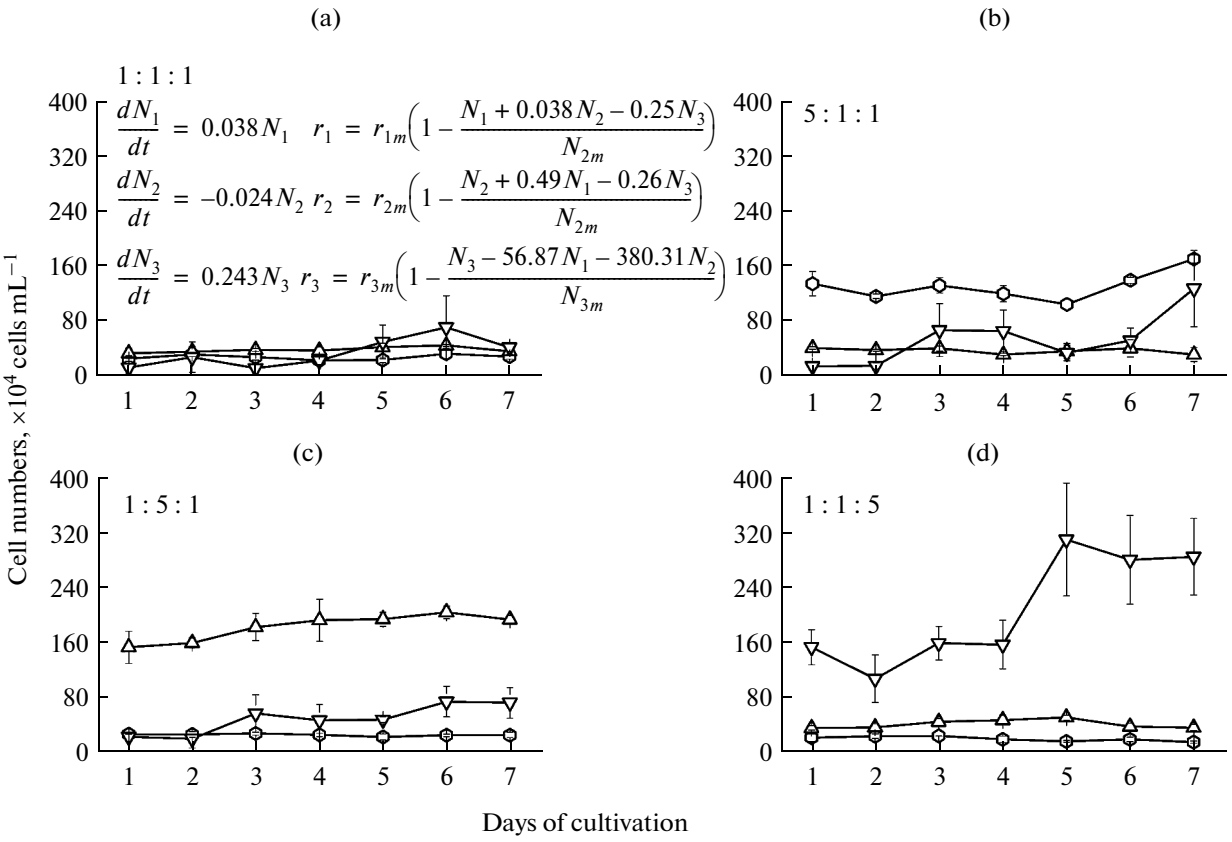

Fig. 3. Growth curves of *Chlorella* sp. HQ (hexagon), *Chlamydomonas reinhardtii* (up triangle) and *A. flos-aquae* (down triangle) under 1 : 1 : 1, 5 : 1 : 1, 1 : 5 : 1 and 1 : 1 : 5 inoculum ratio (*Chlorella* to *Chlamydomonas* and *Anabaena*), means ±SD, $n = 3$.

