Start-up of a spiral periphyton bioreactor (SPR) for removal of COD and the characteristics of the associated microbial community

Haidong Shangguan^{a, 1}, Junzhuo Liu^{a, b, 1}, Yan Zhu^a, Zhengong Tong^c, Yonghong Wu^{a, *}

^a State Key Laboratory of Soil and Sustainable Agriculture, Institute of Soil Science, Chinese Academy of Sciences, No. 71 East Beijing Road, Nanjing 210008, P. R. China

^b Laboratory of Protistology and Aquatic Ecology, Department of Biology, Ghent University, Krijgslaan 281-S8, 9000 Ghent, Belgium

^c School of Civil Engineering, East China Jiaotong University, 808 Shuang Gang East Road, Nanchang, Jiangxi 330013, P. R. China

*Corresponding author. Tel./Fax.: +86 25 86881330. E-mail address: yhwu@issas.ac.cn

¹ These authors contributed equally to this work.

Abstract

Periphyton-based bioreactors are widely accepted for removing various pollutants from wastewater; however, the slow start-up and low efficiency in widely fluctuating temperatures limit its application. A spiral periphyton bioreactor (SPR) was developed and its COD removal capability and the associated microbial communities investigated. This SPR can be easily backwashed to stimulate periphyton growth and efficiently remove COD at temperatures ranging from 4-30 °C. The species richness and evenness of the periphyton community increased during domestication, while its functional diversity and organic carbon metabolic vitality were higher after 30 days domestication. Cyanobacteria were the main components of the SPR and produced an aerobic environment, while *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria* and *Sphingobacteria* were the microorganisms responsible for COD removal. This study provides valuable insights into changes in pivotal microorganisms of the periphyton community during domestication, and indicates that SPR is simple to operate and efficient in COD removal.

Keywords: SPR, COD, Room/low temperature, Microbial diversity

1. Introduction

The exponential human population increase and rapid industrialization have produced tremendous volumes of wastewater containing various organic pollutants since the middle of the 20th century (Demirel et al., 2005; Li et al., 2012). Consequently, the surface and ground water ecosystems have been greatly impacted or threatened by organic pollution (Ali et al., 2012). Among the conventional biological wastewater treatments, activated sludge plants and anaerobic treatment are commonly employed in organic pollutant removal, but their large sludge production and high energy consumption are significant drawbacks (Demirel et al., 2005). Therefore, innovative bio-measures with low energy requirements and environmentally benign technologies are urgently needed to treat organic pollution.

Periphyton, mainly composed of benthic microorganisms including photoautotrophic algae, bacteria, fungi, protozoa and small multicellular animals, is an important ecological component of surface water and plays a major role in primary productivity, nutrient transformation and food source biomass (Bere & Tundisi, 2012; Chetelat et al., 1999; Larned, 2010; Wu et al., 2014b). In recent years, periphyton has become attractive in wastewater treatment and the bioremediation of polluted natural water ecosystems as it can be easily contrived and/or incorporated in bioreactors, such as Algal Turf Scrubber (Adey et al., 2013; Wu et al., 2014b). Periphyton-based bioreactors have been investigated for the removal of various contaminants including heavy metals and nutrients, and the degradation of organic pollutants (Adey et al., 2013; Bere & Tundisi, 2012; Hamelin et al., 2015; Wu et al., 2014b). The periphyton-based bioreactors were easy to construct, resistant to external environmental changes, produced no secondary pollution

or toxic secretions (Craggs et al., 1996; Larned, 2010; Wu et al., 2010). Moreover, the autotrophs of periphyton biofilm can produce oxygen through algal photosynthesis and restore the DO levels of the wastewater to prompt aerobic degradation of organic matter by heterotrophic bacteria. Consequently, no aeration is needed for COD removal (Craggs et al., 1996; Wu et al., 2014a).

Despite these advantages, periphyton-based systems have limitations. For instance, periphyton community compositions are not stable and nutrient and COD removal efficiency can be negatively influenced by low temperature and solar irradiance (Craggs et al., 1996; Larras et al., 2013; Wu et al., 2014b). Consequently, it is not practical to use these systems during cold seasons. In addition, the pollutant removal efficiency increases slowly during the start-up phase of a periphyton bioreactor and sloughing of the periphyton biofilm has negative effects on effluent water quality (Adey et al., 2013; Sandefur et al., 2011). Therefore, new types of periphyton bioreactors able to operate under a broad temperature range and with less periphyton shedding are needed to quickly adapt to different types of wastewater, to remove multiple organic pollutants, and use less energy (Larned, 2010; Wu et al., 2014b).

