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Title page

Enhanced treatment of shale gas fracturing waste fluid through plant – microbial synergism

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Abstract: Cost-efficient and environment-friendly treatment of hydraulic fracturing effluents is of great significance for the sustainable development of shale gas exploration. We investigated the synergistic effects of plant – microbial treatment of shale-gas fracturing waste fluid. The results showed that illumination wavelength and temperature are direct drivers for microbial activity which affected the removal effects of COD_{Cr} and BOD_5, while little effects were observed for nitrogen compounds, TDS, EC, SS and microbial species and composition. Plant-microbial synergism could significantly enhance the removal of pollutants compared with removal efficiency without plant enhancement. Additionally, the relative abundance and structure of microorganisms in the hydraulic fracturing effluents greatly varied with the illumination wavelength and temperature under plant-microbial synergism. 201.24 g Water Dropwort and 435 mg/L activated sludge with illumination of 450-495 nm (blue) at 25 ℃ was proved as the best treatment condition for shale-gas fracturing waste fluid samples, which showed the highest removal efficiency of pollutants and the lowest algal toxicity in treated hydraulic fracturing effluents. The microbial community composition (36.73% *Flavobacteriia*, 25.01% *Gammaproteobacteria*, 18.55% *Bacteroidia*, 9.3% *Alphaproteobacteria*, 4.1% *Cytophagia* and 2.83% *Clostridia*) was also significantly different from other treatments. The results provide a potential technical solution for improved treatment of shale gas hydraulic fracturing effluents.

Keywords: Shale gas; Hydraulic fracturing effluents; Plant-microbial synergism; Aquatic ecotoxicity; Microbial community.

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Authors' contributions

Mei He: Supervision; Project administration; Funding acquisition; Resources; Conceptualization; Methodology; Writing-Review & Editing.

Yan Lin: Writing-Review & Editing.

Bo Shao: Formal analysis; Investigation; Data Curation; Visualization; Writing-Original Draft.

Lei Tian: Resources and investigation.

Wen-Jie Chen, *Xu Tan* and *Ju-Long Li*: Investigation.

Highlights

- Combination of 201.24 g Water Dropwort and 435 mg/L activated sludge showed best treatment efficiency.
- Illumination and temperature important drivers for pollutant removal and microbial abundance.
- Blue light illumination at 25 ℃ was best conditions for enhancing treatment of FW.
- Plant-microbial synergism is proven to be an effective treatment method for FW.

Declarations

- *Ethics approval and consent to participate*

Not applicable.

- *Consent for publication*

Not applicable.

- *Availability of data and materials*

All data generated or analysed during this study are included in this published article and its supplementary information files.

- *Competing interests*

The authors declare that they have no competing interests.

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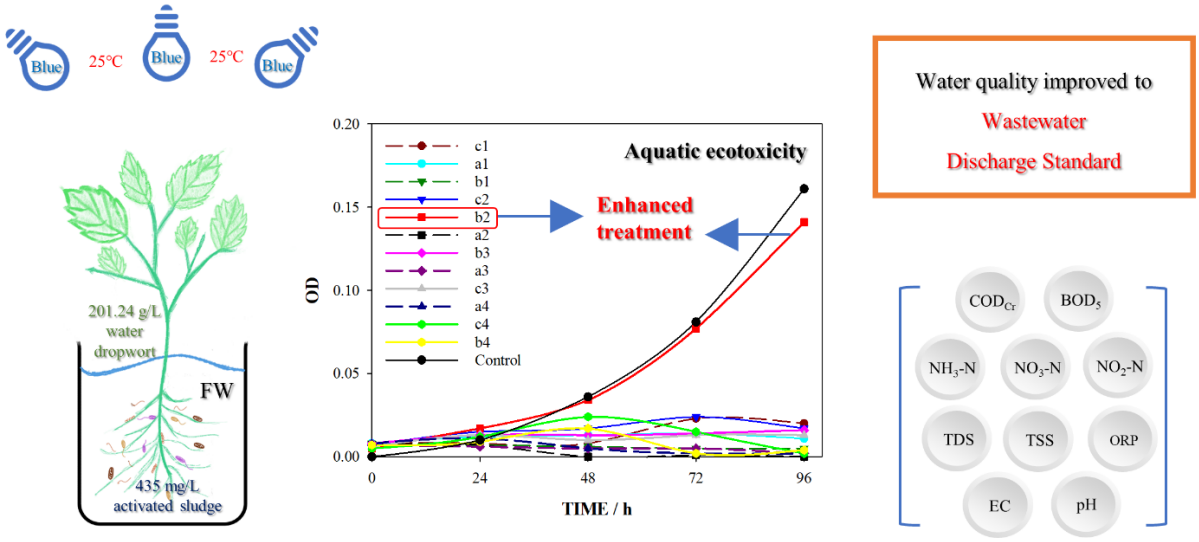
89 **Graphical abstract**



90

1. Introduction

The application of horizontal drilling and hydraulic fracturing techniques has made it possible for the cost-efficient extraction of shale gas. However, shale gas production process has also caused many environmental issues (Zhang and Yang, 2015), such as the large consumption of freshwater resources (Vandecasteele et al., 2015; Chen and Carter, 2016), the adverse impacts of regional water, air, and soil quality (Vidic et al., 2013; Chen et al., 2017; Gordalla et al., 2013; Rish and Pfau, 2017; Entrekin et al., 2011; Purvis et al., 2019; Vinciguerra et al., 2015), the increase of road traffic, waste management and noise impacts (Graham et al., 2015; Sun et al., 2019), and the adverse health impacts (Durant et al., 2016; Blewett et al., 2017; Casey et al., 2015; Stacy, 2017). Among these issues, water-related environmental issues in the shale gas production have aroused greater concerns. Compared to the extraction of conventional natural gas, high volumes of freshwater (1000~30,000 m^3/well/year) are required during hydraulic fracturing operations of shale gas (Chen and Carter, 2016) which subsequently lead to the production of a large amount of effluents (5~70% of the injected fluid) (Vandecasteele et al., 2015). These effluents can be distinguished as two types: flowback water (FW) from the fracturing stage and produced water (PW) from the gas production stage. The pollutants in the effluents are complex and ever-changing which include a variety of toxic chemicals (such as heavy metals, salts, soluble organic/inorganic compounds, etc.) from the formation and the additives of injected fluids (Lester et al., 2015). Thus, unqualified treatment and discharge of effluents can lead to irreversible environmental pollutions and then pose a high risk to human health.