of cultivation, cell numbers of *Chlorella* were higher than those of *Anabaena* and lower than those of *Chlamydomonas* at 1 : 1 : 1 inoculum ratio (Fig. 3a). After the fifth day, *A. flos-aquae* achieved the highest cell numbers, indicating its strong capacity for growth. Furthermore, *Anabaena* densities in co-cultures inoculated with different cell numbers of all components with 1×10^6 cells mL^{-1} inoculum density were higher than other microalgae with the same inoculum density in different co-cultures in the latter 3 days of cultivation (Figs. 3b–3d). It indicated that the growth ability of *A. flos-aquae* was stronger than those of *Chlorella* sp. HQ and *Chlamydomonas reinhardtii* in co-cultures.

At 1 : 1 : 1 inoculum ratio, *Chlorella* sp. HQ was more readily stimulated than other microalgae and cyanobacteria, as were its strains in co-cultures inoculated with different cell numbers of all components (Figs. 4a and 4b). On the other hand, effects of stimulation started to decline after the 3rd cultivation day. This could be attributed to the interference from other microalgae and cyanobacteria. *A. flos-aquae* were more readily inhibited in co-cultures (Fig. 4c). However, cell numbers of *Anabaena* were higher than those of the other microalgae in all four co-cultures since the 5th cultivation day. As a result the growth potential of *A. flos-aquae* in co-cultures was stronger than the other two microalgae.

Water extract from the roots of a macrophyte *Thalia dealbata* significantly inhibited the growth of *A. flos-aquae* and induced a decrease in its chlorophyll a content, which was an allelopathic effect [19]. *M. aeruginosa* cell-free filtrate caused a maximum decrease in the growth and content of pigments of *Anabaena* PCC.7120, which was also an allelopathic effect [20]. Chlorophylls are colored pigments [21], and the present study found a less intense pigmentation of *Anabaena* cells in co-cultures compared with the mono-cultures (photos not shown). It is speculated that *Chlorella* and *Chlamydomonas* released allelochemicals to inhibit *Anabaena* growth. In lakes with prolonged nitrogen limitation, *Anabaena* species were found to be common [22]. Wood et al. reported that the apparent increase in nitrogen fixation capacity appeared to be an important prerequisite to bloom formation for *Anabaena planktonica* in a reservoir [23]. It is speculated from the present finding that *A. flos-aquae* might utilize its nitrogen-fixing ability to adapt to the nutrient deficiency in the latter 3 cultivation days, thus gaining growth advantage.

Growth of *Chlorella* sp. HQ with *Selenastrum capricornutum* and *M. wesenbergii*. Figure 5a shows the