Most previous research on pollutant removal from wastewater using periphyton-based systems focused on their performance at the community level (Adey et al., 2013; Hamelin et al., 2015). Little attention has been paid to the taxonomic components of the periphyton community and their role in the metabolic activities of the associated microbial community (Bere & Tundisi, 2012). Therefore, a more thorough investigation of the common species in periphyton communities and identification of their roles in carbon metabolic processes can improve the COD removal capability and sustainability of a

periphyton bioreactor (Wu et al., 2014b).

Accordingly, the primary objectives in this study were to: (1) design a back-washable periphyton bioreactor to efficiently remove COD from wastewater over a broad temperature range; (2) domesticate the periphyton community to remove high concentration COD at low temperatures; (3) investigate microorganism characteristics (functional diversity, species composition and their roles in organic carbon degradation) of the periphyton community during domestication of high COD loading and low temperature. This study provides a promising periphyton bioreactor to quickly remove organic matter at different temperatures and more in depth knowledge of the relationship between microbial diversity and the functional microorganisms during the domestication process.

2. Material and methods

2.1. Spiral periphyton bioreactor (SPR)

The core components of the spiral periphyton bioreactor (SPR) in this study were one spiral PE pipe with periphyton attached (total length: 49.4 m; diameter: 2 mm), one pump, one clarification tank and one influent tank (Fig. 1). During the operation, the wastewater or growth medium is pumped from the influent tank to the spiral PE pipe and clarification tank then discharge. To prevent pipe blockage and periphyton shedding, the SPR should be regularly backwashed, cleaned and regenerated.

2.2. Experimental design

In this study, two stages were carried out: periphyton formation on the PE pipe

and the trial operation stage, which were run from October to November 2014 and November 2014 to January 2015, respectively.

For the periphyton formation stage, lake water rich in algae and bacteria was collected from Xuanwu Lake, Nanjing, China, which was eutrophic, and added to the PE pipe. Seven days later, the lake water was refreshed. Seven days after that, a green periphyton community was observed by the naked eye in the PE pipe and the SPR was started with WC medium at a speed of 309 $\mu\text{l}/\text{min}$ for 14 days. The spiral PE pipe was set-up in an artificial climatic box (QHX-300BS-III) at different temperatures (from 4 to 30 $^{\circ}\text{C}$) with a light intensity of 2500-5000 lux and light/dark cycle of 12 h: 12 h.

For the operation stage, the SPR was set-up under the same conditions as the formation stage and lasted for 44 days. To investigate the periphyton shedding of SPR, distilled water was employed in the first eight days, constituting phase I (day -8 to 0). Then, to verify the ability of the SPR to remove organic matter from wastewater of various concentrations, the influent COD of a synthetic wastewater was gradually increased from 107.3 mg/L on day 0 to 375.2 (day 8), 562.6 (day 18) and 738.2 mg/L (day 28) constituting phases II, III, IV and V, respectively. The synthetic wastewater in this study was mainly composed of CH_3COONa (0 to 945.75 mg/L), NH_4Cl (0 to 77.39 mg/L), and KH_2PO_4 (0 to 39.55 mg/L). Trace elements of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (22.6 mg/L), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (2.8 mg/L), $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ (0.2 mg/L) from Li et al. (2012) were added to the synthetic wastewater. NaHCO_3 was used to regulate the pH of the synthetic wastewater at 7.2-7.6. The synthetic wastewater was pumped through the spiral PE pipe at a constant rate of 463.5 $\mu\text{l}/\text{min}$ (hydraulic retention time: 5.6 h) and then out to another tank (Fig 1). From phase II onwards, after every two days the influent wastewater was replaced by the effluent accumulated in the previous two days until the end of each phase.

Two days was considered as one cycle. At the end of stage II, the temperature of the influent tank was gradually decreased from 30 °C on day -8 to 4 °C on day 36 by employing a water bath or fridge. To minimize periphyton shedding and stimulate periphyton growth, the SBR was backwashed with distilled water for 30 to 60 min in the opposite direction to the influent and cleaned every seven days. The speed of the backwash water was controlled between 1.63-2.03 µl/min, with the intensity of the backwash dependent on the amount of periphyton shed.

