Purification Effect Evaluation of the Designed New Volcanic Soil Adsorption Material Containing Bioreactor for Eutrophic Water Treatment

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Research Article

Keywords: Eutrophication, volcanic soil, bioreactor, viable but non-culture (VBNC) bacteria, resuscitation-promoting factor (Rpf)

Posted Date: February 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-222333/v1

Purification effect evaluation of the designed new volcanic soil adsorption material containing bioreactor for eutrophic water treatment

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Abstract

To investigate the purification effect of the new adsorption material containing bioreactor and the critical role of non-culture (VBNC) bacteria in eutrophication ecosystem, major water quality parameters and microbial community were determined and analyzed in prepared eutrophic water for 2 years monitoring. The results showed that removal rates of total phosphorus (TP), total nitrogen (TN), chlorophyll-a (Chl-a) and chemical oxygen demand (COD) ranged of 90.7% - 95.9%, 84.5% - 92.4%, 87.9% - 95.8% and 68.3% - 82.7%, respectively, indicating the designed bioreactor possessed high efficiency in eutrophic water treatment. Although the bioreactor had been operated for 2 years, water from treatment group was more clearer and odorless than control group, exhibiting the long service life of the bioreactor. Stopping operation in August caused the removal rates of major water quality parameters significantly decrease ($p < 0.05$), revealing stopping operation and high temperature in Summer exerted dual effect on the bioreactor, whereas the impact could be minimized when the bioreactor was running. According to most probable number (MPN) method, the total bacteria under +Rpf treatment were higher than under -Rpf treatment, implying Rpf could resuscitate VBNC bacteria in eutrophication ecosystem. Nine VBNC bacteria were isolated based on the BLAST results of 16S rRNA gene, and these bacteria might contribute to eutrophic water treatment based on their functions, such as phosphorus-collecting and denitrification. Those results provided new insights for engineering technology innovation and had benefit in eutrophic water treatment.

Keywords Eutrophication · volcanic soil · bioreactor · viable but non-culture (VBNC) bacteria · resuscitation-promoting factor (Rpf)

Introduction

In recent decades, aquatic ecosystem has suffered much more serious problems of water eutrophication due to anthropogenic pollution and climate change (Le et al. 2010; Smith 2003; Kosten et al. 2012; Andersen et al. 2020a; Freeman et al. 2020). Eutrophication is often accompanied with rapid occurrences of harmful algal blooms (HABs), especially of chlorella, cyanobacteria (Wang et al. 2019a), and diatoms (Paerl et al. 2016, 2019; Huisman et al. 2018; Woolway et al. 2019; Kim et al. 2020), which threaten other aquatic life and change the color and/or odor of the water body (Vollenweider and Kerekes 1982). Therefore, eutrophic water will finally lose its original functionality, such as aquatic food production and safe-drinking water supply (Huisman et al. 2018). Meanwhile, eutrophication is worldwide environmental problems and have been found in many countries, such as Israel (Geisler et al. 2020), Mexico (Caballero and Vazquez 2020) and USA (Tomasko et al. 2020). It is summarized that over 75% of the closed water bodies (e.g., lakes, ponds and reservoirs) in Africa, Asia and Latin America have deteriorated largely and encountered some extents of eutrophication since the 1990s (UN-Water 2018).

Evaluating the trophic state of eutrophic water have become a research hotspot in the water ecology community based on several water quality parameters, including total phosphorus (TP), total nitrogen (TN), chlorophyll-a (Chl-a) and chemical oxygen demand (COD) (Chao Rodriguez et al. 2014; Smith and Schindler 2009; Carlson 1977). Previous studies demonstrated that the excessive accumulation of N and P in aquatic

ecosystem is closely related to eutrophication, which, on the one hand, causes greater primary production (Ahlgren et al. 2005; Feuchtmayr et al. 2009) and, on the other hand, hinders the restoration process of the eutrophic ecosystem (Banerjee 2016), thus results in hyper-eutrophication and subsequent water quality deterioration (Ahlgren et al. 2005). At the same time, Chl-a and COD are the key indicators of eutrophic water, which reflect the eutrophication level and the biomass of HABs, and further uncover the intrinsic essence of eutrophication. Therefore, systematically monitoring the trophic state of aquatic ecosystem can effectively evaluate the degree of eutrophication and assess the feasibility of new technical for eutrophic aquatic ecosystem restoration.

Former experiences in eutrophic aquatic ecosystem restorations specifically emphasizing reductions of external nutrient inputs might fail to alleviate the eutrophication (Paerl et al. 2016; Wang et al. 2019a; Lurling and Mucci 2020). As plenty of studies announce that effective removal of excessive N and P are of concern for the aquatic ecosystem balance (Gruber and Galloway 2008; Domangue and Mortazavi 2018; Stoliker et al. 2016), the potential impacts from internal nutrient eliminating should be taken into considerations for eutrophic water treatment (Paerl et al. 2019; Wang et al. 2019a; Qin et al. 2020). Physical (Schauser et al. 2003), chemical (Walpersdorf et al. 2004; Wauer et al. 2005) and biological (Xu et al. 2011; Liu et al. 2012; Lu et al. 2014; Petersen et al. 2014; Wu et al. 2015) methods were adopted in extensive researches during the past decades. However, as the reason of financial and labor-intensive challenge, limited to biotic and abiotic restriction and high risk of

83 secondary pollution, these methods were difficult to meet the high frequency for
84 eutrophic water treatment, especially for projects with wide distributions (Liang et al.
85 2014). Hence, new ecological techniques are considered to be the most promising
86 technology depending on its unique features.

87 Nowadays, numerous studies have reported that new volcanic soil adsorption
88 materials and microbial communities also played important roles in water purification
89 (Ding et al. 2009, 2011; Zhou and Wang 2010). Simultaneously, viable but
90 non-culturable (VBNC) bacteria are frequently found in dyeing workshop (Jin et al.
91 2017), pharmaceutical waste water (Li et al. 2014) and polychlorinated biphenyls (PCBs)
92 (Su et al. 2014) polluted soils in recent years. The VBNC bacteria can survive under
93 extreme environments by passing into the viable but non-culturable state, and can
94 become culturable under the condition of adding resuscitation promoting factor (Rpf)
95 cultivation (Ding et al. 2011; Mukamolova et al. 2002; Serpaggi et al. 2012). Previous
96 studies have certified a number of VBNC bacteria could be recovered by Rpf , including
97 high G+C gram-positive bacteria, low G+C gram-positive bacteria and several
98 gram-negative bacteria (Ding et al. 2011; Yu et al. 2015). However, whether VBNC
99 bacteria surviving in eutrophic water and weather VBNC bacteria possessing certain
100 environmental purification function had not been reported to the best of our knowledge.

101 In this study, a new volcanic soil adsorption material containing multi-stage
102 tandem type bioreactor has been designed. The purification effect of eutrophic water (TP,
103 TN, Chl-a and COD) and the functional microbial communities, especially VBNC

bacteria, have been assessed and analyzed based on seasonal changing. This work has benefit in further understanding the microbial clustering in sewage treatment system and provide new insights for enhancing the efficiency of eutrophic water treatment by improving the engineering technology.

Materials and methods

Preparation of eutrophic water

The water sample in the bioreactor was composed of synthetic nutrient matrix mixed with raw water from a eutrophic lake (Xinyue lake, Jinhua, China), and the ratio of synthetic nutrient matrix and raw water was 97:3 (V/V). The initial compositions of synthetic nutrient matrix were as follows: beef extract (1.000 g), yeast extract (1.000 g), K_2HPO_4 (0.272 g), KH_2PO_4 (0.456 g), water (1.0 L). The quality parameters of water sample were TP 22.4 mg/L, TN 16.5 mg/L, Chl-a 218.5 μg/L and COD 202.4 mg/L. The water sample (100 L) recycling treatment in the bioreactor was denoted as treatment group, and the other water sample (100 L) placement under natural condition was denoted as control group.

The novel adsorption material

The novel adsorption material in the bioreactor was a mixture of several efficient adsorption materials, which made from volcanic soil. It had granular appearance and its diameter ranged from 2 to 4 mm (Fig. 1) (Ding et al. 2009). The surface area of 1 L novel adsorption material can be up to 21,476 m^2 and is suit for microbial habitat as its

124 micron-scale holes. Therefore, when sewage flowed through the bioreactor, the new
125 adsorption materials could effectively absorb chemicals and enrich microbial doubly.

126 **Construction and operation of the bioreactor**

127 The self-designed bioreactor was composed of 9 tower tanks (volume of tower
128 tank was 550 mL), and every 3 of them were in series (Fig. 2). The water sample flowed
129 into the bottom of the first tower tank and transferred into the bottom of the subsequent
130 tower tank from the top of the former tower tank after flowing through the filter plate
131 and the new adsorption material (200 g). Then the bioreactor treated water returned into
132 reservoir through reflux pipe and merged for next purification.

133 Based on eutrophication preferred to occur in spring, summer, autumn and less
134 outbreak in winter, 3 running and 2 stopping stages were designed during the
135 experiment. Two years stable operation scheme of the bioreactor was enacted as follows:
136 1 year running before summer - stopping in summer - running in autumn - stopping in
137 winter - running after winter. The details for actual operation and the environmental
138 temperature were listed in Table 1.

139 **Water quality parameters detection**

140 The major water quality parameters of water samples were detected about every 3
141 months both in treatment and control group. The TP, TN, Chl-a and COD were
142 measured according to ammonium molybdate spectrophotometric method, alkaline
143 potassium persulfate digestion UV spectrophotometric method, spectrophotometric

144 method and HACH method, respectively. The removal rates were calculated through the
145 following equation: $R = (1 - C/C_o) \times 100\%$, where R was the removal rate (%) of major
146 water quality parameter, C and C_o were the concentrations of major water quality
147 parameter in treatment and control group, respectively. Three replicates were set for
148 control and treatment group of water samples. T-test was performed using SPSS 19.0
149 software. The results were expressed as the mean ± SD (standard deviation).

150 **TP detection**

151 The water sample was adjusted to neutral and then 25 mL water sample was
152 transferred into a 50 mL plug scale tube. About 4 mL $K_2S_2O_8$ solution was added into
153 the plug scale tube for digestion in a autoclave. Two hours later, when temperature
154 dropped to 80°C, the plug scale was took out and diluted to the tick mark with ultrapure
155 water. Ascorbic acid (1 mL) was added to the diluted digestion solution and then mixed
156 completely. Thirty seconds later, 2 mL molybdate solution were added into the plug
157 scale and placed for 15 min at room temperature. The absorbance was determined at the
158 wavelength of 700 nm. The content of TP was determined according to the following
159 formula:

160 $$TP\ (mg/L) = m/V;$$

161 where “m” was the P content in water sample (μg), “V” was the volume of water
162 sample (mL).

