Improvement of wastewater treatment capacity using the microalga *Scenedesmus* sp. and membrane bioreactors

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Abstract

Primary urban wastewater was processed using the microalga *Scenedesmus* sp. in an outdoor pilot-scale raceway reactor connected to an ultrafiltration membrane. The goal was to separate cellular retention time from hydraulic retention time. This strategy led to a 129.3% increase in the daily volume of wastewater treated per square meter, and to a 48.7% increase in biomass productivity to a final value of 22.2 ± 1.9 $g \cdot m^{-2} \cdot day^{-1}$. Nutrient removal was highly influenced by permeate rate, allowing to remove up to 0.65 $mg \cdot m^{-2} \cdot day^{-1}$ of phosphates. Over 99% of ammonia was removed when the ultrafiltration membrane was used, although this was partially due to nitrate production by nitrifying bacteria: higher permeate rates led to higher relative abundance of the nitrifying bacterial. The amplification and sequencing of the microalgae-bacteria samples led to the detection of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria, such as *Bradyrhizobiaceae*, *Nitrospiraceae*, *Nitrosomonadaceae*, and *Chromatiaceae*. The most abundant families detected in the microalgae-bacteria biomass were Rhodobacteraceae and Comamonadaceae.

Keywords: *Scenedesmus* sp., bioremediation, raceway reactor, taxonomic classification, nitrification, biomass, ultrafiltration.

1. Introduction

Worldwide, one in three people do not have access to safe drinking water. A 40% deficit in freshwater resources expected during the next decade, together with an increasing global population, have our planet heading towards a global water crisis. Moreover, many of our water resources are contaminated and there is a lack of technologies to reclaim used water, enlarging even more the water problem. The 2030 Agenda for Sustainable Development, adopted by all United Nations Member States, highlights this problem as Goal 6: Ensure access to water and sanitation for all.

Although nitrogen and phosphorus occur naturally, most of the nitrogen and phosphorus in our water streams come from human activities and sources (fertilisers, urban and industrial wastewater, animal waste, etc.). Despite being essential for plant growth, overabundance of nutrients in water, primarily nitrogen and phosphorus, leads to eutrophication: approximately 40-50% of lakes and water reservoirs are eutrophic in North America, Asia, Europe, and South America, and 28% in Africa [1]. Conventional wastewater treatment processes have several limitations, which include high-energy requirements, poor nutrient removal yields, and high environmental impact, the latter mainly caused due to the emission of greenhouse gases [2,3]. The development of sustainable processes is of key importance for the future, and microalgae-based processes are called to play a key role in coming years. Microalgae are attracting increased attention in the context of bioeconomy, especially in terms of bioremediation because of their dual role: they can “clean” wastewater while producing valuable biomass with applications in agriculture and other industrial applications [4,5]. Also, the feasibility of using microalgae for wastewater treatment has been reported by a large number of research groups [6–8] and these valuable microorganisms are well accepted by consumers [9].

Several different photobioreactors have been proposed for microalgae production, but only a small number of designs are being used for mass production. Because of their ease of use and low costs, raceways are the most commonly used bioreactors. Raceway designs have another advantage in terms of wastewater treatment: because of their relatively high culture depth (generally between the range 0.10-0.30 m), they allow to process large volumes of wastewater per surface area. The use of membrane bioreactors, which is a combination of bioreactors and membranes, has been proposed as an innovative strategy to process even larger amounts of water, increasing hydraulic retention times while keeping cellular retention times constant [10].

Production of microalgae in wastewater involves the association of microalgae with aerobic and anaerobic microorganisms present in the media. Microalgae are photosynthetic microorganisms and therefore, produce oxygen. Oxygen produced by microalgae supports not only the growth of heterotrophic microorganisms, namely bacteria, but also the degradation of organic compounds [11]. The composition of the microalgae-bacteria consortia is important to achieve high nutrient removal rates. However, data on the effect of operational and environmental conditions on the composition of the microalgae-bacteria consortium are scarce [12,13]. These data are important for the correct design of the process, as certain factors can promote the growth of unwanted microorganisms such as nitrifying bacteria, which reduces microalgal consumption of nitrogen.