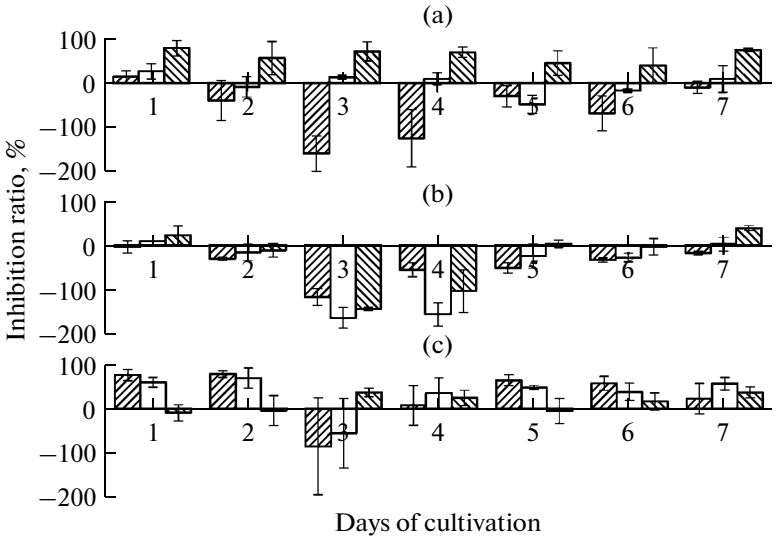

Fig. 4. Inhibition/stimulation ratios of *Chlorella* sp. HQ, *Chlamydomonas reinhardtii* and *A. flos-aquae* in co-cultures. (a)—1 : 1 : 1 inoculation, *Chlorella*: squares with left diagonal; *Chlamydomonas*: blank squares; *Anabaena*: squares with right diagonal (b)—*Chlorella* ISR under other inoculum ratios, (c)—*Anabaena* ISR under other inoculum ratios, *Chlorella* to *Chlamydomonas* and *Anabaena*, 5 : 1 : 1: squares with left diagonal; 1 : 5 : 1: blank squares; 1 : 1 : 5: squares with right diagonal, means ±SD, $n = 3$.

growth curves of *Chlorella* sp. HQ, *Selenastrum capricornutum* and *M. wesenbergii* under 1 : 1 : 1 inoculum ratio. Cell numbers of *Chlorella* kept declining for the first 5 cultivation days and rose up on the 6th cultivation day, suggesting its gradual adaptation. This trend was also observed, albeit for the first four days of cultivation, in the case of co-cultures with heavier *Chlorella* inoculum (1×10^6 cells mL^{-1}) (Fig. 6b). It indicated that higher inoculum density helped *Chlorella* to adapt faster to the co-existence conditions. *Chlorella* ISR value kept rising in the first 5 cultivation days under 1 : 1 : 1 inoculum ratio, showing good linear correlation with t (ISR (%) = $13.52t - 11.96$, $r = 0.980$), suggesting an initiative to gain growth advantages (Fig. 6c). Furthermore, ISR values of *Chlorella* strains in co-cultures with different inoculum ratios all showed a growing trend in the 6th to 7th cultivation day, showing a good growth potential.

Comprehensive comparison on the growth relationships. Table 3 shows the fitted parameters of the logistic and co-existence models for *Chlorella* and other algae and cyanobacteria in co-cultures at 1 : 1 : 1 inoculum ratio. In Combination III *Chlorella* showed the highest carrying capacity ($N_m = (5.20 \pm 4.03) \times 10^8$ cells mL^{-1}) and specific growth rate in the exponential phase ($\mu = (0.198 \pm 0.078)$ day^{-1}), suggesting a good growth potential of *Chlorella* when co-cultured with *Selenastrum capricornutum* and *M. wesenbergii*. These results are in agreement with the analysis on growth curves (see above). *A. flos-aquae* and *M. wesenbergii* exhibited higher maximum population densities in the co-cultures than the green algae. *Chlorella* specific growth rates during the exponential growth phase were always higher than those of other green algae but lower than those of cyanobacteria. According to our calculations, the type of co-existing species has a significant effect on *Chlorella* specific growth rates in the exponential phase, but not on its carrying capacities and specific growth rates throughout the whole period of growth (analyzed by one-way ANOVA, $P < 0.05$).

Fitted parameters a_{mn} (where m is the type of co-existing algal species and n is the inoculum ratio) from the co-existence model showed that *Chlorella* sp. HQ exerted an inhibitory effect on cyanobacterial growth in different co-existence environments under 1 : 1 : 1 inoculum ratio ($a_{51} = -0.41$, $a_{52} = -56.87$, $a_{53} = -24.03$), which is also apparent from the positive ISR values of cyanobacteria, while other common green algae exhibited a synergistic effect on the revelant cyanobacteria, except for *Chlamydomonas reinhardtii* to *A. flos-aquae* ($a_{62} = -380.31$). It is concluded that the algal co-existence model after data mining from cell number monitoring can identify the specific growth inhibitor among three co-existing species. Observation of a_{mn} parameters showed synergistic interaction between *Chlorella* and *Scenedesmus obliquus, Chlorella* and *Chlamydomonas reinhardtii, Scenedesmus obliquus* and *M. aeruginosa*, and *Selenastrum capricornutum* and *M. wesenbergii*. The b_{mn} parameters were then fitted from the transformed co-existence model (Eq. (6)) shown in Table 4, and c_{mn} values were substituted for the corresponding a_{mn} values in different co-cultivation environments. Our results showed that the b_{mn} values were all negative, indicating the occurrence of synergism in the transformed co-existence model. Moreover, the same growing and declining trend was found between the absolute values of b_{mn} and c_{mn}, indicating a good correlation between the