2.3. Samples and analyses

COD of the influent and effluent was determined every two days by collecting 10 ml water samples according to the standard potassium dichromate digestion method (GB11914-89) of the Ministry of Environmental Protection of China (Wei, 2002). To determine the periphyton biomass, 5 mm of the PE pipe was cut every four to eight days and the periphyton was washed in an Erlenmeyer flask and diluted to 10 ml using distilled water. The biomass COD was determined following the method of Bullock et al. (1996) and the periphyton biomass on the PE pipe was expressed as mg BioCOD/mm.

To determine the functional diversity of the microbial community during the operation process, periphyton samples were collected by cutting 10 mm of the PE pipe and washing with 10 ml distilled water on day -8 and 22 of the operation stage, constituting the non-domesticated and 30 day domesticated microbial community, respectively. The community-level substrate utilization was assayed using a BiologTM ECO Microplate (Hayward, CA, USA). The ECO microplate contains three replicate wells of 31 carbon substrates (Choi & Dobbs, 1999). The wells contain a redox-sensitive tetrazolium dye which turns purple following respiratory electron transport in metabolically active cells.

Therefore, the plate color is proportional to microbial respiratory activity and can reflect the characteristics of the microbial community (Choi & Dobbs, 1999; Li et al., 2012). A 150 µl aliquot was added to each well of the ECO microplate. The ECO microplate was then incubated at 25 °C and the average well color development (AWCD) was determined using a Biolog Microplate Reader at 590 nm every 24 h for seven days.

Periphyton samples collected by cutting and washing the 10 mm of PE pipe on day -8, 7 and 22 of the operation stage were used for microbial diversity level analysis via MiSeq sequencing. First, DNA was extracted from the water with washed periphyton sample using E.Z.N.A.[®] Water DNA Kit (D5525-02) and the A260/280 value of the extracted DNA was kept around 2.0. Then, primers 515F (5'-GTGCCAGCMGCCGCGG-3') from Turner et al. (1999) and 907R (5'-CCGTCAATTCMTTTRAGTTT-3') from Morales and Holben (2009) were used for PCR amplification of 16S rRNA in ABI GeneAmp[®] 9700 (ABI, USA). Each 20 µl reaction mixture for PCR consisted of 4 µl 5× FastPfu Buffer, 2 µl dNTPs (2.5 mM), 0.4 µl forward primer (5 µM), 0.4 µl reverse primer (5 µM), 0.4 µl FastPfu Polymerase, 20 ng template DNA and 12.8 µl ddH₂O. The PCR product was then checked with agarose gel electrophoresis and extracted with an AxyPrep DNA gel extraction kit (AxyGen, China). The PCR product was subsequently quantified on QuantiFluor[™] -ST (Promega, USA) and sequenced using the MisSepence. These were done by Shanghai Majorbio (<http://www.majorbio.com/>).

After sequencing, the raw sequence data were processed and the PE reads were separated and overlapped to assemble the final sequences with a minimum overlap length of 10 bp of each two PE reads. The PE reads with more than two mismatches

within 10 bp region were removed during the overlap step. The sequence trim was processed using Trimmomatic. Operational taxonomic unit (OTU) grouping was then determined at a similarity of 97 % using Usearch. The taxonomy assignment of tags was performed on OTUs using RDP classifier (<http://sourceforge.net/projects/rdp-classifier/>) and the taxonomic structure compositions of the periphyton community were calculated and expressed as relative abundances. Based on OTUs, the Rank-abundance distribution curve and Shannon-Wiener Index curve were analyzed using Mothur to evaluate the community diversity levels (Wang et al., 2012). A Venn diagram was analyzed using the R statistical program to investigate the diversity similarity of the three periphyton communities.

3. Results and discussion

3.1. Periphyton shedding and COD removal of SPR

The periphyton biomass in the PE pipe increased gradually from 3.63 to 10.03 mg BioCOD/mm from phase I to V (Fig. 2A). After the influent COD loading increased from 107.28 to 738.15 mg/L, the maximal COD removal rate of each phase varied between 33.79 and 335.4 mg/L. The highest COD removal quantity of 335.4 mg/L in day 18 was achieved in phase IV with a COD loading of 562.6 mg/L and a temperature of 15 °C.

