Hydrogen oxidizing bacteria are capable of removing orthophosphate to ultra-low concentrations in a fed batch reactor configuration

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Abstract

This paper proposes the use of hydrogen oxidizing bacteria (HOB) for the removal of orthophosphate from surface water as treatment step to prevent cyanobacterial blooms. To be effective as an orthophosphate removal strategy, an efficient transfer of hydrogen to the HOB is essential. A trickling filter was selected for this purpose. Using this system, a removal rate of 11.32 ± 0.43 mg PO_4^{-3}-P/L.d was achieved. The HOB biomass, developed on the trickling filter, is composed of 1.25% phosphorus on dry matter, which suggests that the orthophosphate removal principle is based on HOB growth. Cyanobacterial growth assays of the untreated and treated water showed that *Synechocystis* sp was only able to grow in the untreated water. Orthophosphate was removed to average residual values of 0.008 mg/L. In this proof of principle study, it is shown that HOB are able to remove orthophosphate from water to concentrations that prevent cyanobacterial growth.

Keywords

Hydrogen oxidizing bacteria; HOB; phosphate removal; eutrophication

1. Introduction

Eutrophication of natural water bodies has become a global threat (Sinha et al., 2017). The phenomenon – enrichment of water bodies with nutrients – promotes the growth of autotrophic organisms such as algae and cyanobacteria. Algal blooms lead to the fouling of water intakes and waterways and to low-oxygen (hypoxic) or oxygen-free (anoxic) water bodies, resulting in the disruption of food webs and fish death. Also, some cyanobacteria are known to produce potent toxins. Hence, their presence can hinder the supply of irrigation and drinking water, fisheries and recreational amenities and gives rise to substantial economic losses (Dodds et al., 2009). Further expansion and intensification of cyanobacteria blooms are expected, as the combined effect of temperature increase and concentration of humic substances in water has been shown to give cyanobacteria an advantage over other phytoplankton organisms (Paerl and Huisman, 2008).

Phosphorus has long been reported to play a key role in eutrophication of surface waters, originating mostly from agriculture and urban run offs and industrial discharges (Carpenter et al., 1998). In Europe, the current treated water discharge regulations require total phosphorus levels to be between 1 and 2 mg/L, depending on the population equivalent (EuropeanCommission, 2017). However, several studies show that concentrations above 0.1 mg P/L can already support algal growth (Carvalho et al., 2013, Lurling and van Oosterhout, 2013, Richardson et al., 2007) even when nitrogen availability is low, due to the presence of nitrogen-fixing cyanobacteria (Paerl, 2017, Schindler et al., 2008). Taking this into account, there has been an increasing pressure to actively decrease phosphorus loads into water bodies to levels as low as 0.01 mg P/L (Ashekuzzaman, 2017). Decreasing phosphorus concentrations from effluents of normal

sewage treatments plants or water storage basins below 0.01 mg P/L remains a challenge (Gu et al., 2011, USEPA, 2000).

The aim of the present study is to provide a proof of concept for the use of hydrogen oxidizing bacteria (HOB) to remove soluble phosphorus (orthophosphate) to levels below 0.010 mg/L. HOB use hydrogen as their electron donor and oxygen as their electron acceptor to fixate carbon dioxide. This reaction yields a substantial amount of energy that has been explored for decades for the production of proteins, for instance for the animal feed industry (Repaske and Mayer, 1976). Their use of CO2 as carbon source offers an advantage in the treatment of water with insufficient bioavailable organics, such as groundwater, drinking water, and secondary treated wastewater (Karanasios et al., 2010). H_2 is inherently acceptable as an electron donor to be added to surface water, i.e. it does not persist in the treated water, therefore not posing any potential threat to fauna and flora nor to the consumer of the water. Moreover, H2 can be easily produced from water electrolysis, powered by renewable energy sources (Hosseini and Wahid, 2016, Mohsin et al., 2018). The biological trickling filter was chosen as treatment system as it allows the decoupling of solids retention time (SRT) and hydraulic retention time (HRT) (Naz et al., 2015). In biological trickling filters microorganisms are attached to a fixed surface forming a biofilm while the water to be treated flows downwards in direct contact with the biofilm, and the gas with the electron donor and acceptor is fed from the bottom and flows upward (Nadell et al., 2016, Wik, 2003). This system allows for all nutrients to be directly available to a large surface of biofilm even at low concentrations, a feature which is particularly relevant for biological orthophosphate removal from water bodies as orthophosphate is present in very low concentrations. In addition to the operational simplicity, its size can easily be expanded by providing additional surface area.

In this study, a proof of concept for the hydrogenotrophic removal of orthophosphate to ultra-low concentrations is presented. A trickling filter operated in fed batch was used to enrich for hydrogen oxidizing bacteria. Using this system, the lowest residual orthophosphate concentration achievable and the operational conditions in which orthophosphate removal can be maximized were determined.

2. Materials and methods

2.1. Experimental setup

The experiments were carried out in a sealed PVC tube of 1 L containing plastic biofilm carriers (900 m2/m3 specific total surface area, Aqwise). The reactor was continuously flushed with an excess mixture of hydrogen (45%), oxygen (10%) and carbon dioxide (15%) (Matassa et al., 2016). A feed solution, hereafter referred to as the recycling liquid, consisting of tap water enriched with 1 mg PO4−3-P/L (added as KH_2PO_4), 20 mg NH4/L (added as NH_4Cl), 0.5 mg/L ferric ammonium citrate and 0.2 mL/L of trace element solution (0.6 g H_3BO_3, 0.4 g $CoCl_2 \cdot 6H_2O$, 0.2 g $ZnSO_4 \cdot 7H_2O$, 0.06 g NaMoO4·2H2O, 0.04 g $NiCl_2 \cdot 6H_2O$ and 0.02 g $CuSO_4 \cdot 5H_2O$) was recirculated through the reactor at a flow of 0.45 L/min (wetting rate of 22 $m^3/m^2.h$). Initial soluble orthophosphate concentration in the recycling liquid (10 L) was 1 mg PO_4^{-3}-P/L to mimic realistic phosphorus discharge concentration of water treatment plants (EuropeanCommission, 2017). To guarantee phosphorus was the only limiting element, the maximum expected biomass was estimated assuming that all P is converted to biomass and biomass is composed of at least 1% of P. Nitrogen and trace elements required were then calculated based on stoichiometric proportions.

