Chemosphere 122 (2015) 162–167

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Effects of aqueous stable fullerene nanocrystal (nC_{60}) on *Scenedesmus obliquus*: Evaluation of the sub-lethal photosynthetic responses and inhibition mechanism

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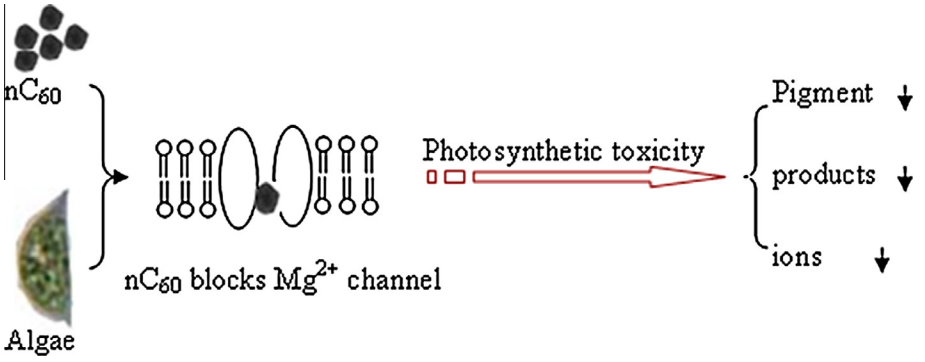
HIGHLIGHTS

- nC_{60} inhibited the growth rate of *S. obliquus*.
- nC_{60} at the sub-lethal concentration decreased algal photosynthetic products.
- nC_{60} at the sub-lethal concentration decreased algal chlorophylls contents.
- nC_{60} decreased algal chlorophyll center ion Mg^{2+} content by inhibiting Mg^{2+} uptake.
- nC_{60} aggregated to algal surface and partly decreased Mg^{2+}-ATPase activity.

GRAPHICAL ABSTRACT

nC_{60} particles are adsorbed on algal surface, and block the Mg^{2+} channels, consequently result in photosynthetic toxicity: decrease the photosynthetic pigment, photosynthetic products and other photosynthetic concerned ions, Mg^{2+}.



ARTICLE INFO

Article history:
Received 25 December 2013
Received in revised form 9 November 2014
Accepted 14 November 2014
Available online 3 December 2014

Handling Editor: J. de Boer

Keywords:
C_{60}
Fullerene
S. obliquus
Sub-lethal

ABSTRACT

Understanding sub-lethal effects of nanomaterial may be particularly important to determining ecosystem responses as current levels of nanomaterial release are low compared to levels projected for the future. In this work, the sub-lethal effects of water stable, nanocrystalline fullerenes as C_{60} (termed nC_{60}) were studied on *Scenedesmus obliquus*, a globally distributed phytoplankton. Sub-lethal concentration for *S. obliquus* was firstly determined as 0.09 mg L^{-1} using the standard 72 h exposure tests (OECD Guideline 201). Subsequent sub-lethal experiment of nC_{60} on the *S. obliquus* was carried out for 60 d and focused on the photosynthesis processes. The results demonstrate that upon sub-lethal exposure, the photosynthetic products of polysaccharide, soluble protein and total lipid were decreased with exposure time. The photosynthetic pigments of chlorophyll a and chlorophyll b were negatively impacted. Further investigations indicate that the decrements in photosynthetic products and pigments were mainly due to the algal Mg^{2+} decrement (by 40%) at the sub-lethal concentration (0.09 mg L^{-1}) of nC_{60}. The decrement in Mg^{2+} of *S. obliquus* was due to the inhibition of Mg^{2+}-ATPase activity caused by

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http://dx.doi.org/10.1016/j.chemosphere.2014.11.035

Photosynthesis
Mg^{2+}-ATPase

nC_{60}. Sum up, these results not only describe the sub-lethal effects but also provide the probably mechanism for sub-lethal effects of nC_{60} on exposed *S. obliquus*.