During phase I, the effluent had a significantly higher COD concentration of 86.12-100.08 mg/L than the influent which was only 2-4 mg/L. This was most probably due to the shedding of periphyton from the PE pipe. The shedding of biofilm including periphyton, during growth is a common phenomenon (Horn et al., 2003; Sandefur et al.,

2011) resulting in the overestimation of the effluent COD concentration. In the study of Horn et al. (2003), the detached biofilm biomass was positively correlated with biofilm density. In this study, the periphyton biomass of the PE pipe showed a gradual increase during the operation stage, so the minimum periphyton shedding happened during phase I, which was 86.12-100.08 mg BioCOD/L. Therefore, the shed periphyton biomass should be subtracted from the effluent COD when calculating the COD removal efficiency; 86.12 mg BioCOD/L was used in this study.

From phase II onwards, the periphyton biomass gradually increased and the maximal growth was observed during phase III from 5.35 to 8.42 mg BioCOD/mm. Consequently, the maximal COD removal rate increased from 130 mg/L in phase III to 335 mg/L in phase IV. During phase IV and V, although the temperature decreased from 18 °C to 10 °C and then to 4 °C, the periphyton maintained a high COD removal rate of 114-293 mg/L at a hydraulic retention time of 5.6 h. Moreover, in contrast to the high COD concentration of the effluent in phases I and II, the effluent COD of phase V was as low as 43.9 mg/L. This is due to the periodical backwashing of the SPR backwash. The SPR backwash could remove the old periphyton biomass and stimulate further periphyton growth. Thus, the SPR backwash reduced the influence of shedding periphyton biomass on effluent COD and prompted a continuous COD removal rate even at 4-10 °C.

After subtracting the periphyton shedding biomass of 86.12 mg BioCOD/L, the COD removal efficiency at a hydraulic retention time of 5.6 h was 64.1-100 % from phase II to IV (Fig. 2B). During phase V, although the COD removal efficiency of the first cycle slightly decreased (51.33 %) compared to phase IV, the COD removal effi-

ciency reached 85.9 % after two cycles (equal to a HRT of 11.2 h) and 100 % after three cycles (equal to a HRT of 16.8 h backwash. These results imply that the periphyton has a quick adaptability to low temperatures of 4 °C and maintained a high COD removal capability. Although previous studies [such as Feng et al. (2009) and Li et al. (2012)] have reported that microorganisms cultured at low temperature could have a high COD removal rate, these high COD removal efficiencies only appeared under low temperature conditions (such as 4 °C). In this study, the COD removal by SPR was highly effective under continuous temperature shifts from 30 to 4 °C. Therefore, this SPR could efficiently remove COD of 375-738 mg/L from wastewater at a broad temperature range of 4-30 °C with a hydraulic retention time of 5.6-16.8 h after a short domestication and periodical backwash.

3.2 Functional diversity of the periphyton community

3.2.1. Comparison of Average Well Color Development (AWCD)

The AWCD of the periphyton from day -8 and 22 showed no visible changes in the first 24 h (Fig. 3A). This was probably due to the low initial microbial density and minimal carbon utilization. After 24 h, the AWCD increased quickly until day 4 and thereafter at a relatively slow rate. Furthermore, the periphyton from day 22 had a higher AWCD than that of day -8 and thus a higher utilization capability of carbon sources. In the first 120 h the periphyton from day 22 had an S-shaped growth curve, while the periphyton from day -8 did not have a distinct limiting point. This was probably because the periphyton community diversity was highly dependent on the wastewater composition (Wu et al., 2011). Specifically, the periphyton collected on day -8 was not adapted to the various carbon sources, while after 30 days domestication the periphyton was

adapted to various organic carbon sources and pollutants. In addition, the AWCD experiment was carried out at a lower incubation temperature (25 °C) than day -8 of the operation stage (30 °C). The periphyton from day 22 had already been domesticated at a broad temperature range and could quickly adapt to temperature changes. The periphyton collected on day -8 needed time to adapt to the sudden temperature decrease and grew at slower rates than the periphyton from day 22. In addition, microorganisms grown at a low temperature have higher metabolic activity and adaptability than those growing at high temperature (Li et al., 2012). Therefore, after 30 days domestication to increasing COD loading and decreasing temperature, the periphyton community had a higher functional diversity and could more quickly adapt to the single carbon source environment than non-domesticated periphyton. This was in accordance with the contrasting COD removal performance of the SPR during phases I and II and phases IV and V.

