Skeletonema cf. *costatum* biogenic silica production rate determinated by PDMPO method

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Abstract .This paper introduces the basic mechanism of the formation of diatom silica walls and the new way of research silicic acid metabolism-PDMPO (2-(4-pyridyl)-5-((4-(2-dimethylaminoethylaminocarbamoyl) methoxy) pHenyl) oxazole) fluorescent dye method, which can combine with silicic acid to form complex eventually into the Si deposition within diatom cells, under a fluorescence microscope after excitation with bright green fluorescence, the advantage of this method is that it can monitor the metabolism of silicate after added PDMPO. Experiment, collecting samples in each of the specified time points, samples were determination of the unuse silicic acid, silica dissolution intracellular and Si deposition within diatom cells are not wet-alkaline digestions method but also PDMPO fluorescent dye method. Experiments show that PDMPO fluorescent and silicate have a good linear relationship, but the resulting of BSi: PDMPO is low compared with other reported.

Key words : biogenic silica; PDMPO Method; *Skeletonema* cf. *costatum*

1 Introduction

Silicon in the earth's crust as the second most abundant element, is widely present in the silicate and silica, less in elemental form [1]. Silicon is a very important element of diatoms, sponges, mollusks and higher plants etc [2,3]. In oceans, diatoms are one of the most typical biological groups who uses silicon elements to form itself structure, having more than 30 000 species (Jun Sun personal communications), and having more from nano- to micro- silica cell wall structure [7]. Siliceous mineralization of diatom can produce material structure is over chemical synthesis process, making bio-based nano-materials manufacturing technology applications possible [8-11]. Thus, it is particularly important that the perspective of the internal structure of diatom and molecular biology to understand diatom cell wall formation mechanism.

In water, silicate concentrations of the general environment for diatoms is usually below 100 μM [1,12], higher in estuaries and coastal regions, lower in ocean. However, intracellular has a very high concentration of dissolved silicic acid which can be achieved 19-340 mM [1,13]. It is supersaturated. Diatom priority with silicon transporters (SITS) to absorb $Si(OH)_4$ from ambient water [14-18]. Physiological studies show that silicic acid transmembrane transport dependent Na^+ ion and selective Si active transport system [2,18-23]. Silicic acid transport to cellular, diatoms will form silicon transport vesicles (STVS) to storage silicic acid [14,24]. Then silicic acid is transported to an organelle membrane structures called silica deposition vesicle (SDV). SDV is constantly extended shell surface and synthetize daughter-cell

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frustules [2,25-27]. Diatom wall structure is complex and exquisite, and has a specificity between different species. Many studies focus on the relationship between the various aspects of the formation of diatom frustules as well as the diatom silicate metabolism response mechanism affected with environment factors. Recently, combined with the corresponding development of fluorescent probes and electronic imaging technology, fluorescent dye PDMPO (2-(4-pyridyl)-5-((4-(2-dimethylaminoethylaminocarbamoyl) methoxy) pHenyl) oxazole) is introduced for the studying of diatoms biogenic silica forming.

PDMPO which stands for compound 2-(4-pyridyl)-5-((4-(2-dimethylaminoethylaminocar bamoyl) methoxy)pHenyl)oxazole, is a pH fluorescent indicator (Figure 1). PDMPO can be detected pH change within the living cell [28-31]; the pH is low (pH < 5.0), have the yellow emitting florescence, and the pH is high (pH> 6.0), have the blue emitting florescence [28]. Because stokes shift is larger and light stability is stronger, it become ideal tool for pH fluorescent indicator in vivo [28].

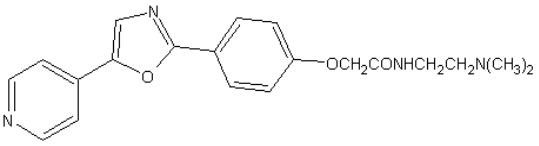

Figure 1 PDMPO chemical structure [2,28]

Silicon can be formed complex with PDMPO, along with silicic acid into the interior of cell, and finally deposited in the newly formed frustules which can be distinguished with the old formation of frustules [32], making it become an ideal fluorescent tracer for silicon [2]. The complex is formed between polymeric silicic acid with PDMPO, which has a unique fluorescent properties, bright green fluorescence emission [2]. It has a positive significance to study the process of diatom biogenic silica forming.

2 Materials and methods

2.1 Instruments and reagents

The fluorescence was measured using a spectrofluorophotometer (model RF-5301, SHIMADZU, Japan). The fluorescent PDMPO (LysoSensorTM yellow/blue DND-160) was used as an indicator dye, at a concentration of 1 mM.