1. Introduction

Carbon fullerenes have been used in the applications of drug delivery, energy conversion, and other fields (Langa and Nierengarten, 2011). The increase in the production and applications of fullerenes inevitably result in the increasing release into the aqueous environment (Gottschalk et al., 2009). Fullerenes are widespread in a variety of environments, e.g. commercial cosmetics (from 0.04 to 1.1 $\mu g\, g^{-1}$) (Benn et al., 2011) and wastewater-treatment plant effluents across Europe (much higher than the estimated values) (Gottschalk et al., 2009; Tiede et al., 2009; Farré et al., 2010). Log *n*-octanol–water partition coefficient (Log K_{ow}) of molecular C_{60} is 6.67 (Jafvert and Kulkarni, 2008), and so molecular C_{60} tends to associate with environmental hydrophobic substances (e.g., natural organic matter and living organisms). Fullerenes (C_{60}) in aquatic system also tend to form the colloid (nC_{60}) via several mechanisms (Andrievsky et al., 1995). Previous toxic studies of nC_{60} have been studied on aqueous organisms of bacteria (Fortner et al., 2005; Lyon and Alvarez, 2008), *Daphnia magna* (Zhu et al., 2009; Tao et al., 2009), and other organisms (Oberdöster et al., 2006), but little is known about the toxic effects on the primary producer, *Scenedesmus obliquus* (*S. obliquus*).

S. obliquus is a producer at the first level of trophic chain, and is being used to produce biodiesel (Mandal and Mallick, 2012). Due to its important role in the equilibrium of aquatic ecosystems, *S. obliquus* is recommended as a standard test alga in growth inhibition tests for toxic compounds (OECD, 2006). Algal photosynthesis is the principal process in exploiting solar energy for algal growth, and is also the most sensitive process to assay many pollutants (Brack and Frank, 1998). There are currently some studies on the influence that nanoparticles (TiO_2, carbon black, carbon nanotubes and SiO_2) have on algae cultures (Hund-Rinke and Simon, 2006; Nielsen et al., 2008; Matorin et al., 2010; Wei et al., 2010), but little is known about the sub-lethal effects of fullerene on the photosynthesis of *S. obliquus*.

Mg^{2+} is the metallic ion at the center of chlorophylls, which are green pigments in the algal chloroplasts involved with photosynthesis. Mg^{2+} is also a necessary activator for two essential enzymes in carbon fixation: ribulosbiphophate carboxylase (RuBisCo) (Stec, 2012) and phosphoenolpyruvate carboxylase (PEPC) (Wedding and Black, 1988). Therefore, Mg^{2+} is an essential macronutrient for normal plant (algae) growth (Hüner and Hopkins, 2008); low amounts of Mg^{2+} will lead to decreases in photosynthetic and enzymatic activity. The algal uptake of Mg^{2+} is greatly involved with the activity of Mg^{2+}-ATPase, which is an ATPase that pumps Mg^{2+}. Previous studies shows that nC_{60} adjusts the uptake of metal ion (Tao et al., 2013), but little information is available about the algal Mg^{2+} uptake in the presence of nC_{60}.

We here focused on the sub-lethal toxicity of nC_{60} on the *S. obliquus* photosynthesis. In these studies, sub-lethal nC_{60} concentration was first determined for sequent experiments. The *S. obliquus* was repetitively exposed 20 times to nC_{60} at the sub-lethal concentration. The algal growth inhibition, photosynthesis products (polysaccharide, soluble protein and total lipid), photosynthesis pigments (chlorophyll a and chlorophyll b), Mg^{2+} absorption (key ion for photosynthesis), and Mg^{2+}-ATPase activity (transportation protein of Mg^{2+}) were measured to assess the sub-lethal toxic effects of aqueous stable fullerene nanocrystal (nC_{60}) on the photosynthesis process of *S. obliquus*.

2. Materials and methods

2.1. Materials

The C_{60} (99.9% purified through sublimation) was purchased from the Materials Electronics Research Corporation (USA). Tetrahydrofuran (THF) (spectro-analyzed, >99.99%), nitric acid, potassium dichromate and other chemicals (Analytic grade) were purchased from Fisher Scientific (USA). The *Scenedesmus obliquus* (*S. obliquus*) was gifted from Prof. Huang of Shanghai Ocean University. The water used was ultra-purified to >18 MΩ (Millipore® Synergy System).