3.2.2 Metabolic capability of different types of carbon sources

Many studies have demonstrated that the morphology, staining and metabolic activity of microorganisms are very typical at the later stage of the exponential phase (Subbalakshmi & Sitaram, 1998). In this study, the periphyton community from day 22 entered its stationary phase around 96 h (Fig. 3A), and consequently, the 96 h Biolog data was selected for detailed metabolic capability analysis of carbon sources. As described in Choi and Dobbs (1999), the 31 carbon substrates of the ECO microplate can be classified into six main types: amine acid, amino acid, carbohydrate acid, carboxylic acid, phenolic acid and polymer. The metabolic capability of the carbon sources were evaluated on the level of these six main types (Li et al., 2012).

The periphyton from day 22 had greatly higher utilization efficiency of all six types of carbon sources than periphyton from day -8 (Fig. 3B). The microorganisms from the 0 and 30 days domesticated periphyton communities showed a little difference in the first utilized carbon sources. The periphyton community from day 22 had an equal metabolic capability of using phenolic acid and amino acid and then carbohydrate, while the periphyton of day -8 first utilized amino acid and then carbohydrate and phenolic acid. For the other three types of carbon sources, both of the periphyton communities from day -8 and 22 had the similar utilization pattern. Again, it implied that the 30 day domesticated periphyton had higher metabolic capability in utilizing carbons than the periphyton without domestication.

3.3 Microbial diversity levels of periphyton community

3.3.1. Taxonomic compositions of periphyton

In this study, the MiSeq sequencing technology was used to analysis the different diversity levels of the microbial communities based on 44,701,497 bases of 112,992 sequences of three periphyton community samples from day -8, 7 and 22. Cyanobacteria were the largest components of the periphyton community and their relative abundance increased from 50 to 80.4 % from day -8 to 7 and then decreased to 12.7 % on day 22 (Fig. 4A). Cyanobacteria are therefore the main contributor to the high effluent COD during phases I and II (Fig. 2). This also indicated that the periphyton had sufficient oxygen produced by cyanobacteria (Berman-Frank et al., 2003), and thus promoted the growth of the aerobic microbial community and their activities in degrading organic matter. Consequently, the periphyton bioreactor is suitable for the aerobic treatment of wastewater and could also reduce the energy consumption for aeration.

The growth of other microorganisms were relatively suppressed during day -8 to 7 due to the large quantity of cyanobacteria in the periphyton community, because cyanobacteria assimilated most of the nutrients from wastewater through photosynthesis (Dodds, 2003). *Proteobacteria* including *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* were the second largest components after Cyanobacteria in the periphyton community with a relative abundance of 14.9-66.9 % (Fig. 4A). Specifically, *Alphaproteobacteria* had its highest relative abundance of 8.6 % in the periphyton of day 7. *Betaproteobacteria* was the only group with a relative abundance higher than 5 % (5.9-19.4 %) during the whole process. *Gammaproteobacteria* exhibited great changes in its relative abundance with an initial from 20 % to 0.46 % and then an increase to 46.19 % during the 30 days. Moreover, *Sphingobacteria* showed a gradually increasing relative abundance of 0.8-11.5 % from day -8 to 22. In summary, following the increase in COD loading and decrease in temperature, aerobic bacteria were more relatively abundant by day 22, which was in accordance with the COD removal performance in phases IV and V (Fig. 2). This indicates that the domestication selected the most functional bacterial species to grow and thus remove COD. Moreover, it also explained the differences in AWCD and the metabolic utilization of the six types of carbon sources by the 0 and 30 days domesticated periphyton communities (Fig. 3).

Proteobacteria has been widely reported as the dominant bacteria in municipal wastewater and activated sludge (Baudišová et al., 2012) and *Betaproteobacteria* play a critical role in degradation of organic matter in sewage treatment plants (Liu et al., 2007). Some members of *Gammaproteobacteria* have a wide temperature tolerance (Williams et al., 2010) and some members such as *Acinetobacter* and *Pseudomonas* are efficient in COD removal (Olukanni et al., 2006). In the work of Sinkko et al. (2013),

Sphingobacteria, *Alphaproteobacteria* and *Gammaproteobacteria* showed strong positive correlations with the organic carbons in coastal surface sediments and *Sphingobacteria* was important in the degradation of biopolymer sedimentary organic matter. Therefore, *Proteobacteria* including *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* and *Sphingobacteria* may be important functional microorganisms in COD removal by the SPR and could be further regulated to optimize the SPR COD removal efficiency.