2.2. Inoculation and acclimation

Aerobic activated sludge from a municipal wastewater treatment plant (Leeuwarden, The Netherlands) was used to inoculate the trickling filter and acclimated to the process conditions of gas-water contact for a period of 11 days.

2.3. Microbial community analysis preparation sequencing

Pellets were obtained by centrifuging liquid samples for 15 min at 13,000 g. Subsequently, total DNA was extracted by using the PowerBiofilm DNA isolation Kit (Quiagen, USA) according to the manufacturer's protocol. The extracted DNA was quantified using the QuantiFluor dsDNA kit and a QuantusTM 2.0 fluorometer (Promega, USA), and DNA purity and quality were confirmed by measuring the absorbance at 260 and 280 nm (Nanodrop 2000, Thermo Scientific, Waltham, MA, USA) and via agarose gel electrophoresis, respectively.

2.4. Amplicon sequencing and data processing

The DNA extracts were sent to MrDNA (www.mrdnalab.com, Shallowater, TX, USA) for PCR amplification of the V4-V5 hypervariable region of the 16S rRNA gene was performed using bacterial primers 515F (Parada et al., 2016) and 926R (Quince et al., 2011). Sequence data processing comprised quality control and amplicon sequence variant (ASV) calling using the DADA2 (Callahan et al., 2016) implementation in QIIME2 (Bolyen et al., 2019). Taxonomy was assigned to representative sequences of each ASV using a naive Bayesian classifier trained on full 16S sequences of the curated SILVA database v.132 (Bokulich et al., 2018, Pedregosa et al., 2011). Raw sequence data has been deposited in EMBL-EBI under project number PRJEB38088. Bacterial ASVs

137 comprising at least 0.1% of reads in a sample were used to calculate relative abundance at
138 the genus level.
139
140 **2.5. Biostability and re-growth assays**
141 The experiments were performed in 24 well plates. Untreated water and treated
142 water samples were inoculated with Synechocystis sp (PCC 6803) and incubated at room
143 temperature under controlled light conditions (100 µmol/m2.s). Prior to inoculation, cells
144 were washed three times with filtered tap water to remove residual orthophosphate. Tap
145 water and BG11 (Stanier et al., 1971) were used as negative and positive controls,
146 respectively. Optical density (680 and 750 nm) was measured every day for a period of
147 one week (Victor3 1420 Multilabel Counter, Perkin Elmer, USA). All samples were
148 tested in triplicate. To confirm that phosphorus was the growth limiting factor, the treated
149 water samples were supplemented with 1 mg PO_4^{-3}-P/L before being inoculated with
150 Synechocystis sp (PCC 6803) and cultivated under the conditions described above for
151 five instead of seven days.
152
153 **2.6. Analytical methods**
154 Samples taken from the recycling liquid were filtered (0.20 µm) prior to analysis.
155 Orthophosphate (PO4−3-P) was measured with inductive coupled plasma (ICP) (Perkin
156 Elmer Optima 5300 DV equipped with an optical emission spectrometer). Treated water
157 samples that were below the ICP detection limit of 0.020 mg PO_4^{-3}-P/L were analyzed
158 using a continuous flow analyzer (Skalar; Breda; the Netherlands). For this measurement,
159 molybdate and antimony potassium tartrate react with phosphate to give a phosphate-
160 complex. The continuous flow analyzer is an automated flowcell with an optical path

length of 50 cm that detects at 660 nm the reduction of the phosphate-complex by ascorbate. For each measurement, a calibration curve that consisted of seven standard solutions was used. In this manner, phosphate is accurately determined in the range of 1 to 50 µg/L. Ammonium was determined by ion chromatography (Metrohm Compact IC 761 equipped with a conductivity detector, using the pre-column Metrohm Metrosep A Supp 4/5 Guard and the column Metrohm Metrosep A Supp 5, 150/4.0 mm). Gas samples were analyzed with gas chromatography (Varian, CP-4900 equipped with a thermal conductivity detector using a Mol Sieve 5 Å PLOT 10 m column at 80 °C and a PoraPlot U 10 m column at 65 °C, and argon as carrier gas at 1.47 mL/min). To determine the P content of the biomass, 0.5 g were digested at 180 °C for 15 min (Milestone ETHOS 1) using 8 mL HNO3 (68%). After this digestion, the total P concentration was measured using ICP. The C, H, S and N content of the biomass was measured with an elemental analyzer (EA 1110, ThermoQuest CE Instruments, USA) utilizing a vertical quartz tube (combustion tube) maintained at 1000 °C with a constant flow of helium at 120 mL/min, an oxidation catalyst (WO3) zone, a copper zone followed by a Porapack PQS column maintained at 60 °C and finally, followed by a TCD detector.

2.7. Calculations

Orthophosphate removal (mg PO_4^{-3}-P/L.d) rate calculated as a function of the volume of treated water

$$\frac{P_i - P_o}{V \times T}$$

Orthophosphate removal rate (mgPO_4^{-3}-P/m^2.d) calculated as a function of the available biofilm surface area

184 $$\frac{P_i - P_o}{BSA \times T}$$

185 Pi Initial Orthophosphate concentration (mg PO_4^{-3}-P/L)

186 Po Final Orthophosphate concentration (mg PO_4^{-3}-P/L)

187 V Volume of treated water (L)

188 T Operational time (d)

189 BSA Biofilm surface area (m^2)

190

191 **3. Results and discussion**

192 **3.1. Proof of concept: reaching ultra-low orthophosphate concentrations**

193 At the start-up of the enrichment process, activated sludge was used to inoculate
194 the tricking filter reactor. This inoculum was chosen for its high microbial diversity
195 (Wang et al., 2019) therefore increasing the probability of selecting and enriching for a
196 robust biofilm community capable of removing orthophosphate under hydrogenotrophic
197 conditions. The reactor was continuously flushed with a mixture of hydrogen, oxygen and
198 carbon dioxide (Matassa et al., 2016). The recycling liquid, i.e. tap water enriched with
199 orthophosphate, ammonium, iron and trace elements, was circulated over the carrier
200 material and refreshed daily to select for hydrogen oxidizing bacteria. The residual PO_4^{-3}-
201 P concentration after the first day of operation decreased to 0.88 mg/L (i.e. removal of
202 13.7%). After three inoculation cycles, a visible biofilm colonized the carrier material. In
203 order to promote the establishment and further expansion of the biofilm, the recycling
204 liquid was refreshed daily for a period of 11 days. This action also allowed for the
205 removal of detached and planktonic biomass and to assure the selection for a HOB
206 community. After 11 days, the desired orthophosphate concentration was achieved
207 (below 0.020 mg PO_4^{-3}-P/L; removal of 99.2%) (Fig. 1).