2.2. nC_{60} preparation and purification

The nC_{60} preparation method of THF makes better crystal and better nano-scale particles (Brant et al., 2006; Lyon et al., 2006), which lead THF-nC_{60} to be higher toxic than other solvent-nC_{60} (Zhu et al., 2006; Lovern and Klaper, 2006). This higher toxicity allows THF-nC_{60} to be a good experimental object, which can provide the lower potential threshold value than other sovelent-nC_{60} for the aqueous animal protection. Therefore, the nC_{60} in this research was prepared according to the method of THF (Fortner et al., 2005; Tao et al., 2009). The residual THF and its derivatives were removed 10 times by YM 20000 membranes under nitrogen pressure (Fortner et al., 2007). Final residual organic concentrations in nC_{60} stock solution were lower than the detection limit (<1 $\mu g\, L^{-1}$) of a GC–MS (Agilent 6890/5793 GC/MS equipped with a HP-5MS (a 30 m × 0.25 mm i.d. column)). The *Z*-size of nC_{60} was 98 nm (Zetasizer Nano, Malvern Instruments Ltd., USA) (Tao et al., 2011).

2.3. Culture of S. obliquus

The *S. obliquus* was cultured in 800 mL liquid HB-4 medium contained in the Erlenmeyer flasks of 2000 mL volume. *S. obliquus* was incubated at 25 ± 1 °C under light at 3000 Lux with a light–dark period of 12:12 h. The pH was kept within 7–8. The Erlenmeyer flasks were shaken three times a day at 8:00, 12:00 and 20:00. *S. obliquus* cells were calculated based on the optical density (OD) measured by UV/Visible spectrometer at 670 nm.

2.4. Sub-lethal concentration determination

The algal growth inhibition test was conducted to determine the inhibition concentration of 50% growth rate (IC_{50}) and the maximal no observed effect concentration (NOEC) for nC_{60} according to the updated OECD guideline 201 (Freshwater Algal and Cyanobacterial Growth Inhibition Test). *S. obliquus* at the exponential growth phase was inoculated at an initial concentration of 2000 algae mL^{-1}. The nC_{60} concentrations in growth rate inhibition test were 0, 0.045, 0.09, 0.18, 0.36, 0.72 and 1.44 mg L^{-1}, respectively. The Erlenmeyer flask cultured *S. obliquus* were covered with a semi-membrane for 72 h under 25 ± 1 °C, with light cycle (day:-night = 12 h:12 h, 10000 lux). *S. obliquus* cells were calculated based on the optical density (OD) measured by UV/Visible spectrometer at 670 nm. All treatments, including control, were conducted in triplicate. The *S. obliquus* of control were increased 16 times more within 72 h. The reference toxicant for this test was

potassium dichromate. The IC_{50} of nC_{60} for *S. obliquus* growth rate was calculated using the method in OECD 201. The maximal no observed effect concentration (NOEC) of *S. obliquus* was deduced by compared treatment means using analysis of variance (ANOVA) techniques followed by Dunnett's test (OECD, 2005). The NOEC of nC_{60} for *S. obliquus* was used as the sub-lethal toxic concentration in all subsequent experiments.

2.5. Photosynthetic effects

2.5.1. Sub-lethal exposure experiment

The sub-lethal toxicity experiment of nC_{60} was carried out using *S. obliquus* repetitive exposure to 0.09 mg L^{-1} nC_{60} 20 times. The *S. obliquus* was inoculated at the density of 2000 algae mL^{-1}. The experimental incubation conditions of *S. obliquus* were the same as those for stock culture. The samples were taken at the 4th day, and then the exposed *S. obliquus* was re-inoculated to fresh cultural medium with nC_{60} at the density of 2000 algae mL^{-1}. The sub-lethal repetitive exposures to nC_{60} medium were carried out 20 times (60 d). The polysaccharide, soluble protein, total lipid, chlorophyll a, chlorophyll b, Mg^{2+} absorption and Mg^{2+}-ATPase activity were measured in the samples to assay sub-lethal toxic effects of nC_{60} on *S. obliquus*.

2.5.2. Measurements of polysaccharide, soluble protein and total lipid

The *S. obliquus* cells filtered from 10 mL samples were ground in a mortar with phosphate buffer (pH = 7.8). The concentrations of polysaccharide, soluble protein and total lipid were determined using Phenol-sulfate method, G-250 staining method and chloroform–methanol extraction method (C–M extraction method), respectively.