3.3.2. Microbial community diversity indices

Microbial community diversity concerns both taxon richness and evenness (Wang et al., 2012), so the Shannon-Wiener Index and Rank-abundance distribution curve were used in this study to investigate species richness and evenness of the periphyton community (Bates et al., 2013). The Shannon-Wiener Index (Fig. 4B) showed that species richness decreased from day -8 to 7 probably due to the increasing COD loading and decreasing temperature. After 15 days acclimation to the wastewater, the periphyton community started to restore its species diversity and higher species richness was observed on day 22 than day 7. This indicates that the periphyton bacterial community may need 15 to 30 days to adapt to wastewater and that the periphyton has a strong ability to adapt to the environment and restore its diversity and functions. Furthermore, results suggest that the species richness of periphyton is not strongly influenced by decreasing temperature, even to 4 °C. This is in accordance with the results of Li et al. (2012).

The species evenness of the periphyton community increased greatly following the domestication process (Fig. 4C). Although the species evenness on day 7 was slight-

ly higher than day -8, it was significantly higher on day 22 than days -8 and 7. Furthermore, the species richness of the periphyton community on day 22 was lower than on day -8 (Fig. 4B), but the species evenness on day 22 was much higher than on day -8 (Fig. 4C). This is probably due to the periodical increase in COD loading of the influent and/or the gradual decrease of temperature

A Venn diagram is a measure of the similarities of different communities (Fouts et al., 2012). In this study, the similarity analysis of microbial communities was done on the OUTs. The numbers of OUTs of the periphyton communities from day -8, 7 and 22 were 148, 85 and 135, respectively (Fig. 4D). This was in accordance with the species richness determined by the Shannon-Wiener Index (Fig. 4B). The periphyton communities of day -8 and 7 had 40 OTUs in common, while the periphyton of day 7 and 22 shared 64 OTUs. This indicates that the periphyton community had greater changes from day -8 to 7 than from day 7 to 22. This was probably because the periphyton community gradually adapted to the wastewater in the first 15 days and subsequently restored the microbial functional diversity and vitality.

4. Conclusion

SPR could be quickly started up after a short domestication and easily back-washed to stimulate periphyton growth for efficient and continuous COD removal at 4-30 °C. After domestication, the periphyton community had higher metabolic activities of utilizing organic carbons and thus COD removal capabilities than before domestication. Cyanobacteria were the main components of SPR and produced an aerobic environment, while *Proteobacteria* including *Alphaproteobacteria*, *Betaproteobacteria* and *Gammaproteobacteria* and *Sphingobacteria* were the pivotal microorganisms degrading

organic matter. A better understanding of the diversity and key microorganisms of periphyton communities can significantly contribute to the optimization of COD removal by SPR.

Acknowledgements

We sincerely appreciate Dr. Clare Morrison from School of Environment, Griffith University, Australia for her valuable comments and revision of this manuscript. This work was jointly supported by the State Key Development Program for Basic Research of China (2015CB158200), the National Natural Science Foundation of China (41422111 and 41171363) and “Grand Challenges Exploration” of the Bill & Melinda Gates Foundation.

References

1. Adey, W.H., Laughinghouse, H.D., Miller, J.B., Hayek, L.A.C., Thompson, J.G., Bertman, S., Hampel, K., Puvanendran, S., 2013. Algal turf scrubber (ATS) flowways on the Great Wicomico River, Chesapeake Bay: productivity, algal community structure, substrate and chemistry. *J. Phycol.* 49, 489-501.
2. Ali, I., Asim, M., Khan, T.A., 2012. Low cost adsorbents for the removal of organic pollutants from wastewater. *J. Environ. Manag.* 113, 170-183.
3. Bates, S.T., Clemente, J.C., Flores, G.E., Walters, W.A., Parfrey, L.W., Knight, R., Fierer, N., 2013. Global biogeography of highly diverse protistan communities in soil. *The ISME Journal* 7, 652-659.
4. Baudišová, D., Benáková, A., Wanner, F., 2012. Changes in prokaryotic community composition in the small wastewater treatment plant of Zbytiny during treatment processes. *Silva Gabreta* 18, 79-90.