The cost-efficient and environment-friendly dispose of hydraulic fracturing effluents is a major challenge for shale gas sustainable development. Deep well injection and partial treatment and reuse are the available conventional measures to minimize the environmental impacts caused by the effluents

in the shale gas industry (Torres et al., 2016). Physical pretreatments (filtration, pH adjustment, sedimentation and degreasing/deoiling), chemical precipitation methods and biological treatments are the common partial treatment options. Physical pretreatments can effectively remove total suspended solids and reduce the salinity of the effluents, but the treatment efficiency is limited and volatile pollutants may escape into the atmosphere during the treatment (Torres et al., 2016). Desalination technologies such as membrane separation/distillation (Cho et al., 2018), forward osmosis (Coday and Cath, 2014), mechanical vapor compression (Riley et al., 2016), electrocoagulation (Sardari et al., 2018; Lobo et al., 2016), advanced oxidation (Igunnu and Chen, 2014), and hybrid membrane bio-systems (Riley et al., 2016) are usually served to process these effluents for agricultural irrigation, livestock water and landscape water-use. Chemical precipitation methods, including coagulation, sedimentation, filtration lime softening water treatment processes, sodium hydroxide alkalization, and potassium permanganate oxidation, can effectively minimize the hardness, total organic carbon (TOC) and iron concentrations of the effluents (Lester et al., 2015; Mao et al., 2018; Torres et al., 2016), but these treatments are often expensive and the addition of chemicals may bring secondary pollutions. In the biological treatments, organic matter in the effluents could be removed effectively through aerobic degradation of activated sludge or lake water microbial consortia (Kekacs et al., 2015; Lester et al., 2013; He et al., 2019), but high total dissolved solids (TDS) concentrations of the effluents usually hinder microbial activity and thus affect treatment efficiency (Mao et al., 2018; Torres et al., 2016). Therefore, high-efficient and low-cost innovative treatments are required for the reuse or discharge of hydraulic fracturing effluents.

Plant-microbial synergism treatment has already been considered as a good option which takes the full advantages of plant and microbes' capability and presents good potential for a low-cost solution for

135 disposing these effluents in our previous studies (He et al., 2019). In the plant-microbial synergism
136 treatment, water dropwort and activated sludge synergism presented high treatment efficiency of COD
137 (39.5~51.4% reduction), TN (62.9~78.0% reduction), TP (4.4~96.5% reduction), and significantly
138 increased the Shannon-Winner index, improved microbial community structure and reduced the aquatic
139 ecotoxicity of these effluents (He et al., 2019). However, the treatment efficiency requires further
140 improvement. Microbial and plant biomass is a major factor affecting the treatment efficiency (Ncibi et
141 al., 2017; Su et al., 2012). Treatment conditions such as temperature and illumination are also usually
142 considered as important factors influencing the treatment efficiency of biological treatment.
143 Temperature can significantly affect biological enzyme activities and metabolic rates and hence the
144 treatment efficiency of microorganisms and plants (Çetin and Sürücü, 1990; Gillooly et al., 2001).
145 Previous reports have indicated that temperature caused differences in microbial number and
146 composition of activated sludge (Eikelboom, 2000). Illumination conditions can affect the treatment
147 efficiency of the plant-microbial synergism through the effects on photosynthetic efficiency of plants
148 (Li et al., 2010; Li et al., 2016). Blue light illumination promotes vegetative growth through strong root
149 growth and intense photosynthesis, while red light illumination promotes stem growth, flowering and
150 fruit production (Ma et al., 2015; Xu et al., 2012; Wu et al., 2018). In this study, effects of biological
151 effect and environmental condition (illumination, temperature) on water dropwort-activated sludge
152 synergism treatment of shale-gas fracturing waste fluid were investigated to identify the optimum
153 conditions, which can provide efficient biological treatment for shale-gas fracturing waste fluid.

154 **2. Materials and methods**

155 *2.1 Fracturing waste fluid collection*

The fracturing waste fluid samples (FW) were collected from Well 201-H1 in Changning Shale Gas Mining Area (Sichuan, China) and transported to the laboratory under low temperature and dark conditions. All FW samples were stored in the dark at 4 ℃ and centrifuged for solid-liquid separation before treatments. Then the supernatant aliquot was used for the experiment.

2.2 Plant-microbial synergism treatment

Activated sludge and water dropwort were applied in the plant-microbial synergism treatment of FW samples. Activated sludge was collected from a domestic sewage treatment plant in Caidian District, Wuhan, China and subjected to overnight aeration operation before treatments. The mixed liquor suspended solids (MLSS) concentration of the activated sludge was measured following APHA Standard Methods for the Examination of Water and Wastewater (Rice et al., 2012). Water dropwort was collected from a vegetable production base in Caidian District, Wuhan, China and washed with ultrapure water before treatment. Approximate root volumes of water dropwort were considered to have the same biomass. The root volume of water dropwort was determined as the increased volume of water when the plant root was completely immersed in clean ultrapure water.

The effects of different biomasses of activated sludge and water dropwort, temperature and illumination on the treatment efficiency of the plant-microbial synergism treatment were explored. As shown in Table 1, different combinations of microbial MLSS concentration, plant biomass, temperature and illumination wavelength were conducted to identify the optimum conditions for the treatment of FW samples. 1 L FW was transferred into a 5 L glass container for a 12-day treatment and all the illuminations were adjusted to the same illumination intensity with different number of illumination lights in all the treatments. Each treatment was conducted in triplicate. Water quality indicators, algal

177 toxicity and microbial community diversity and structure were analyzed before and after these
178 treatments to evaluate their treatment effects.

179 *2.3 Water quality parameters analysis of FW*

180 Nitrogen-containing compounds, organic matters and other five indicators (TDS, pH, ORP, EC,
181 TSS) were used for evaluating the treatment effects of different treatments. TDS, pH, electrical
182 conductivity (EC), redox potential (ORP) were measured in HACH HQ30d portable meter with
183 corresponding IntelliCAL^{TM} electrode, and biochemical oxygen demand (BOD_5) was measured in
184 HACH HQ30d dissolved oxygen meters through the changes in the dissolved oxygen concentration in
185 a 5-day duration. Nitrogen compounds concentrations (NH_4-N, NO_2-N, NO_3-N) and chemical oxygen
186 demand (COD_{Cr}) of FW were analyzed in the HACH DR2800 Spectrophotometer following the
187 measuring procedures for water or wastewater analysis (HACH Company, 2007). The determination of
188 total suspended solids (TSS) was similarly measured as MLSS following APHA Standard Methods for
189 the Examination of Water and Wastewater (Rice et al., 2012).

190 *2.4 Aquatic ecotoxicity determination of FW*

191 Algal aquatic ecotoxicity evaluation is carried out based on OECD method: Freshwater Alga and
192 Cyanobacteria, Growth Inhibition Test (OECD, 2011), which can effectively assess the overall and
193 integrative environmental impact of multiple aquatic pollutants. In this study, a unicellular green algae
194 *Scenedesmus obliquus* was used to analyze the algal aquatic ecotoxicity of FW before and after the
195 treatments according to the influences of FW on growth and reproduction of algal within 96 h. The
196 algal aquatic ecotoxicity were determined according to the OECD Test Guideline 201 (OECD, 2011).
197 Briefly, algae *Scenedesmus obliquus* were first activated and pre-cultured to the exponential growth

198 phase in an algal medium described in our previous study (He et al., 2019). Then, the test and control
199 flasks were prepared and compared for aquatic ecotoxicity determination. In the control flasks, sterile
200 distilled water was used to prepare the algal medium and the algae in logarithmic growth was then
201 transferred to 100 mL of prepared medium for further cultivation in a light incubator of 20 ± 2 ℃ with
202 an initial algal cell concentration of 10^4 cells/mL. The preparation of the test flasks was the same as
203 control, using FW sample instead of distilled water (He et al., 2019). Finally, the optical density (OD)
204 of the algae in the control and test flasks were measured at 0, 24, 48, 72, and 96 hours, respectively.
205 The algal ecotoxicity of FW was quantified through the reduction rate of OD in the control and test
206 flasks during the 96-h incubation. Each aquatic ecotoxicity analysis were conducted in triplicate.