2.5.3. Measurements of chlorophyll a and chlorophyll b

The *S. obliquus* cells filtered from 10 mL were used to measure the chlorophyll a and chlorophyll b. The chlorophylls in algae were extracted for 24 h in dark at 4 °C after adding 5 mL acetone (80%). The extracted mixtures were centrifuged at 8000 rpm, and then supernatants were measured using spectrometer at 665 nm and 649 nm, respectively (Lichtenthaler, 1987). Chlorophyll a (chl a) and chlorophyll b (chl b) contents were calculated by the Eq. (1).

$$\text{Chl a} = 13.95A_{665} - 6.88A_{649};\ \text{Chl b} = 24.96A_{649} - 7.32A_{665} \quad (1)$$

where chl a and chl b are the contents for chlorophyll a and chlorophyll b, respectively; A_{665} and A_{649} are the optical absorption values at 665 nm and 649 nm, respectively.

2.6. Mg^{2+} uptake

2.6.1. Mg^{2+} in algal cells

The *S. obliquus* cells filtered from 10 mL were digested by adding 10 mL HNO_3 and 1 mL $HClO_4$, and then were heated in high beaker covered with a watch glass. The digested solution was brought to the volume of 20 mL with 2% HNO_3 solution. The Mg^{2+} concentration was measured using graphite furnace atomic absorption spectrometer at 285.2 nm.

2.6.2. Mg^{2+} in medium

The Mg^{2+} concentration in medium within the first exposure cycle (72 h) was conducted using batch experiment. The samples of 5 mL were taken at 0, 12, 24, 36, 48, 60, and 72 h, respectively. The algal cells in samples were removed using 0.45 μm cellulose acetate filter. The filtrate was acidized adding with nitric acid, and final nitric acid was 2%. The Mg^{2+} concentration in acidized filtrate was measured using graphite furnace atomic absorption spectrometer at 285.2 nm.

2.7. Mg^{2+}-ATPase activity

The *S. obliquus* cells filtered from 10 mL were ground using mortar and pestle at 4 °C after adding 3 mL STN buffer (0.05 mol L^{-1} sugar, 0.01 mol L^{-1} NaCl, pH = 7.8). The grinding mixture was centrifuged at 8000 rpm for 10 min at 4 °C, and then the supernatant was used for the Mg^{2+}-ATPase determination. Mg^{2+}-ATPase activities were measured using the measure kit for Mg^{2+}-ATPase (Nanjing Jiancheng Biological Engineering Institute, China).

2.8. Statistical analysis

Data were analyzed statistically by using SPSS 19.0 software. The NOEC was confirmed by compared treatment means using analysis of variance (ANOVA) techniques followed by Dunnett's test (OECD, 2005). The significant differences in polysaccharide, soluble protein, total lipid, Mg^{2+} absorption and Mg^{2+}-ATPase activity were tested by one-way ANOVA. The means of them were compared by Tukey Kramer HSD.

3. Results and discussion

3.1. nC_{60} inhibited the growth rate of *S. obliquus*

Growth rate of *S. obliquus* was decreased with the increments in nC_{60} concentration (Fig. 1). The growth rate was significantly inhibited by nC_{60} at the concentration $\geqslant$0.18 mg L^{-1} ($P < 0.05$). At the exposure time of 72 h, inhibition concentration of nC_{60} for *S. obliquus* 50% growth rate was calculated to be 1.94 mg L^{-1}. This value is quite similar to that of Seki et al. (2008) (72 h IC_{50} = 2.27 mg L^{-1}). The maximal NOEC of 72 h was deduced as 0.09 mg L^{-1} using statistical analysis of Dunnett's multiple comparison test ($P < 0.05$). Other study shows the fullerenes decreases the algal growth (Kubatova et al., 2013), which coincides with our results. In this experiment, the lowest concentration of nC_{60} (0.045 mg L^{-1}) did not significantly inhibit the growth rate of *S. obliquus* compared to the control ($P > 0.05$), and no hormetic effect here (Chen et al., 2012). This may be due to higher selected concentrations of nC_{60} than that of hormetic concentration. Previous study indicates growth inhibition was not due to the light intensity (Baun et al., 2008), but due to the membrane damage (Kamat et al., 2000) and the physical effects of aggregated nC_{60} particles (Baun et al., 2008; Seki et al., 2008). In this experiment, growth rate inhibition at high concentrations of nC_{60} ($\geqslant$0.18 mg L^{-1}) could be similar as those noted above. The 0.09 mg L^{-1} of nC_{60} was chosen to be used in subsequent sub-lethal toxicity experiments to further study sub-lethal toxic effects and probably toxic mechanism.