163 **TN detection**

The pH of water sample was adjusted to 5 - 9 and 10 mL water sample was transferred into a 25 mL colorimetric tube. About 5 mL alkaline potassium persulfate solution was added into the colorimetric tube for digestion in a autoclave. Thirty minutes later, when pressure dropped to 80℃, the colorimetric tube was took out and cooled down. Hydrochloric acid (1 mL) was added into the digestion solution and then diluted to the tick mark with ultrapure water. About 5 mL diluted solution were added to cuvette. The absorbance was determined at the wavelength of 200 and 275 nm, and the absorbance $A = A_{220} - A_{275}$. The content of TN was determined according to the following formula:

$$TN\ (mg/L) = m/V;$$

where “m” was the N content in water sample (μg), “V” was the volume of water sample (mL).

Chl-a detection

Water sample (1000 mL) and 1% $MgCO_3$ (5 mL) were mixed completely. About 200 mL mixtrue was filtered with 0.45 um glass fiber filter film. When filtering finished, the filter film was clamped out with clean tweezers. After folded in half twice, the filter film was put into a centrifuge tube with 10 mL 95% ethanol and incubated at 4℃ for 20 h. Following the centrifuge tube were 3000 rpm centrifuged for 15 min and then determined the absorbance at the wavelength of 649, 665 and 750 nm. The content of Chl-a was determined according to the following formula:

$$Chl\text{-}a = (13.7 * (A_{665} - A_{750}) - 5.76 * (A_{649} - A_{750})) * (E/F) * L;$$

where “E” was the extraction volume (mL), “F” was the filtration volume (L), “L” was the width of cuvette (mm).

COD detection

Water sample (2.5 mL), $K_2Cr_2O_7$ solution (1.5 mL) and Ag_2SO_4 solution (3.5 mL) were mixed completely in HACH. Then the mixture was digested under 150℃ for 2 h and cooled down for 30 min. The absorbance was determined by DR1010.

Most probable number (MPN) method for bacteria identification in the bioreactor

Bacteria were isolated from the bioreactor using the most probable number (MPN) method (McCrady et al. 1915). The VBNC bacteria were isolated by adding Rpf protein coming from *Micrococcus luteus* culture supernatant (Ding et al. 2012; Su et al. 2014). The liquid medium consisted of peptone 5.0 g, yeast extract 0.5 g, glucose 5.0 g, sodium chloride 2.5 g, distilled water 1.0 L, pH 7.0-7.2, including the active Rpf (+Rpf) and inactive Rpf (-Rpf), respectively, where the volume ratio was 4:1. Three replicates were set for adding +Rpf and -Rpf treatments, respectively. The bacteria solution was prepared by mixing weighted 1 g novel volcanic soil adsorption material and 9 mL 0.9% normal saline. Bacteria solution (0.25 mL) and liquid medium (2.25 mL) was mixed. Then, the mixture was gradually diluted from 10^{-1} to 10^{-7} with the prepared liquid medium. The bacteria solution with different dilutions were incubated at 30℃ and the turbidity of bacteria solution was measured by microplate reader at the wavelength of 660 nm (OD_{660}). When the flora was in stationary phase, turbidity or non-turbidity of

the cultivate tube was recorded as positive or negative, respectively. The total number of bacteria in +Rpf and -Rpf treatments were determined by the MPN table (Fig. S1).

Rpf effect and VBNC bacteria status evaluation

V_R was the ratio of total amount of bacteria in +Rpf treatment and in -Rpf treatment, and was used to evaluate the activity abundance of Rpf. V_R greater than 5 indicated that there had dominant bacteria in VBNC state, which was sensitive to Rpf.

Denaturing Gradient Gel Electrophoresis (DGGE) of the diluted bacteria solution from 10^{-1} to 10^{-6} in +Rpf and -Rpf treatments were also carried out to identify the presence of VBNC bacteria. Genomic DNA was extracted using the MiniBEST Bacterial Genomic DNA Extraction Kit Ver.2.0 (Takara, Dalian, China), and 16S rDNA V3 region was amplified by the following primers: F, 5'-CGCCCGCCG CGCGCGGCGGGCGGGGCGGGGGCACGGGGGGCCTACGGGAGGCAGCAG-3' and R, 5'-ATTACCGCGGCTGCTGG-3'. The PCR amplified procedure was shown in Table S1.

The PCR product was detected by 1% agarose gel electrophoresis (AGE) and purified. The qualified PCR products was separated by denaturing gradient gel electrophoresis (DGGE) with 8% polyacrylamide and 40%-60% denaturing solution.

Constructing the phylogenetic trees of the isolated bacteria

16S rRNA gene of the bacteria, which isolated from the bioreactor, was amplified by the primers: F, 5'-AGAGTTTGATCCTGGCTCAG-3', and R, 5'-GGCTACCTTGTT

ACGA-3'. The sequences of 16S rRNA gene were blasted in NCBI (http://www.ncbi.nlm.nih.gov/) and EzTaxon server (http://www.eztaxon.org/) to identify the genrea of bacteria. The phylogenetic tree of all isolated bacteria was constructed by MEGA 5.0. The neighbor-joining method was selected in the instruction of phylogenetic tree (Saitou and Nei 1987). The evolutionary distance matrix was calculated using Kimura's two-parameter method (Kimura 1980). And the bootstrap values were set for 1000 replications (Felsenstein 1985).

Results

Removal effect of TP in the bioreactor

The TP concentrations in water sample (both in control and treatment groups) and the removal rate of the bioreactor were shown in Fig. 3. The TP concentrations ranged of 0.91 - 2.06 mg/L and 20.09 - 23.38 mg/L in treatment group and control group, respectively. All of the TP concentrations in treatment group were significantly lower than in control group ($p < 0.05$), no matter the bioreactor was running or stopping. The TP removal rates of the bioreactor ranged from 90.7% to 95.9%, indicating the bioreactor could efficiently reduce the TP concentration of eutrophic water. The TP removal rate in February of TY was 92.8% and was significantly lower than in November of SY (94.2%) and May of TY (94.4%) ($p < 0.05$), showing bioreactor stopping operation affected TP elimination. Meanwhile, the TP removal rate in August of SY was 90.7% and was significantly lower than other detection months ($p < 0.05$),

exhibiting the bioreactor stopping and the high temperature were the two key factors for influencing TP removing. What's more, the TP removal rate in March of SY (95.9%) were significantly higher than the other detection months ($p < 0.05$), implying the bioreactor had the best purification effect in Spring.

Removal effect of TN in the bioreactor

The TN concentrations in water sample (both in control and treatment groups) and the removal rate of the bioreactor were shown in Fig. 4. The TN concentrations ranged of 1.32 - 2.44 mg/L and 13.86 - 17.50 mg/L in treatment group and control group, respectively. All of the TN concentrations in treatment group were significantly lower than in control group ($p < 0.05$), no matter the bioreactor was running or stopping. The TN removal rates of the bioreactor ranged from 84.5% to 92.4%, indicating the bioreactor could effectively reduce the TN concentration of eutrophic water. The TN removal rate in February of TY was 85.8% and was significantly lower than in November of SY (89.6%) and May of TY (91.8%) ($p < 0.05$), showing the bioreactor stopping operation affected TN elimination. Meanwhile, the TN removal rate in August of SY was 84.5% and was significantly lower than other detection months ($p < 0.05$), exhibiting the bioreactor stopping and the high temperature also influenced TN removing. Simultaneously, the TN removal rate in March of SY (92.4%) were significantly higher than the other detection months, except in September of FY (91.9%) and May of TY (91.8%), suggesting the bioreactor had the better purification effect in Spring and Autumn.

Removal effect of Chl-a in the bioreactor

The Chl-a concentrations in water sample (both in control and treatment groups) and the removal rate of the bioreactor were shown in Fig. 5a. The Chl-a concentrations ranged of 8.41 - 28.46 μg/L and 200.11 - 234.38 μg/L in treatment group and control group, respectively. All of the Chl-a concentrations in treatment group were significantly lower than in control group ($p < 0.05$), no matter the bioreactor was running or stopping. The Chl-a removal rates of the bioreactor ranged from 87.9% to 95.8%, indicating the bioreactor could efficient reduce the Chl-a concentration of eutrophic water. The Chl-a removal rate in February of TY was 93.7% and was significantly lower than in November of SY (95.2%) and May of TY (95.8%) ($p < 0.05$), showing bioreactor stopping operation affected Chl-a elimination. Meanwhile, the Chl-a removal rate in August of SY was 87.9% and was significantly lower than other detection months ($p < 0.05$), exhibiting the bioreactor stopping and the high temperature exerted doubly effect on Chl-a removing. Meanwhile, the Chl-a removal rate in March of SY (95.6%) were significantly higher than the other detection months, except in November of SY (95.2%) and May of TY (95.8%), suggesting the bioreactor had the better purification effect in Spring and Autumn. The real comparison of water sample between control and treatment group was showed in Fig. 5b.

Removal effect of COD in the bioreactor

The COD concentrations in water sample (both in control and treatment groups) and the removal rate of the bioreactor were shown in Fig. 6. The COD concentrations

ranged of 32.13 - 45.11 mg/L and 185.26 - 208.42 mg/L in treatment group and control group, respectively. All of the COD concentrations in treatment group were significantly lower than in control group ($p < 0.05$), no matter the bioreactor was running or stopping. The COD removal rates of the bioreactor ranged from 68.3% to 82.7%, indicating the bioreactor could efficiently reduce the COD concentration of eutrophic water. The COD removal rate in February of TY was 78.6% and was significantly lower than in November of SY (82.7%) and May of TY (81.3%) ($p < 0.05$), showing bioreactor stopping operation affected COD elimination. Meanwhile, the COD removal rate in August of SY was 68.3% and was significantly lower than other detection months ($p < 0.05$), exhibiting the bioreactor stopping and the high temperature severely affected COD cleaning. Meanwhile, the COD removal rate in March of SY (82.5%) was significantly higher than the other detection months, except in November of SY (82.7%), suggesting the bioreactor had the better purification effect in Spring and Autumn.