207 *2.5 Determination of microbial diversity and structure in FW*

208 Microbial community diversity (Shannon index and Chao1 index) and structure of FW were
209 determined before and after treatment, based on microbial communities analyses through
210 high-throughput sequencing method at the Chengdu Institute of Biology, Chinese Academy of Sciences.
211 200 ml of FW sample in each treatment were 0.22 μm-filtered under aseptic condition for microbial
212 communities collecting. Subsequently, the MO BIO Power Soil DNA Extraction Kit (MO BIO
213 Laboratories, Carlsbad, CA, USA) was used to extract the genomic DNA of the FW sample. the purity
214 and concentration of the extracted DNA were then separated by agarose gel electrophoresis and
215 measured by NanoDrop Spectrophotometer. Qualified genomic DNA was used as a template,
216 Polymerase Chain Reaction (PCR) was performed on the V4 hypervariable region of the 16S rRNA
217 gene using specific primers 515F (5'-GTGCCAGCMGCCGCGGTAA-3') and 909R
218 (5'-CCCCGYCAATTCMTTTRAGT-3') with a 12-nt unique barcode (Caporaso et al., 2012; Caporaso
219 et al., 2011). Two PCR reactions were performed on each sample and the amplified products were

combined and then detected by 1% agarose gel electrophoresis. A SanPrep DNA gel extraction kit (Sangon Biotech, China, catalog number SK8132) was used to excise and purify the product bands, and then the concentration and quality were determined by Nanodrop. The purification amplified products were then analysed with the v2 sequencing kit (2×250 bp) through an Illumina Miseq system. QIIME Pipeline-Version 1.7.0 (http://qiime.org/) was used to process the obtained raw sequence data. Microbial diversity and structure in different treatments were analyzed according to the relative abundance of microorganisms based on the sequence data.

2.6 Statistical analysis

Statistical analysis was performed using SPSS 16.0, data drawing was performed using Sigmaplot 14.0, Principal component analysis (PCA) and heatmap of microbial communities was analyzed based on relative abundance data of bacterial 16S rRNA gene at class level in the different treatments using Origin 2020 and TBtools, respectively.

3. Results and Discussion

3.1 Water quality improvement

3.1.1 Organic compounds

Before water dropwort-activated sludge synergism treatment, COD_{Cr} and BOD_5 concentration of FW samples were 1323 and 7.36 mg/L, respectively. COD_{Cr} level was 2.65 times higher than the lowest effluent standard for petrochemical enterprises defined by the Integrated Wastewater Discharge Standard of China (MEP, 1996), but BOD_5 level did not exceed the maximum allowable emission concentration (MEP, 1996). However, the value of BOD_5/COD_{Cr} in FW samples as low as 0.0033 also

indicated that most of the organic compounds in FW sample such as macromolecules (surfactants, phenolics, et al.) (Lester et al., 2015) were difficult to be biodegraded and utilized.

The plant-microbial synergism showed more significant effects in the removal of organic pollutants than illumination and temperature conditions for treatment of FW samples. Removal of organic pollutants with COD_{Cr} and BOD_5 as indicators were presented in Fig. 1 and Fig. S1. Illumination wavelength and temperature directly affected the treatment effects of COD_{Cr} and BOD_5 in the FW samples. Without biological functions, the removal efficiency of COD_{Cr} and BOD_5 was 47.8% and 25.4% for natural illumination at 20 ℃ (a1), 61.5% and 32.1% for blue light illumination at 25 ℃ (b1), and 22.9% and 45.7% for red light illumination at 30 ℃ (c1). Biotreatment has played a major role in the COD_{Cr} and BOD_5 removal of FW samples. Plant-microbial synergism on BOD_5 removal efficiency was highly improved to 71.3~95.0%, while COD_{Cr} removal in some treatments was lower than that without plant-microbial effects, which indicated that plant-microbial synergism presented better performance in BOD_5 removal than COD_{Cr} removal. Under the effects of plant-microbial synergism, it presented a better treatment performance in COD_{Cr} removal under natural illumination at 20 ℃ (a2, a3, a4) and blue light illumination at 25 ℃ (b2, b3, b4), compared with red light illumination at 30 ℃ (c2, c3, c4). Previous reports have found that blue light illumination promoted the growth of plant roots and thus enhanced the treatment effects (Xu et al., 2012), but high temperatures (30 ℃) might inhibit plant growth and microbial metabolism and then reduced the biotreatment (Gillooly et al., 2001). However, no significant difference was observed for BOD_5 removal with different illumination wavelength and temperature conditions.

With comprehensive comparison of the removal efficiency of COD_{Cr} and BOD_5, treatment b2 showed the highest removal efficiency for COD_{Cr} (85.6%) and BOD_5 (94.4%), followed by treatment

a2 (COD_{Cr} and BOD_5 removal efficiency 71.2 % and 90.9%) and b3 (COD_{Cr} and BOD_5 removal efficiency 51.6% and 90.1%). After the treatment treatments, the COD_{Cr} and BOD_5 levels of the FW sample meet the effluent standard defined by the Integrated Wastewater Discharge Standard of China (MEP, 1996).

3.1.2 Nitrogenous compounds

NH_4-N concentration in FW samples before treatments was 9.81 mg/L, which was 1.23 times higher than the emission standard of pollutants for petroleum chemistry industry defined by China Ministry of Environmental Protection (MEP, 2015). Direct discharge of untreated FW wastewater containing high concentrations of ammonia may cause eutrophication and water quality deterioration of aquatic environments, and also present harmful effects to organisms in the environments. The biotransformation of high concentration of ammonia is beneficial for the reduction of its environmental hazards. The results showed that the NH_4-N levels were higher than NO_2-N (0.02 mg/L) and NO_3-N (1.93 mg/L) for untreated FW samples, indicating that native microbes in the FW samples had little effect on the biotransformation of these nitrogenous compounds.

As shown in Fig. 1 and Fig. S1, different illumination wavelength and treatment temperature conditions (treatments a1, b1, c1) didn't cause too much difference to the removal efficiency of nitrogenous compounds in FW samples. However, the plant-microbial synergism played a key role in removing the nitrogenous compounds of FW samples. Among these treatments, the treatment b2 showed the highest removal efficiency respectively for NH_4-N (93.1%), NO_2-N (77.7%), and NO_3-N (90.6%), followed by treatment b3 (with a removal efficiency of 88.7%, 33.3% and 57.9% for NH_4-N, NO_2-N and NO_3-N) and treatment a4 (with a removal efficiency of 74.5%, 11.1% and 52.2% for NH_4-N, NO_2-N and NO_3-N). The obtained results indicated that the increase in microbial concentration

was advantageous for removing nitrogen pollutants in FW samples under low treatment temperature and natural illumination, as reflected by the removal efficiency in the treatment a2, a3 and a4. However, the removal efficiency of nitrogen pollutants in FW samples didn't increase with the increase of microbial concentration under higher treatment temperature conditions (25 ℃ and 30 ℃) with blue light and red light illuminations.