2.2 Experimental setup

Seawater (collected from Seats station in South China Sea) is filtrated with 0.2 μm pore-size PC (polycarbonate) membrane (Millipore, USA), and sterilized in the laboratory. *Skeletonema* cf. *costatum* is isolated from Baicheng sea areas of Xiamen city. Culture medium according to the f/2 formula prepared, and PDMPO added to a final concentration of 0.125 μM in the culture medium. Since a molecule of silicic acid can be combined with 3230 molecules of PDMPO [32], a maximum concentration of silicic acid of 403.75 μM can be combined with 0.125 μM PDMPO. It has done experiments shows: silicate concentration is about 175.92 μM in f/2 medium, so PDMPO can all be combined with silicic acid; preparation of adding only the other f/2 formula, no PDMPO medium.

30 ml of *Skeletonema* cf. *costatum* stock cultures were transferred into 125 mL culture flasks in four replicates. Three out the four flasks were then added with PDMPO solution and diluted to 125 ml using 0.2 μm filtered seawater, the fourth flasks were only diluted to 125 mL without addition of PDMPO. All the flasks were mixed well and incubated at 26 ℃, under irradiance of 25-28 umol/(m^2×s) in the incubator (GXZ-1000c Jiangnan, China). The light/ dark cycle of the incubation was 12h/ 12h. The samples for fluorescence measurements were collected at time points of 0 h, 2 h, 4 h, 10 h, 22 h and 34 h.

At each sampling time, 10ml medium from flask 1-4, respectively, filtrated with 0.6 μm PC membrane, taking 5 ml filtrate is determined unutilized silicate; membrane rinsed with 1 ml 10% HCl, repeated twice, then immediately with 1 ml milli-Q water rinse, repeat three times, taking its solution measured intracellular dissoluble silicate; membrane is biogenic silica which deposited on the

walls of *Skeletonema* cf. *costatum*. Unutilized silicate and intracellular dissoluble silicate diluted 5-fold is used silicomolybdate complex assay. Determination of biogenic silica which deposited on the walls of *Skeletonema* cf. *costatum* use hot alkali extraction method (0.2M NaOH) and silicomolybdate complex assay.

1-3 flasks at each time point, filtrate 5 ml medium with 0.6 μm PC membrane again, take 5 ml of filtrate is measured unutilized silicate; membrane rinsed with 1 ml 10% HCl, repeated twice, and then immediately with 1 ml mili-Q water rinse, repeated three times, taking its solution measured intracellular dissoluble silicate; membrane is biogenic silica which deposited on the walls of *Skeletonema* cf. *costatum*. Unutilized silicate and intracellular dissoluble silicate using fluorescence spectrofluorophotometer is determined (excitation wavelength of 375 nm, emission wavelength of 530 nm); biogenic silica which deposited on the walls of *Skeletonema* cf. *costatum* using hot alkaline extraction method (0.2M NaOH) and fluorescence spectrofluorophotometer is measured (excitation wavelength of 375 nm, emission wavelength of 530 nm).

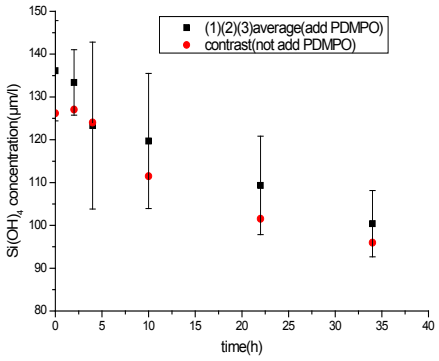

Figure 2 The determination of unutilized silicate with hot alkali extraction method

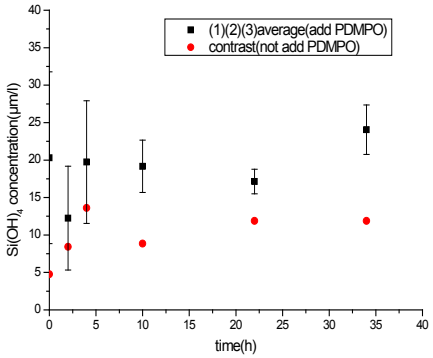

Figure 3 The determination of intracellular dissoluble silicate with hot alkali extraction method

3 Results and analysis

3.1 The results of determinate *Skeletonema* cf. *costatum* biogenic silica(BSi) with hot alkali extraction and PDMPO dyeing method

At 34 h of culture time, measured by hot alkali extraction method, unutilized silicate decreased from (136.128 ± 11.700) μm/l to (100.403 ± 7.735) μm/l, is significantly lower (Figure 2); intracellular dissoluble silicate from (20.311 ± 11.458) μm/l fluctuations to (24.056 ± 3.301) μm/l, there is a small increase (Figure 3); biogenic silica which deposited on the walls of *Skeletonema cf. costatum* from (15.846 ± 3.028) μm/l to (42.351 ± 7.356) μm/l, is significantly increases (Figure 4). In recovery, the average recovery rate at each time point is 95.644%.