3.2. The orthophosphate removal process depends on hydrogen as sole electron donor

To confirm the hydrogenotrophic character of the orthophosphate removal process, the reactor was operated under the same conditions as described in section 3.1 for three cycles of 24 h, followed by three cycles of 24 h in which hydrogen was replaced by nitrogen. In the three final cycles, the reactor was flushed with nitrogen (75%), oxygen (10%) and carbon dioxide (15%). As expected, during the first three cycles orthophosphate was removed within 24 h to a concentration below 0.020 mg PO_4^{-3}-P/L (Fig. 2) comparable to those reported in section 3.1. In the absence of hydrogen, the orthophosphate concentration of the untreated water (0.950 ± 0.08 mg PO_4^{-3}-P/L) and the treated water (0.920 ± 0.011 mg PO_4^{-3}-P/L) were very similar. These results confirm that hydrogen is essential for the removal of orthophosphate by the tricking filter.

3.3. Orthophosphate removal is biologically driven

Orthophosphate removal in the reactor might be driven by other removal principles than through biological activity. For instance, the iron which is present in the recycling liquid to stimulate hydrogen oxidation has a phosphate adsorbing capacity (Schink and Schlegel, 1978). To further confirm that orthophosphate was biologically removed, the total amount of removed orthophosphate (PO_4^{-3}-P) was compared with the total amount of phosphorus present in the HOB biomass. Because the amount of biomass present on the trickling filter cannot be accurately measured, nitrogen consumption was used to predict the amount of produced biomass. Nitrification was not detected, and hence ammonium and nitrate removal can only be explained by cell uptake. Elemental analysis was performed to determine nitrogen (1 mol biomass = 0. 177 mol N) and

phosphorus (biomass = 1.25% P) present in the biomass. A determined biomass formula was used over other empirical formula to allow for accurate estimations. The relation between the amount of phosphorus that was removed and the estimated amount of phosphorus present in the biomass was investigated. The linear relation between the two parameters supports the hypothesis of a biologically driven removal process. Further evidence that supports this expectation was provided by the observation that without biofilm or with inactivated biofilm on the carrier material, the orthophosphate removal capacity is inevitably hampered (orthophosphate removal below 5%).

3.4. Increased biofilm surface /water volume ratio results in faster removal

Based on the assumption that the efficiency of the phosphorus removal process is directly related to the amount of active biofilm – expressed in this section as the available surface area for biofilm growth – a set of experiments was performed to determine the effect of the ratio of biofilm surface area to recycling liquid volume on the orthophosphate removal rate. Fig. 3 shows the average removal of orthophosphate (PO_4^{-3}-P/L) by the hydrogenotrophic biofilm for a fed batch water contact volume of 10, 5 and 2 L which corresponds to 22, 43 and 108 m^2/m^3 water, respectively. For all tested ratios, a concentration below 0.020 mg PO_4^{-3}-P/L was reached within a period of 24 h, while the set orthophosphate concentration of the untreated water was not changed. Orthophosphate removal rates, however, increased up to a factor 10 from 0.94 ± 0.01 to 11.32 ± 0.43 mg PO_4^{-3}-P/L.d as the recycling volume decreased (Table 1). By increasing the ratio of biofilm surface to the treated water volume, the operational time was decreased to two hours whilst the residual orthophosphate concentration was kept below the detection limit. It therefore appears that the ratio of recycling liquid to biofilm surface

area is a parameter that can be used for process optimization according the required demands. It could also be that the wetting efficiency of the biomass was not optimal. Under the tested conditions, the lower the volume of liquid the higher was the contact opportunity between the liquid and the biofilm.

3.5. Bacterial community composition

For all experiments, the bacterial community composition of the biofilm was determined using Illumina MiSeq sequencing. After sequence data processing, an average (±standard deviation) of 40305 ± 4888 reads were obtained per sample distributed across 1143 ASVs. The subsequently rarefied dataset filtered to retain bacterial ASVs comprising > 0.1% in at least one of the samples comprised 330 ASVs across samples, covering 19 phyla, 34 classes, 69 orders, and 98 families. The acquired sequencing depth was sufficient to sample the bacterial diversity in all four samples (Fig. 4). Dominant bacterial families appear enriched from non-dominating members of the activated sludge community. This microbial analysis shows a selection towards a specialized community that evolved to stability as the reactor system continued to be fed batch operated over time. The specialized community is dominated by members of the families Burkholderiaceae, Flavobacteriaceae, Rhodocyclaceae. and Aquaspirillaceae, three of which have been identified as hydrogen oxidizers by (Schink and Schlegel, 1978) in their study on hydrogen metabolism.

3.6. Treated water does not support cyanobacterial growth

Experiments were conducted to (1) confirm that the treated water no longer supports cyanobacterial growth and (2) explore whether this inability to grow can be

explained by the absence of phosphorus. For this purpose, seven treated water samples that were obtained from independent trickling filter experiments were inoculated with the cyanobacteria Synechocystis sp (PCC 6803). As depicted in Fig. 5, all of the tested treated water samples as well as the tap water control samples did not support cyanobacterial growth. Growth was only observed in untreated water samples. The addition of 1 mg PO_4^{-3}-P/L to the treated water samples, which restored the orthophosphate concentration to that of the untreated water, did not promote growth either. This observation suggests that besides phosphorus another nutrient or nutrients were removed from the water matrix to levels that prevent algal growth. Indeed, 70% of the available iron and 100% of the available copper were removed during the orthophosphate removal process. Although their importance as a limiting factor of cyanobacterial growth is unclear, there is evidence such metals are used in a wide range of cell processes and thus we hypothesized their removal might explain the absence of cyanobacterial growth even when the treated water was re-spiked with orthophosphate.

