Study on method and mechanism of deep well circulation for the growth control of *Microcystis* in aquaculture pond

Haibing Cong, Feng Sun, Jun Wu, Yue Zhou, Qi Yan, Ao Ren and Hu Xu

**ABSTRACT**

In order to control the growth of *Microcystis* in aquaculture ponds and reduce its adverse effect on water quality and aquaculture, a production-scale experiment of deep well circulation treatment was carried out in an aquaculture pond with water surface area of 63,000 m² and water depth of 1.6–2.0 m. Compared with the control pond, the experiment pond had better water quality as indicated by 64.2% reduction in chlorophyll *a*, and 81.1% reduction in algal cells. The chemical oxygen demand, total nitrogen, and total phosphorus concentration were reduced by 55.1%, 57.5%, and 50.8%, respectively. The treatment efficiency is mainly due to the growth control of *Microcystis* (i.e. cell reduction of 96.4%). The gas vesicles collapsing because of the water pressure was suggested to be the mechanism for *Microcystis* suppression by the deep well circulation treatment. The *Microcystis* lost its buoyancy after gas vesicles collapsed and it settled to the bottom of the aquaculture pond. As a result, the algae reproduction was suppressed because algae could only grow in the area with enough sunlight (i.e. water depth less than 1 m).

**Key words** | aquaculture pond, deep well circulation, growth control, *Microcystis*

**INTRODUCTION**

The main goal of aquaculture is high yield, which needs high density of aquatic animals and a large amount of feed. The excessive dosing of feed and the wastes of aquatic animals lead to a nutrient-rich environment, such as nitrogen, phosphorus, and organic matters, which provides adequate nutrition sources for the reproduction of algae (Ahmed et al. 2008). There will be cyanobacterial blooms during summer in the aquaculture water of *Macrobenthicium rosenbergii* at Gaoyou City, Jiangsu Province, China. A large amount of cyanobacteria floating on the surface of the water gets rotten after being exposed to the sun. The process will consume the dissolved oxygen in water, release algal toxins to the water, and therefore seriously affect the aquaculture and threaten the safety of products (Chellappa et al. 2008). In the whole aquaculture production process, half of the water needs to be replaced by water from the surrounding river every 20 days. The cyanobacterial slurry will also be discharged to the river during the cyanobacterial blooms. The aquaculture area of Gaoyou City is up to 93.3 km², and produces a large amount of cyanobacteria-containing waste water draining into the river of the eastern route of the south-to-north water transfer project and contaminating the water source.

The common methods used in aquaculture water for algae control include chemical, biological and ecological methods. The chemical method uses algaecide such as CuSO₄, NaClO, and ClO₂ to deactivate algae. Cu²⁺ transfers into the algal cells and replaces the Mg²⁺ of chlorophyll molecules to make the algae become poisoned and make chlorophyll lose its photosynthetic capacity (Vavilin et al. 1995; Qian et al. 2010). Cu²⁺ can be sustained in water for 10–60 d for sustained control of algae. However, Cu²⁺ can cause the release of intracellular toxins of algae (Iwinski et al. 2016), and can be enriched in the viscera of aquatic animals (Whitaker et al. 1978). NaClO and ClO₂ as oxidants can oxidize the cell wall of algae and cause cell disintegration (Fan et al. 2013; Zhou et al. 2013). The effective chlorine dose of 1–3 mg/L will deactivate the algae after 30 min (Wert et al. 2014). The algae oxidized will decline and rot rapidly, resulting in the algal toxins and intracellular substances being released into and contaminating the water (Li et al. 2013). Also, the oxidants decay rapidly in water and thus have no continuous algae control ability. The biological method for algae control relies on the water-borne animals to use algae as their food. The typical water-borne animals
include silver carp (Radke & Kahl 2002), bighead (Miura 1990), protozoa (Oladoja et al. 2015), and shellfish (Li et al. 2015). The ecological method integrates nutrient management, animal grazing and environment adjustment to inhibit the algae growth. The nitrogen and phosphorus could be absorbed and removed by the water-borne plants (Porrello et al. 2003) and microbial agents (Ramanan et al. 2016). A constructed wetland could also be used to remove the nutrients in ponds (Lin et al. 2002). The phytoplankton could be used to block the sunlight and inhibit the algae growth. The chemical method will change the physical and chemical indicators of water, and break the ecological balance of water (Song et al. 2011; Song & Wang 2015). In comparison to the chemical method, biological and ecological methods do not produce secondary pollution and can increase comprehensive benefits. However, the relationship between pollutant loading and aquatic organisms in the food chain is difficult to determine and the subsequent processing of aquatic organisms is complicated, which make the management of biological and ecological methods relatively difficult (Martins et al. 2010).