- 374 5. Bere, T., Tundisi, J.G., 2012. Cadmium and lead toxicity on tropical freshwater
375 periphyton communities under laboratory-based mesocosm experiments.
376 *Hydrobiologia* 680, 187-197.
- 377 6. Berman-Frank, I., Lundgren, P., Falkowski, P., 2003. Nitrogen fixation and
378 photosynthetic oxygen evolution in cyanobacteria. *Res. Microbiol.* 154, 157-164.
- 379 7. Bullock, C.M., Bicho, P.A., Zhang, Y., Saddler, J.N., 1996. A solid chemical oxygen
380 demand (COD) method for determining biomass in waste waters. *Water Res.* 30,
381 1280-1284.
- 382 8. Chetelat, J., Pick, F., Morin, A., Hamilton, P., 1999. Periphyton biomass and
383 community composition in rivers of different nutrient status. *Can. J. Fish. Aquat.*
384 *Sci.* 56, 560-569.
- 385 9. Choi, K.-H., Dobbs, F.C., 1999. Comparison of two kinds of Biolog microplates (GN
386 and ECO) in their ability to distinguish among aquatic microbial communities. *J.*
387 *Microbiol. Methods* 36, 203-213.
- 388 10. Craggs, R.J., Adey, W.H., Jessup, B.K., Oswald, W.J., 1996. A controlled stream
389 mesocosm for tertiary treatment of sewage. *Ecol. Eng.* 6, 149-169.
- 390 11. Demirel, B., Yenigun, O., Onay, T.T., 2005. Anaerobic treatment of dairy
391 wastewaters: a review. *Process Biochem.* 40, 2583-2595.
- 392 12. Dodds, W.K., 2003. The role of periphyton in phosphorus retention in shallow
393 freshwater aquatic systems. *J. Phycol.* 39, 840-849.
- 394 13. Feng, H., Hu, L., Mahmood, Q., Fang, C., Qiu, C., Shen, D., 2009. Effects of
395 temperature and feed strength on a carrier anaerobic baffled reactor treating
396 dilute wastewater. *Desalination* 239, 111-121.
- 397 14. Fouts, D.E., Szpakowski, S., Purushe, J., Torralba, M., Waterman, R.C., MacNeil,

- M.D., Alexander, L.J., Nelson, K.E., 2012. Next generation sequencing to define prokaryotic and fungal diversity in the bovine rumen. *PloS One* 7, e48289.
15. Hamelin, S., Planas, D., Amyot, M., 2015. Mercury methylation and demethylation by periphyton biofilms and their host in a fluvial wetland of the St. Lawrence River (QC, Canada). *Sci. Total Environ.* 512, 464-471.
16. Horn, H., Reiff, H., Morgenroth, E., 2003. Simulation of growth and detachment in biofilm systems under defined hydrodynamic conditions. *Biotechnol. Bioeng.* 81, 607-617.
17. Larned, S.T., 2010. A prospectus for periphyton: recent and future ecological research. *J. North. Am. Benthol. Soc.* 29, 182-206.
18. Larras, F., Lambert, A.-S., Pesce, S., Rimet, F., Bouchez, A., Montuelle, B., 2013. The effect of temperature and a herbicide mixture on freshwater periphytic algae. *Ecotoxicol. Environ. Saf.* 98, 162-170.
19. Li, T., Bo, L., Yang, F., Zhang, S., Wu, Y., Yang, L., 2012. Comparison of the removal of COD by a hybrid bioreactor at low and room temperature and the associated microbial characteristics. *Bioresour. Technol.* 108, 28-34.
20. Liu, X., Zhang, Y., Min, Y., Wang, Z., Lv, W., 2007. Analysis of bacterial community structures in two sewage treatment plants with different sludge properties and treatment performance by nested PCR-DGGE method. *J. Environ. Sci.* 19, 60-66.
21. Morales, S.E., Holben, W.E., 2009. Empirical Testing of 16S rRNA Gene PCR Primer Pairs Reveals Variance in Target Specificity and Efficacy Not Suggested by In Silico Analysis. *Appl. Environ. Microbiol.* 75, 2677-2683.
22. Olukanni, O., Osuntoki, A., Gbenle, G., 2006. Textile effluent biodegradation

422 potentials of textile effluent-adapted and non-adapted bacteria. *Afr. J. Biotechnol.*
423 5, 1980-1984.