3.7. Comparison with other orthophosphate removal strategies

Orthophosphate concentrations obtained in this study (in average 0.008 mg PO_4^{-3}-P/L), are comparable to the ones obtained by chemical precipitation. Newcombe et al. (2008) for instance have reported an average concentration of 0.011 mg PO_4^{-3}-P/L by co-precipitation with iron. Physical adsorption strategies have also been reported to reach concentrations lower than 0.01 mg PO_4^{-3}-P/L (Genz et al., 2004, Luo et al., 2016). hen enhanced biological phosphorus removal (EPBR) (Blackall et al., 2002, Boelee et al., 2011, De Vleeschauwer et al., 2019) is used, residual orthophosphate concentrations of 0.500 to 0.100 mg PO_4^{-3}-P/L are reported. These values are at least 10 times higher

304 relative to the residual concentrations reported in this work. Algae base technologies have
305 the potential to reach ultra-low nutrient loading (Gardner-Dale et al., 2017). Submerged
306 aquatic based vegetation wetlands have been shown to reach concentrations as low as
307 0.023 mg PO_4^{-3}-P/L (Dierberg et al., 2002, Healy et al., 2007).
308
309 **3.8. Towards circularity: Recovery and reuse of phosphorus**
310 The major driver for the development and implementation of innovative
311 technologies capable of removing phosphorus to extremely low concentrations, such as
312 the one presented in this work, is undoubtedly environmental preservation i.e. preventing
313 eutrophication. As paradoxical as it might seem, phosphorus is also a critical,
314 geographically concentrated and nonrenewable resource crucial to support global food
315 production. Hence, there is an added value in technologies that combine a highly efficient
316 removal process with a recovery step (Mayer et al., 2016). While recovery of the
317 removed phosphorus is not the focus of this research, it is a possibility that requires
318 further investigation. In the proposed system, phosphorus is removed from the recycling
319 liquid and immobilized within the microbial biomass. Since the process is dependent on
320 the presence of active microbial biomass, the outer part of the biofilm is regularly
321 removed by a back-wash treatment procedure. This harvested biomass could, for
322 instance, be used as an organic fertilizer. Indeed, several studies address the use of
323 microbial biomass as an organic slow-release fertilizer (Coppens et al., 2016, Yuan et al.,
324 2012).
325
326 **3.9. Practical implications and outlook**

This paper presents a novel biological strategy to remove orthophosphate down to levels below 0.010 mg/L, with the ultimate goal of preventing the regrowth of cyanobacteria in surface water. It should be noted that the scope of this study is limited to the proof-of-concept for the removal of soluble reactive phosphorus (orthophosphate). Phosphorus, however, is not always present in water matrices in the soluble reactive form (eg. 1 mg PO_4^{-3}-P /L as used in this proof of concept). Non-reactive forms of phosphorus can indeed be converted to reactive forms leading to new eutrophication events. In addition, scenarios with high process flow rates (e.g., large rivers) and low initial total phosphorus concentrations present a considerable challenge. As such, the practically of the hydrogenotrophic phosphorus removal should be further explored by monitoring both soluble and total phosphorus and by testing the reactor with decreasing phosphorus concentrations in continuous mode of operation over a long time.

4. Conclusions

Hydrogen oxidizing bacteria are shown to have the ability to remove orthophosphate down to levels below 0.01 mg PO4−3-P/L. An orthophosphate removal efficiency of 98 ± 2% and an orthophosphate removal rate of 11.32 ± 0.43 mg/L.h were reached for a ratio of biofilm surface area to treated liquid of 108 m^2/m^3. Treated water is shown not to support algae growth even when re-spiked with orthophosphate. The microbial analysis shows a selection towards a specialized HOB community that evolved to stability.

CRediT authorship contribution statement

Raquel G. Barbosa: Conceptualization, Investigation, Formal analysis, Writing - review & editing. Tom Sleutels: Conceptualization, Supervision, Review & editing. Willy

Verstraete: Conceptualization, Supervision, Review & editing. Nico Boon: Conceptualization, Supervision, Review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

1. Ashekuzzaman, J.Q, 2017. Improving the removal phosphate in secondary effluent of domestic wastewater treatment plant. CEST.
2. Blackall, L.L., Crocetti, G.R., Saunders, A.M., Bond, P.L., 2002. A review and update of the microbiology of enhanced biological phosphorus removal in

wastewater treatment plants. Antonie Van Leeuwenhoek. 81, 681–691. https://doi.org/10.1023/a:1020538429009

3. Boelee, N.C., Temmink, H., Janssen, M., Buisman, C.J.N., Wijffels, R.H., 2011. Nitrogen and phosphorus removal from municipal wastewater effluent using microalgal biofilms. Water Res. 45, 5925–5933. https://doi.org/https://doi.org/10.1016/j.watres.2011.08.044

4. Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M., Bolyen, E., Knight, R., Huttley, G.A., Gregory Caporaso, J., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6, 90. https://doi.org/10.1186/s40168-018-0470-z

5. Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugam, M., Asnicar, F., Bai, Y., Bisanz, J.E., Bittinger, K., Brejnrod, A., Brislawn, C.J., Brown, C.T., Callahan, B.J., Caraballo-Rodríguez, A.M., Chase, J., Cope, E.K., Da Silva, R., Diener, C., Dorrestein, P.C., Douglas, G.M., Durall, D.M., Duvallet, C., Edwardson, C.F., Ernst, M., Estaki, M., Fouquier, J., Gauglitz, J.M., Gibbons, S.M., Gibson, D.L., Gonzalez, A., Gorlick, K., Guo, J., Hillmann, B., Holmes, S., Holste, H., Huttenhower, C., Huttley, G.A., Janssen, S., Jarmusch, A.K., Jiang, L., Kaehler, B.D., Kang, K. Bin, Keefe, C.R., Keim, P., Kelley, S.T., Knights, D., Koester, I., Kosciolek, T., Kreps, J., Langille, M.G.I., Lee, J., Ley, R., Liu, Y.X., Loftfield, E., Lozupone, C., Maher, M., Marotz, C., Martin, B.D., McDonald, D., McIver, L.J., Melnik, A. V., Metcalf, J.L., Morgan, S.C., Morton, J.T., Naimey, A.T., Navas-Molina, J.A., Nothias, L.F., Orchanian, S.B., Pearson, T., Peoples, S.L., Petras, D., Preuss, M.L., Pruesse, E., Rasmussen, L.B., Rivers, A., Robeson, M.S., Rosenthal, P., Segata, N., Shaffer, M., Shiffer, A.,