*Microcystis aeruginosa* is the dominant species in the aquaculture water of *Macrobrachium rosenbergii* at Gaoyou City. The gas vesicles existing in *Microcystis* cells provide buoyancy and make the cells float on the water surface to utilize sunlight for reproduction (Konopka et al. 1987). The gas vesicles will collapse when the external pressure is more than the critical pressure at 0.4–0.7 MPa. As a result, the *Microcystis* will lose buoyancy and sink to the bottom (Kinsman et al. 1991). According to this principle, a deep well circulation treatment technology through pressure promoting sedimentation for *Microcystis* removal was developed (Cong & Huang 2015). The deep well circulation treatment was applied practically on the growth control of *Microcystis* in an aquaculture pond. The aquaculture water was introduced into the deep well with double channels, flowing from one channel to the bottom of the well and back to the breeding water body from the other channel. The gas vesicles in *Microcystis* were broken by the water pressure at the bottom of deep well, and the *Microcystis*, losing buoyancy of gas vesicles, will settle to the bottom when they return to the aquaculture pond, thus inhibiting the growth and reproduction of *Microcystis*. The aim of this study was: (1) to evaluate the treatment efficiency of deep well circulation for the growth control of *Microcystis* in aquaculture ponds; (2) to explore the impact of deep well circulation treatment on the algae species in the aquaculture ponds; (3) to investigate the impact of deep well circulation treatment on the water quality of the aquaculture ponds.

**MATERIALS AND METHODS**

**Study object**

This study was carried out at the *Macrobrachium rosenbergii* aquaculture pond of Gaoyou City, Jiangsu Province, from July 26 to September 17, 2015. Five aquaculture ponds with length of 180 m, width of 70 m and water depth of 1.6–2.0 m were tested in parallel. Four ponds were selected as experiment ponds and the remaining pond was used as control pond. The initial culture density and breeding method of the five ponds were the same: water was injected into the ponds and postlarvae were put into the ponds at June 8 and the aquaculture was finished at September 18; the initial culture density of *Macrobrachium rosenbergii* was 25/m² and equal feeds were evenly given twice every day for each pond; fishing operations were carried out twice and the water was drained out during the fishing period to control the water depth of the pond at ca. 1 m; the river water was refilled after the fishing operation. The water temperature of the aquaculture ponds was 24.2–31.0 °C and the pH was 7.84–8.45 during the testing period.

**Experimental device**

The experimental device shown in Figure 1 included a deep well, inflow pipe, outflow pipe, and supply pipe. The deep well was installed in the middle ridge of the four experiment ponds. The aquaculture water was sent to the deep well through the outflow pipe installed at one end of each experiment pond, and flowed out to the other end of the experiment pond through the supply pipe after being circulated within the deep well. The treated aquaculture water was then delivered to each experiment pond through the four outlets (Ø150 mm) with valves at each inflow pipe. The deep well was composed of two concentric wells, with the bottom of the outer well closed and the bottom of the inner well open, and the top of both the inner and outer well closed. The outer well was connected with the outflow pipe and the inner well was connected with the supply pipe. The aquaculture water was pumped to the supply pipe by the pump installed within the inner well, forming a circulating water flow from one end of the experiment pond, to the deep well, and then to the other end of the experiment pond. The diameter of the outer well and inner well was 650 mm and 360 mm respectively. The depth of the deep well was 80 m. The pump was selected as 250QSZ-4.5-7.5 type axial flow pump (Tianjin Blue Pump Co., Ltd, China).
with power of 7.5 kW, outlet diameter of 250 mm and head lift of 4.5 m.

