Environmental Pollution 220 (2017) 1282–1288

Contents lists available at ScienceDirect

Environmental Pollution

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

Toxic effects of microplastic on marine microalgae *Skeletonema costatum*: Interactions between microplastic and algae☆

Cai Zhang, Xiaohua Chen, Jiangtao Wang*, Liju Tan

Key Laboratory of Marine Chemistry Theory and Technology, Ministry of Education, Ocean University of China, Qingdao 266100, China

ARTICLE INFO

Article history:
Received 21 August 2016
Received in revised form
26 September 2016
Accepted 2 November 2016
Available online 18 November 2016

Keywords:
Microplastic
Particle size
Algal inhibition test
Shading test
Microalgae

ABSTRACT

To investigate toxic effects of microplastic on marine microalgae *Skeletonema costatum*, both algal growth inhibition test and non-contact shading test were carried out, and algal photosynthesis parameters were also determined. The SEM images were used to observe interactions between microplastic and algae. It was found that microplastic (mPVC, average diameter 1 μm) had obvious inhibition on growth of microalgae and the maximum growth inhibition ratio (IR) reached up to 39.7% after 96 h exposure. However, plastic debris (bPVC, average diameter 1 mm) had no effects on growth of microalgae. High concentration (50 mg/L) mPVC also had negative effects on algal photosynthesis since both chlorophyll content and photosynthetic efficiency (ΦPSII) decreased under mPVC treatments. Shading effect was not one reason for toxicity of microplastic on algae in this study. Compared with non-contact shading effect, interactions between microplastic and microalage such as adsorption and aggregation were more reasonable explanations for toxic effects of microplastic on marine microalgae. The SEM images provided a more direct and reasonable method to observe the behaviors of microplastic.

1. Introductions

Plastic has been a kind of essential materials in society due to its excellent properties. It is reported that global plastic manufacture and consumption have exceeded 300 million tons annually (Andrady and Neal, 2009). However, about 10% of plastic products end up in the ocean through various channels, constituting between 60 and 80% of all marine debris (Kaposi et al., 2014; Setälä et al., 2014). Plastic debris has been a worldwide contaminant found throughout the marine environment (Eriksen et al., 2013). A large number of marine species is known to be harmed by plastic debris increasing year by year (Derraik, 2002). Larger plastic debris can become small fragments by photodegradation and abrasion due to wave action (Cole et al., 2013). Meanwhile, some plastic products themselves are small for their properties such as facial cleansers, cosmetic preparations and textile fibers (Moore, 2008). These small plastic debris — microplastic (diameter <1 mm) (Browne et al., 2011) — as an emerging pollution has attracted enough attention from researchers (Barnes et al., 2009; Ivar do Sul and Costa, 2014).

Microplastic has a worldwide distribution on shorelines and in the ocean (Browne et al., 2011; Derraik, 2002; Ng and Obbard, 2006). For example, the microplastic density was 0.167 ± 0.138 n/m^{-3} in the East China Sea (offshore sea) (Zhao et al., 2014). Meanwhile, average plastic abundance in the Northeast Atlantic (open ocean) was calculated as 2.46 particles m^{-3} (Lusher et al., 2014). Except for wide distribution, microplastic of small size make them available to a wide range of marine biota (Barnes et al., 2009). There were many reports focusing on toxic effects of microplastic on marine animals (Collignon et al., 2012; Derraik, 2002) such as sea urchins (Kaposi et al., 2014), mussels, crabs (Farrell and Nelson, 2013) and fishes (Rochman et al., 2014). The major toxic mechanism was ingestion of microplastic and sorbent for toxic organics (Besseling et al., 2013). However, there were few studies about toxicity of microplastic on marine phytoplankton (Besseling et al., 2014; Bhattacharya et al., 2010; Long et al., 2015; Sjollema et al., 2016).

Microalgae have a tremendous potential to interact with microplastic in the ocean. As a primary producer and a matter of marine trophic chain, microalgae are the indispensable part to maintain marine ecosystem balance (Harris, 2012). In addition, microalgae have much more advantages for the eco-toxicology assay including short growth period, easy operation, easy

☆ This paper has been recommended for acceptance by Maria Cristina Fossi.

* Corresponding author.
E-mail address: jtwang@ouc.edu.cn (J. Wang).

http://dx.doi.org/10.1016/j.envpol.2016.11.005
0269-7491/

observation and sensitivity to toxic substance (Clément et al., 2013). Without ingestion process, microalgae are a better choice to detect environmental threat caused by microplastic pollution.

To investigate toxic effects of microplastic on marine microalgae, algal growth inhibition tests were carried out to compare toxicity between two kinds of microplastic on *Skeletonema costatum*. Shading test with a special vessel was used to detect shading effect of microplastic under the condition of non-contact with algae. Chlorophyll content and photosynthetic efficiency were determined to evaluate impact of microplastic on algal photosynthesis. Interactions between microplastic and algae were clearly observed with SEM technique. The SEM images of interactions between microplastic and algae provided a more direct and reasonable method to explain toxic effects of microplastic on microalgae.

2. Materials and methods

2.1. Chemicals

Microplastic (mPVC), pure polyvinyl chloride without additives was white spherical powder (average diameter 1 μm) and obtained by a milling process. Bulk plastic debris (bPVC) was white block (average diameter 1 mm) and obtained by cutting thin PVC plates into small pieces. Two kinds of plastic were both pristine chemicals and were not environmentally aged particles. They were purchased from Shanghai Youngling Electromechanical Technology Co. Ltd., China. The density of PVC was 1.4 g/cm^{-3}. The SEM image of mPVC and the photo of bPVC were provided in Fig. 1.