399 Sinha, R., Song, S.J., Spear, J.R., Swafford, A.D., Thompson, L.R., Torres, P.J.,
400 Trinh, P., Tripathi, A., Turnbaugh, P.J., Ul-Hasan, S., van der Hooft, J.J.J., Vargas,
401 F., Vázquez-Baeza, Y., Vogtmann, E., von Hippel, M., Walters, W., Wan, Y., Wang,
402 M., Warren, J., Weber, K.C., Williamson, C.H.D., Willis, A.D., Xu, Z.Z., Zaneveld,
403 J.R., Zhang, Y., Zhu, Q., Knight, R., Caporaso, J.G., 2019. Reproducible, interactive,
404 scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol.
405 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9

406 6. Callahan, B.J., Mcmurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes,
407 S.P., 2016. DADA2 : High-resolution sample inference from Illumina amplicon data
408 Nat. Methods. 13, 581-583. https://doi.org/10.1038/nmeth.3869

409 7. Carpenter, S.R., Caraco, N.F., Correll, D.L., Howarth, R.W., Sharpley, A.N., Smith,
410 V.H., 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen.
411 Ecol. Appl. 8, 559–568. https://doi.org/10.1890/1051-
412 0761(1998)008[0559:NPOSWW]2.0.CO;2

413 8. Carvalho, L., McDonald, C., de Hoyos, C., Mischke, U., Phillips, G., Borics, G.,
414 Poikane, S., Skjelbred, B., Solheim, A.L., Van Wichelen, J., Cardoso, A.C., Cadotte,
415 M., 2013. Sustaining recreational quality of European lakes: minimizing the health
416 risks from algal blooms through phosphorus control. J. Appl. Ecol. 50, 315–323.
417 https://doi.org/10.1111/1365-2664.12059

418 9. Coppens, J., Grunert, O., Van den Hende, S., Vanhoutte, I., Boon, N., Haesaert, G.,
419 De Gelder, L., 2016. The use of microalgae as a high-value organic slow-release
420 fertilizer results in tomatoes with increased carotenoid and sugar levels. J. Appl.
421 Phycol. 28, 2367–2377. https://doi.org/10.1007/s10811-015-0775-2

10. De Vleeschauwer, F., Caluwé, M., Dobbeleers, T., Stes, H., Dockx, L., Kiekens, F., D'Aes, J., Copot, C., Dries, J., 2019. Performance and stability of a dynamically controlled EBPR anaerobic/aerobic granular sludge reactor. Bioresour. Technol. 280, 151–157. https://doi.org/https://doi.org/10.1016/j.biortech.2019.02.052

11. Dierberg, F.E., DeBusk, T.A., Jackson, S.D., Chimney, M.J., Pietro, K., 2002. Submerged aquatic vegetation-based treatment wetlands for removing phosphorus from agricultural runoff: response to hydraulic and nutrient loading. Water Res. 36, 1409–1422. https://doi.org/https://doi.org/10.1016/S0043-1354(01)00354-2

12. Dodds, W.K., Bouska, W.W., Eitzmann, J.L., Pilger, T.J., Pitts, K.L., Riley, A.J., Schloesser, J.T., Thornbrugh, D.J., 2009. Eutrophication of U.S. freshwaters: analysis of potential economic damages. Env. Sci Technol 43, 12–19. https://doi.org/10.1021/es801217q

13. EuropeanCommission, 2017. 9th Technical assessment of information on the implementation of Council Directive https://doi.org/10.1016/j.apgeochem.2013.05.015

14. Gardner-Dale, D.A., Bradley, I.M., Guest, J.S., 2017. Influence of solids residence time and carbon storage on nitrogen and phosphorus recovery by microalgae across diel cycles. Water Res. 121, 231–239. https://doi.org/https://doi.org/10.1016/j.watres.2017.05.033

15. Genz, A., Kornmuller, A., Jekel, M., 2004. Advanced phosphorus removal from membrane filtrates by adsorption on activated aluminium oxide and granulated ferric hydroxide. Water Res. 38, 3523–3530. https://doi.org/10.1016/j.watres.2004.06.006

16. Gu, A.Z., Liu, L., Neethling, J.B., Stensel, H.D., Murthy, S., 2011. Treatability and fate of various phosphorus fractions in different wastewater treatment processes. Water Sci. Technol. 63, 804–810. https://doi.org/10.2166/wst.2011.312

17. Healy, M.G., Rodgers, M., Mulqueen, J., 2007. Treatment of dairy wastewater using constructed wetlands and intermittent sand filters. Bioresour. Technol. 98, 2268–2281. https://doi.org/https://doi.org/10.1016/j.biortech.2006.07.036

18. Hosseini, S.E., Wahid, M.A., 2016. Hydrogen production from renewable and sustainable energy resources: Promising green energy carrier for clean development. Renew. Sustain. Energy Rev. 57, 850–866. https://doi.org/https://doi.org/10.1016/j.rser.2015.12.112

19. Karanasios, K.A., Vasiliadou, I.A., Pavlou, S., Vayenas, D. V, 2010. Hydrogenotrophic denitrification of potable water : A review. J. Hazard. Mater. 180, 20–37. https://doi.org/10.1016/j.jhazmat.2010.04.09

20. Luo, X., Wang, X.R., Bao, S.P., Liu, X.W., Zhang, W.C., Fang, T., 2016. Adsorption of phosphate in water using one-step synthesized zirconium-loaded reduced graphene oxide. Sci. Rep. 6. https://doi.org/Artn 3910810.1038/Srep39108

21. Lurling, M., van Oosterhout, F., 2013. Controlling eutrophication by combined bloom precipitation and sediment phosphorus inactivation. Water Res. 47, 6527–6537. https://doi.org/10.1016/j.watres.2013.08.019

22. Matassa, S., Verstraete, W., Pikaar, I., Boon, N., 2016. Autotrophic nitrogen assimilation and carbon capture for microbial protein production by a novel enrichment of hydrogen-oxidizing bacteria. Water Res. 101, 137–146. https://doi.org/10.1016/j.watres.2016.05.077

467 23. Mayer, B.K., Baker, L.A., Boyer, T.H., Drechsel, P., Gifford, M., Hanjra, M.A.,
468 Parameswaran, P., Stoltzfus, J., Westerhoff, P., Rittmann, B.E., 2016. Total value of
469 phosphorus recovery. Env. Sci Technol. 50, 6606–6620.
470 https://doi.org/10.1021/acs.est.6b01239

471 24. Mohsin, M., Rasheed, A.K., Saidur, R., 2018. Economic viability and production
472 capacity of wind generated renewable hydrogen. Int. J. Hydrogen Energy. 43, 2621–
473 2630. https://doi.org/https://doi.org/10.1016/j.ijhydene.2017.12.113