The main goal of the current study was to assess the potential of the microalga *Scenedesmus* sp. as a biological system to recover nutrients, namely nitrogen and phosphorus, from urban wastewater using a raceway reactor. The current study also aimed at evaluating the potential use of membrane technologies to increase the

amount of water processed daily and to identify the effect of operational conditions on the composition of the microalgae-bacteria consortia by illumine sequencing.

2. Materials and methods

2.1. Microorganism and culture media

The selected strain was the freshwater *Scenedesmus* sp. This strain has been widely utilised for outdoor production because of its high tolerance to adverse conditions [14]. Briefly, *Scenedesmus* sp. was produced in 3.0 m^3 capacity tubular photobioreactors operated under continuous more. The culture medium used was a modification of the Arnon medium [15], prepared following industrial practices and using commercial-grade fertilisers instead of pure chemicals. The inoculum was transferred to pilot-scale raceways pre-filled with primary domestic wastewater, obtained from a sewage treatment plant operated by FCC AQUALIA S.A. in Almeria (Spain). The average composition of the culture medium and the wastewater used during the experiments is listed in Table 1.

2.2. Experimental set-up

Experiments were conducted with a raceway reactor located at the pilot plant facilities of Las Palmerillas Research Centre of the Cajamar Foundation in Almería, Spain. The area of the reactor was 32 m^2 and culture depth was kept constant at 0.12 m (4.4 m^3). The pH, temperature, and dissolved oxygen concentration of the culture were measured using 5083T and 5120 probes (Crison Instruments, Spain) connected to an MM44 control-transmitter unit (Crison Instruments, Spain). Labview data acquisition software (National Instruments, Texas, US) provided complete data monitoring and control of the installation. The gas flow rate entering the reactor was measured by a

PFM 725S-F01-F mass flow meter (SMC, Tokyo, Japan). The pH of the culture was controlled at 8.0 ± 0.2 by on-demand injection of CO_2 at 10 $L \cdot min^{-1}$. Air was supplied to the reactor using a blower providing 350 mbar overpressure, through a fine bubble diffuser AFT2100 (ECOTEC, Barcelona, Spain), at 100 $L \cdot min^{-1}$ when no CO_2 is demanded and dissolved oxygen overpasses 250%Sat. The reactor was operated in continuous mode with a dilution rate of 0.2 day^{-1}, which means that 20% of the culture was daily replaced with fresh medium (in this case, wastewater). Only data from the steady-state conditions were used. Evaporation inside the reactor was compensated by daily addition of fresh water.

As the wastewater used had a low nutrient content, a 10 m^2 Bio-Cell BC10 ultrafiltration membrane (Ecotec S.A., Spain) was used to separate cell residence time, or cellular retention time (CRT), from the hydraulic retention time (HRT) (Figure 1). The membrane was installed inside the sump of the reactor and was used to separate the water from the cells, keeping the latter in the reactor and allowing the release of clean water outside of the reactor. The culture was passed through the membrane using an HP 6/11 magnetically coupled plastic centrifugal pump (Harton Anlagentechnik GmbH, Germany) operating at a flowrate of 6.2 $L \cdot min^{-1}$. A MEDO LA-120 aerator compressor (Nitto Kohki Ltd., US) was also connected to the membrane to minimise the accumulation of microalgal cells on the membranes' surface and minimise the water flux loss. The airflow of the compressor was set at 120 $L \cdot min^{-1}$ and the compressor worked continuously during the filtration process. The permeate flux varied from 0 (control) to 40% of the volume of the culture. A permeate flux of 40% means that 40% of the total volume of the culture was filtered, the permeate removed from the system, and replaced with fresh medium. The HRT in this case would be 0.6 day^{-1} (dilution rate plus permeate rate) while the CRT would be 0.2 day^{-1}. Taking into

account that the dilution rate was 0.2 day^{-1}, a permeate rate of 40% means that it takes 1.67 days to renew the whole culture volume.