The OD_{660} value of bacteria solution in +Rpf and -Rpf treatment

In present study, bacteria were cultivated by using MPN method. The OD_{660} values in the stationary phase of bacteria solution were listed in Table 2. No significant difference of OD_{660} values were observed between +Rpf and -Rpf treatment under the dilution of 10^{-1} and 10^{-3}, respectively. However, the OD_{660} values under the dilution from 10^{-4} to 10^{-6} were continuously higher in +Rpf treatment than in -Rpf treatment, revealing the number, species or number and species of bacteria was increased under the adjunction of Rpf. The total number of bacteria attached on the novel volcanic soil

adsorption material were 2.4×10^9 and 1.1×10^8 cells/g in +Rpf and -Rpf treatments, respectively. The V_R value was 21.8, indicating there had dominant bacteria in VBNC state, which were sensitive to Rpf.

The DGGE analysis of bacteria solution in +Rpf and -Rpf treatment

The PCR results of 16S rDNA V3 region in MPN culture system (same as the above experiment) were showed in Fig. 7. The target bands were clear and bright, indicating the PCR product could be used for DGGE analysis after purification.

For DGGE analysis, the highlight bands in the lanes of +Rpf treatment were more than -Rpf treatment, especially under the dilution from 10^{-2} to 10^{-6} (Fig. 8), which were in accordance with the OD_{660} values, indicating Rpf had a certain effect on promoting bacteria resuscitation in the eutrophic water treatment system. What's more, the bands under the dilution of 10^{-3}, 10^{-5} and 10^{-6} +Rpf treatment appeared on the top of the lanes, whereas the bands under the dilution of 10^{-2} +Rpf treatment appeared at the bottom of the lane, suggesting the microbial would compete with each other and would not both take function under the same dilution.

Blast results of 16s rDNA PCR products

In this study, 24 strains of bacteria were isolated from the bioreactor (Table 3). The isolated bacteria were belonged to 11 genera, including *Bacillus*, *Brevibacillus*, *Burkholderia*, *Enterobacter*, *Lysinibacillus*, *Microbacterium*, *Micrococcus*, *Ochrobactrum*, *Paenibacillus* and *Pseudomonas*. Most of the isolated bacteria were

328 belonged to Low GC gram-positive (50%), followed by gram-negative bacteria (42%)
329 and poor in high GC gram-positive bacteria (8%). Therefore, Rpf not only promoted the
330 resuscitation of gram-positive bacteria, but also activated gram-negative bacteria in the
331 bioreactor. At the same time, 9 strains of VBNC bacteria were native from 4 genrea,
332 including *Bacillus* (3), *Burkholderia* (2), *Enterobacter* (2) and *Pseudomonas* (2). The
333 phylogenetic tree was constructed and showed in Fig. 9.

334 **Discussion**

335 **Efficient purification capacity of the volcanic soil containing bioreactor**

336 Based on former researches, TP, TN, Chl-a and COD were the major water quality
337 parameters for evaluating the trophic state of eutrophic water (Chao Rodriguez et al.
338 2014; Smith and Schindler 2009, Carlson 1977). Excessive accumulation of N and P in
339 aquatic ecosystem not only caused greater primary production (Ahlgren et al. 2005,
340 Feuchtmayr et al. 2009), but also slowed down the restoration process of water
341 ecosystem (Banerjee 2016), thus resulted in eutrophication (Ahlgren et al. 2005) and
342 accompanied with occurrences of HABs. Simultaneously, Chl-a and COD were the key
343 indicators for reflecting the degree of eutrophication. Therefore, systematically
344 monitoring the trophic state of eutrophic water could effectively assess the purification
345 effect of the designed multi-stage tandem type bioreactor. In this study, 2 years
346 systematically monitoring job were carried out by real-time detection the 4 major water
347 quality parameters. The results showed that the self-designed bioreactor could efficient

348 removal TP, TN, Chl-a and COD, no matter the bioreactor was running or stopping. The
349 average removal rates of TP, TN, Chl-a and COD reached to 93.6%, 89.6%, 93.4% and
350 78.5%, respectively. In addition, regardless the high efficient removal rate of the major
351 water quality parameters, the bioreactor had a long service life. Until now, although the
352 bioreactor has been operated for more than 2 years, the water from treatment group was
353 clear and odorless, whereas the water from control group was cloudy and odorous.
354 What's more, as the reason of vulnerable influenced by temperature and light intensity,
355 the removal rate of other reported bioremediation methods could easily been affected,
356 such as the aquatic plant and macrophytic algae restoration system (Zuo et al. 2014; Xu
357 et al. 2011) and the *Ipomoea aquatica* with low-energy ion implantation method (Li et
358 al. 2009). However, the removal rate of the designed bioreactor were little influenced
359 when the bioreactor was running, especially for TP, TN and Chl-a, which the removal
360 rates were approximately or greater than 90%. Furthermore, when compared with other
361 eutrophic water treatment system, the bioreactor also revealed the better purification
362 effect than them, such as the constructed wetlands system (Zhao et al. 2012), the
363 integrated floating island system (Lu et al. 2014), the coordinated restoration of animals
364 and plants system (Hua et al. 2008), and the combination treatment of bacteria and
365 plants system (Hua et al. 2010). Therefore, the designed bioreactor could meet the
366 demand for large-scale eutrophication ecosystem restoration.

367 **Season-changing and stopping operation affect the removal rate of the bioreactor**

368 Numerous researches had indicated the climate alteration as well as season

369 changing were the key factors for water eutrophication (Le et al. 2010; Smith 2003). At
370 the same time, water temperature and nutrient availability were believed to be two of
371 the most important factors in facilitating occurrences of HABs (Beaulieu et al. 2013;
372 Rigosi et al. 2014; Tong et al. 2019a). Therefore, understanding the potential impacts of
373 eutrophication and water temperatures and their interactions with seasonal changing was
374 crucial to assess the designed bioreactor. In present study, a continuously running
375 process of the bioreactor was designed in the first year, whereas two stopping status
376 (Summer and Winter) were designed in the next year, so that we could comprehensivly
377 evaluate the effect of season changing on the purification of the bioreactor. According to
378 the results, the removal rates of the 4 major water quality parameters in February of TY
379 were significantly lower than in November of SY and in May of TY ($p < 0.05$),
380 indicating stopping operation could significantly influence the purification effect of the
381 bioreactor, despite eutrophication was less likely to occur in Winter. What's more, the
382 removal rates of the 4 major water quality parameters in August of SY were
383 significantly lower than the other detection months ($p < 0.05$), exhibiting stopping
384 operation and the high temperature in Summer could doubly affect the removal rates of
385 the bioreactor. Warming in surface waters was the most direct response of water body in
386 summer (Rigosi et al. 2014; Piccolroaz et al. 2020), where was usually the place for
387 phytoplankton living. As the overall function of aquatic ecosystems alteration in
388 Summer, such as biochemical transformations of nutrients (Wu et al. 2017; Ding et al.
389 2018; Jenny et al. 2020), there might create an environment which is particularly

beneficial for phytoplankton surviving (e.g. cyanobacteria) and result in HABs (Paerl et al. 2016; Freeman et al. 2020). From the results, although stopping operation and high temperature could affect the removal rates of the bioreactor, there had little influence on the purification effect when the bioreactor was running, showing the good effect of the bioreactor. Meanwhile, the removal rates of the 4 major water quality parameters in March and December of SY were always higher than the other detection months, suggesting the bioreactor had the better purification effect in Spring and Autumn.

Potential ability of VBNC bacteria in eutrophication ecosystem restoration

It has been reported that the eutrophication level was well associated with the activities of bacteria in aquatic ecosystem, such as nitrogen-fixing bacteria, nitrifying bacteria and denitrifying bacteria (Fosso-Kankeu and Mulaba-Bafubiandi 2014). Meanwhile, the nitrogen absorbed and utilized by microbial and algae would dissolve back into the water body again with death and decomposition, resulted in nitrogen concentration increasing. In the Lake Taihu, China, NO_3^--N concentration in Spring over 10 times higher than in summer (Xu et al. 2010, 2015; Wang et al. 2019b), and had confirmed that nitrification rates were highest in March and lowest in July based on stable-isotope techniques (Hampel et al. 2018), which implied microbial could positive take part in eutrophic water treatment. In this study, the total bacteria count in +Rpf treatment group was obviously higher than in -Rpf treatment group, which were in consistent with the OD_{660} values and the DGGE results of MPN culture system, indicating adding active Rpf could efficiently facilitate microbial resuscitation. What's

more, the VR value was 21.82, implying there had dominant VBNC bacteria in eutrophic water treatment system, which was sensitive to Rpf. Meanwhile, 24 bacteria were isolated from the novel volcanic soil adsorption material, and were native from 11 genera. Nine isolated strains belonged to VBNC bacteria based on the BLAST results of 16S rDNA gene, and were annotated into 4 genera, including genera *Bacillus*, *Burkholderia*, *Enterobacter* and *Pseudomonas*. Numerous researches had certified that bacteria from genrea *Bacillus* owned the ability of algae-lysing and heterotrophic nitrification (Kim et al. 2005). Whereas bacteria from *Pseudomonas* possessed the ability of phosphorus-collecting and participated in sewage treatment as their function of nitrification and denitrification (Li et al. 2015; Srinandan et al. 2011). In addition, *Bacillus* and *Pseudomonas* are two crucial genrea in heavy metals (HMs) treatment as their high adsorption and transformation ability for heavy metal ions, such as Co^{2+}, Ni^{2+} and Pb^{2+} (Giridhar Babu et al. 2013; Haroun et al. 2017). Meantime, *Burkholderia* and *Enterobacter* bacteria were reported to purify sewage and to promote organic matter degradation (Mcneely et al. 2009; Tiar et al. 2018), respectively. Therefore, the verified VBNC bacteria of the 4 genera potential had the functions of degrading organic matter, denitrification, phosphorus-collecting and algae-lysing, which had benefit in eutrophic water treatment. Although the definite function of the isolated bacteria remained limitation to the best of our knowledge, the role VBNC bacteria provide new insights for eutrophication ecosystems restoration.

Conclusion

In present study, the purification effect of the designed bioreactor was investigated by detection the major water quality parameters for 2 years. We found that, first of all, the bioreactor revealed high removal rates of TP, TN, Chl-a and COD, indicating the efficient purification ability of the volcanic soil containing bioreactor. Secondly, although stopping operation and high temperature in Summer affected purification effect, the impact could be minimized when the bioreactor was running. Thirdly, the bioreactor had a long service life, which meet the demand for long period treatment. Fourthly, Rpf could resuscitate the VBNC bacteria in eutrophication ecosystem, and these bacteria could potential participate in eutrophic water treatment. These results had benefit in new engineering technology innovation for aquatic ecosystems restoration, and provided new insights for water environment treatment by VBNC bacteria.