474 25. Nadell, C.D., Drescher, K., Foster, K.R., 2016. Spatial structure, cooperation and
475 competition in biofilms. Nat. Rev. Microbiol. 14, 589–600.
476 https://doi.org/10.1038/nrmicro.2016.84

477 26. Naz, I., Saroj, D.P., Mumtaz, S., Ali, N., Ahmed, S., 2015. Assessment of biological
478 trickling filter systems with various packing materials for improved wastewater
479 treatment. Environ. Technol. 36, 424–434.
480 https://doi.org/10.1080/09593330.2014.95140

481 27. Newcombe, R.L., Strawn, D.G., Grant, T.M., Childers, S.E., Moller, G., 2008.
482 Phosphorus removal from municipal wastewater by hydrous ferric oxide reactive
483 filtration and coupled chemically enhanced secondary treatment: part II--mechanism.
484 Water Env. Res. 80, 248–256. https://doi.org/10.2175/106143007x220987

485 28. Paerl, H., 2017. The cyanobacterial nitrogen fixation paradox in natural waters.
486 F1000Res. 6, 244. https://doi.org/10.12688/f1000research.10603.1

487 29. Paerl, H.W., Huisman, J., 2008. Climate. Blooms like it hot. Science. 320, 57–58.
488 https://doi.org/10.1126/science.1155398320/5872/57

30. Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ. Microbiol. 18, 1403–1414. https://doi.org/10.1111/1462-2920.13023

31. Pedregosa, F., Ga, Varoquaux, l, Gramfort, A., Michel, V., Thirion, B., Grisel, O., Blondel, M., Prettenhofer, P., Weiss, R., Dubourg, V., Vanderplas, J., Passos, A., Cournapeau, D., Brucher, M., Perrot, M., #201, Duchesnay, douard, 2011. Scikit-learn: Machine Learning in Python. J. Mach. Learn. Res. 12, 2825–2830

32. Quince, C., Lanzen, A., Davenport, R.J., Turnbaugh, P.J., 2011. Removing noise from pyrosequenced amplicons. BMC Bioinformatics 12, 38. https://doi.org/10.1186/1471-2105-12-38

33. Repaske, R., Mayer, R., 1976. Dense autotrophic cultures of Alcaligenes eutrophus. Appl Env. Microbiol 32, 592–597.

34. Richardson, C.J., King, R.S., Qian, S.S., Vaithiyanathan, P., Qualls, R.G., Stow, C.A., 2007. Estimating ecological thresholds for phosphorus in the Everglades. Env. Sci Technol 41, 8084–8091. https://doi.org/10.1021/es062624w

35. Schindler, D.W., Hecky, R.E., Findlay, D.L., Stainton, M.P., Parker, B.R., Paterson, M.J., Beaty, K.G., Lyng, M., Kasian, S.E.M., 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. Proc. Natl. Acad. Sci. U. S. A. 105, 11254–11258. https://doi.org/10.1073/pnas.0805108105

510 36. Schink, B., Schlegel, H.-G., 1978. Hydrogen metabolism in aerobic hydrogen-
511 oxidizing bacteria. Biochimie 60, 297–305.
512 https://doi.org/https://doi.org/10.1016/S0300-9084(78)80826-
513 37. Sinha, E., Michalak, A.M., Balaji, V., 2017. Eutrophication will increase during the
514 21st century as a result of precipitation changes. Science. 357, 405–408.
515 https://doi.org/10.1126/science.aan2409
516 38. Stanier, R.Y., Kunisawa, R., Mandel, M., Cohen-Bazire, G., 1971. Purification and
517 properties of unicellular blue-green algae (order Chroococcales). Bacteriol. Rev. 35,
518 171–205.
519 39. USEPA, 2000. Nutrient Criteria Technical Guidance Manual-Lakes and Reservoirs.
520 40. Wang, R., Wilfert, P., Dugulan, I., Goubitz, K., Korving, L., Witkamp, G.-J., van
521 Loosdrecht, M.C.M., 2019. Fe(III) reduction and vivianite formation in activated
522 sludge. Sep. Purif. Technol. 220, 126–135.
523 https://doi.org/https://doi.org/10.1016/j.seppur.2019.03.024
524 41. Wik, T., 2003. Trickling Filters and Biofilm Reactor Modelling. Rev. Environ. Sci.
525 Biotechnol. 2, 193–212. https://doi.org/10.1023/B:RESB.0000040470.48460.bb
526 42. Yuan, Z.G., Pratt, S., Batstone, D.J., 2012. Phosphorus recovery from wastewater
527 through microbial processes. Curr. Opin. Biotechnol. 23, 878-883 https://doi.org/
528 10.1016/j.copbio.2012.08.001
529
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Tables and Figures