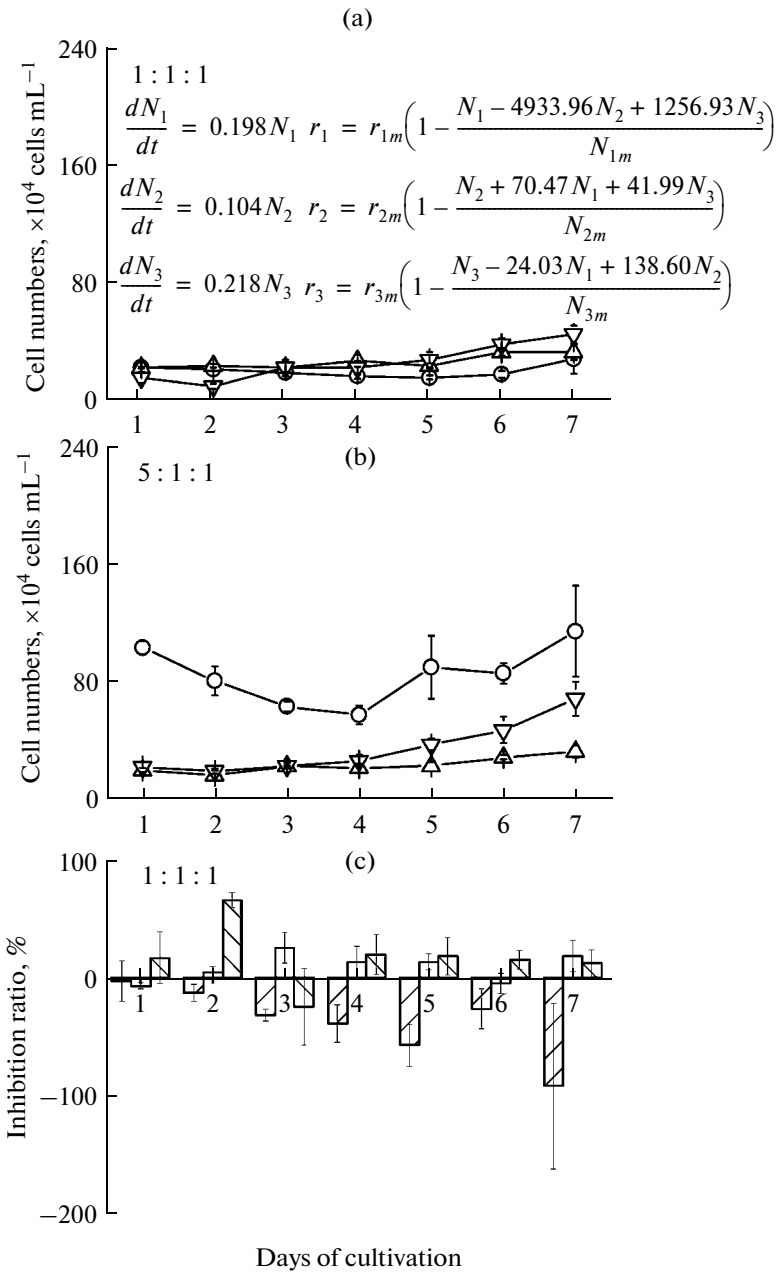

Fig. 5. Growth of *Chlorella* sp. HQ (hexagon), *Selenastrum capricornutum* (up triangle) and *M. wesenbergii* (down triangle) in co-cultures. (a)—1 : 1 : 1 inoculation, (b)—5 : 1 : 1 inoculation (*Chlorella* to *Selenastrum* and *Microcystis*), (c)—inhibition/stimulation ratios under 1 : 1 : 1 inoculum ratio, *Chlorella*: squares with left diagonal; *Selenastrum*: blank squares; *Microcystis*: squares with right diagonal, means ±SD, $n = 3$.

transformed and non-transformed co-existence model.