Experimental methods

Operation of experimental device

The aquaculture water was drained into the outer well through the outflow pipe, flowing down to the bottom of the outer well and then into the inner well. The up-flow water in the inner well was then sent to the supply pipe and inflow pipe passing the water pump and delivered to the other end of the aquaculture pond. The total flow rate of the supply pipe was measured at 395 m$^3$/h by using an ultrasonic flowmeter, and then the flow rate of the inflow pipe was controlled by regulating the outlet valves to make the volume of water in each pond equal. In this way, the water in the four experiment ponds could be circulated once every 6 days.

Comparison of water quality between experiment pond and control pond

The four experiment ponds with same culture density and feed amount were connected in parallel with water mixed in the deep well, which suggested the water quality of the four ponds was basically the same, and thus one of the four experiment ponds was selected as representative for sampling and monitoring. In order to ensure the water sample represented the average values, a total of nine water samples from nine evenly distributed spots in three rows and three columns of each pond were mixed to derive the average water quality of the pond. The water sample of each sampling point was taken with a columnar sampler, an organic glass tube with a diameter of 50 mm, which was provided with a one-way valve in the bottom that only allowed water to flow in and prevented water from flowing out. The columnar sampler was vertically inserted into the water to the bottom of the pond, letting the columnar water enter into the columnar sampler via the one-way valve, and the nine water samples were mixed into one sampling bucket for laboratory tests. Sampling was generally completed at 7–9 a.m. once a day for the tests of chlorophyll $a$, chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP) of the experiment pond and control pond, and the algae identification and counting were carried out about every 15 days.

Vertical distribution of algae and its productivity

The vertical distribution of algae was derived by measuring the concentration of chlorophyll $a$ sampling at 0.1, 0.3, 0.5, 0.7, 0.9, 1.1, 1.3, and 1.5 m under the water surface.

The water samples drawn from the depth of 0.1, 0.5, 0.9, 1.3 and 1.6 m were used to measure the chlorophyll $a$ $(Chl-a; \mu g/L)$, and placed into transparent glass bottles without air bubbles inside for measuring the initial dissolved oxygen $(DO_b; mg O_2/L)$. The bottles with different water samples were re-suspended in their corresponding water depth for 24 h, and then tested for their dissolved oxygen $(DO_e; mg O_2/L)$. The net productivity of algae was calculated as $P_n = DO_e - DO_b$ (mg $O_2/L$), and the net
productivity of unit chlorophyll \(a\) mass of algae was calculated as \(P = (P_n/\text{Chl-}a)\) (mg \(O_2/\mu g\) Chl-\(a\)).

**Observation of the cell structure of Microcystis**

The algae of the experiment pond at the outlet of the inflow pipe and the control pond were collected with 40 mesh screens for ultrathin slice observation with a transmission electron microscope (TEM).

**Testing indices**

The dissolved oxygen was tested with the HQ30 d dissolved oxygen sensor (Hach, USA). The chlorophyll \(a\) was tested according to the recommended method in *Water and Wastewater Monitoring and Analysis Methods* (4th edition) (Walsby 1972), with acetone extraction for 24 h and measured by a UV722S spectrophotometer (Shanghai Yidian Analysis Instrument Co., Ltd, China). The COD, TN, and TP were tested with the DR900 + DRB200 multi-parameter measuring instrument (Hach, USA). The algae counting was carried out with the BM-37XB-D microscope (Shanghai BM Optical Instruments Manufacture Co., Ltd, China). The ultrathin slices of algal cells were analyzed with a CM100 TEM (Philips, The Netherlands).