Ethical Approval

Not applicable.

Consent to Participate

Not applicable.

Consent to Publish

Not applicable.

Authors Contributions

Huiling Fu: Conceptualization, Methodology, Writing - original draft.

Linxian Ding: Conceptualization, Supervision, Writing-Review & Editing.

Jingyu Zhai: Methodology, Investigation, Formal analysis, Visualization.

Xuesong Wang: Conceptualization, Supervision, Writing-Review & Editing.

Funding

The study was supported by the Public Welfare Technology Application Research Project of Zhejiang Science and Technology Department (2017C33046).

Competing Interests

The authors declare that they have no competing interests.

Availability of data and materials

The datasets used or analyzed during this study are available from the corresponding author on reasonable request.

Acknowledgments

We gratefully acknowledge Prof. Akira Yokota for his kindly instruction on our research.

References

Ahlgren J, Tranvik L, Gogool A, Waldeback M, Markides K, Rydin E (2005): Depth attenuation of biogenic phosphorus compounds in lake sediment measured by 31PNMR. Environ Sci Technol 39, 867e872.

Andersen IM, Williamson TJ, Gonzalez MJ, Vanni MJ (2020): Nitrate, ammonium, and phosphorus drive seasonal nutrient limitation of chlorophytes, cyanobacteria, and diatoms in a hyper-eutrophic reservoir. Limnol Oceanogr 65(5), 962-978.

Banerjee S (2016): Network analysis reveals functional redundancy and keystone taxa amongst bacterial and fungal communities during organic matter decomposition in an arable soil. Soil Biol Biochem 97, 188-198.

Beaulieu M, Pick F, Gregory-Eaves I (2013): Nutrients and water temperature are significant predictors of cyanobacterial biomass in a 1147 lakes data set. Limnol Oceanogr 58(5), 1736-1746.

Caballero M, Vazquez G (2020): Mixing patterns and deep chlorophyll a maxima in an eutrophic tropical lake in western Mexico. Hydrobiologia doi.org/10.1007/s10750-020-04367-y.

Carlson RE (1977): A trophic state index for lakes1 Limnology and oceanography. Limnol Oceanogr 22, 361-369.

Chao Rodriguez Y, el Anjoumi A, Dominguez Gomez JA, Rodriguez Perez D, Rico E (2014): Using Landsat image time series to study a small water body in Northern

Spain. Environ Monit Assess 186, 3511-3522.

Ding LX, Hirose T, Yokota A (2009): Four novel Arthrobacter species isolated from filtration substrate. Int J Syst Evol Micr 59, 856-862.

Ding LX, Su XM, Yokota A (2011): Research progress of VBNC bacteria. Acta Microbiologica Sinica 51(7), 858-862.

Ding LX, Zhang PH, Hong HC, Lin HJ, Yokota A (2012): Cloning and expression of Micrococcus luteus IAM 14879 Rpf and its role in the recovery of the VBNC state in *Rhodococcus* sp. DS471. Acta Microbiologica Sinica 52(1), 77-82.

Ding S, Chen M, Gong M, Fan X, Qin B, Xu H, Gao S, Jin Z, Tsang DCW, Zhang C (2018): Internal phosphorus loading from sediments causes seasonal nitrogen limitation for harmful algal blooms. Sci Total Environ 625, 872-884.

Domangue RJ, Mortazavi B (2018): Nitrate reduction pathways in the presence of excess nitrogen in a shallow eutrophic estuary. Environ Pollut 238, 599e606.

Felsenstein J (1985): Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39, 783-791.

Feuchtmayr H, Moran R, Hatton K, Connor L, Heyes T, Moss B, Harvey I, Atkinson D (2009): Global warming and eutrophication: effects on water chemistry and autotrophic communities in experimental hypertrophic shallow lake mesocosms. J Appl Ecol 46, 713e723.

Fosso-Kankeu E, Mulaba-Bafubiandi AF (2014): Implication of plants and microbial metalloproteins in the bioremediation of polluted waters: a review. Phys Chem

507 Earth 67-69, 242-252.
508 Freeman EC, Creed IF, Jones B, Bergström AK (2020): Global changes may be
509 promoting a rise in select cyanobacteria in nutrient-poor northern lakes. Global
510 Change Biol 26, 4966-4987.
511 Geisler E, Bogler A, Bar-Zeev E, Rahav E (2020): Heterotrophic Nitrogen Fixation at
512 the Hyper-Eutrophic Qishon River and Estuary System. Front Microbio 11, 1370.
513 Giridhar Babu A, Kim JD, Oh BT (2013): Enhancement of heavy metal
514 phytoremediation by Alnus firma with endophytic *Bacillus thuringiensis* GDB-1. J
515 Hazard Mater 250-251, 477-483.
516 Gruber N, Galloway JN (2008): An Earth-system perspective of the global nitrogen
517 cycle. Nature 451, 293e296.
518 Hampel JJ, McCarthy MJ, Gardner WS, Zhang L, Xu H, Zhu G, Newell SE (2018):
519 Nitrification and ammonium dynamics in Taihu lake, China: seasonal competition
520 for ammonium between nitrifiers and cyanobacteria. Biogeoscience. 15(3),
521 733-748.
522 Haroun AA, Kamaluddeen KK, Alhaji I, Magaji Y, Oaikhena EE (2017): Evaluation of
523 Heavy Metal Tolerance Level (MIC) and Bioremediation Potentials of
524 *Pseudomonas aeruginosa* Isolated from Makera-Kakuri Industrial Drain in Kaduna,
525 Nigeria. Eur Exp Biol 7(5), 28.
526 Hua MH, Ao YS, Yang XE, Li TQ (2008) Treating eutrophic water for nutrient
527 reduction using an aquatic macrophyte (*Ipomoea aquatica* Forsskal) in a deep flow

technique system. Agr Water Manage 95(5), 607-615.

Hua MH, Yuan JH, Yang XE, He ZL (2010) Effects of temperature on purification of eutrophic water by floating eco-island system. Acta Ecologica Sinica 30, 310-318.

Huisman J, Codd GA, Paerl HW, Ibelings BW, Verspagen JMH, Visser PM (2018): Cyanobacterial blooms. Nat Rev Microbiol 16(8), 471-483.

Jenny JP, Anneville O, Arnaud F, Baulaz Y, Bouffard D, Domaizon I, Bocaniov SA, Chèvre N, Dittrich M, Dorioz JM, Dunlop ES, Dur G, Guillard J, Guinaldo T, Jacquet S, Jamoneau A, Jawed Z, Jeppesen E, Krantzberg G, Lenters J, Leoni B, Meybeck M, Nava V, Nõges T, Nõges P, Patelli M, Pebbles V, Perga ME, Rasconi S, Ruetz CR, Rudstam L, Salmaso N, Sapna S, Straile D, Tammeorg O, Twiss MR, Uzarski DG, Ventelä AM, Vincent WF, Wilhelm SW, Wängberg SA, Weyhenmeyer GA (2020): Scientists' warning to humanity: rapid degradation of the world's large lakes. J Great Lakes Res 46(4), 686-702.

Jin Y, Gan G, Yu X, Wu D, Zhang L, Yang N, Hu J, Liu Z, Zhang L, Hong H, Yan X, Liang Y, Ding L, Pan Y (2017): Isolation of Viable but Non-culturable Bacteria from Printing and Dyeing Wastewater Bioreactor Based on Resuscitation Promoting Factor. Curr Microbiol 74(7), 787-797.

Kim JK, Park KJ, Cho KS, Nam SW, Park TJ, Bajpai R (2005): Aerobic nitrification–denitrification by heterotrophic *Bacillus* strains. Bioresour Technol 96(17), 1897-1906.

Kim K, Mun H, Shin H, Park S, Yu C, Lee J, Yoon Y, Chung H, Yun H, Lee K, Jeong

549 G, Oh JA, Lee I, Lee H, Kang T, Ryu HS, Park J, Shin Y, Rhew D (2020):
550 Nitrogen stimulates microcystis-dominated blooms more than phosphorus in river
551 conditions that favor non-nitrogen-fixing genera. Environ Sci Technol 54 (12),
552 7185-7193.
553 Kimura M (1980): A simple method for estimating evolutionary rates of base
554 substitutions through comparative studies of nucleotide sequences. J mol Evol
555 16(2), 111-120.
556 Kosten S, Huszar VLM, Becares E, Costa LS, van Donk E, Hansson LA, Jeppesenk E,
557 Kruk C, Lacerot G, Mazzeo N, De Meester L, Moss B, Lurling M, Noges T, Romo
558 S, Scheffer M (2012): Warmer climates boost cyanobacterial dominance in shallow
559 lakes. Global Change Biol 18(1), 118-126.
560 Le C, Zha Y, Li Y, Sun D, Lu H, Yin B (2010): Eutrophication of lake waters in China:
561 cost, causes, and control. Environ Manage 45, 662-668.
562 Li C, Yang J, Wang X, Wang E, Li B, He R, Yuan H (2015): Removal of nitrogen by
563 heterotrophic nitrification-aerobic denitrification of a phosphate accumulating
564 bacterium *Pseudomonas stutzeri* YG-24. Bioresour Technol 182, 18-25.
565 Li M, Wu YJ, Yu ZL, Sheng GP, Yu HQ (2009): Enhanced nitrogen and phosphorus
566 removal from eutrophic lake water by *Ipomoea aquatica* with low-energy ion
567 implantation. Water Res 43, 1247-1256.
568 Li SH, Jin Y, Cheng J, Park DJ, Kim CJ, Hozzein WN, Wadaan MAM, Shu WS, Ding
569 LX, Li WJ (2014): *Gordonia jinhuaensis* sp. nov. a novel actinobacterium, isolated