Figure 6 shows the values of abilities to utilize high inoculum density (Δ*hu*) for all the microalgae and cyanobacteria inoculated with 1×10^6 cells mL^{-1} in co-cultures. *Chlorella* Δ*hu* values were all positive in three combinations (except for the 5th cultivation day in Combination I), suggesting its highest ability to utilize higher inoculum density compared with six other microorganism. *Scenedesmus* Δ*hu* values were all negative, suggesting its lowest ability of utilizing the higher inoculum density, which agrees with the former analysis of the growth curves. The declining trend of *Anabaena* Δ*hu* values suggested possible allelopathic interaction or resource competition with other microalgae in co-cultures, while its cell numbers were still the highest on those cultivation days, which indicated a substantially stronger capacity for growth. The type of co-existing species had no significant effect on *Chlorella* Δ*hu* values (analyzed by One-Way ANOVA,

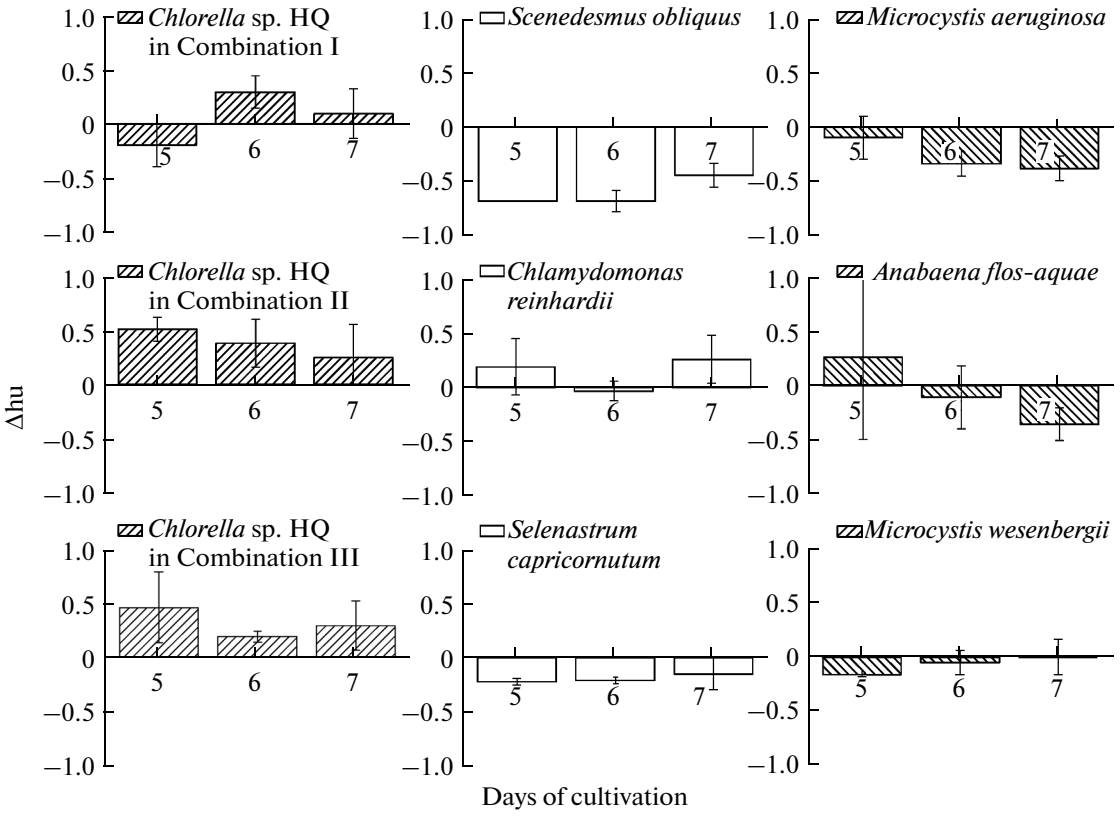

Fig. 6. Algal abilities to utilize high inoculum density (Δ*hu*) from the 5th to 7th cultivation day (1×10^6 cells mL^{-1} inoculum density), means ±SD, $n = 3$.

$P > 0.05$). This again verifies a good growth potential of *Chlorella* sp. HQ in co-cultures.

Mass cultivation of lipid-producing algae in open pond systems results in a complex symbiotic system of microalgae, cyanobacteria, bacteria, and zooplankton where the target organisms should dominate [24]. Since open ponds are under direct exposure to the atmosphere, and the growing algal cells usually get essential nutrition from treated or untreated wastewater, lipid-producing algae may be contaminated not only by other microalgae and cyanobacteria, but also by bacteria, zooplankton (grazing), viruses, etc. [9]. Eight bacterial strains isolated from a long-term laboratory culture of green alga *Chlorella ellipsoidea* were reported to promote the growth of *Chlorella ellipsoidea* in co-cultures [25]. Yin et al. observed from co-culture experiments that the presence of *Clamydomonas sajao* inhibited the growth potential of *Chlorella ellipsoidea*, while introduction of herbivorous *Daphnia magna* enhanced the growth of *Chlorella ellipsoidea*, since *D. magna* preferred *Clamydomonas sajao* to *Chlorella ellipsoidea* regardless of the relative abundance of these two algae [26].