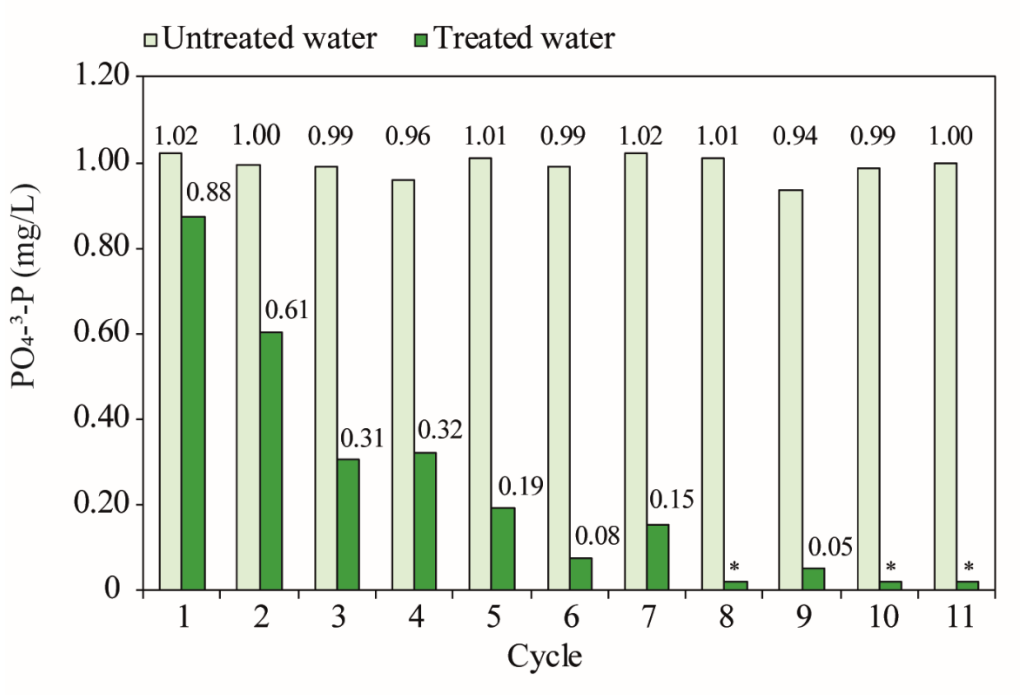


Figure 1. Establishment of the hydrogen oxidizing biofilm. Untreated water (light green) and treated water (dark green) orthophosphate concentrations (mg PO_4^{3-}-P/L) over a period of 11 fed batches (1 batch = 1 day). Fresh liquid was prepared daily and subjected to treatment. orthophosphate concentrations (single measurements) below the normal detection limit (< 0.020 mg PO_4^{3-}-P /L) are marked with asterisks.

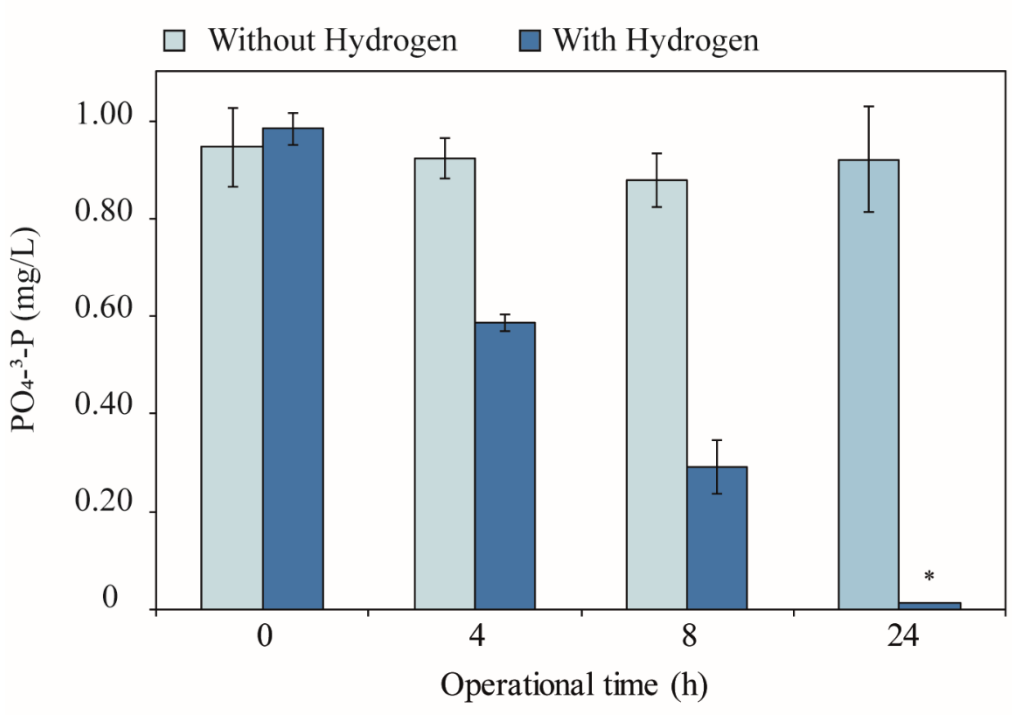
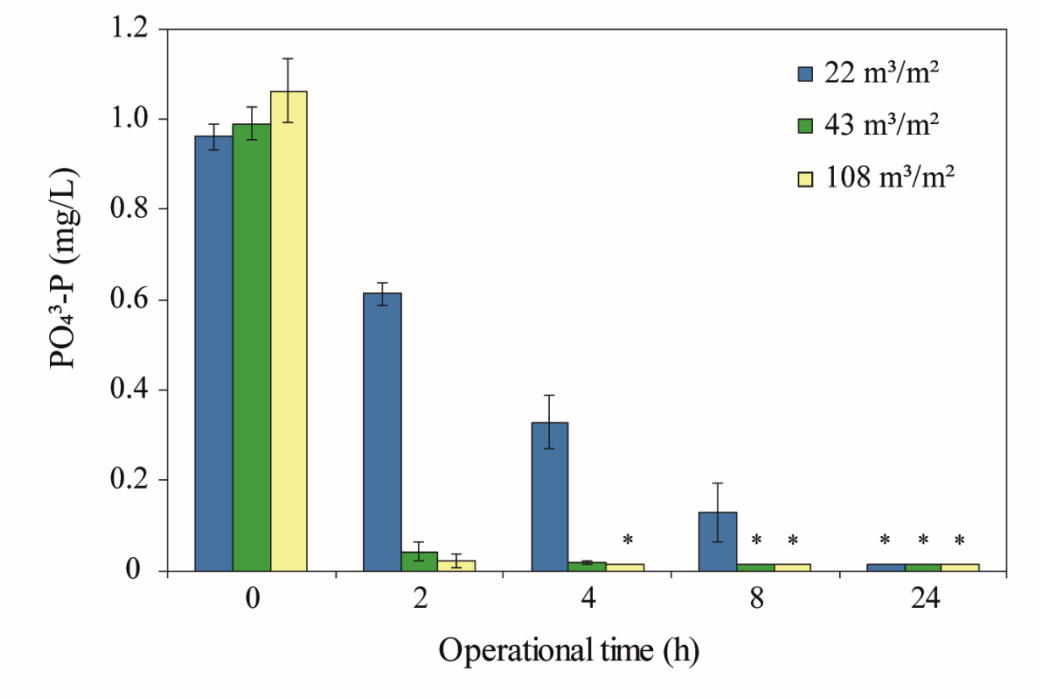


Figure 2. Residual orthophosphate (mg PO_4^{-3}-P/L) concentrations in the presence (dark blue) and absence (light blue) of hydrogen. Assays were performed over a period of 24 h, in triplicate. Orthophosphate concentrations below the normal detection limit (< 0.020 mg PO_4^{-3}-P/L) are marked with asterisks. Error bars represent standard deviation.



547

548 Figure 3. Residual orthophosphate concentration (mg PO_4^{-3}-P/L) over time.

549 Experiments were performed in fed batch mode for three distinct ratios of biofilm surface

550 area/ water volume to be treated, 22 (blue), 43 (green) and 108 (yellow) m^2/m^3 (n= 6).