**RESULTS AND DISCUSSION**

**Comparison of water quality between experiment pond and control pond**

The experiment began in July 26, 2015, and ended in September 17, 2015. The results of chlorophyll \(a\), COD, TP, and TN tested every day are shown in Figure 2. At the beginning, the chlorophyll \(a\) of the experiment pond and the control pond was similar (Figure 2(a)). During the entire period, the chlorophyll \(a\) of the control pond was in the range of 28.2–367.0 \(\mu g/L\) with average concentration of 114.2 \(\mu g/L\), while the chlorophyll \(a\) of the experiment pond was in the range of 3.4–103.2 \(\mu g/L\) with average concentration of 40.9 \(\mu g/L\), corresponding to 64.2% reduction in comparison to the control pond. The chlorophyll \(a\) of the control pond fluctuated greatly with the change of temperature, and increased up to the peak value of 367.0 \(\mu g/L\) in August 27 due to the algal bloom occurring from August 22 to September 1, which caused a large amount of visible algae particles floating on the surface of water, decaying and emitting a stench after exposure to the sun. In order to reduce the impact of algae decay on aquaculture, the farmers discharged the algal slurry on the water surface into the river with the pump on August 25 and August 28, decreasing the concentration of chlorophyll \(a\) in the control pond significantly. It could be concluded that the concentration of chlorophyll \(a\) in the control pond was higher...
than the tested value. However, the concentration of chlorophyll $a$ in the experiment pond was relatively stable with no visible algae floating on the surface of the water.

It is shown in Figure 2(b) that the COD of control pond was in the range of 47–199 mg/L with average concentration of 96.2 mg/L, while the COD of experiment pond was 12–86 mg/L with average concentration of 43.2 mg/L, which decreased by 55.1% comparing with the control pond. The COD was derived from the feeds, the waste of shrimps and the photosynthetic organic compounds of algae. The added feeds and cultured shrimps of experiment pond and control pond were equal, making the influence of feed residuals and shrimp wastes on COD of the two ponds identical. The difference between experiment pond and control pond was the concentration of algae. The photosynthetic organic compounds were stored in algal cells with algae absorbing CO$_2$ from the air. Due to the high concentration of algae in control pond, the high quantity of photosynthetic organic compounds was the main reason for the increased COD of control pond.

Figure 2(c) shows that the TP of the control pond was 0.25–1.70 mg/L with average concentration of 0.755 mg/L, while the TP of experiment pond was 0.22–0.78 mg/L with average concentration of 0.372 mg/L, which decreased by 50.8% comparing with the control pond. Phosphate as an important nutrient for the growth of algae and shrimps was mainly derived from the feeds. The phosphorus-containing feeds were partially used by the shrimps, partially dissolved in water, and mostly precipitated at the bottom of the water in the form of particles of feed residuals and shrimp wastes. Because of the few algae in experiment pond, the TP tested in the water sample of experiment pond was mainly composed of dissolved and suspended phosphorus. However, the large amount of algae in control pond absorbed the dissolved phosphorus and promoted the precipitated particulate phosphorus to dissolve in water and be enriched in algae, resulting in the higher TP from the water sample of the control pond, which also contained much phosphorus enriched in algae besides the dissolved and suspended phosphorus.

Figure 2(d) shows that the TN of control pond was 0.8–17.6 mg/L with average concentration of 6.12 mg/L, while the TN of experiment pond was 1.1–5.1 mg/L with average concentration of 2.60 mg/L, which decreased by 57.5% comparing with the control pond. Similarly, due to the absorption and accumulation of nitrogen by algae, the precipitated nitrogen was dissolved and enriched in algae, resulting in the higher TN in the water sample of control pond than that of experiment pond.

### Identification of the dominant species of algae

#### Identification of algae species

A total of three phyla, Cyanophyta, Chlorophyta, and Bacillariophyta, including 23 genera and 39 species in the aquaculture pond were identified and are listed Table 1. The dominant species were *Microcystis aeruginosa* and *Oscillatoria princeps* of Cyanophyta, and *Chlorella vulgaris* and *Scenedesmus obliquus* of Chlorophyta.