In this study, the algae and cyanobacteria differed widely in their cell length and width. *Chlorella* outgrew *Scenedesmus obliquus* and *M. aeruginosa* in the co-existence environment under 1 : 1 : 1 inoculum ratio during the whole experimental period, while its volume ratio of inoculum biomass was lower compared with the other alga and cyanobacteria. *Chlorella* sp. HQ is a smaller algal species (length: 3.05 ± 0.54 μm, width: 2.55 ± 0.44 μm, $n = 20$) compared with *Scenedesmus obliquus* (length: 7.78 ± 1.25 μm, width: 5.06 ± 0.75 μm, $n = 20$) and *M. aeruginosa* (length: 5.17 ± 1.13 μm, width: 3.53 ± 0.80 μm, $n = 20$). However, the fact that *Chlorella* still outgrew one green alga and one cyanobacterium with lower inoculum biomass suggested its substantial strong growth ability.

Qian et al. found that co-existing relationship between *Chlorella pyrenoidosa* and *M. aeruginosa* might be complex because of an allelopathic effect, and nutrient competition was not the only factor determining species dominance in the phytoplankton assemblage [27]. DellaGreca et al. reported that the composition and amount of allelochemicals released by *Chlorella vulgaris* and *Pseudokirchneriella subcapitata* in co-cultures might depend on phosphate concentration in the culture medium [28]. Li et al. reported that allelopathy and nutrient competition were both possible mechanisms in determining the dominant species of three coastal harmful dinoflagellates, *Prorocentrum minimum, Prorocentrum donghaiense* and *Karlodinium veneficum*, and one off-shore dinoflagellate, *Karenia brevis* in co-cultures [29]. We

Table 3. Fitted parameters of logistic model and co-existence model for *Chlorella* sp. HQ, other algae and cyanobacteria co-existing under 1 : 1 : 1 inoculum ratio, means ±SD, $n = 3$