570 from a vbnc (viable but non-culturable) state in pharmaceutical wastewater. Anton
571 Leeuw 106(2), 347-56.
572 Liang X, Zhu S, Ye R, Guo R, Zhu C, Fu C, Tian G, Chen Y (2014): Biological
573 thresholds of nitrogen and phosphorus in a typical urban river system of the Yangtz
574 delta, China. Environ Pollut 192, 251-258.
575 Liu X, Huang SL, Tang T, Liu XG, Scholz M (2012): Growth characteristics and
576 nutrient removal capability of plants in subsurface vertical flow constructed
577 wetlands. Ecol Eng 44, 189-198.
578 Lu H, Yuan Y, Campbell DE, Qin P, Cui L (2014): Integrated water quality, emergy
579 and economic evaluation of three bioremediation treatment systems for eutrophic
580 water. Ecol Eng 69, 244-254.
581 Lurling M, Mucci M (2020): Mitigating eutrophication nuisance: in-lake measures are
582 becoming inevitable in eutrophic waters in the Netherlands. Hydrobiologia
583 doi:10.1007/s10750-020-04297-9.
584 McCrady MH (1915): The numerical interpretation of fermentation-tube results. J Infect
585 Dis 17, 183-212.
586 Mcneely D, Moore JE, Elborn JS, Millar BC, Dooley JSG (2009): Isolation of
587 *Burkholderia cenocepacia* and *Burkholderia vietnamiensis* from human sewage.
588 Int J Environ Heal R 19(2), 157-162.
589 Mukamolova GV, Turapov OA, Kazarian K, Telkov M, Kaprelyants AS, Kell DB,
590 Young M (2002): The rpf gene of *Micrococcus luteus* encodes an essential secreted

591 growth factor. MolMicrobiol 46(3), 611-621.
592 Paerl HW, Gardner WS, Havens KE, Joyner AR, McCarthy MJ, Newell SE, Qin B,
593 Scott JT (2016): Mitigating cyanobacterial harmful algal blooms in aquatic
594 ecosystems impacted by climate change and anthropogenic nutrients. Harmful
595 Algae 54, 213-222.
596 Paerl HW, Havens KE, Xu H, Zhu G, McCarthy MJ, Newell SE, Scott JT, Hall NS,
597 Otten TG, Qin B (2019): Mitigating eutrophication and toxic cyanobacterial
598 blooms in large lakes: the evolution of a dual nutrient (N and P) reduction
599 paradigm. Hydrobiologia doi:10.1007/s10750-019-04087-y.
600 Petersen JK, Hasler B, Timmermann K, Nielsen P, Torring DB, Larsen MM, Holmer M
601 (2014): Mussels as a tool for mitigation of nutrients in the marine environment.
602 Mar Pollut Bull 82(1-2), 137-143.
603 Piccolroaz S, Woolway RI, Merchant CJ (2020): Global reconstruction of twentieth
604 century lake surface water temperature reveals different warming trends depending
605 on the climatic zone. Clim Change 160(3), 427-442.
606 Qin B, Zhou J, Elser JJ, Gardner WS, Deng J, Brookes JD (2020): Water depth
607 underpins the relative roles and fates of nitrogen and phosphorus in lakes. Environ
608 Sci Technol 54(6), 3191-3198.
609 Rigosi A. Carey CC, Ibelings BW, Brookes JD (2014): The interaction between climate
610 warming and eutrophication to promote cyanobacteria is dependent on trophic state
611 and varies among taxa. Limnol Oceanogr 59(1), 99-114.

612 Saitou N, Nei M (1987): The neighbor-joining method: a new method for reconstructing
613 phylogenetic trees. Mol Biol Evol 4(4), 406-425.
614 Schauser I, Lewandowski J, Hupfer M (2003): Decision support for the selection of an
615 appropriate in-lake measure to influence the phosphorus retention in sediments.
616 Water Res 37(4), 801-12.
617 Serpaggi V, Remize F, Recorbet G, Gaudot-Dumas E, Sequeira-Le Grand A, Alexandre
618 H (2012): Characterization of the "viable but nonculturable" (VBNC) state in the
619 wine spoilage yeast Brettanomyces. Food Microbiol 30(2), 438-447.
620 Smith VH (2003): Eutrophication of freshwater and coastal marine ecosystems a global
621 problem. Environ Sci Pollut R 10, 126-139.
622 Smith VH, Schindler DW (2009): Eutrophication science: where do we go from here?
623 Trends Ecol Evol 24, 201-207.
624 Srinandan CS, Shah M, Patel B, Nerurkar AS (2011): Assessment of denitrifying
625 bacterial composition in activated sludge. Bioresour Technol 102, 9481-9489.
626 Stoliker DL, Repert DA, Smith RL, Song B, LeBlanc DR, McCobb TD, Conaway CH,
627 Hyun SP, Koh DC, Moon HS (2016): Hydrologic controls on nitrogen cycling
628 processes and functional gene abundance in sediments of a groundwater
629 flow-through lake. Environ Sci Technol 50, 3649e3657.
630 Su X, Liu Y, Hu J, Ding L, Shen C (2014): Optimization of protein production by
631 *Micrococcus luteus* for exploring pollutant-degrading uncultured bacteria.
632 SpringerPlus 3(1), 117.

633 Tiar A, Askarne L, Ait addi E, Assabbane A, Boubaker H (2018): Decolorization of the
634 azo dye methyl red by an isolated bacterium *Enterobacter hormaechei* strain
635 CUIZ1. J Mater Environ Sci 9(10), 2822-2830.
636 Tomaskoa D, Aldersonb M, Burnesc R, Heckerd J, Iadevaiad N, Leveroneb J,
637 Raulersone G, Sherwood E (2020): The effects of Hurricane Irma on seagrass
638 meadows in previously eutrophic estuaries in Southwest Florida (USA). Mar Pollut
639 Bull 156, 111247.
640 Tong Y, Xu X, Zhang S, Shi L, Zhang X, Wang M, Qi M, Chen C, Wen Y, Zhao Y,
641 Zhang W, Lu X (2019): Establishment of season-specific nutrient thresholds and
642 analyses of the effects of nutrient management in eutrophic lakes through statistical
643 machine learning. J Hydrol 578, 124079.
644 UN-Water Paris (2018): The United Nations World Water Development Report 2018:
645 Nature-Based Solutions for Water. UNESCO.
646 Vollenweider R, Kerekes J (1982): In: Eutrophication of Waters. Monitoring,
647 Assessment and Control. Organization for Economic Co-Operation and
648 Development (OECD), Paris p. 156.
649 Walpersdorf E, Neumann T, Stüben D (2004): Efficiency of natural calcite precipitation
650 compared to lake marl application used for water quality improvement in an
651 eutrophic lake. Appl Geochem 19(11), 1687-1698.
652 Wang J, Fu Z, Qiao H, Liu F (2019a): Assessment of eutrophication and water quality
653 in the estuarine area of Lake Wuli, Lake Taihu, China. Sci Total Environ 650,

654 1392-1402.
655 Wang M, Xu X, Wu Z, Zhang X, Sun P, Wen Y, Wang Z, Lu X, Zhang W, Wang X,
656 Tong Y (2019b): Seasonal pattern of nutrient limitation in a eutrophic lake and
657 quantitative analysis of the impacts from internal nutrient cycling. Environ Sci
658 Technol 53(23), 13675-13686.
659 Wauer G, Gonsiorczyk T, Kretschmer K, Casper P, Koschel R (2005): Sediment
660 treatment with a nitrate-storing compound to reduce phosphorus release. Water Res
661 39(2-3), 494-500.
662 Woolway RI, Merchant CJ (2019): Worldwide alteration of lake mixing regimes in
663 response to climate change. Nat Geosci 12(4), 271-276.
664 Wu HL, Huo YZ, Hu M, Wei ZL, He PM (2015): Eutrophication assessment and
665 bioremediation strategy using seaweeds co-cultured with aquatic animals in an
666 enclosed bay in China. Mar Pollut Bull 95(1), 342-349.
667 Wu Z, Liu Y, Liang Z, Wu S, Guo H (2017): Internal cycling, not external loading,
668 decides the nutrient limitation in eutrophic lake: a dynamic model with temporal
669 Bayesian hierarchical inference. Water Res 116, 231-240.
670 Xu D, Gao Z, Zhang X, Qi Z, Meng C, Zhuang Z, Ye N (2011): Evaluation of the
671 potential role of the macroalga *Laminaria japonica* for alleviating coastal
672 eutrophication. Bioresour Technol 102, 9912-9918.
673 Xu H, Paerl HW, Qin B, Zhu G, Gao G (2010): Nitrogen and phosphorus inputs control
674 phytoplankton growth in eutrophic Lake Taihu, China. Limnol Oceanogr 55(1),

675 420-432.

676 Xu H, Paerl HW, Qin B, Zhu G, Hall NS, Wu Y (2015): Determining critical nutrient
677 thresholds needed to control harmful cyanobacterial blooms in eutrophic Lake
678 Taihu, China. Environ Sci Technol 49(2), 1051-1059.

679 Yu XY, Zhang L, Ren B, Yang N, Liu M, Liu XT, Zhang LX, Ding LX (2015):
680 *Arthrobacter liuii* sp nov., resuscitated from Xinjiang desert soil. Int J Syst Evol
681 Micr 65, 896-901.

682 Zhao F, Xi S, Yang X, Yang W, Li J, Gu B, He Z (2012): Purifying eutrophic river
683 waters with integrated floating island systems. Ecol Eng 40, 53-60.

684 Zhou XH, Wang GX (2010): Nutrient concentration variations during *Oenanthe*
685 *javanica* growth and decay in the ecological floating bed system. J Environ Sci
686 22(11):1710-1717.

687 Zuo SP, Wan K, Ma SM (2014): Environmental restoration effects of *Ranunculus*
688 *sceleratus* L. in a eutrophic sewage system. Biochem Syst Ecol 55, 34-40.