<table>
<thead>
<tr>
<th>Phylum</th>
<th>Genus</th>
<th>Species</th>
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<tr>
<td><strong>Cyanophyta</strong></td>
<td><em>Microcystis</em></td>
<td><em>Microcystis aeruginosa</em>, <em>Microcystis flos-aquae</em>, <em>Microcystis wesenbergii</em></td>
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<td><em>Oscillatoria</em></td>
<td><em>Oscillatoria princeps</em>, <em>Oscillatoria ornate</em></td>
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<td><em>Anabaena</em></td>
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<td><em>Spirulina</em></td>
<td><em>Spirulina maxima</em>, <em>Spirulina platensis</em></td>
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<td><em>Dactylococcopsis acicularis</em></td>
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<td><em>Chlorella</em></td>
<td><em>Chlorella vulgaris</em></td>
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<td><em>Scenedesmus</em></td>
<td><em>Scenedesmus obliquus</em>, <em>Scenedesmus bijugus</em>, <em>Scenedesmus dimorphus</em>, <em>Scenedesmus quadricauda</em>, <em>Scenedesmus acuminatus</em></td>
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<td><em>Pleodorina californica</em></td>
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<td><em>Cyclotella bodanica</em></td>
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<td><em>Synedra</em></td>
<td><em>Synedra affinis</em></td>
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Changes of algal cells

The experimental system was run from July 26, and sampled on July 28, August 17, August 31 and September 15 for algal cell counting with a microscope. The amount of algal cells in experiment pond and control pond are shown in Figure 3. After 2 days of running (July 28), the cell amount of experiment pond was slightly less than that of control pond (98.4 × 10^6 cells/L). Thereafter, the algal cells in control pond gradually increased to 197.0 × 10^6–235.0 × 10^6 cells/L, while the algal cells in experiment pond decreased to 20.4 × 10^6–68.3 × 10^6 cells/L, which had a reduction of 81.1% compared with control pond.

The dominant genera in the aquaculture pond were *Microcystis*, *Oscillatoria*, *Chlorella*, and *Scenedesmus*. The total amount of the four genera in experiment pond and control pond were 87.1% and 95.3%, respectively. The changes of the cell number and proportion of the four genera are shown in Figure 4.

In the control pond, the cell number of *Microcystis* showed an absolute predominance and gradually increased with the temperature, showing an average amount of 184.86 × 10^6 cells/L and cell percentage of 85.7%. *Chlorella* was the second most abundant genus in the control pond and decreased with the temperature from 19.0% to 5.0%. *Oscillatoria* and *Scenedesmus* were relatively less in the control pond with cell percentage of 0–1.0% and 2.0–3.0%, respectively. *Microcystis*, which is suitable to grow in aquaculture water in summer with temperature of 23–30°C and high nitrogen content, had a growth competition with *Oscillatoria*, *Chlorella*, and *Scenedesmus*, showing a converse changing trend with them. Another reason for the fast growth and reproduction of *Microcystis* was its buoyancy regulation mechanism. The gas vesicles existing in *Microcystis* cells provide buoyancy and make the cells float on the water surface to accept sunlight for photosynthesis. Also, *Microcystis* grows in gregarious form in water, with thousands of cells adhering together through the extracellular gelatin substances and forming large *Microcystis* particles with diameter up to 1 mm, which makes the *Microcystis* have great buoyancy and resistance to the mixing effect of water flows and waves, to rapidly return to the surface of water.

In the experiment pond, the cell number of *Microcystis* decreased after 2 days of running but still occupied the dominant position with the cell percentage of 43.0%. With the treatment of deep well circulation, the *Microcystis* decreased sharply to 8.0–20.9% with average amount of 6.66 × 10^6 cells/L. The percentage of *Chlorella* was 36.0–24.0%. The proportion of *Oscillatoria* was greatly increased from 6% to 41.0–46.0% with the average number of 17.27 × 10^6 cells/L. There was no obvious change for *Scenedesmus*, which was in the range of 2.0–3.0%. The above data showed that the growth of *Microcystis* was inhibited after the deep well circulation treatment, decreasing 96.4% comparing with that in the control pond, whereas the *Oscillatoria* obtained the chance to grow without the competition of *Microcystis*, and became the dominant genus, which was 26.2 times the amount in the control pond.