2.2. Organisms

The algal strain *Skeletonema costatum* (Bacillariophyta) was obtained from the Algal Center of Key Laboratory of Marine Chemistry Theory and Technology, Ocean University of China. The algae were collected from Jiaozhou Bay of China and cultured in the laboratory over one year. Glassware was acid-soaked, cleaned with milli-Q water, and then autoclaved for culture. The microalgae were cultivated in f/2 medium (Guillard and Ryther, 1962) made with sterile seawater (filtered by 0.45 μm membrane) from Qingdao, China. The microalgae were cultivated in 5 L Erlenmeyer flask at 20 ± 1 °C under cool continuous white fluorescent lights (4000 lx) with a 12-h light/dark cycle, and manually shaken twice a day to prevent the sedimentation of algae. The incubation was lasted 5–7 days until log phase growth prevailed. Cell density was measured by hemacytometer under inverted microscope (Leica, DM4000B) with 400 magnification times. The initial algal density was 50×10^4 cell/mL in the beginning of the experiment.

2.3. Preparation of mPVC suspensions

Stock solution of mPVC was prepared through dispersing dry mPVC particles into milli-Q water to the concentration of 1000 mg/L. The well-dispersed stock solution through shaking and sonicating could be kept in storage for a week at room temperature. The stock solution was diluted with milli-Q water to the final concentrations of 200, 100 and 20 mg/L, and then ultrasound bathed 15 min at 50 W for dispersion before the toxicity assay.

2.4. Algal growth inhibition test

All tests in the study complied with OECD (Organization for Economic Cooperation and Development) Guidelines 201. Glassware was acid-soaked, cleaned with milli-Q water, and then autoclaved before test. 100 mL pre-cultured algal cells in log phase growth were transferred into 250 mL Erlenmeyer flask, then mPVC suspensions (5 mL) under different concentrations were added into the test flask in triplicate respectively. The final concentrations of mPVC in algal growth inhibition tests were 0, 1, 5, 10 and 50 mg/L. The bPVC debris was directly added into 100 mL pre-cultured algal solution in 250 mL Erlenmeyer flask to the bPVC concentrations of 0, 50, 500, 1000 and 2000 mg/L and shaken well. Every bPVC treatment was in triplicate, too. The test flasks were randomly placed in the growth incubator for 4 days under the condition in accordance with pre-cultured condition. The cell density was counted every 24 h and the 4 days growth curve was available. Growth inhibition ratio (IR) was calculated as: $IR(\%)=(1-T/C)\times 100\%$, where T and C were cell density in experimental group and control group respectively.

2.5. Shading test

Shading test was carried out in a special vessel (Aruoja et al., 2009) (Fig. 2). The upper portion of the vessel was light-proof except bottom. 100 mL pre-cultured algal cells were cultivated in the upper portion. The lower portion was completely transparent

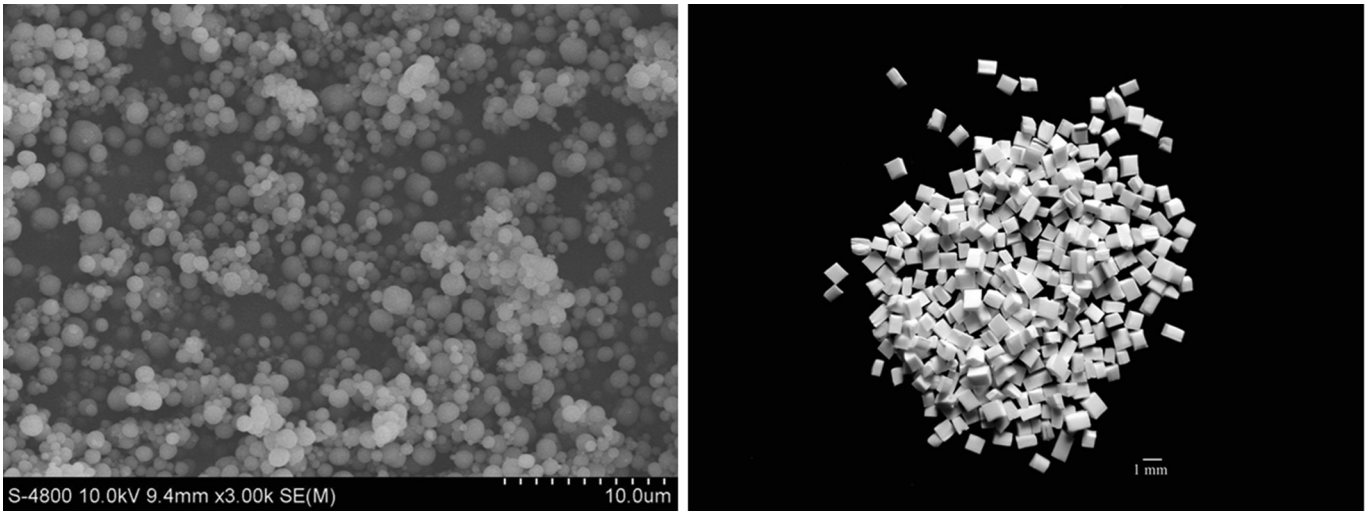

Fig. 1. The SEM image of mPVC and the photo of bPVC.

