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Effects of nitrogen, phosphorus, iron and silicon on growth of five species of marine benthic diatoms

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ABSTRACT

The effects of nitrogen, phosphorus, iron and silicon on growth of five species of marine benthic diatoms, namely *Navicula patrickae*, *Nitzschia panduriformis*, *Navicula thienemannii*, *Nitzschia longissima* and *Navicula atomus* were studied by single factor experiments and the optimal concentration ratios of the four nutrient elements beneficial for diatoms growth were screened out separately using the $L_9\,(3^4)$ orthogonal design. The results highlighted that nitrogen, phosphorus, iron and silicon all had highly significant effects on growth of five diatoms while the diatoms growth rates reached the highest averagely in the 2nd to the 6th culture day. In addition, the optimal concentrations (mg/L) of four nutrients suitable for diatoms growth were found higher than that in f/2 medium except that *Nitzschia longissima* had the same concentration of nitrogen as that in f/2 medium which is optimal for growth. Moreover, the optimal growth concentrations of four elements for five diatoms varied in the range of 12.36–74.16 mg/L for nitrogen, 1.70–3.98 mg/L for phosphorus, 2.00–4.00 mg/L for iron, 23.01–69.03 mg/L for silicon, respectively. By means of the orthogonal test of four nutrients for five benthic diatoms, the optimal concentration ratios N:P:Fe:Si (mg/L) were obtained as follows: 74.16:2.27:3.33:23.01 for *N. patrickae*; 37.08:3.98:4.00:11.50 for *N. panduriformis*; 49.44:3.98:3.33:34.51 for *N. thienemannii*; 12.36:1.70:4.00:11.50 for *N. longissima*; 74.16:2.27:4.00:69.03 for *N. atomus*.

Artificial culturing microalgae play an important role in marine animal culture. As the main source of nourishment in larval developmental phase of many marine animals in aquatic ecosystem microalgae can also improve the animals' nutritional value. Marine benthic diatoms as the important food of sea cucumber and abalone larva in stage of diet conversion can induce the larval settlement as well as metamorphosis [1,2]. Benthic diatoms can be cultured and grow rapidly in certain conditions relating to the basic ecological factors such as illumination, water temperature and so on [3–5]. Without nitrogen, phosphorus, iron and silicon, which are benefit for the diatom metabolism, diatoms cannot grow normally during the mass culture [6]. Many studies on effects of nutrients on growth of *Navicula* and *Nitzschia* have been reported [7–10]. However, there are no effective media used for mass culture of microalgae especially benthic diatoms. Based on the f/2 medium [11], we studied effects of nitrogen, phosphorus, iron and silicon on growth of three strains of Navicula (*Navicula patrickae*, *Navicula thienemannii* and *Navicula atomus*) and two strains of Nitzschia (*Nitzschia panduriformis* and *Nitzschia longissima*) by means of single factor experiments. Moreover, a series of orthogonal experiments were performed to confirm the optimal concentration ratios of the four nutrient elements suiTab. for diatoms growth, which could be beneficial to exploit effective nutritional media boosting the rapid growth and mass production of benthic diatoms.

1. Materials and methods

1.1. Source of benthic diatoms

We isolated 21 strains of marine benthic diatoms by means of the micropipette, dilution, plate streaking and spreading techniques from October 2011 to March 2012 in tidal zone of Heishijiao in Dalian, China. The monospecific benthic diatoms were domesticated gradually and cultured in illumination incubator with sTab. conditions. Finally five strains with relative higher growth rates were selected to conduct the present study. With acidizing and observation under a microscope and SEM [12–14], they were identified *Navicula patrickae* (5#), *Nitzschia panduriformis* (6#), *Navicula thienemannii* (7#), *Nitzschia longissima* (8#) and *Navicula atomus* (9#), respectively, maintained at the key laboratory of hydrobiology in Liaoning province. Figure 1 describes the morphological characteristics of five diatoms under the Olympus microscope.

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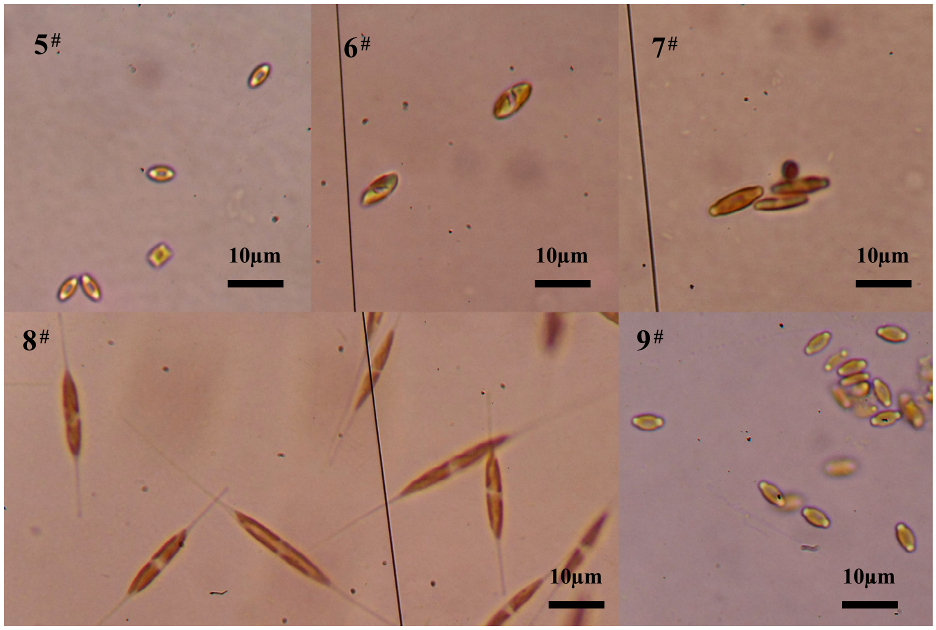