3.2. nC_{60} decreased algal contents of polysaccharide, soluble protein, and total lipid

Compared to the control, polysaccharide, soluble protein and total lipid in *S. obliquus* exposed to 0.09 mg L^{-1} nC_{60} were decreased with the increment in inoculation times (Fig. 2). The polysaccharide, soluble protein and total lipid in *S. obliquus* were decreased by 7%, 6% and 6%, respectively, after 20 repetitive exposures. The *S. obliquus* exposed to nC_{60} was decreased in nutritional contents, which means the photosynthesis was probably partly inhibited. Wei et al. (2010) reported that silica nanoparticles ($nTiO_2$) also partly inhibited algal photosynthesis. As we known, the sub-lethal effects need certain constant concentration and exposure time. Within a short exposure time (72 h, one exposure cycle), the soluble protein content and total lipid content were not significantly different between *S. obliquus* in the presence/absence of nC_{60} ($P > 0.05$); and after a long exposure time

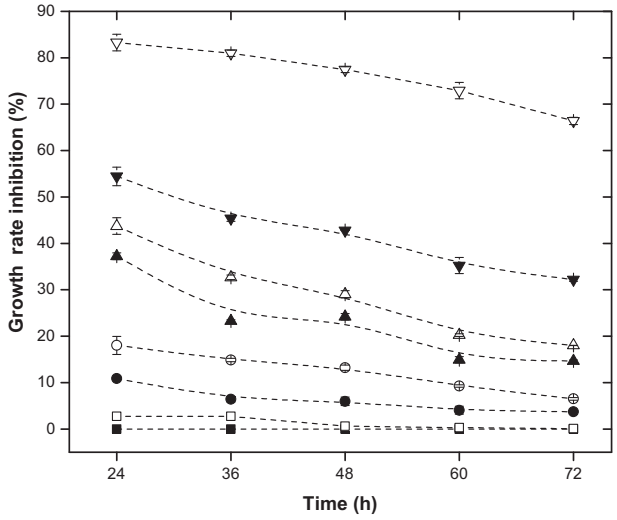

Fig. 1. Growth rate inhibition of *S. obliquus* in the presence of nC_{60}. (0 mg L^{-1}, ■; 0.045 mg L^{-1}, □; 0.09 mg L^{-1}, ●; 0.18 mg L^{-1}, ○; 0.36 mg L^{-1}, ▲; 0.72 mg L^{-1}, Δ; 1.44 mg L^{-1}, ▼; 2.50 mg L^{-1}, ∇.) Error bars represent one standard deviation of results conducted in triplicate. At the exposure time of 72 h, the nC_{60} concentration for 50% growth rate inhibition was calculated to be 1.94 mg L^{-1}. The maximal NOEC of 72 h was 0.09 mg L^{-1}.

(>28 d), the sub-lethal toxic effects appeared significantly in three nutritional contents ($P < 0.05$). Repetitive exposures of *S. obliquus* to nC_{60} (20 times) lead to a long exposure time and a relative high nC_{60} concentration (to algal cells), which would lead to a lasting stress for a period (60 d) on algae cells and result in the nutrition decrements. Thus, researchers should not only focus on the acute toxicity of nanoparticles, but also focus on sub-lethal toxicity of repetitive exposure in the environment to better understand their potential risk in the aqueous environment.

3.3. nC_{60} decreased contents of algal chlorophyll a and chlorophyll b

Both chlorophyll a and chlorophyll b were decreased with the increment in exposure times (Fig. 3). The chlorophyll a and chlorophyll b were decreased significantly by nC_{60} since the 6th and the