After plant-microbial synergism treatment, the NH_4-N level of the treated FW sample meets the effluent standard defined by the Integrated Wastewater Discharge Standard of China (MEP, 1996) and the emission standard of pollutants for petroleum chemistry industry (MEP, 2015), significantly reducing the risk of eutrophication and deterioration of water quality.

3.1.3 Others water quality indicators

Five water quality indicators including pH, oxidation-reduction potential (ORP), total dissolved solids (TDS), electrical conductivity (EC) and total suspended solids (TSS) were determined after the treatment of the plant-microbial synergism. Different treatments showed significant differences in these tested water quality parameters. Fig. 1 and Fig. S1 showed the variations of the five water quality indicators in different treatments. Compared with the untreated FW sample with a pH of 7.39, a small increase in pH (7.6~8.4) was observed for FW samples after the treatments, except for the treatments under red light illumination at 30 ℃ (c2, c3 and c4). The pH of treatment c1 was lower than other treatments, probably attributed to the enhancement of the ionization in the FW samples due to the influence of higher temperature and red light illumination. Treatments c2, c3 and c4 further decreased the pH of FW samples than c1, which indicated that plant-microbial synergism presented a significant removal effect on negatively charged ions in the FW samples. The increase in acidity was not suitable for the survival and activities of effective microorganisms and plants, and thus directly affected the

306 treatment effects of other pollutants in the FW samples. The ORP of the treated FW wastewater (except
307 for the c2, c3, and c4 treatments) was higher than 0 mv indicated that the FW samples in these
308 treatments was in an oxidizing environment which was beneficial for the growth of aerobic
309 microorganisms thus increasing the degradation of pollutants. However, ORP of the FW samples after
310 c2, c3, and c4 treatments was lower than 0 mv, indicating a reducing environment against the treatment
311 of the pollutants.

312 The variation of illumination wavelength and treatment temperature showed little effect on the
313 TDS, EC and SS removal from the FW samples, without the role of plant and microbes. Comparatively,
314 a reduction of TDS (45.7~83.9%), EC (24.3~70.0%) and TSS (3.6~59.1%) was observed after the
315 plant-microbial synergism treatments. Treatment b2 presented the best performance in removing TDS
316 (83.9%) and EC (70.0%) from FW samples, followed by b3 and a3 (with a removal efficiency of 78.7%
317 and 72.0%, respectively). However, the removal efficiency of TSS in the FW sample followed the
318 pattern: treatment b2 > b3 > a2.

319 *3.2 Aquatic ecotoxicity of fracturing waste fluid after treatments*

320 Aquatic ecotoxicity of FW samples before and after treatments was compared based on the growth
321 reduction and reproduction impairment of green algae *Scenedesmus obliquus* in 96h. Fig. 2a shows the
322 algal ecotoxicity of FW samples after treated by different concentration of activated sludge. The results
323 showed that 435, 904 and 1339 mg/L activated sludge didn’t produce obvious difference on the algal
324 growth and reproduction inhibition rate of in a 96-h exposure, compared with the untreated FW
325 samples. The algal growth and reproduction were almost completely inhibited in the FW samples after
326 treated by different concentration of activated sludge.

As shown in the impacts of treated FW samples on the algal growth and reproduction curves (Fig. 2b), the treated FW samples still presented high inhibition effects on the algal growth and reproduction, except for the treatment b2. The ecotoxicity of FW sample was significantly reduced after b2 treatment, in which the growth and reproduction inhibition rate at 48, 72 and 96-h were 5.56%, 4.94% and 12.42%, respectively. Compared with the healthy algae in the control, the treated FW sample had a very small negative impacts on the growth and reproduction of these algae, showing a good treatment effects on the removal of pollutants in FW samples which was consistent with the results of the water quality indicators. However, the 96-hour algal growth and production inhibition rate of the treated FW samples in other treatments was ranged from 87.58% to 100%, which was still showing a high algal ecotoxicity.

3.3 Changes in the microbial community diversity and structure of FW

3.3.1 Variations of Microbial community diversity

Biodiversity indicators refers to the richness and uniformity of the organisms in a specific ecosystem. In this study, two alpha diversity indices (Shannon Index and Chao1 Index) were calculated to evaluate the variations of microbial community diversity and richness of FW samples after different treatments (Fig. 3). Before the treatments, the Shannon index and Chao 1 index significantly increased after the addition of 435, 904, 1339 mg/L activated sludge into FW in the treatments N2, N3, N4, compared to the untreated FW samples (treatment N1). The increases of the diversity and richness of the microbial community indicated that the addition of activated sludge provided a large amount of exogenic microorganisms into the plant-microbe synergism treatment system. After a 12-days treatment, the microbial species and populations showed a significant response to the changes in temperature,

illumination wavelength, addition of plants and then reached a new balance, as reflected by the decrease of the Shannon index and Chao1 index (compared to the treatments N1, N2, N3, N4) in different treatments.

As shown in Fig. 3, no significant difference was observed for the Shannon Index in the FW after treatments from the untreated FW samples. The Shannon Index of FW in some treatments was even lower than the untreated FW samples. However, the Chao1 Index showed significant difference between the treated and untreated FW samples. Compared to untreated FW samples, the Chao1 index in FW were significantly enhanced from 878.11 to 2115.77 after 12 days of plant-microbial synergism treatment, except the treatment b1 (with a Chao1 Index of 616.08). The low Chao1 Index in treatment b2 was consistent with the results of water quality improvement and algal ecotoxicity as describe before. Higher Chao1 Index of FW samples in treatment c2, c3 and c4 indicated that the plant-microbial synergism in red light illumination at 30 ℃ was beneficial for the microbial growth and reproduction so that the richness and biodiversity of the microbial community was highly improved. However, our results of water quality improvement and algae ecotoxicological effects showed that the highest biodiversity index was not observed in the treatment b2 which presented the best treatment performance in FW samples. Thus it can be seen that microbial community diversity and abundance were not the unique determinants for the treatment efficiency of FW samples. The microbial species and composition were also very important for the plant-microbial synergism treatment of FW samples, which has been reported in previous studies (He et al., 2019).

3.3.2 Species and composition of microbial community

Taxonomic composition distribution histograms and heatmap in each treatment were shown in Fig. 4 and Fig. S2, based on the relative abundance of the microbial community at the class level.

370 *Alphaproteobacteria* (45.61%), *Gammaproteobacteria* (23.35%), *Flavobacteriia* (16.89%) *and*
371 *Anaerolineae* (5.41%) were the dominant microbial species, followed by *Betaproteobacteria* (2.52%),
372 *Methanomicrobia* (1.27%), *Bacteroidia* (1.09%) *and Clostridia* (1.02%) in the untreated FW sample
373 (treatment N1). The microbial species and composition did not change in the FW sample, however, the
374 proportion and structure of the microorganisms greatly changed with the illumination wavelength and
375 temperature (treatments a1, b1, c1). As shown in Fig. 4, Gammaproteobacteria was the most dominant
376 species, followed by *Alphaproteobacteria, Flavobacteriia, Anaerolineae, Bacteroidia*.