689

Figures

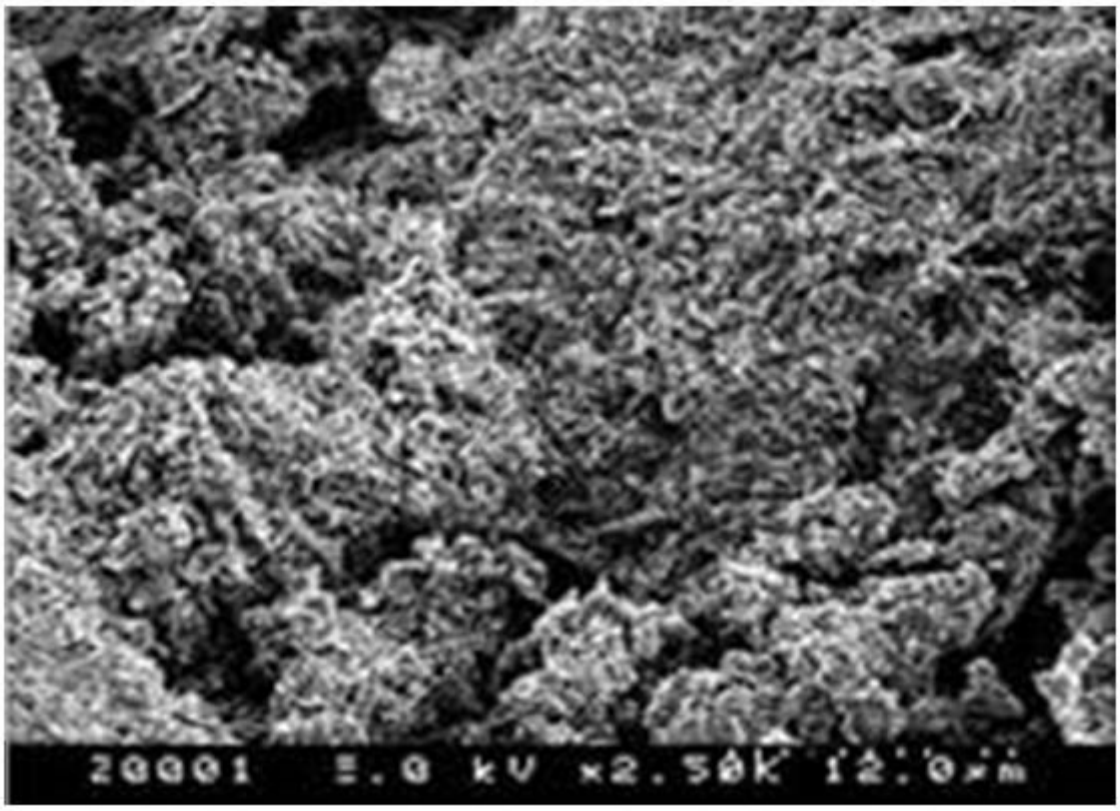


Figure 1

Scanning electron microscope (SEM) image of new adsorption material

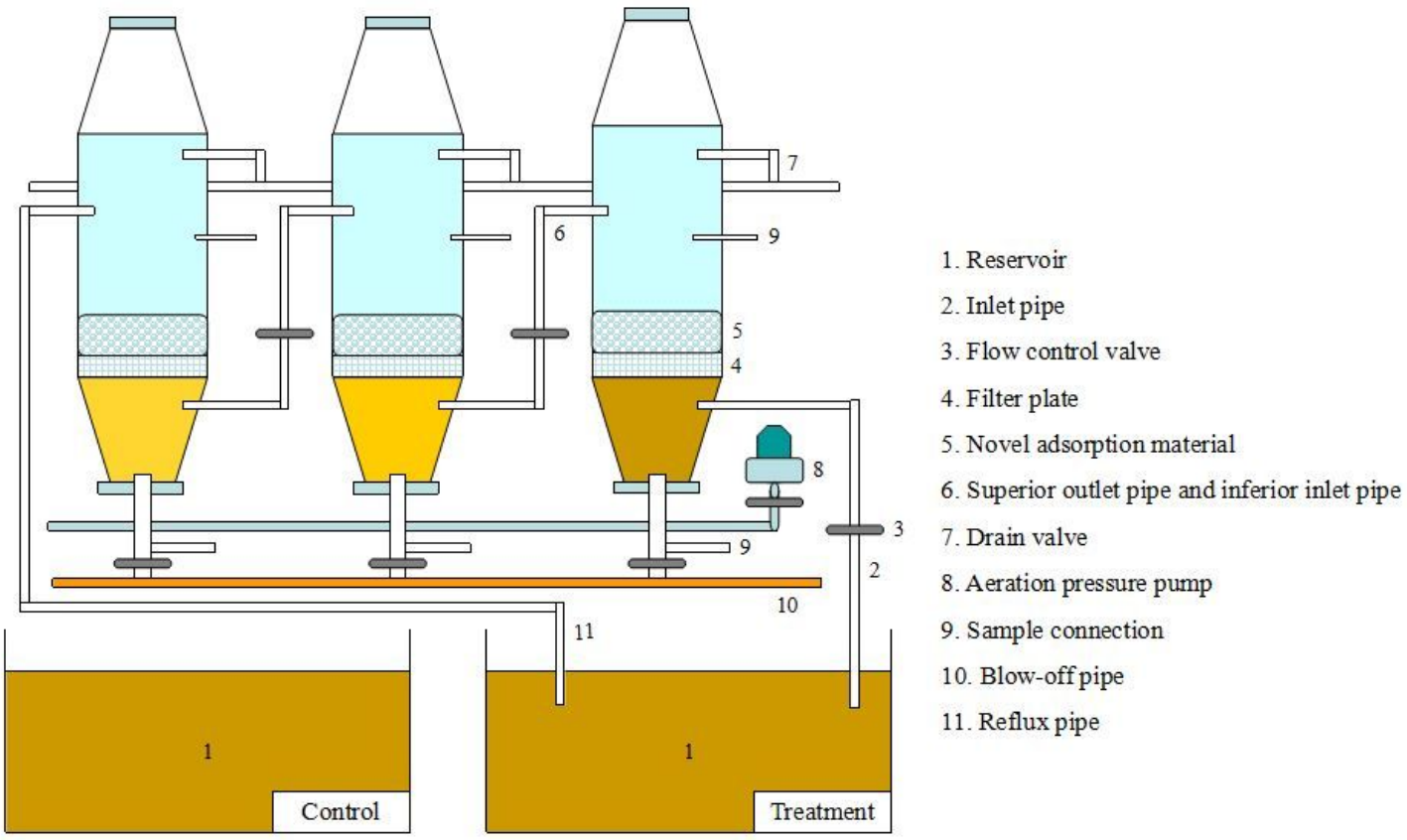


Figure 2

Design diagram of the bioreactor

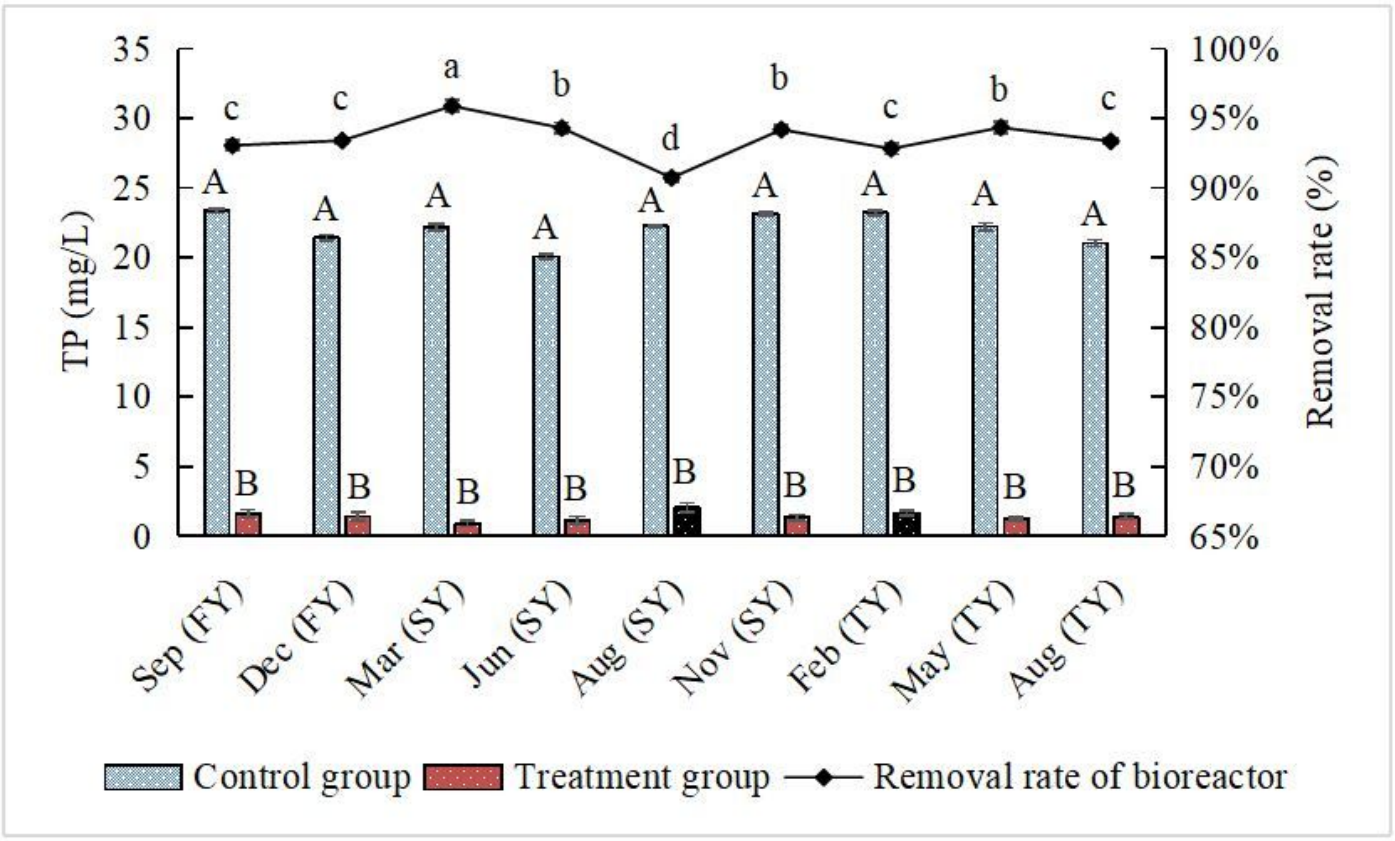


Figure 3

The TP concentrations in control and treatment group as well as the TP removal rate of the bioreactor. Different small letters indicate significant differences at $p < 0.05$ level of LSD test of the removal rates under different detection months. Different capital letters indicate significant differences at $p < 0.05$ level between control and treatment groups at the same detection month. The black bar in treatment group represented the TP concentrations of water sample when the bioreactor was stopping.