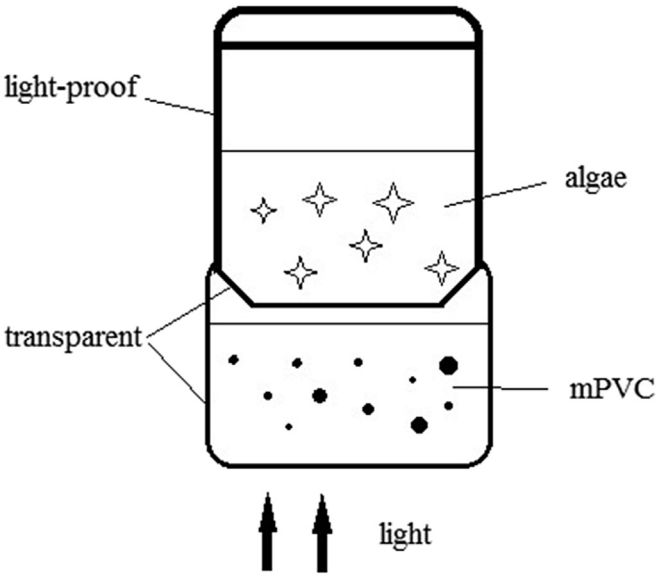

Fig. 2. The sketch of the special vessel in shading test.

and lights could pass through the lower portion into the upper portion. The mPVC of different concentrations (0, 5 and 50 mg/L in triplicate) was well dispersed in f/2 medium at the lower portion. The condition of culture was same as pre-cultured condition.

2.6. Determination of chlorophyll and photosynthetic efficiency (ΦPSII)

Chlorophyll content and photosynthetic efficiency (ΦPSII) were determined by Algae Lab Analyser based on fluorescent technique (bbe Moldaenke GmbH, Germany). All data from the instrument were converted to percentage of control (% control) for easy comparison. Chlorophyll content reflected the content of algal photosynthesis and ΦPSII represented the efficiency of algal photosynthesis. The ability of algal photosynthesis was calculated as: $A\,(\%) = C \times E$, where C and E were chlorophyll content and ΦPSII under the same treatment.

2.7. Sample preparation for SEM

The interaction between microplastic and algae was observed with scanning electron microscope (SEM, Hitachi S-4800 cold field-emission) (Zhang et al., 2016). After 24 h exposure under 50 mg/L mPVC treatment, algal cells were collected by centrifugation (3000 rpm, 10 min), and cells were fixed by 2.5% glutaraldehyde at 4 °C overnight. The samples were then washed with 0.1 mol phosphate buffer solution (PBS, pH 7.4) by centrifugation (3000 rpm, 10 min) three times. Algal cells were fixed by 1% osmium tetroxide at 4 °C for 1 h, and then were washed with 0.1 mol PBS (pH 7.4) by centrifugation (3000 rpm, 10 min) three times. Samples were dehydrated successively through a series of alcohol solutions of 30%, 50%, 70%, 80%, 90%, 95% and 100% for 20 min. After dehydration, samples were fixed with tert butyl alcohol and then freeze-dried for final observation.

2.8. Data analysis

One-way analysis of variance with Duncan comparisons (used software SPSS 19) was applied in order to test significant differences in effects among treatments (Unless otherwise noted, significance level was set at $p < 0.05$). Data were expressed as average ± standard deviation in the study.

3. Results and discussion

3.1. Algal growth inhibition test

Two kinds of microplastic (mPVC and bPVC) were used to investigate size effect of microplastic on toxicity to microalgae. Meanwhile, Chlorophyll content and ΦPSII under mPVC treatment were determined to explore effects of mPVC on algal photosynthesis.

The algae density decreased with mPVC concentration increasing in Fig. 3A. All of treatments had a significant difference ($p < 0.05$) with the corresponding control in every time except 1 mg/L treatments at 24 and 48 h. The most obvious inhibitory effects ($p < 0.05$) of mPVC appeared at 96 h, at which the maximum IR was 39.7% under 50 mgL/L treatment. The IR increased with mPVC concentration increasing, but didn't increase with time. For example, the IR of 50 mg/L mPVC concentration at 24, 48, 72 and 96 h was 37.9, 36.5, 35.4 and 39.7%, respectively. Some other particles such as zinc oxide and titanium dioxide particles also inhibited the growth of microalgae, but growth inhibition effects of these particles increased with time (Klaine et al., 2008; Zhang et al., 2016). It was different from those particles (Klaine et al., 2008) that the mPVC didn't have toxic accumulation effects on algae. However, all of algae density didn't have a significant difference ($p > 0.05$) with control under bPVC treatments (Fig. 3B). Even under 2000 mg/L treatments of bPVC, the algae had the same cell density with control in every time. The bPVC didn't have toxic effects on algal growth within the range of concentrations in this study. The size could affect the toxicity of microplastic on algae. Sjollema et al. (2016) also found small sized microplastic (0.05 μm polystyrene) could obviously inhibit the growth of microalgae *D. tertiolecta*, but large microplastic (6 μm polystyrene) didn't have a significant effect on algae. The bPVC could keep out some light from above with floating on the surface of the medium, but light could go through the transparent wall of flask into the medium. Light from both sides of the incubator also satisfied the needs of algal growth. Moreover, the mPVC could exist in the medium under a stable and uniform condition. The floating bPVC could not enter into the medium and interact with algae just as mPVC. It could be one of the major reasons why the bPVC didn't have toxicity on algae.