377 After the addition of activated sludge (treatments N2, N3, N4), a large amount of new
378 microorganisms such as *Cytophagia, Nitrospira, Acidimicrobiia, Anaerolineae, Saprospirae, Mollicutes,*
379 *Gemm-1*, *Sphingobacteriia, Planctomycetia, Thermoleophilia and Deltaproteobacteria* were
380 introduced into the FW samples to make the microbial community structure in the treatments complex
381 and abundant. With the treatment of the plant-microbial synergism, the relative abundance of microbes
382 such as *Bacteroidia*, *Flavobacteriia*, *Gammaproteobacteria*, *Alphaproteobacteria*, *Cytophagia* and
383 *Clostridia* greatly changed (treatments a2, a3, a4, b2, b3, b4, c2, c3, c4). Microbial species and
384 composition in the treatments c2, c3 and c4 were different from other treatments. *Bacteroidia* was
385 found to be the main microbe in the treatments c2, c3 and c4, followed by *Gammaproteobacteria*，
386 *Alphaproteobacteria*，*Clostridia. Bacteroidia* (phylum: *Bacteroidetes*) and *Clostridia* (phylum:
387 *Firmicutes*) were found to be good at survival strategies such as producing endospores, using oxygen,
388 and using toxic halogenated organics as electron acceptors, tolerating high temperatures, and using
389 light for photosynthesis to live in the condition of red light illumination at 30 ℃(Mor and Kwon, 2015).
390 In contrast, a higher relative abundance of *Flavobacteriia* and *Gammaproteobacteria* but a lower
391 abundance of *Anaerolineae* and *Bacteroidia* were found in the treatments a2, a3, a4, b2, b3 and b4.

392 *Flavobacteriia* had a potential intracellular circulation of the glycogen/starch pathway (Liu et al., 2019),
393 which may serve as a survival strategy for starvation in FW samples. The presence of abundant toxin
394 exporting, transcription and signal transduction related genes in *Flavobacteriia* also may further help to
395 survive in the extreme conditions of FW (Liu et al., 2019). In addition, *Flavobacteriia* and
396 *Gammaproteobacteria* can produce diverse carbohydrate-active hydrolytic enzymes with a good
397 removal efficiency of organic pollutants (Martin et al., 2016), which might be the primary reason for
398 the good performance in water quality treatment of FW samples in these treatments. High abundance of
399 *Flavobacteriia* (36.73%) and moderate abundance of *Bacteroidia* (18.55%), *Gammaproteobacteria*
400 (25.01%), *Alphaproteobacteria* (9.30%), *cytophagia* (4.10%) and *Clostridia* (2.83%) were observed in
401 the FW sample after the treatment b2 with the best treatment performance (Fig. 4 and Fig. S2). The
402 relative abundance of *Flavobacteriia*, *Bacteroidia*, *Gammaproteobacteria* and *Clostridia* were
403 significantly enhanced compared to the untreated FW samples, supporting that these native
404 microorganisms in the FW sample rather than the exogenic microorganisms from the activated sludge
405 were positively activated and played an important treatment effects on the FW sample after the
406 treatment b2.

407 A principal component analysis (PCA) was conducted to the microbial community in FW under
408 different treatments (Fig. 5). The analysis results showed that the microbial community composition of
409 FW in the treatment a1 and b1 showed significant difference with the untreated FW samples (N1),
410 indicating that the influence of natural light and blue light illuminations at lower temperature (20 ℃ and
411 25 ℃) on the microbial community composition of FW was greater than red light illumination at 30 ℃.
412 In addition, the plant-microbe synergistic treatments (a2, a3, a4, b2, b3, b4, c2, c3, c4) remarkably
413 changed the microbial community composition of FW samples.

Our previous study has reported that the microbial community composition was closely associated with its treatment efficiency of shale-gas fracturing flowback and produced water (He et al., 2019). In this study, treatment b2 showed the best performance in improving the water quality and reducing the ecotoxicity of FW samples. The composition of the microbial community in the treatment b2 significantly differed from other plant-microbe synergistic treatments. *Flavobacteriia* (36.73%) *and Gammaproteobacteria* (25.01%) were the dominant microbial species, followed by *Bacteroidia* (18.55%), *Alphaproteobacteria* (9.3%), *Cytophagia* (4.1%), *Clostridia* (2.83%). As shown in the PCA analysis, *Bacteroidetes* (*Flavobacteria*, *Cytophagia*), *Proteobacteria* (*Alphaproteobacteria*, *Gammaproteobacterial*, *Deltaproteobacteria*), *Cyanobacteria (Chloroplast)*, *Firmicutes (Erysipelotrichi)*, and *Verrucomicrobia* (*Opitutae*, *Verrucomicrobiae*) were the dominant microorganisms which might be directly associated with the treatment efficiency of treatment b2. These microbes can first survive by their adapting and surviving strategies in FW and then play treatment effects on FW through their high decomposition and degradation abilities of pollutants. For example, *Flavobacteriia* can produce diverse carbohydrate-active hydrolytic enzymes with original biochemistry to remove organic matters; *Proteobacteria* (*Alphaproteobacteria*, *Gammaproteobacteria*, *etc*.) was also identified as significant contribution in the fixation and degradation of contaminants due to their diverse metabolic properties and wide variety in metabolism types; *Actinobacteria* could play a good synergistic effect with plants, living symbiotically with plants whose roots fixed nitrogen for plants in exchange for access to some of the plant's saccharides, which act as fungi to decompose organic matter so that the pollutant molecules can be taken up anew by plants(Servin et al., 2008).

4 Conclusions

435 The treated FW samples could all meet the Integrated Wastewater Discharge Standard of China
436 and the discharge standard of pollutants for petroleum chemistry industry. Illumination wavelength and
437 temperature are direct drivers for the microbial treatment effects of COD_{Cr} and BOD_5 in the FW
438 samples, but showed little effect on the TDS, EC and TSS and nitrogenous compounds removal from
439 the FW samples. With the plant-microbial synergism, enhanced effects in the removal of FW pollutants
440 were observed. The best treatment solution for FW samples is 435 mg/L activated sludge enhanced by
441 201.24 g Water Dropwort under blue illumination of 450-495 nm at 25 ℃.

442 The relative abundance of microbes and the composition of the microorganisms greatly varied
443 with the illumination wavelength and temperature under plant-microbial synergism, and the relative
444 abundance were significantly enhanced under the water dropwort enhanced treatment which indicates
445 positive effects in promoting the microbial activities.

446

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455

Compliance with Ethical Standards

The authors declare that we have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper, and the manuscript does not report on or involve the use of any animal or human data or tissue. This manuscript is approved by all the authors, and it has not been submitted to more than one journal for simultaneous consideration. The manuscript content has not been published before. The submitted work has not received any financial support from a third party, and there is no financial relationship with any of the entities. All of the financial organizations associated with this work have been disclosed. There is no patent, whether planned, pending or issued, that is broadly relevant to the submitted work.