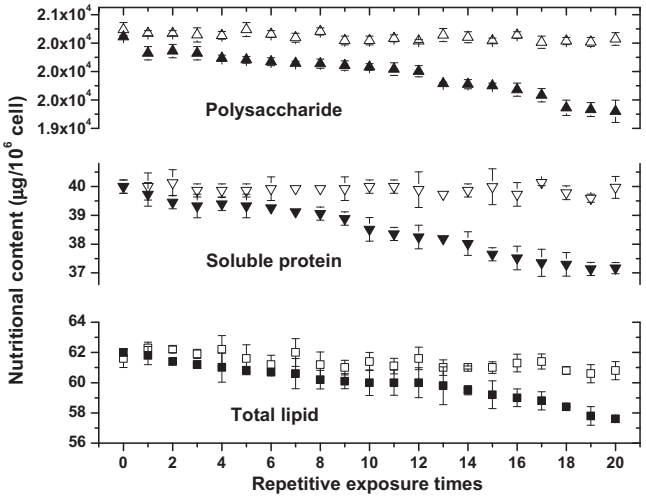

Fig. 2. Sub-lethal effects of nC_{60} on nutritional contents of *S. obliquus*. (Polysaccharide: control, Δ; 0.09 mg L^{-1} nC_{60}, ▲. Soluble protein: control, ∇; 0.09 mg L^{-1}, ▼. Total lipid: control, □; 0.09 mg L^{-1} nC_{60}, ■.) Error bars represent one standard deviation of results conducted in triplicate. Compared to the control, nC_{60} of sub-lethal concentration decreased the polysaccharide, soluble protein and total lipid in *S. obliquus* after 20 successive inoculations exposure.

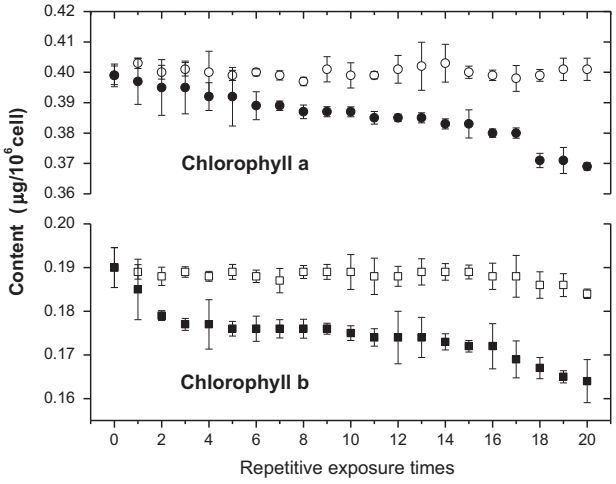

Fig. 3. Sub-lethal effects of nC_{60} on chlorophyll a and chlorophyll b. (Chlorophyll a: control, ○; 0.09 mg L^{-1} nC_{60}, ●; Chlorophyll b: control, □; 0.09 mg L^{-1} nC_{60}, ■.) Error bars represent one standard deviation of results conducted in triplicate. The results showed that nC_{60} of sub-lethal concentration inhibited the contents of chlorophyll a and chlorophyll b.

3rd exposure, respectively ($P < 0.05$). Previous researches point out the chlorophyll a and b in alga are obviously decreased when exposed to fullerenes (Kubatova et al., 2013), $nSiO_2$ (Wei et al., 2010) and $nTiO_2$ (Chen et al., 2012), which are consistent with our results. Previous study has presented an increase in chlorophyll a when alga is exposed to nickel oxide nanoparticles (Gong et al., 2011). The resulting variation could be a result of differences in properties of nanomaterials (the fullerene and nickel oxide) and the different exposure way (repetitive exposure and single exposure). The photosynthetic pigments are essential for photosynthesis because of their role as primary electron donors in the electron transport chain (Raven et al., 2004); thus the decrements in chlorophyll a and chlorophyll b would lead to the decrements in absorption of light, and indirectly result in the photosynthesis production decrements (polysaccharide, soluble protein and total lipid) and growth inhibitions. The reason for algal chlorophylls decrements may be due to the deficiency of Mg^{2+}, which is the key metallic ion at the center of chlorophylls.