Microplastic had some influence on algal photosynthesis in previous studies (Besseling et al., 2014; Bhattacharya et al., 2010). Negative effects of mPVC on algal photosynthesis were investigated under 5 and 50 mg/L mPVC treatments (Fig. 4). Chlorophyll content just decreased 7% under 5 mg/L mPVC treatment at 72 h and 96 h (Fig. 4A). However, chlorophyll content decreased 20% from 24 h to 96 h under 50 mg/L mPVC treatment. Algal chlorophyll content under mPVC treatment of high concentration (50 mg/L) had greater decrease (independent sample t test, $p < 0.05$) than that under low concentration (5 mg/L). About ΦPSII, it decreased 5% under 5 mg/L mPVC treatment at 1 and 24 h (Fig. 4B). ΦPSII decreased 32% at 1 h under 50 mg/L mPVC treatment. The value of ΦPSII increased with time but still lower than control (100%). As the same to results of chlorophyll content, high concentration of mPVC had more effects on algal ΦPSII. The similar result was found in previous study that 0.5 μm microplastic of 25 and 250 mg/L had a negligible effects on algal ΦPSII after 72 h exposure (Sjollema et al., 2016). It was probably the same situation with this study. The variation trend of two parameters of photosynthesis was different, so the ability of photosynthesis — product of chlorophyll content and ΦPSII — was calculated to show effects of mPVC on algal photosynthesis (Table 1). Minor effects (< 8%) of mPVC under 5 mg/L treatment on

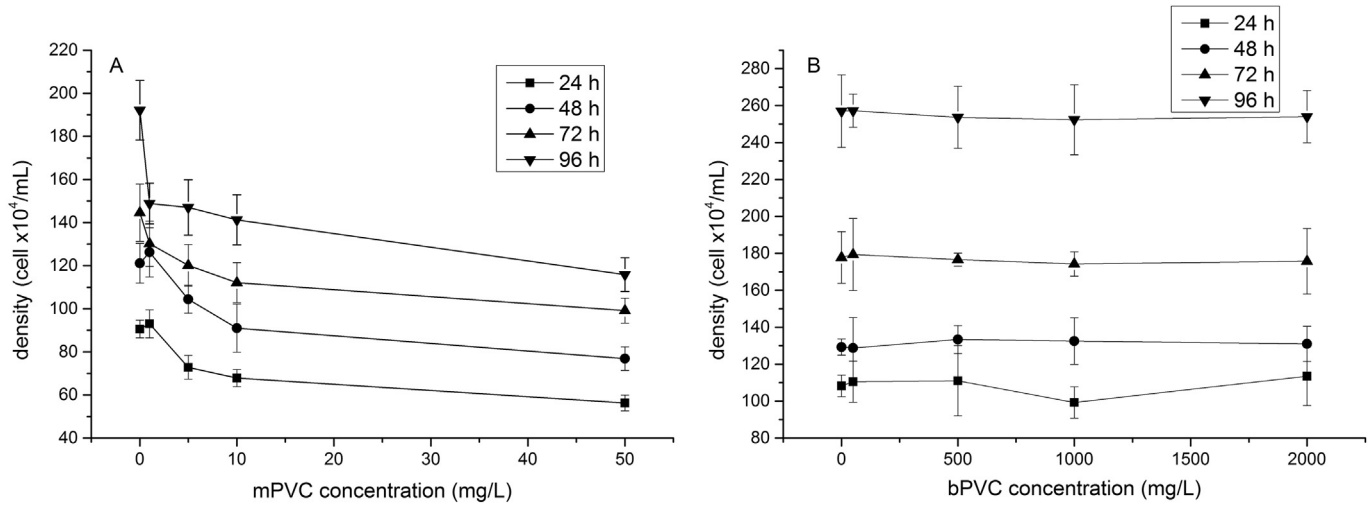

Fig. 3. Toxic effects of mPVC and bPVC of different concentrations on growth of microalgae with time. Square, circle, triangle and inverted triangle represented cell density under different mPVC concentrations at 24 h, 48 h, 72 h and 96 h, respectively. A: mPVC B: bPVC.