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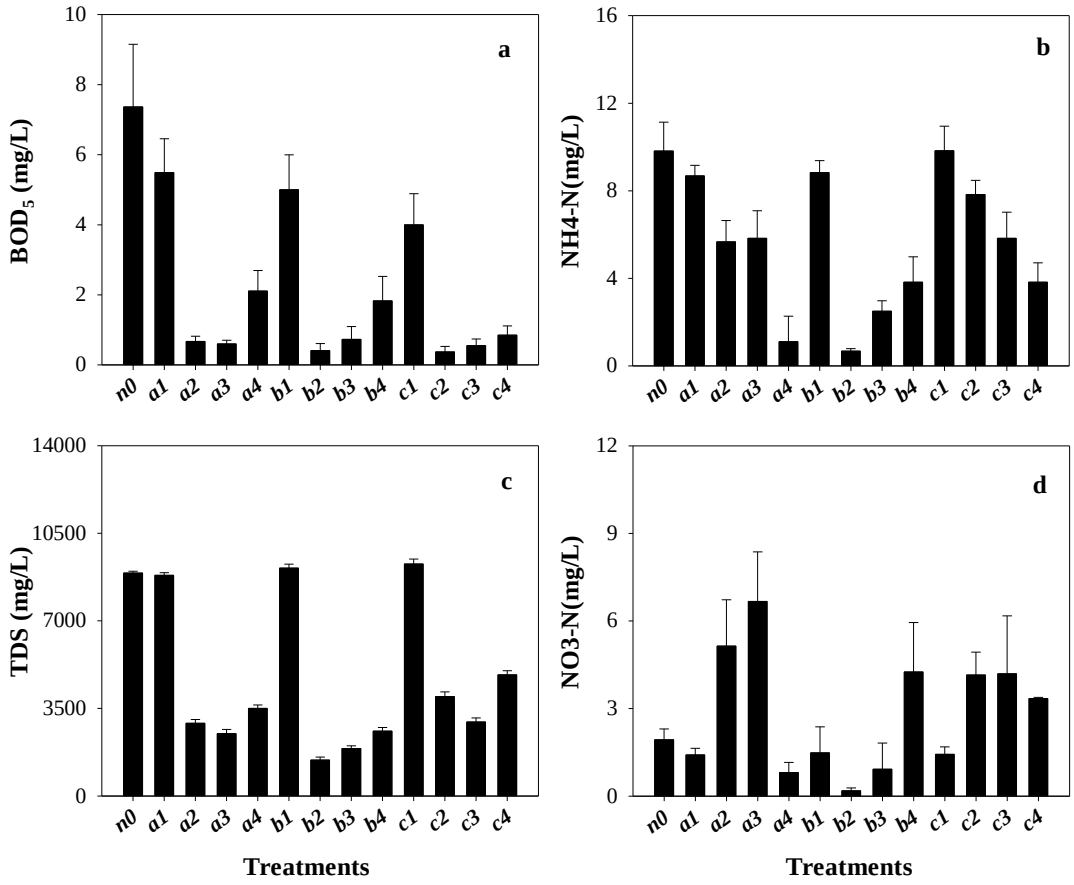
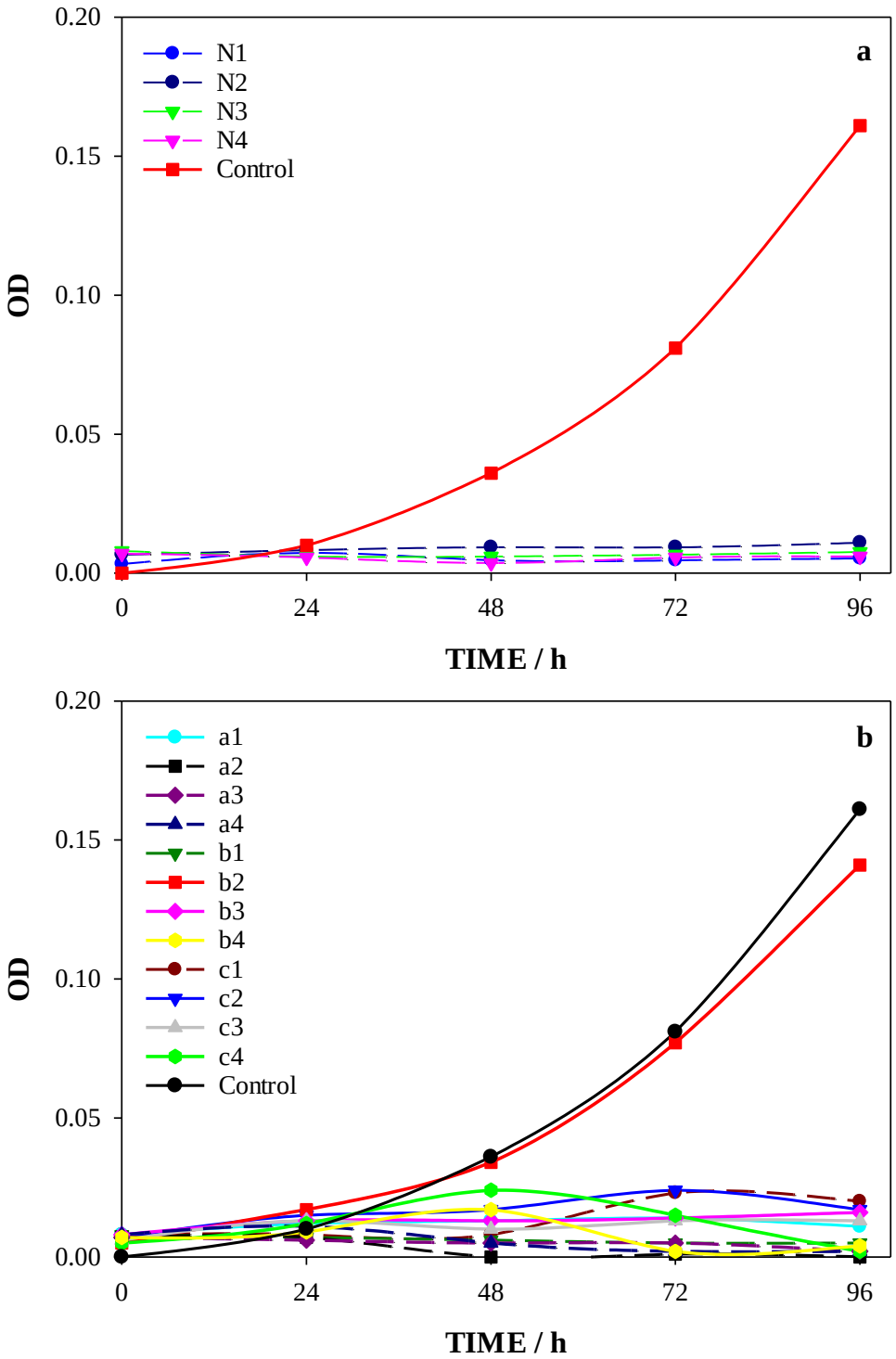
Figure 1-5



Notes: n0, untreated FW samples; a1, a2, a3, a4, b1, b2, b3, b4, c1, c2, c3, c4, FW samples after treated by the treatments as describe in table 1.