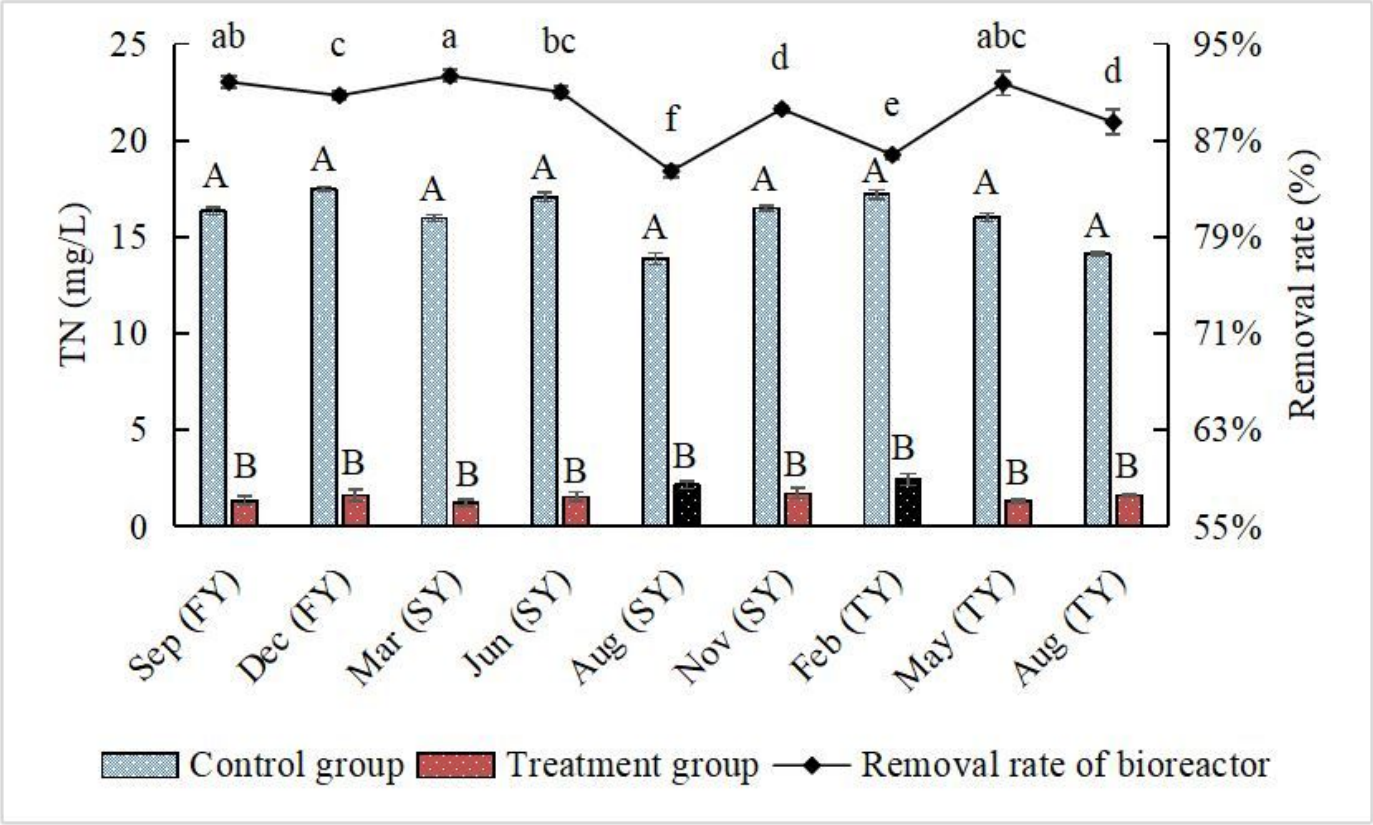
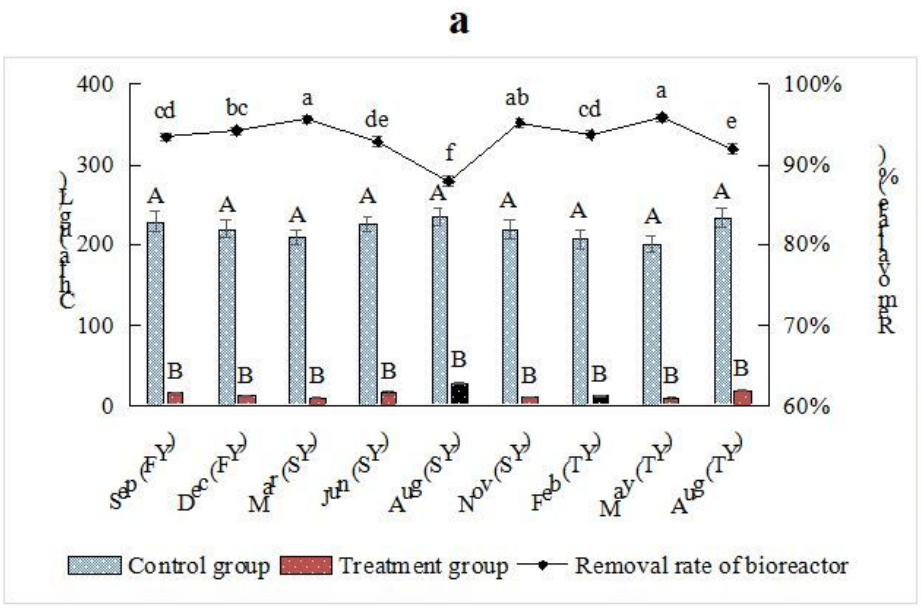


Figure 4

The TN concentrations in control and treatment group as well as the TN removal rate of the bioreactor. Different small letters indicate significant differences at $p < 0.05$ level of LSD test of the removal rates under different detection months. Different capital letters indicate significant differences at $p < 0.05$ level between control and treatment groups at the same detection month. The black bar in treatment group represented the TN concentrations of water sample when the bioreactor was stopping.



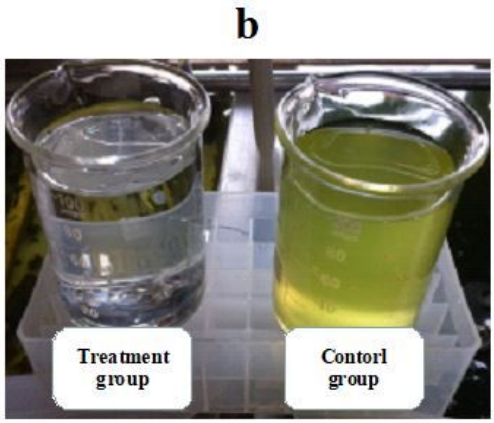


Figure 5

a) The Chl-a concentrations in control and treatment group as well as the Chl-a removal rate of the bioreactor; b) The real comparison between control and treatment group. Different small letters indicate significant differences at $p < 0.05$ level of LSD test of the removal rate under different detection months. Different capital letters indicate significant differences at $p < 0.05$ level between control and treatment groups at the same detection month. The black bar in treatment group represented the TN concentrations of water sample when the bioreactor was stopping.

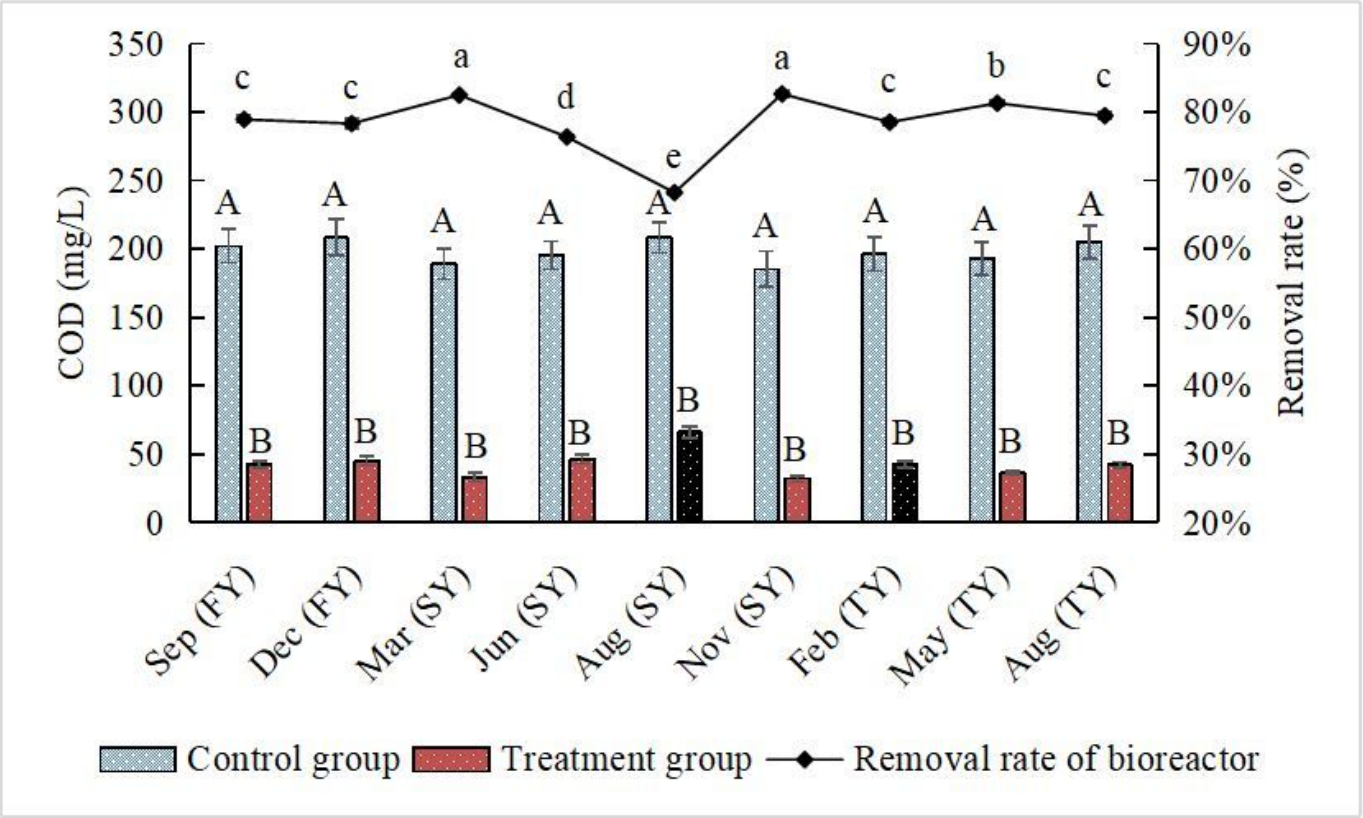


Figure 6

The COD concentrations in control and treatment group as well as the COD removal rate of the bioreactor. Different small letters indicate significant differences at $p < 0.05$ level of LSD test of the removal rate under different detection months. Different capital letters indicate significant differences at $p < 0.05$ level between control and treatment groups at the same detection month. The black bar in treatment group represented the TN concentrations of water sample when the bioreactor was stopping.