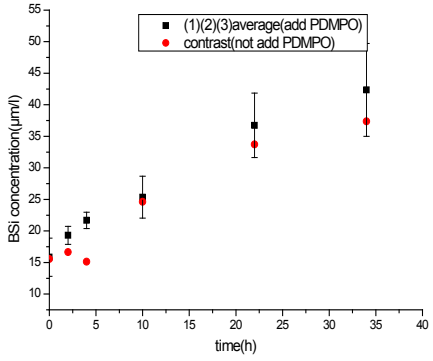

Figure 4 The determination of biogenic silica which deposited on the walls of *Skeletonema* cf. *costatum* with hot alkaline extraction method

In the 34 h of culture time, PDMPO concentration measured of intracellular dissoluble silicate from (1.448 ± 1.437) nm/l fluctuations to (4.175 ± 0.576) nm/l, is a small increase (Figure 5); biogenic silica

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which deposited on the walls of *Skeletonema* cf. *costatum* from (13.139 ± 0.769) nm/l to (51.158 ± 3.421) nm/l, is significantly increased (Figure 6).

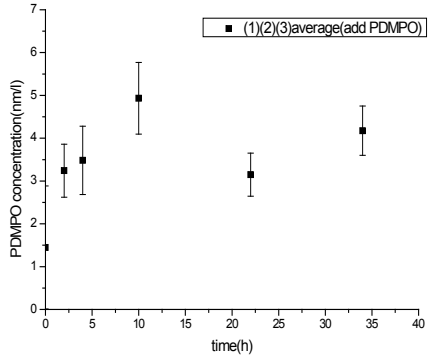

Figure 5 The determination of intracellular dissoluble silicate with PDMPO dyeing method

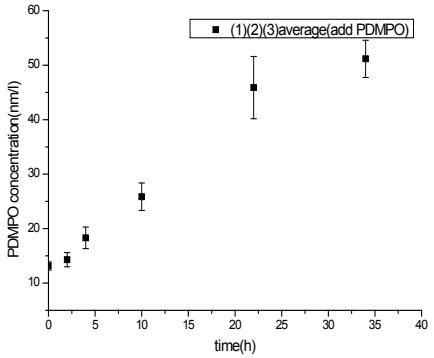

Figure 6 The determination of biogenic silica which deposited on the walls of *Skeletonema* cf. *costatum* with PDMPO dyeing method

A relationship curve between PDMPO and BSi (Figure 7) whose data from determination of biogenic silica deposited on the walls of *Skeletonema* cf. *costatum* with hot alkaline extraction and PDMPO dyeing method have a good linear relationship. The value of BSi: PDMPO which is (1244.269 ± 1005.235), compared with the BSi: PDMPO value (3230 ± 660) from Leblanc and Hutchins reported is low [32].

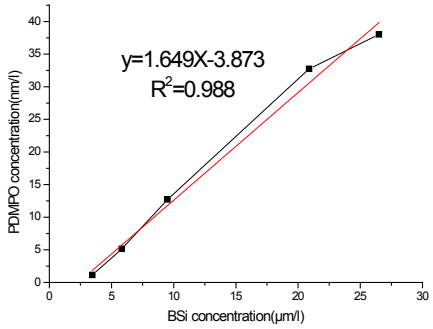

Figure 7 The relationship curve between PDMPO and BSi

4 Conclusion

At 34 h of culture time, it can be seen from figure 2-4 that adding PDMPO have not negative effect to the growth of *Skeletonema* cf. *costatum*; the concentrations of unutilized silicate and biogenic silica which deposited on the walls of *Skeletonema* cf. *costatum* were significantly reduced and increase. But intracellular dissoluble silicate has been maintained at a concentration of 22.184 μm/l, is no significant increase. The concentration of intracellular dissoluble silicate with PDMPO dyeing method have a slight increase during 0-10 h incubation time. There is no significant increase in 10-34 h incubation time similar to the trend with hot alkali extraction method. In terms of the relationship PDMPO and BSi, the present study using *Skeletonema* cf. *costatum* as subjects, the result of PDMPO: BSi is (1244.269 ± 1005.235), is lower than Leblanc and Hutchins reported BSi: PDMPO value (3230 ± 660). Karine Leblanc and David A. Hutchins studies indicate that PDMPO with concentration of 0.125 μM have not negative effects of diatom growth and frustules formation in over 96 h culture time [32]

At each time point, the determination of unutilized silicate, intracellular dissoluble silicate and biogenic silica which deposited on the walls of *Skeletonema* cf. *costatum* with hot alkali extraction and PDMPO dyeing method, respectively. It

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measured the relationship of input and output flux with *Skeletonema* cf. *costatum*.

Leblanc and Hutchins [32] consider that all the potential to study biogenic silica with PDMPO has not been reflected in the study of marine biogeochemical cycle, and this technology still needs further improvement, its development having benefit for diatoms research. With the continuous development of potential fluorescent dye, such as HCK-123 and 5-isothiocyanate (FITC) [33] with its advantages will be more widely used in biological research of diatom in the future.

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