Fig. 1 Variations of BOD_5, NH_4-N, NO_3-N and TDS levels in different treatments (fig1a: BOD_5; fig.1b: NH_4-N fig.1c: TDS; fig.1d: NO_3-N)



Notes: N1, untreated FW sample; N2, untreated FW added with a microbial concentration of 435 mg/L; N3, untreated FW added with a microbial concentration of 904 mg/L; N4, untreated FW added with a microbial concentration of 1339 mg/L; a1, a2, a3, a4, b1, b2, b3, b4, c1, c2, c3, c4, FW samples after treated by the treatments as describe in table 1.

Fig. 2 Algal ecotoxicity of FW samples in different treatments (fig.2a: FW samples after treated by different concentration of activated sludge; fig.2b: FW samples after treated by various Plant-microbial synergism treatments)

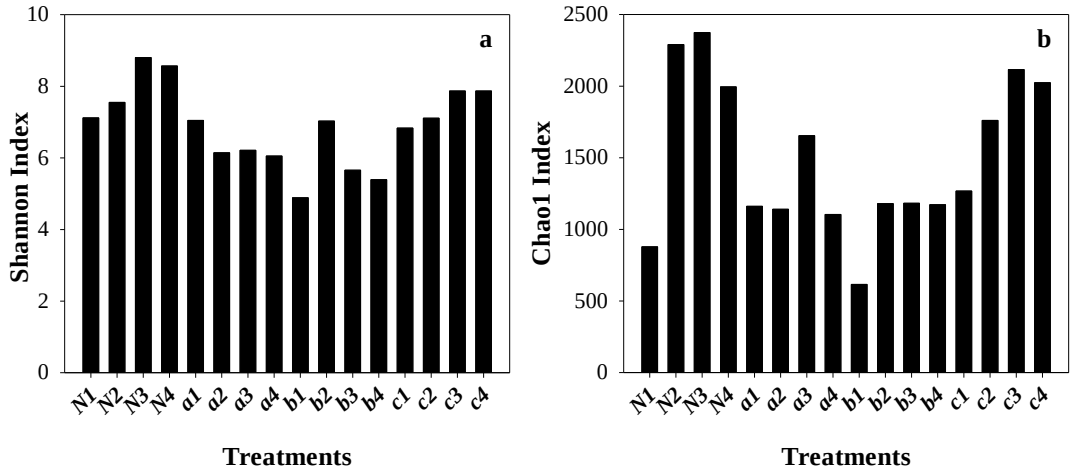


Fig. 3 Variations of microbial community diversity of FW in different treatments (fig.3a: Shannon Index of FW samples after treated by different treatments; fig.3b: Chao1 Index of FW samples after treated by different treatments)

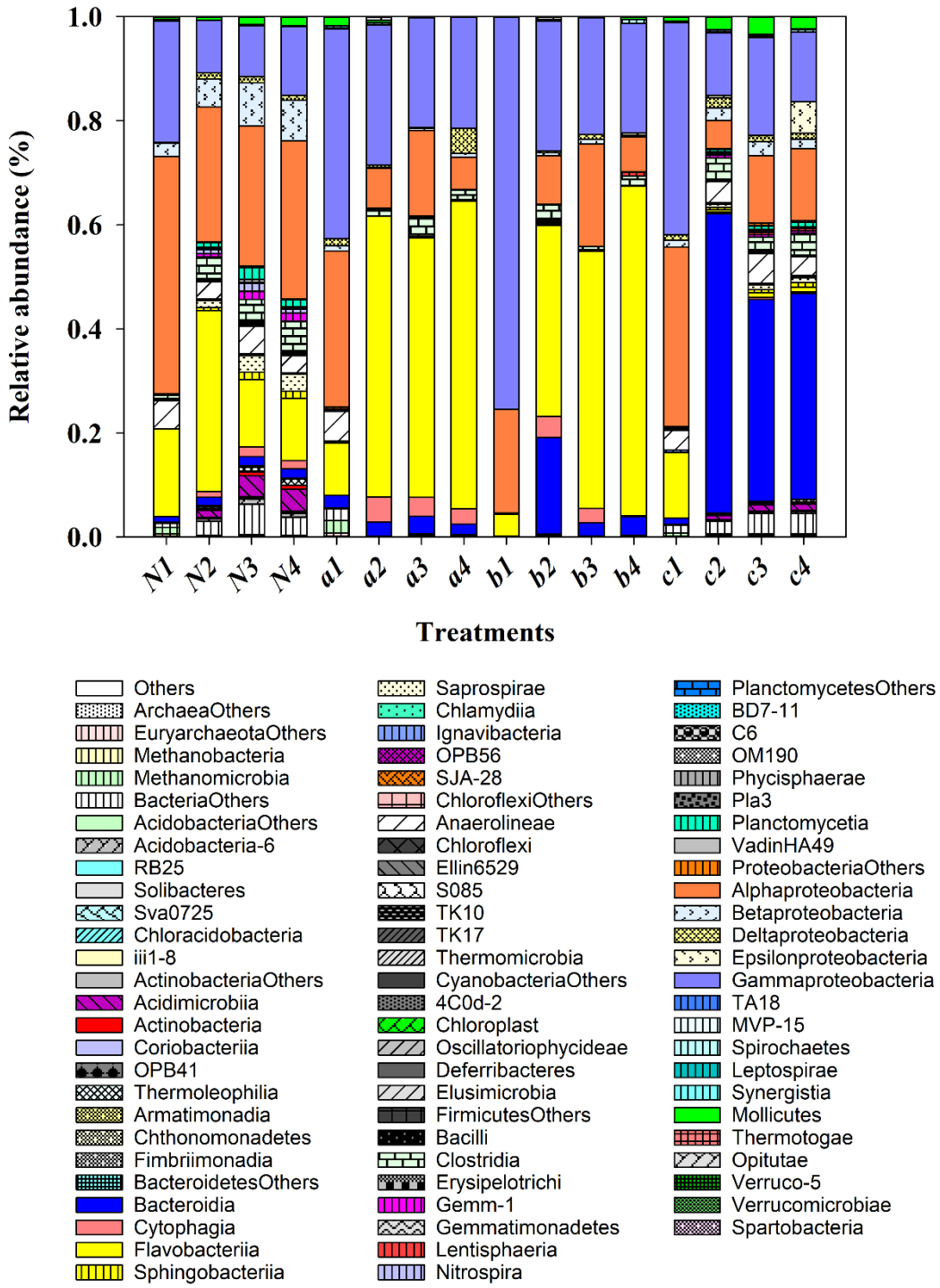
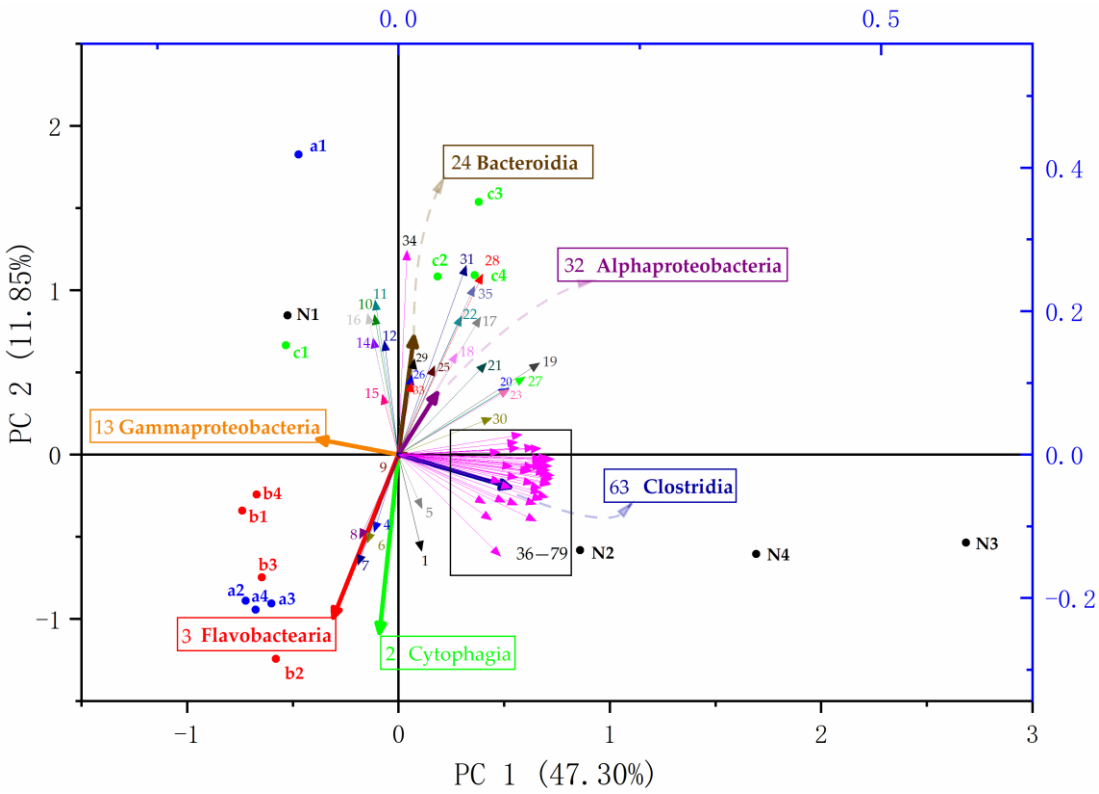