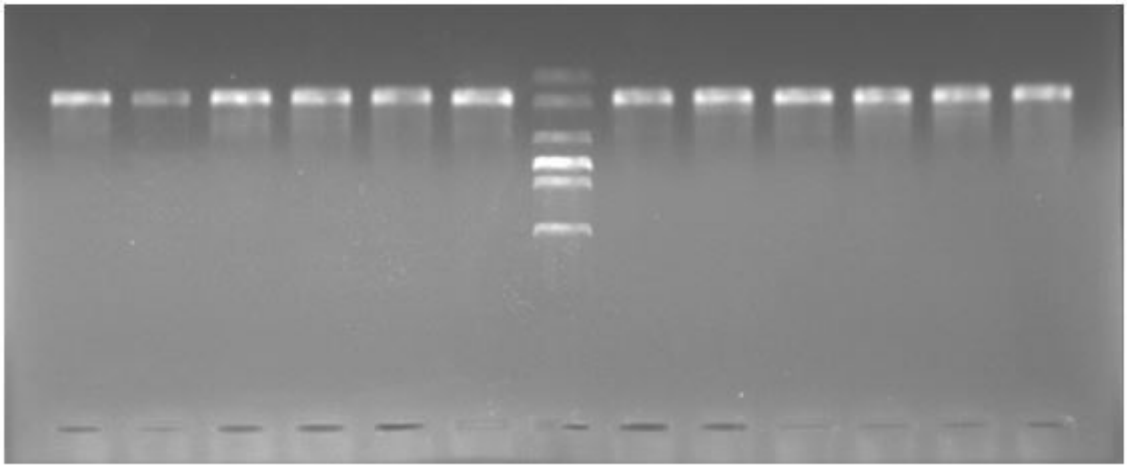


Figure 7

AGE analysis of the PCR products in MPN culture system of an experiment. The middle band was DNA Marker 2000; the left and right bands were the PCR products of dilution bacteria liquid from 10-1 to 10-6 with +Rpf and -Rpf treatments, respectively.

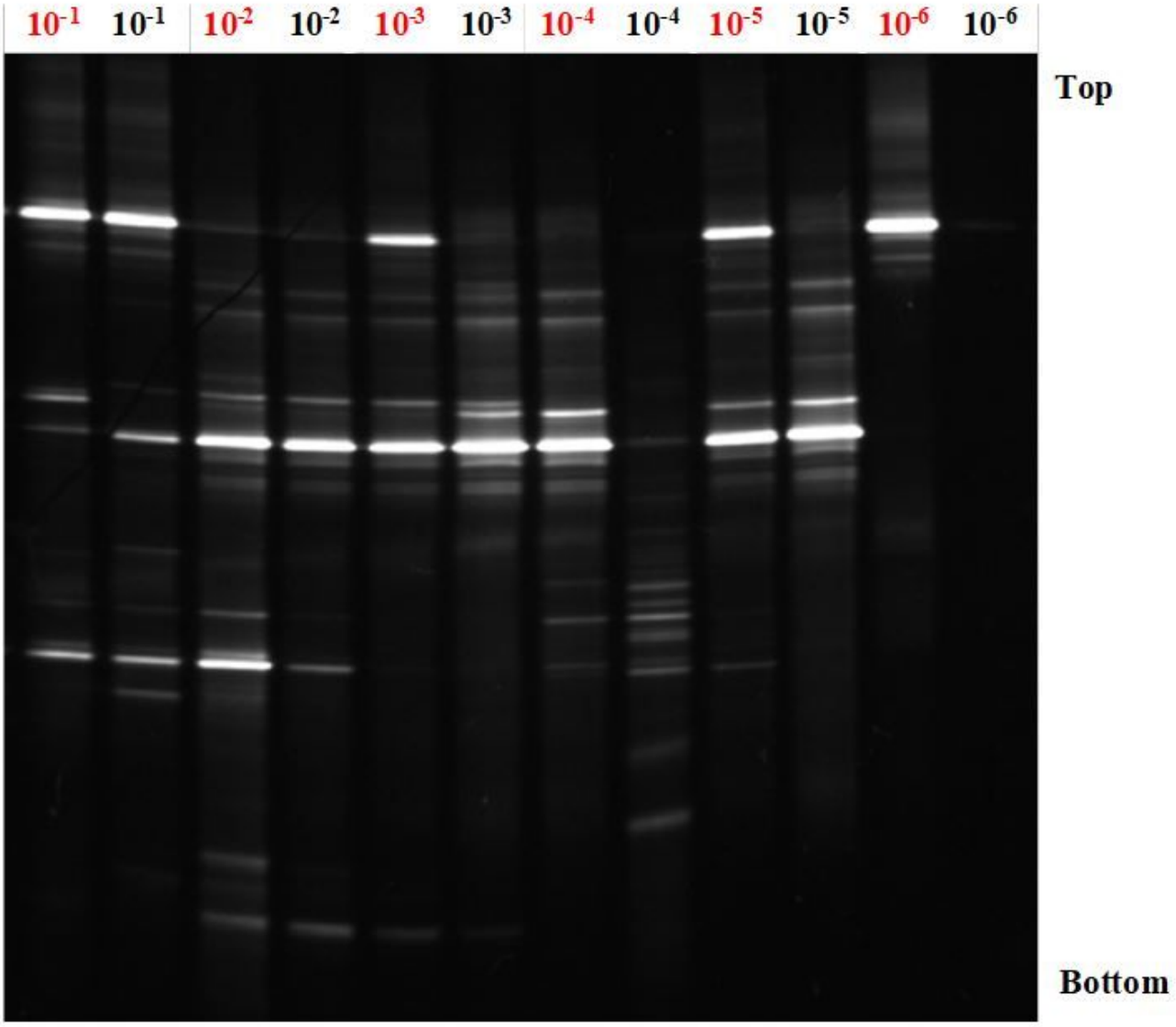


Figure 8

DGGE profiles of 16S rDNA V3 region PCR products (dilution bacteria liquid from10-1 to 10-6). The red 10-1 to 10-6 represented the dilution bacteria liquid from10-1 to 10-6 with adding +Rpf treatment; the black 10-1 to 10-6 represented the dilution bacteria liquid from10-1 to 10-6 with adding -Rpf treatment; the blue marked 1 to 10 represented the clear bands for DGGE analysis.

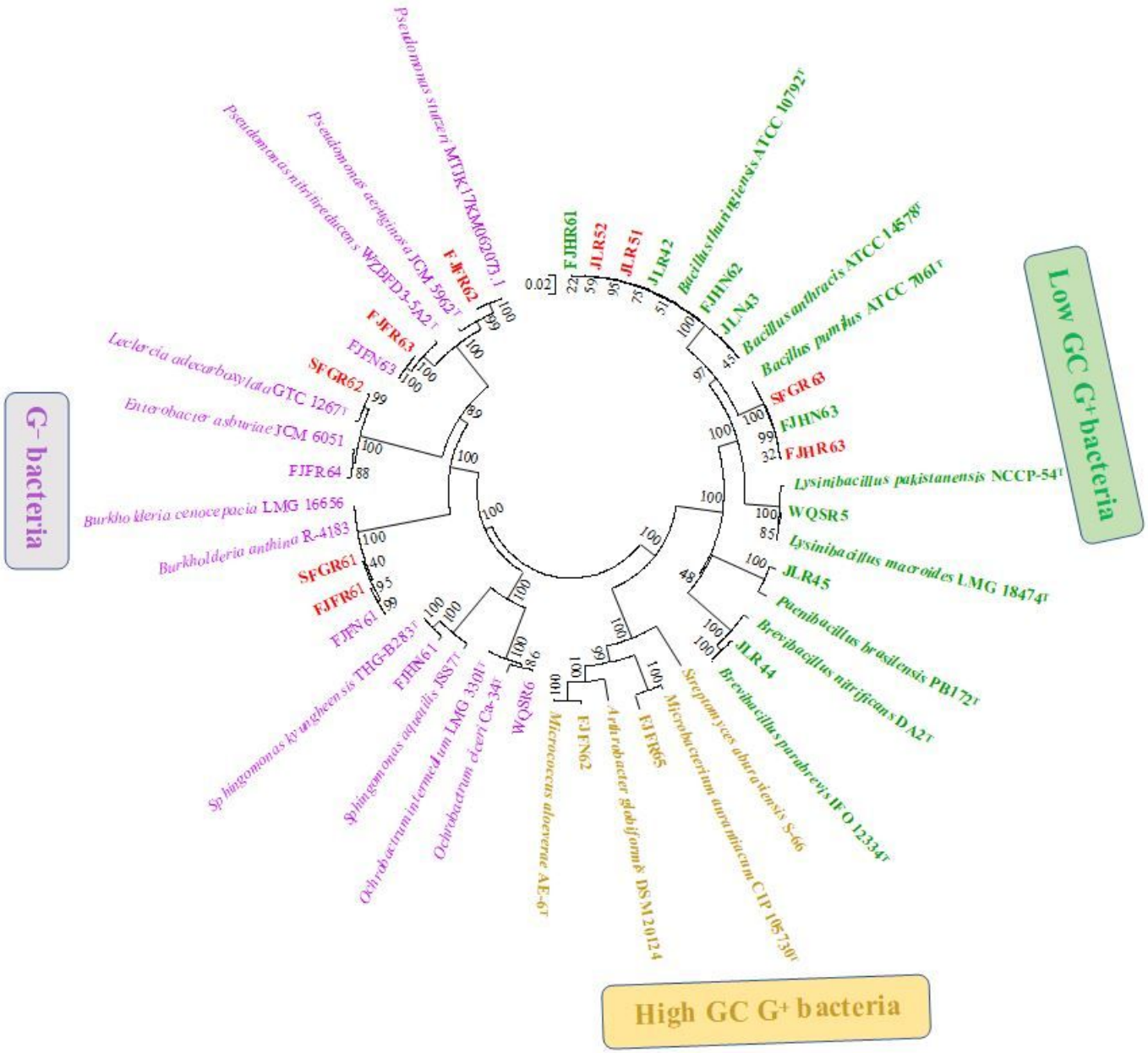


Figure 9

Phylogenetic tree based on the 16S rRNA gene sequences of isolated strains from the bioreactor. Bootstrap values above 60% were shown. Bar (0.02) substituted per nucleotide position. Low GC G+ bacteria represented low G + C gram-positive bacteria; High GC G+ bacteria represented high G + C gram-positive bacteria; G- bacteria represented gram-negative bacteria; the red marked strains belonged to VBNC bacteria.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementarymaterial.docx