3.4. nC_{60} inhibited algal uptake of Mg^{2+}

nC_{60} at sub-lethal concentration decreased the Mg^{2+} concentration in algal cell by inhibiting the uptake of Mg^{2+} (Fig. 4). Compared to that of the control, Mg^{2+} concentration in *S. obliquus* exposed to 0.09 mg L^{-1} nC_{60} was significantly decreased since the 6th exposure ($P < 0.05$). The Mg^{2+} in *S. obliquus* was decreased by 40% after 20 repetitive exposures to fresh nC_{60} medium. The changes in medium Mg^{2+}concentration represented that nC_{60} decreased Mg^{2+} uptake in medium by about 10%. Our previous research has pointed out nC_{60} increases aqueous animal (*D. magna*) takes up metal ion (Cu^{2+}) (Tao et al., 2013), is not similar to this result (aqueous phytoplankton (*S. obliquus*) took up metal ion). The uptake of metals in zooplankton and phytoplankton are different in the presence of nC_{60}, which may due to the different methods of uptake. Zooplankton takes up the complex aggregates of nC_{60} and metals in the intestine (Tervonen et al., 2010), while phytoplankton absorbs the complex aggregates of nC_{60} and metals through the cell wall and membrane (Baun et al., 2008). This research would supplement some information about nC_{60} decreased aqueous phytoplankton (*S. obliquus*) took up Mg^{2+}.

As we known, Mg^{2+} is the metallic ion at the center of chlorophylls, and is an essential macronutrient for normal algal growth

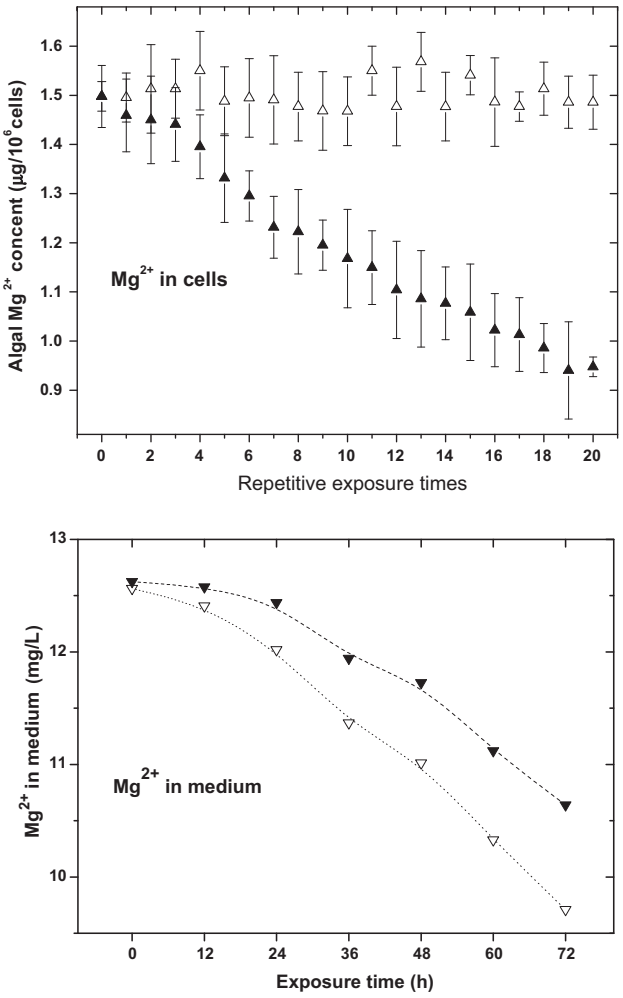

Fig. 4. Sub-lethal effects of nC_{60} on the Mg^{2+} content of *S. obliquus*. (Mg^{2+} in cell: control, △; 0.09 mg L^{-1} nC_{60}, ▲. Mg^{2+} in medium: control, ▽; 0.09 mg L^{-1}, ▼.) Error bars represent one standard deviation of results conducted in triplicate. The results showed that nC_{60} at the sub-lethal concentration decreased the Mg^{2+} content in algal cell by inhibiting the uptake of Mg^{2+}.

(Hüner and Hopkins, 2008); Mg^{2+} is also a necessary activator for essential enzymes in carbon fixation: ribulosbiphophate carboxylase (RuBisCo) and phosphoenolpyruvate carboxylase (PEPC). Therefore, Low amounts of Mg^{2+} would lead to a decrease in photosynthetic and enzymatic activities in algal cells, and indirectly result in the nutrition decrements. The inhibition of Mg^{2+} uptake from medium was probably due to the nC_{60} sub-lethal toxicity to Mg^{2+}-ATPase, which is an ATPase that pumps Mg^{2+} from the medium into algal cell.