5#—*Navicula patrickae*, (6×3)μm; 6#—*Nitzschia panduriformis*, (11×6)μm; 7#—*Navicula thienemannii*, (12×4)μm;

8#—*Nitzschia longissima* (90×6)μm; 9#—*Navicula atomus* (7×3)μm

Fig. 1. Characteristics of five benthic diatoms in micrometers (length × width). 5# – *Navicula patrickae*, 6 × 3 μm; 6# – *Nitzschia panduriformis*, 11 × 6 μm; 7# – *Navicula thienemannii*, 12 × 4 μm; 8# – *Nitzschia longissima*, 90 × 6 μm; 9# – *Navicula atomus*, 7 × 3 μm.

1.2. Algal cultures

Firstly, the diatoms were cultured with natural seawater (salinity 32 and pH 8.3) of Heishijiao in Dalian dealt with sand filtration, precipitation, bolting silk filtration with bore diameter 48 μm and boiling sterilization successively while the background values of nitrogen, phosphorus, iron and silicon of the seawater were 0.15, 0.02, 0.0005, 0.50 mg/L separately. Secondly, the conical flasks and test tubes culturing diatoms were soaked overnight with sodium hypochlorite, scrubbed with a bristled brush, rinsed in hot tap water, dried in a laboratory oven and autoclaved with cotton plug sealing and foil wrapping finally in 121 °C for 15 min. Then, the diatom strains cultured in illumination incubator were inoculated with f/2 medium [11] renewed every 12 days growing in conditions of temperature 20 ± 1 °C and light intensity 60–70 μmol photons $m^{-2} s^{-1}$ with a 12:12 light/dark cycle. And finally, the experimental strains growing in log phase would be quantified with OD value in 680 nm [15].

1.3. Methods

1.3.1. Single factor test of growth on nitrogen, phosphorus, iron and silicon of benthic diatoms

Based on the f/2 medium, eight concentration gradients (mg/L) were designed for nitrogen, phosphorus and iron in forms of $NaNO_3$, $NaH_2PO_4{\cdot}2H_2O$ and $FeC_6H_5O_7{\cdot}5H_2O$ respectively while the silicon element in form of $Na_2SiO_3{\cdot}9H_2O$ was tested designing six concentration gradients (mg/L) (Table 1) and the other nutrient components followed the f/2 medium. The diatom cultures in triplicate were kept in sterile glass test tubes (13 × 100 mm) sealing with cotton plugs (15 test tubes/diatom/concentration) and each diatom growing exponentially was inoculated in test tubes with 3 mL sterile natural seawater. In the meanwhile, five diatoms' optical densities in 680 nm determined by microplate reader in conditions of 20 °C and shaking 1 min/10 s (Multiskan Go) were all controlled at 0.045, approximately equal to the appropriate inoculation density 1 × 10^5 ind./mL [16]. After inoculation and determination, all test tubes were transferred to HPG-280B illumination incubator placing randomly with culture conditions as mentioned earlier maintaining for 48 h. In the stationary culture period, diatom cells were attached to the test tube bottoms little by little. Afterwards, supernatant liquid was discarded and add 3 mL specified medium to corresponding test tubes separately. During the 12 culture days, the triplicate test tubes were randomly taken out respectively in the 2nd, 4th, 6th, 9th and 12th days. Brush their bottoms and walls slowly by wood brushes for 10 circles and keep shaking for 1 min with whirlpool oscillators. Finally, the OD_{680} values were determined in triplicate and then the test tubes were discarded.

1.3.2. Orthogonal experiments of effects of nitrogen, phosphorus, iron and silicon on benthic diatoms

The maximum specific growth rates in signal factor test results were used as a reference to select the three corresponding element concentrations of nitrogen, phosphorus, iron and silicon as the test levels (Table 2) and the L_9 (3^4) design (Table 7) was selected to

Table 1
Concentrations of nitrogen, phosphorus, iron and silicon in single factor design.

Concentration gradient	Element concentration (mg/L)			
	N	P	Fe	Si
1	0.00	0.00	0.00	0.00
2	6.18	0.57	0.33	2.30$^{f/2}$
3	12.36$^{f/2}$	1.14$^{f/2}$	0.65$^{f/2}$	11.50
4	24.72	1.70	1.33	23.01
5	37.08	2.27	2.00	34.51
6	49.44	2.84	2.67	69.03
7	61.80	3.41	3.33	–
8	74.16	3.98	4.00	–

Note: The "f/2" means the corresponding concentration of the element in f/2 medium.