Fig. 4 Relative abundance of microbial community of FW under different treatments in class level



Notes: The class level number corresponds to the following: 1, others; 2, Cytophagia; 3, Flavobacteriia; 4, CyanobacteriaOthers (Classes other than 4C0d-2, Chloroplast and Oscillatoriophycideae in Cyanobacteria); 5, Chloroplast; 6, Erysipelotrichi; 7, Opitutae; 8, Verrucomicrobiae; 9, Deltaproteobacteria; 10, EuryarchaeotaOthers (Classes other than Methanobacteria, and Methanomicrobia in Euryarchaeota); 11, Methanomicrobia; 12, Lentisphaeria; 13, Gammaproteobacteria; 14, MVP-15; 15, Spirochaetes; 16, Thermotogae; 17, ArchaeaOthers (Archaea except Euryarchaeota); 18, Methanobacteria; 19, BacteriaOthers (Bacteria other than those listed in Gemmatimonadetes, Acidobacteria, Firmicutes, etc.); 20, Chloracidobacteria; 21, Coriobacteriia; 22, OPB41; 23, BacteroidetesOthers (Classes other than Bacteroidia, Cytophagia, Flavobacteriia, Sphingobacteriia, and Saprospirae in Bacteroidetes); 24, Bacteroidia; 25, Ignavibacteria; 26, OPB56; 27, ChloroflexiOthers (Classes other than Anaerolineae, Chloroflexi, Ellin6529, S085, TK10, TK17 and Thermomicrobia in Chloroflexi); 28, Anaerolineae; 29, Deferribacteres; 30, Bacilli; 31, ProteobacteriaOthers (Classes other than Alphaproteobacteria, Betaproteobacteria, Deltaproteobacteria, Epsilonproteobacteria, Gammaproteobacteria and TA18 in Proteobacteria); 32, Alphaproteobacteria; 33, Epsilonproteobacteria; 34, Synergistia; 35, Mollicutes; 36, AcidobacteriaOthers (Classes other than Acidobacteria-6, RB25, Solibacteres, Sva0725, Chloracidobacteria, and iii1-8 in Acidobacteria); 37, Acidobacteria-6; 38, RB25; 39, Solibacteres; 40, Sva0725; 41, iii1-8; 42, ActinobacteriaOthers (Classes other than Acidimicrobiia, Actinobacteria, Coriobacteriia, OPB41 and Thermoleophilia in Actinobacteria); 43, Acidimicrobiia; 44, Actinobacteria; 45, Thermoleophilia; 46, Armatimonadia; 47, Chthonomonadetes; 48, Fimbriimonadia; 49, Sphingobacteriia; 50, Saprospirae; 51, Chlamydiia; 52, SJA-28; 53, Chloroflexi; 54, Ellin6529; 55, S085; 56, TK10; 57, TK17; 58, Thermomicrobia; 59, 4C0d-2; 60, Oscillatoriophycideae; 61, Elusimicrobia; 62, FirmicutesOther (Classes other than Bacilli, Clostridia and Erysipelotrichi in Firmicutes); 63, Clostridia; 64, Gemm-1; 65, Gemmatimonadetes; 66, Nitrospira; 67, PlanctomycetesOther (Classes other than Phycisphaerae, Planctomycetia, vadinHA49, Pla3, BD7-11, C6 and OM190 in Planctomycetes); 68, BD7-11; 69, C6; 70, OM190; 71, Phycisphaerae; 72, Pla3; 73, Planctomycetia; 74, VadinHA49; 75, Betaproteobacteria; 76, TA18; 77, Leptospirae; 78, Verruco-5; 79, Spartobacteria.

Fig. 5 Principal component analysis (PCA) of microbial community in FW under different treatment

Table 1 Restoration condition design of twelve treatments for fracturing waste fluid

Treatment	MLSS concentration of activated sludge (mg/L)	Illumination wavelength (nm)	Temperature (℃)	Biomass of Water Dropwort (g)
a1	0	380-750 (natural)	20	0
b1	0	450-495 (blue)	25	0
c1	0	620-750 (red)	30	0
a2	435	380-750 (natural)	20	150.49
b2	435	450-495 (blue)	25	201.24
c2	435	620-750 (red)	30	108.82
a3	904	380-750 (natural)	20	200.54
b3	904	450-495 (blue)	25	108.36
c3	904	620-750 (red)	30	150.67
a4	1339	380-750 (natural)	20	108.44
b4	1339	450-495 (blue)	25	150.57
c4	1339	620-750 (red)	30	200.17