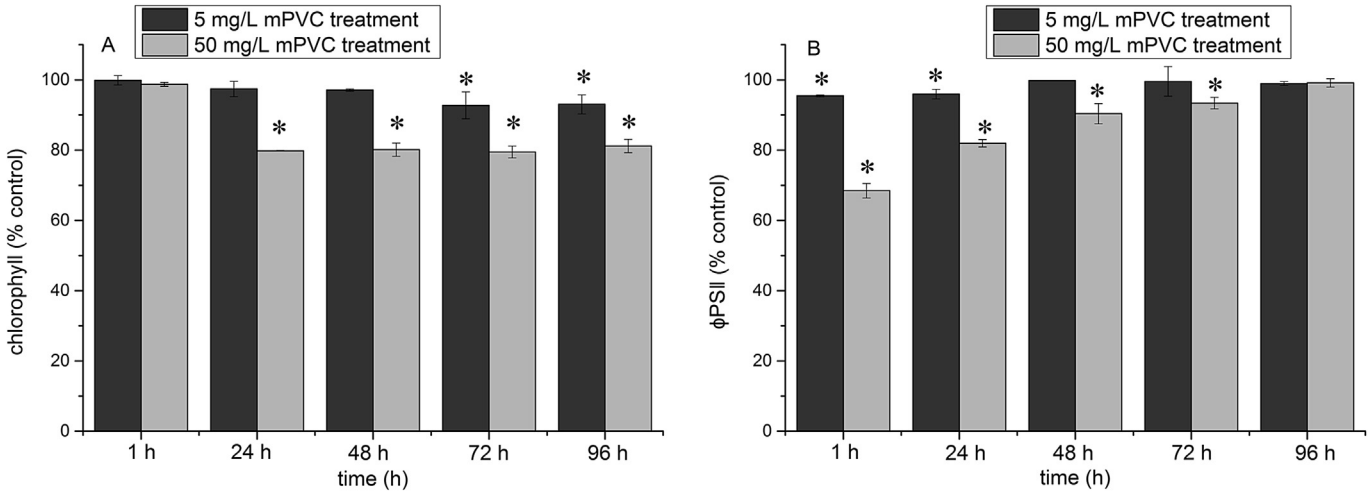

Fig. 4. Effects of mPVC of 5 and 50 mg/L concentrations on photosynthesis of microalgae with time. * represented significant difference ($p < 0.05$) with control (100%). A: chlorophyll B: ΦPSII.

Table 1
The ability of algal photosynthesis with time in algal growth inhibition tests.

mPVC concentration	Photosynthesis ability (%)				
	1 h	24 h	48 h	72 h	96 h
5 mg/L	95.3 ± 1.5[a]	93.4 ± 2.6	96.8 ± 0.8	92.2 ± 3.9	92.0 ± 4.2
50 mg/L	67.6 ± 2.4	65.4 ± 3.1	72.4 ± 1.4	74.2 ± 2.9	80.4 ± 2.4

[a] Results were expressed as average ± standard deviation.

algal photosynthesis ability were observed in every time. Photosynthesis ability under 50 mg/L mPVC treatment had a significant difference (independent sample t test, $p < 0.05$) with control in every time. Effects of 50 mg/L mPVC on algal photosynthesis could not be ignored. However, these negative effects were maximum at first and weakened with time. It was possible that microalgae was vulnerable in initial period of exposure and adaptive to the invasion of microplastic at the end of exposure. Microplastic was not a highly toxic substance. Microalgae might be resistant to toxic effects of microplastic and better grow under microplastic exposure with time. The future study should pay more attention to effects of microplastic on algal photosynthesis in adaptive phase of invasion by microplastic.

In a word, the size of microplastic could affect their toxic effects on microalgae. The mPVC significantly ($p < 0.05$) inhibited the growth of algae while the bPVC had no effects on growth within the range of concentrations in this study. The mPVC had negative effects on algal photosynthesis though the effects weakened with time.

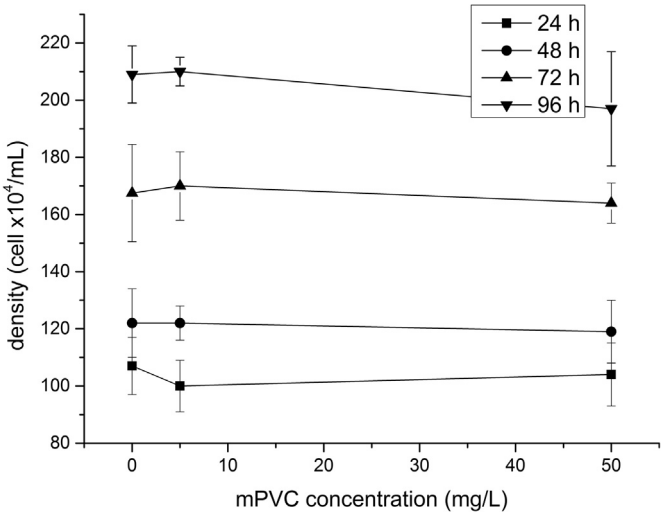

Fig. 5. Shading effects of mPVC on growth of microalgae with time. Square, circle, triangle and inverted triangle represented cell density under different mPVC concentrations at 24 h, 48 h, 72 h and 96 h, respectively.

3.2. Shading test

Shading effect was one of the reasons for toxic effects of particles on algae as usually (Schwab et al., 2011). A special vessel (Fig. 2) keeping non-contact between microplastic and microalgae was used to investigate shading effect of microplastic on algae. The algal density had no significant difference ($p > 0.05$) with control in every time (Fig. 5). Shading effect had no influence on algal growth. Meanwhile, chlorophyll content had no significantly decrease ($p > 0.05$) with control (Fig. 6A). ΦPSII also had no significant decrease ($p > 0.05$) with control under most treatments except under 50 mg/L mPVC treatment at 1 h (Fig. 6B). Shading effect also had negligible influence on algal photosynthesis. The probable reason was that though some light was blocked by microplastic, the rest still satisfied the needs of photosynthesis or the concentration of mPVC was not enough high. Sjollema et al. (2016) found the similar results in their study. Though the light intensity was reduced up to 34% by shading effect of 250 mg/L microplastic, there was no inhibition of photosynthesis found. In a word, shading effect was not one reason for toxic effects of microplastic in this study.