551 Orthophosphate concentrations below the normal detection limit (< 0.020 mg PO_4^{3-}-P/L)

552 are marked with asterisks.

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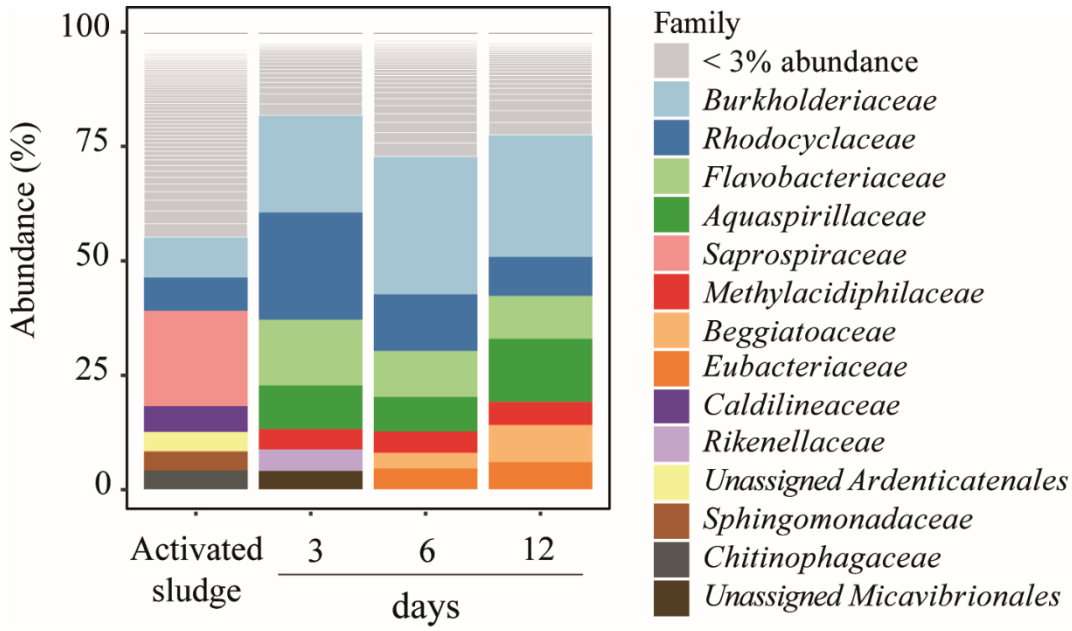

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555 Figure 4. Bacterial community structure. Structure is presented as relative

556 abundance of bacterial families, where families comprising less than 3 % of sequence reads

557 in each sample are pooled and unspecified.

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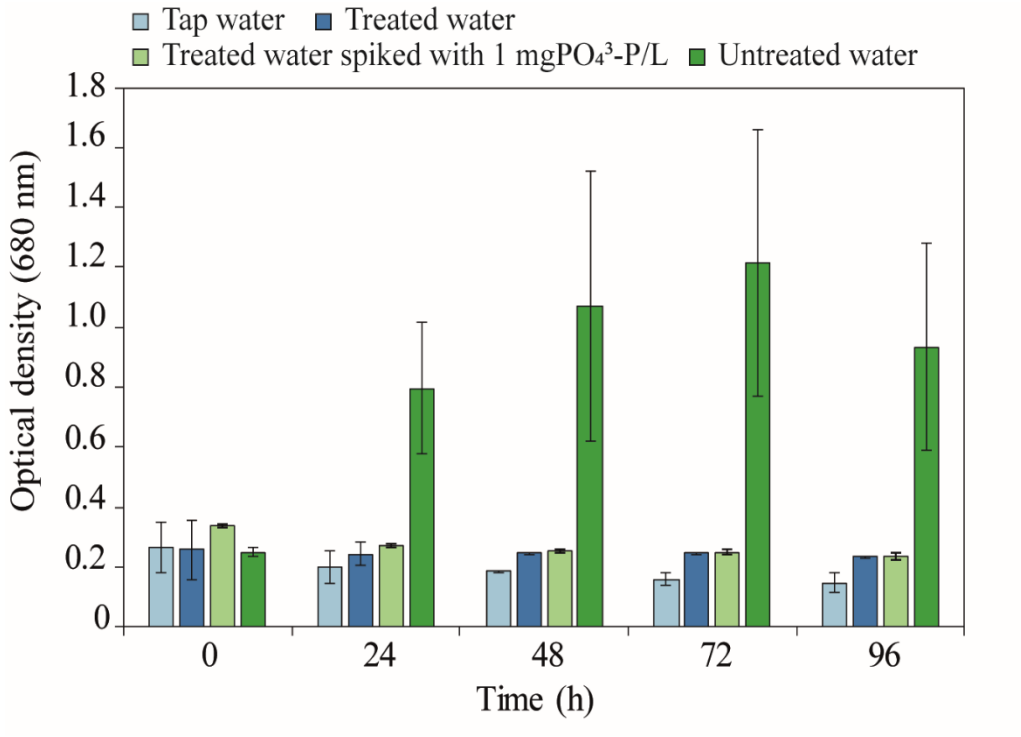

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560 Figure 5. Biostability assay. Tap water (negative control), untreated water (positive

561 control), treated water and treated water spiked with 1mg PO_4^{-3}-P/L were inoculated with

562 cyanobacteria PCC6803. Growth was evaluated by daily optical density measurements at

563 680 nm (n= 7).

564 **Table 1** Main parameters for orthophosphate removal using different specific surface areas

Biofilm surface area/ volume of fed batch (m^2/m^3)	22	43	108
Untreated water P (mg PO_4^{-3}-P/L)	0.946 ± 0.012	0.989 ± 0.037	1.06 ± 0.071
Residual orthophosphate (mg PO_4^{-3}-P/L)	0.008 ± 0.009	0.008 ± 0.009	0.008 ± 0.009
Orthophosphate Removal rate (mg PO_4^{-3}-P/L.d)	0.94 ± 0.01	7.20 ± 0.28	11.23 ± 0.43
Orthophosphate Removal rate (mg PO_4^{-3}-P/m^2.d)	42.90 ± 0.56	193.83 ± 29	104.85 ± 20
Untreated water NH_4 (mg NH_4 /L)	20 ± 1	17 ± 2	20 ± 3
Residual NH_4 (mg NH_4 /L)	10±1	10± 1	11± 2

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