3.5. nC_{60} inhibited the Mg^{2+}-ATPase activity

Mg^{2+}-ATPase activity of *S. obliquus* exposed to nC_{60} of 0.09 mg L^{-1} was decreased with the increment in the exposure times (Fig. 5). The Mg^{2+}-ATPase activity was significantly inhibited since the 2nd exposure ($P < 0.05$), and Mg^{2+}-ATPase activity was decreased by 6% after 20 repetitive exposures. The nC_{60} inhibited the activity of Mg^{2+}-ATPase in *S. obliquus,* which would directly inhibit the Mg^{2+} absorption of *S. obliquus*. Previous investigation has presented polyhydroxylated fullerene decreases the activity of Mg^{2+}-ATPase by 22% (Grebowski et al., 2013), which is compared favorably to our results. The absorption of nC_{60} onto the surface of algae may partly block the ion channel of Mg^{2+} just like fullerene

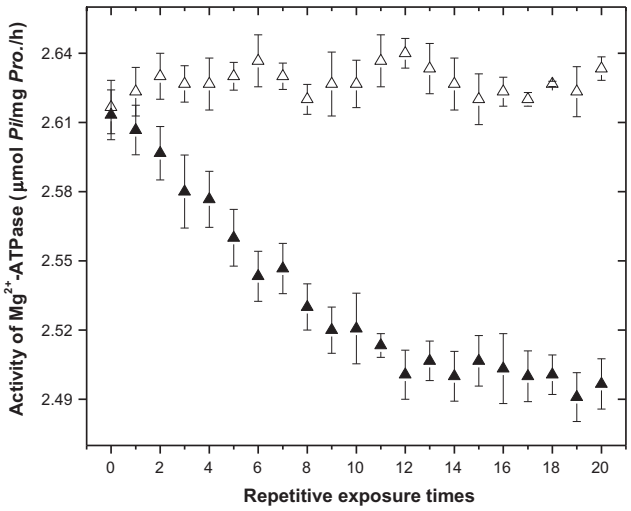

Fig. 5. nC_{60} of sub-lethal concentration inhibited Mg^{2+}-ATPase activity of *S. obliquus*. (Control (0 mg L^{-1}), △; 0.09 mg L^{-1} nC_{60}, ▲.) Error bars represent one standard deviation of results conducted in triplicate. The result indicated that nC_{60} of sub-lethal concentration blocked partial Mg^{2+} ion channels.

blocks K^+ ion channel (Park et al., 2003). Park et al. (2003) provided the blocking mechanism that the fullerene with a diameter of 0.72 nm can fit into the mouth of the selectivity filter and, like a cork in a bottle, obstruct the flow of ions. The inhibition of algal membrane Mg^{2+}-ATPases activity caused by nC_{60} could cause abnormal Mg^{2+} distribution between the outer and inner layer of the algal membrane, thus decreasing the Mg^{2+} concentration in algal cell and further result in chlorophylls amount decrement. This understanding may supplement some information about the inhibition of nonmaterials on algal photosynthesis.

4. Conclusions

These results indicate that under the scenario(s) of sub-lethal exposure, nC_{60} can aggregate to the surface of algal cell, partly block Mg^{2+} channel of and thus decrease the concentration of Mg^{2+} in algal cell, while remaining inhibition of photosynthesis process. Here it is shown to have an adverse photosynthetic impact on a common aquatic phytoplankton species, *S. obliquus*, after repetitive exposures (20 times). Furthermore, nC_{60} decrease algal Mg^{2+} (key metal ion for algal photosynthetic chlorophylls) content by 40%, and inhibit the activity of Mg^{2+}-ATPase (pumping the Mg^{2+} into algal cell). In addition to providing acute toxicity data, this study highlights a critical need for additional data regarding sub-lethal material repetitive exposure, and inhibition in metal ion uptake. To sum up, this understanding could provide some information about the water stable nano-crystal fullerene sub-lethal effects on algal photosynthesis. Ongoing studies are focused on evaluating the biochemical mechanisms of the blocking ion channels.

Acknowledgements

The Shanghai Natural Science Foundation (Grant No. 11ZR1415300), the National Natural Science Foundation of China (Grant No. 41272381), the State Oceanic Administration Public Welfare Project (Grant No. 2015418034), Aquaculture Genetics and Breeding Technology Center in Shanghai Ocean University, the Shanghai University Knowledge Service Platform, Shanghai Ocean University Aquatic Animal Breeding Center (ZF1206) and Shanghai Jiao Tong University supported this research.

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