Parameter category	Co-existing combinations		
	Combination I	Combination II	Combination III
Algal species	*Chlorella* sp. HQ	*Chlorella* sp. HQ	*Chlorella* sp. HQ
N_m (carrying capacity, 10^4 cells mL^{-1})	96.49 ± 37.72	28.99 ±3.16	(5.20 ± 4.03) × 10^4
r_m (specific growth rate, day^{-1})	0.37 ± 0.08	1.91 ± 0.55	0.06 ± 0.06
μ (specific growth rate in exponential phase, day^{-1}	0.078 ± 0.010	0.038 ± 0.031	0.198 ± 0.078
Average values of co-existence model parameters	$a_{11} = 1.01$ $a_{21} = 0.13$	$a_{12} = 0.38$ $a_{22} = -0.25$	$a_{13} = -4933.96$ $a_{23} = 1256.93$
Algal species	*Scenedesmus obiquus*	*Chlamydomonas reinhardtii*	*Selenastrum capricornutum*
N_m (carrying capacity, 10^4 cells mL^{-1})	24.81 ±13.71	40.53 ± 6.02	(0.82 ± 0.36) × 10^4
r_m (specific growth rate, day^{-1})	0.07 ± 0.03	−0.64 ± 0.55	0.16 ± 0.12
μ (specific growth rate in exponential phase, day^{-1})	−0.093 ± 0.048	−0.024 ± 0.010	0.104 ± 0.063
Average values of co-existence model parameters	$a_{31} = 0.34$ $a_{41} = 0.50$	$a_{32} = 0.49$ $a_{42} = -0.26$	$a_{33} = 70.47$ $a_{43} = 41.99$
Algal species	*M. aeruginosa*	*A. flos-aquae*	*M. wesenbergii*
N_m (carrying capacity, 10^4 cells mL^{-1})	91.69 ± 62.40	(5.75 ± 3.89) × 10^4	(2.77 ± 2.47) × 10^5
r_m (specific growth rate, day^{-1})	0.50 ± 0.15	0.19 ± 0.10	0.25 ± 0.02
μ (specific growth rate in exponential phase, day^{-1}	0.134 ± 0.080	0.243 ± 0.093	0.218 ± 0.022
Average values of co-existence model parameters	$a_{51} = -0.41$ $a_{61} = 2.59$	$a_{52} = -56.87$ $a_{62} = -380.31$	$a_{53} = -24.03$ $a_{63} = 138.60$

Table 4. Average transformed and non-transformed co-existence model parameters under 1 : 1 : 1 inoculum ratio between two algal species, $n = 3$

Parameter category	Synergism co-existences	
Algal species	*Chlorella* sp. HQ and *Scenedesmus obliquus*	*Chlorella* sp. HQ and *Chlamydomonas reinhardtii*
Average values of transformed and non-transformed model parameters	$b_{11} = -1.62$, $c_{11} = 1.01$ $b_{21} = -0.30$, $c_{21} = 0.34$	$b_{12} = -6.35$, $c_{12} = 0.38$ $b_{22} = -9.16$, $c_{22} = -0.49$
Algal species	*Scenedesmus obliquus* and *M. aeruginosa*	*Selenastrum capricornutum* and *M. wesenbergii*
Average values of transformed and non-transformed model parameters	$b_{31} = -0.35$, $c_{31} = 0.50$ $b_{41} = -1.78$, $c_{41} = 2.69$	$b_{13} = -305.04$, $c_{13} = 41.99$ $b_{23} = -864.32$, $c_{23} = 138.60$

concluded from this study that *Chlorella* sp. HQ is a promising lipid-producing alga for its application in open pond systems. We also speculated that the growth relationships of *Chlorella* sp. HQ with common microalgae and cyanobacteria in co-cultures are related to allelopathy and nutrient competition. Further research shall be conducted on the mechanisms of the development of growth relationships between *Chlorella* sp. HQ and other microalgae and cyanobacteria (allelochemical composition and concentration in co-cultures, and nutrient utilization extent, etc.).

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