3.3. Interactions between microplastic and algae

Shading effect was not one possible reason for toxicity of microplastic and further microplastic could not release toxic substance to microalgae in a short period. Interactions between microplastic and algae and physical damage may be the probable reason for toxic effects of microplastic on algae. There were many caveolaes on the surface of healthy algal cells and silicic thorns in a round were also fixed on the surface (Fig. 7A). Algae could adsorbed mPVC on the surface of cells and these mPVC could wrap up caveolaes on the surface (Fig. 7B). It could limit the transfer of energy and substance between cells and environment and lead to decrease of nutrition, light, CO_2 and O_2 from medium into cells. The harmful metabolite of algae also had the potential to be locked in the cell to disturb the algal growth. Bhattacharya et al. (2010) also considered adsorption of microplastic hindered algal photosynthesis, because light and air could be blocked by the plastic particles. Meanwhile, the hetero-aggregation of particles and algae could make algae precipitate to affect the algal growth (Ma et al., 2014). The mPVC embedded in the cell wall of algae and even caused physical damage on the surface which also had negative influence on the growth of microalgae (Fig. 7C and D).

The small mPVC particles well dispersed in the medium had high surface reactivity. These small particles would aggregate to a large whole to keep the stable condition. Aggregation of mPVC was observed in Fig. 7E and F. The size of the aggregation was similar to the algal cell (5 μm), which had a potential to increase with time (Jiang et al., 2009). The aggregation could not be adsorbed on the healthy algae for its big size. Small algal fragments generated from damaged and dead algal cells could be adsorbed on aggregate mPVC (Fig. 7E). Hazardous substances adsorbed on aggregate mPVC could precipitate and then leave algal living system with aggregate process, which also indirectly reduced the toxicity of microplastic (Long et al., 2015). As mentioned earlier, the mPVC didn't have toxic accumulation effects on algae and the negative effects of mPVC on photosynthesis decreased with time. Aggregation of mPVC was a possible process resulting in detoxication in above experiments. Redispersion of microplastic by sea wave and degradation of microplastic in a long period which could produce new toxic effects were need be further studied in the future. In brief, compared with non-contact shading effect, interactions between microplastic and microalgae were the major reason for toxic effects of microplastic on algae.

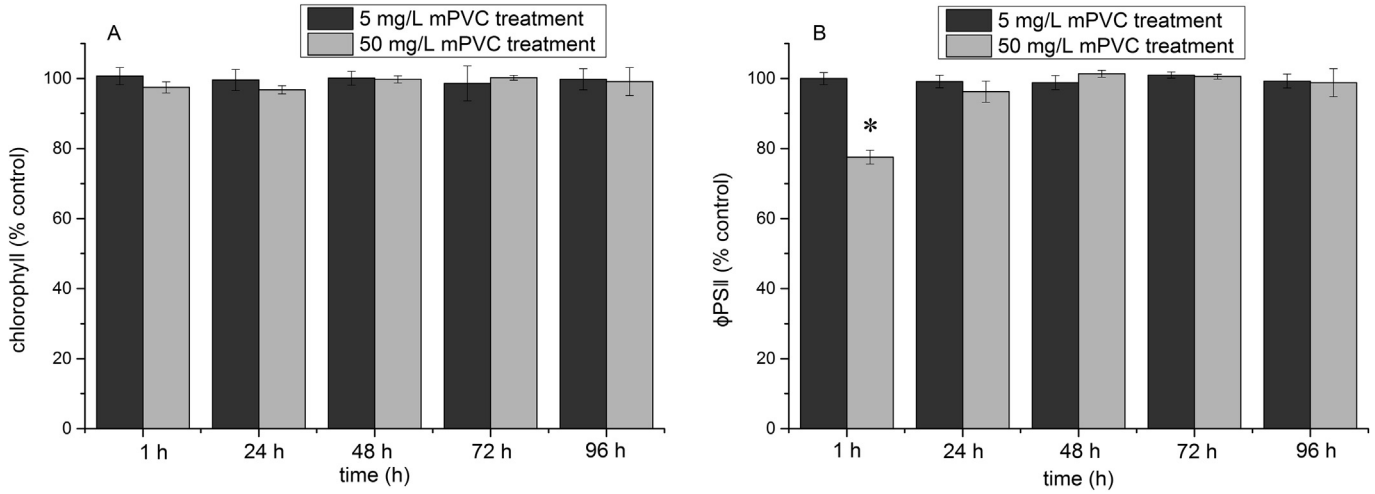

Fig. 6. Effects of mPVC of 5 and 50 mg/L concentrations on photosynthesis of microalgae in shading tests. * represented significant difference ($p < 0.05$) with control (100%). A: chlorophyll B: ΦPSII.