424 23. Sandefur, H.N., Matlock, M.D., Costello, T.A., 2011. Seasonal productivity of a
425 periphytic algal community for biofuel feedstock generation and nutrient
426 treatment. *Ecol. Eng.* 37, 1476-1480.

427 24. Sinkko, H., Lukkari, K., Sihvonen, L.M., Sivonen, K., Leivuori, M., Rantanen, M.,
428 Paulin, L., Lyra, C., 2013. Bacteria Contribute to Sediment Nutrient Release and
429 Reflect Progressed Eutrophication-Driven Hypoxia in an Organic-Rich
430 Continental Sea. *PLoS One* 8, e67061.

431 25. Subbalakshmi, C., Sitaram, N., 1998. Mechanism of antimicrobial action of
432 indolicidin. *FEMS Microbiol. Lett.* 160, 91-96.

433 26. Turner, S., Pryer, K.M., Miao, V.P.W., Palmer, J.D., 1999. Investigating Deep
434 Phylogenetic Relationships among Cyanobacteria and Plastids by Small Subunit
435 rRNA Sequence Analysis1. *J. Eukaryot. Microbiol.* 46, 327-338.

436 27. Wang, Y., Sheng, H.-F., He, Y., Wu, J.-Y., Jiang, Y.-X., Tam, N.F.-Y., Zhou, H.-W.,
437 2012. Comparison of the levels of bacterial diversity in freshwater, intertidal
438 wetland, and marine sediments by using millions of illumina tags. *Appl. Environ.*
439 *Microbiol.* 78, 8264-8271.

440 28. Wei, F., 2002. Monitoring and analysis methods of water and wastewater. China
441 Environmental Science Press, Beijing.

442 29. Williams, K.P., Gillespie, J.J., Sobral, B.W., Nordberg, E.K., Snyder, E.E., Shallom,
443 J.M., Dickerman, A.W., 2010. Phylogeny of gammaproteobacteria. *J. Bacteriol.*
444 192, 2305-2314.

445 30. Wu, Y., He, J., Yang, L., 2010. Evaluating adsorption and biodegradation

mechanisms during the removal of microcystin-RR by periphyton. Environ. Sci. Technol. 44, 6319-6324.

31. Wu, Y., Hu, Z., Kerr, P.G., Yang, L., 2011. A multi-level bioreactor to remove organic matter and metals, together with its associated bacterial diversity. Bioresour. Technol. 102, 736-741.

32. Wu, Y., Xia, L., Liu, N., Gou, S., Nath, B., 2014a. Cleaning and regeneration of periphyton biofilm in surface water treatment systems. Water Sci. Technol. 69, 235-243.

33. Wu, Y., Xia, L., Yu, Z., Shabbir, S., Kerr, P.G., 2014b. In situ bioremediation of surface waters by periphytons. Bioresour. Technol. 151, 367-372.

FIGURE CAPTIONS

Fig. 1 A schematic drawing of the SPR.

Fig. 2 A: Influent and effluent COD (mg/L), temperature (°C) and the associated periphyton biomass of the PE pipe (mg BioCOD/mm) over time; B: Influent COD (mg/L), effluent COD (mg/L) and COD removal efficiency (%) after subtracting a periphyton shedding of 86.12 mg BioCOD/L over time (light intensity: 2500-5000 lux; light/dark cycle: 12 h: 12 h)

Fig. 3 A: Changes in Average Well Color Development (AWCD) of periphyton community with (day 22) and without (day -8) domestication over incubation time at 25 °C on Biolog ECO microplate; B: The metabolic capability of six main types of carbon sources (amine acid, amino acid, carbohydrate, carboxylic acid, phenolic acid and polymer) by the periphyton communities from days -8 and 22 after 96 h incubation at 25 °C on Biolog ECO microplate

Fig. 4 A: Relative abundance of the taxonomic components (%); B: Shannon-Wiener Index; C: Rank-abundance curve; D: Microbial community similarity represented by Venn diagrams of the periphyton community from days -8, 7 and 22 based on microbial diversity level analysis via MiSeq sequencing.

Figures

Fig. 1

Fig. 2

484 Fig. 3

485

486

487 Fig. 4

488