Vertical distribution and productivity change of algae

The vertical distribution of chlorophyll *a* in the experiment pond and control pond are shown in Figure 5. The vertical distribution of chlorophyll *a* in the experiment pond and control pond was basically consistent, with the overall trend of having more chlorophyll *a* in the upper layer and less chlorophyll *a* in the lower layer, and the peak values occurred in the water depth of 0.5 m and 1.3 m respectively. The net productivity of unit chlorophyll *a* mass was calculated, whereas according to the net algae productivity and chlorophyll *a* concentration in different water depth. It is shown in Figure 6 that the algae in control pond had net growth above the water depth of 0.86 m and net decline under the water depth of 0.86 m. The algebraic sum of the area formed by the curve and the longitudinal axis was calculated to obtain the cumulative net productivity of unit chlorophyll *a* mass in the column water body with water depth of 1.6 m as 0.051 mgO2/(μg chl-a-d). The
algae in experiment pond had net growth above the water depth of 1.07 m and net decline under the water depth of 1.07 m. The cumulative net productivity of unit chlorophyll $a$ mass in the column water body of the experiment ponds with water depth of 1.6 m was calculated as 0.086 mgO$_2$/(µg chl-$a$·d). The experiment ponds were more suitable for algae growth and had higher algae productivity of unit chlorophyll $a$ mass than the control pond because of the higher water transparency of experiment pond, leading to the stronger sunlight intensity for illumination in the deeper water. However, the total net productivity of the control pond would be higher than that of the experiment pond due to its higher algae concentration. Therefore, it could be concluded that the control of algae growth by deep well circulation was not because the algae could not grow in the experiment pond but the algae concentration in the light area of the experiment pond was less than that of the control pond, which reduced the breeding base in water and thus lowered the total reproduction.

**Comparison of the structure of gas vesicles in Microcystis cells between the experiment pond and control pond**

The ultrathin slice of Microcystis cell analyzed with TEM is shown in Figure 7, which showed that white honeycomb gas vesicles existed in the control pond (Walsby 1972)
but disappeared in the experiment pond. The gas vesicles have a protein wall which is permeable for gas but not for water, and would collapse when the water pressure outside is more than 0.4–0.7 MPa (Kinsman et al. 1991). The water pressure is 0.8 MPa at the bottom of the deep well, making the water molecules enter through the permeable cell wall and act on the wall of gas vesicles, thus forcing the gas inside the gas vesicles out through their protein walls. The gas vesicles become deflated when the gas diffuses out, resulting in the lost buoyancy and sinking of Microcystis cells. Therefore, the growth control mechanism of Microcystis by deep well circulation treatment is that the Microcystis lost buoyancy of gas vesicles under the water pressure of the deep well, and settled to the bottom when they returned to the aquaculture pond, reducing the breeding base of Microcystis, which could only grow in the light area with water depth of less than 1 m, and thus reducing the total amount of Microcystis reproduction.

**CONCLUSIONS**

1) The deep well circulation treatment was carried out by introducing the aquaculture water into the deep well and back to the breeding water body after circulation. The chlorophyll a, algal cell amount, COD, TN, and TP in the experiment pond of deep well circulation decreased by 64.2%, 81.1%, 55.1%, 57.5%, and 50.8%, respectively, comparing to those in the control pond. The reduction of algae concentration and improvement of other water quality parameters reduced the adverse effects on aquaculture and the pollution to the surrounding water.

2) The dominant species of algae in the experiment pond changed from Microcystis to Oscillatoria after the deep well circulation. The Microcystis and Oscillatoria were 83.7% and 0.5% in the control pond, but 14.3% and 42.7% in the experiment pond. For the cell amount, Microcystis was decreased by 96.4% in the experiment pond, but Oscillatoria was increased to 26.2 times of that in control pond.

3) The growth control mechanism of Microcystis by deep well circulation treatment is that: the gas vesicles of Microcystis collapse under the water pressure of deep well and the Microcystis cells lose buoyancy and settle to the bottom of experiment pond. Therefore, the breeding base of Microcystis in the upper water layer suitable for Microcystis growth was reduced, and the total
amount of Microcystis reproduction was reduced. The Oscillatoria is the dominant genus without the competition of Microcystis.

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