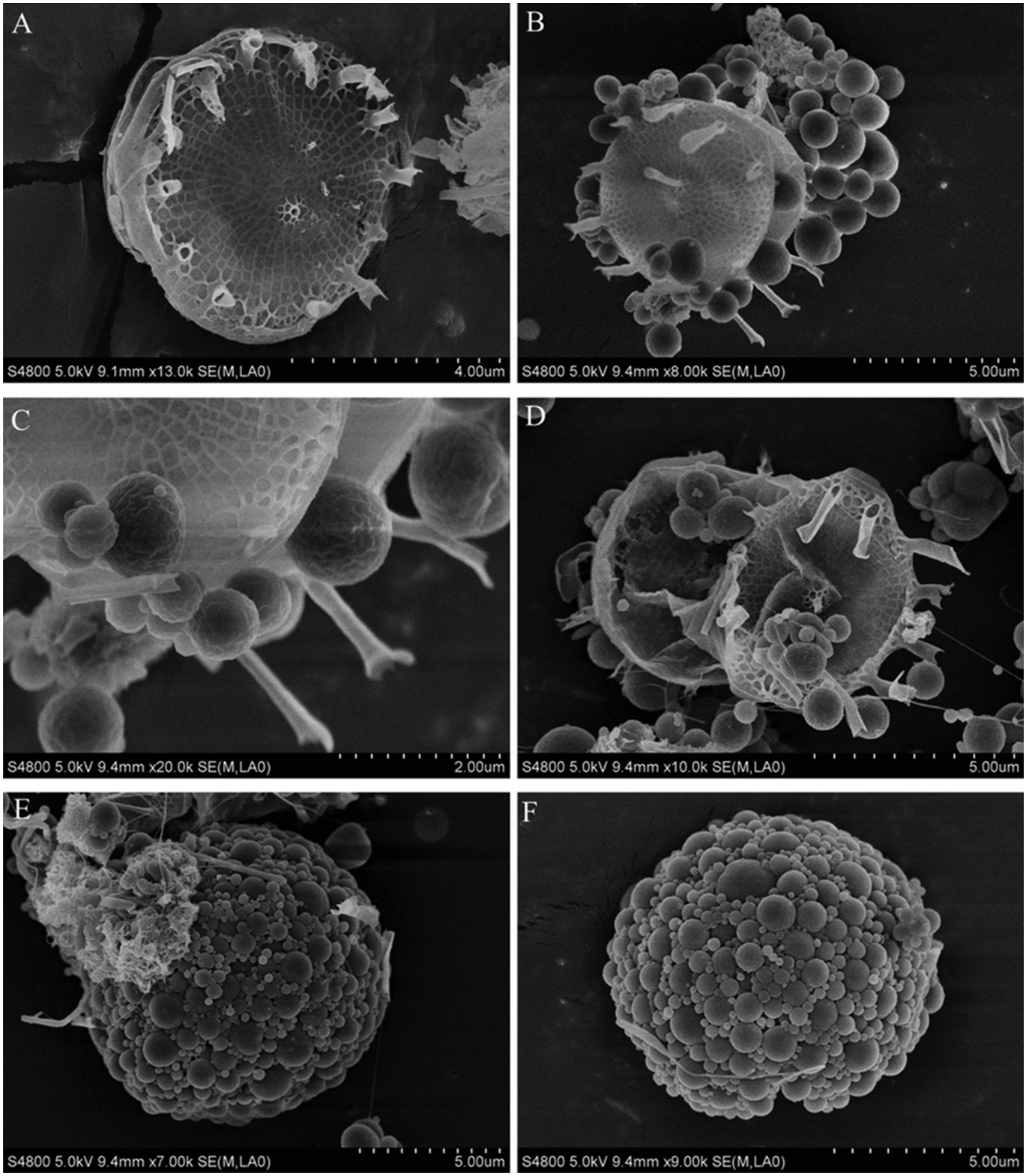

Fig. 7. The SEM images of the interaction between mPVC and microalgae after 24 h exposure. A: control without mPVC B: mPVC adsorbed on algal cells C: mPVC embedded in algal cells D: physical damage on algae by mPVC E: algal fragments adsorbed on mPVC F: aggregation of mPVC.

4. Conclusion

To investigate toxic effects of microplastic on marine microalgae *Skeletonema costatum*, two different sized microplastic (mPVC and bPVC) were applied to conduct algal growth inhibition test. Non-contact shading test was carried to detect shading effect of mPVC, and algal photosynthesis parameters were also determined. The interaction between microplastic and algae was observed with SEM

technique. The mPVC had obvious inhibition on growth of microalgae though the toxicity was not accumulated, and the maximum growth inhibition ratio (IR) reached up to 39.7% after 96 h exposure. However, bPVC had no effects on growth of microalgae. The size of microplastic could influence toxic effects of microplastic on microalgae. High concentration (50 mg/L) mPVC also had negative effects on algal photosynthesis since both chlorophyll content and photosynthetic efficiency (ΦPSII) decreased under mPVC treatment. The negative effects of mPVC on photosynthesis could decrease with time. The mPVC had negligible influence on microalgae in the shading test. Interactions between microplastic and microalage such as adsorption and aggregation were observed in the SEM images. Compared with non-contact shading effect, they were more reasonable explanations for toxic effects of microplastic on marine microalgae.

Acknowledgments

The marine microalgae *Skeletonema costatum* was provided by the Algal Center of Key Laboratory of Marine Chemistry Theory and Technology, Ocean University of China. This study was supported by the Public Science and Technology Research Funds Projects of Ocean (201505034) and the National Programme on Global Change and Air-Sea Interaction (GASI-03-01-02-01).

References

Andrady, A.L., Neal, M.A., 2009. Applications and societal benefits of plastics. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364, 1977–1984.
Aruoja, V., Dubourguier, H.-C., Kasemets, K., Kahru, A., 2009. Toxicity of nanoparticles of CuO, ZnO and TiO_2 to microalgae *Pseudokirchneriella subcapitata*. Sci. total Environ. 407, 1461–1468.
Barnes, D.K.A., Galgani, F., Thompson, R.C., Barlaz, M., 2009. Accumulation and fragmentation of plastic debris in global environments. Philos. Trans. R. Soc. Lond. B Biol. Sci. 364, 1985–1998.
Besseling, E., Wang, B., Lürling, M., Koelmans, A.A., 2014. Nanoplastic affects growth of *S. obliquus* and reproduction of *D. magna*. Environ. Sci. Technol. 48, 12336–12343.
Besseling, E., Wegner, A., Foekema, E.M., van den Heuvel-Greve, M.J., Koelmans, A.A., 2013. Effects of microplastic on fitness and PCB bioaccumulation by the lugworm *Arenicola marina* (L.). Environ. Sci. Technol. 47, 593–600.
Bhattacharya, P., Lin, S., Turner, J.P., Ke, P.C., 2010. Physical adsorption of charged plastic nanoparticles affects algal photosynthesis. J. Phys. Chem. C 114, 16556–16561.
Browne, M.A., Crump, P., Niven, S.J., Teuten, E., Tonkin, A., Galloway, T., Thompson, R., 2011. Accumulation of microplastic on shorelines woldwide: sources and sinks. Environ. Sci. Technol. 45, 9175–9179.
Clément, L., Hurel, C., Marmier, N., 2013. Toxicity of TiO2 nanoparticles to cladocerans, algae, rotifers and plants – effects of size and crystalline structure. Chemosphere 90, 1083–1090.
Cole, M., Lindeque, P., Fileman, E., Halsband, C., Goodhead, R., Moger, J., Galloway, T.S., 2013. Microplastic ingestion by zooplankton. Environ. Sci. Technol. 47, 6646–6655.
Collignon, A., Hecq, J.-H., Glagani, F., Voisin, P., Collard, F., Goffart, A., 2012. Neustonic microplastic and zooplankton in the North Western Mediterranean sea. Mar. Pollut. Bull. 64, 861–864.
Derraik, J.G.B., 2002. The pollution of the marine environment by plastic debris: a review. Mar. Pollut. Bull. 44, 842–852.
Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., Farley, H., Amato, S., 2013. Microplastic pollution in the surface waters of the Laurentian Great Lakes. Mar. Pollut. Bull. 77, 177–182.
Farrell, P., Nelson, K., 2013. Trophic level transfer of microplastic: *Mytilus edulis* (L.) to *Carcinus maenas* (L.). Environ. Pollut. 177, 1–3.
Guillard, R.R., Ryther, J.H., 1962. Studies of marine planktonic diatoms: I. *Cyclotella nana* hustedt, and *Detonula confervacea* (cleve) gran. Can. J. Microbiol. 8, 229–239.
Harris, G., 2012. Phytoplankton Ecology: Structure, Function and Fluctuation. Springer Science & Business Media.
Ivar do Sul, J.A., Costa, M.F., 2014. The present and future of microplastic pollution in the marine environment. Environ. Pollut. 185, 352–364.
Jiang, J., Oberdörster, G., Biswas, P., 2009. Characterization of size, surface charge, and agglomeration state of nanoparticle dispersions for toxicological studies. J. Nanopart. Res. 11, 77–89.
Kaposi, K.L., Mos, B., Kelaher, B.P., Dworjanyn, S.A., 2014. Ingestion of microplastic has limited impact on a marine larva. Environ. Sci. Technol. 48, 1638–1645.
Klaine, S.J., Alvarez, P.J., Batley, G.E., Fernandes, T.F., Handy, R.D., Lyon, D.Y., Mahendra, S., McLaughlin, M.J., Lead, J.R., 2008. Nanomaterials in the environment: behavior, fate, bioavailability, and effects. Environ. Toxicol. Chem. 27, 1825–1851.
Long, M., Moriceau, B., Gallinari, M., Lambert, C., Huvet, A., Raffray, J., Soudant, P., 2015. Interactions between microplastics and phytoplankton aggregates: impact on their respective fates. Mar. Chem. 175, 39–46.
Lusher, A.L., Burke, A., O'Connor, I., Officer, R., 2014. Microplastic pollution in the Northeast Atlantic ocean: validated and opportunistic sampling. Mar. Pollut. Bull. 88, 325–333.
Ma, S., Zhou, K., Yang, K., Lin, D., 2014. Heteroagglomeration of oxide nanoparticles with algal cells: effects of particle type, ionic strength and pH. Environ. Sci. Technol. 49, 932–939.
Moore, C.J., 2008. Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. Environ. Res. 108, 131–139.
Ng, K.L., Obbard, J.P., 2006. Prevalence of microplastics in Singapore's coastal marine environment. Mar. Pollut. Bull. 52, 761–767.
Rochman, C.M., Kurobe, T., Flores, I., Teh, S.J., 2014. Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment. Sci. Total Environ. 493, 656–661.
Schwab, F., Bucheli, T.D., Lukhele, L.P., Magrez, A., Nowack, B., Sigg, L., Knauer, K., 2011. Are carbon nanotube effects on green algae caused by shading and agglomeration? Environ. Sci. Technol. 45, 6136–6144.
Setälä, O., Fleming-Lehtinen, V., Lehtiniemi, M., 2014. Ingestion and transfer of microplastics in the planktonic food web. Environ. Pollut. 185, 77–83.
Sjollema, S.B., Redondo-Hasselerharm, P., Leslie, H.A., Kraak, M.H.S., Vethaak, A.D., 2016. Do plastic particles affect microalgal photosynthesis and growth? Aquat. Toxicol. 170, 259–261.
Zhang, C., Wang, J., Tan, L., Chen, X., 2016. Toxic effects of nano-ZnO on marine microalgae *Skeletonema costatum*: attention to the accumulation of intracellular Zn. Aquat. Toxicol. 178, 158–164.
Zhao, S., Zhu, L., Wang, T., Li, D., 2014. Suspended microplastics in the surface water of the Yangtze Estuary System, China: first observations on occurrence, distribution. Mar. Pollut. Bull. 86, 562–568.