2.3. Analytical determinations

Chlorophyll fluorescence (F_v/F_m) was determined with an AquaPen AP 100 fluorometer (Photon System Instruments, Czech Republic). Microalgae biomass concentration was calculated by dry weight by filtering 100 mL aliquots of the culture through Macherey-Nagel glass fiber MN 85/90 and drying in an oven at 80 ºC for 24 h. Standard official methods approved by the Spanish Ministry of Agriculture were used to analyse the composition of the primary wastewater and the microalgae-bacteria samples (biomass) [16]. Phosphorus was measured by visible spectrophotometry through the phospho-vanado-molybdate complex. In addition, nitrates were quantified spectrophotometrically at 220-275 nm using a GENESYS 10S UV-Vis spectrophotometer (Thermo Fisher Scientific, Spain). Ammonium was measured using the Nessler reactive method and the chemical oxygen demand (COD) was determined by spectrophotometric measurement using Hach-Lange kits (LCI-400).

2.4. Genomic Analysis

Microbial communities present in the cultures were identified by genomic analyses using an Illumina MiSeq system (Illumina, San Diego, CA, US) as described previously [13]. The QIIME pipeline was used to process the sequencing data using the “closed-reference” OTU picking strategy [17].

2.5. Statistical analysis

Differences between measurements, conducted in triplicate, were analysed using analysis of variance (ANOVA) with JMP 13 (SAS Institute Inc., USA). A Tukey pairwise

comparison of the means was conducted to identify where sample differences occurred. The criterion for statistical significance was in all cases $p<0.05$.

3. Results and discussion

3.1. Wastewater processing capacity and biomass productivity

Different types of wastewater and other wastes have been evaluated as potential nutrient sources for microalgae production, including municipal, agricultural, and industrial wastewaters [7]. Microalgae show huge potential for being used as a supplement for tertiary wastewater treatments. However, microalgae-based wastewater treatments have an important limitation: the processing of the extremely high amounts of wastewater produced require very large bioreactors and the use of large surface areas. For example, to process wastewater from a city with approximately 200,000 inhabitants, it would be necessary to use a bioreactor larger than 100 ha. When waste streams have a high nutrient concentration, they can be diluted to the optimum nutrient content for microalgal growth achieving high productivities. However, when wastewater is low in nutrients, biomass productivities are below the optimal. In this sense, it has been suggested that by incorporating membrane technologies into microalgae bioreactors, it would be possible to process larger amounts of water with low nutrient concentration by decreasing hydraulic retention times while keeping cellular retention times constant [10]. In the current study, increasing permeate rates led to lower hydraulic retention times, demonstrating the potential of this strategy to process a larger amount of water per surface area (Figure 2A). Indeed, the volume of water that could be processed when using a permeate rate of 0.4 day^{-1} was 80.6 ± 6.2 $L \cdot m^{-2} \cdot day^{-1}$ while only 35.2 ± 4.1 $L \cdot m^{-2} \cdot day^{-}$

$^{-1}$ could be processed without the use of membranes (Figure 2B). Both, the hydraulic retention time and the amount of wastewater that could be processed were affected by permeate rate (p<0.001).

Increasing permeate rate not only affected the volume of water that could be processed but also biomass productivity. Indeed, biomass productivity increased from 15.2 ± 2.1 to 22.2 ± 1.9 g·m^{-2}·day^{-1} when operating at a permeate rate of 0.0 (control) or 0.3 day^{-1} respectively (p<0.05; Figure 3A). Higher permeation rates led to higher biomass productivity, attributed to higher nutrient availability. However, increasing the permeate rate over 0.2 day^{-1} did not lead to higher productivities, probably because the maximum biomass productivity of the reactor, at a dilution rate of 0.2 day^{-1}, was achieved. Indeed, although the theoretical productivity of raceway reactors can be as high as 40 g·m^{-2}·day^{-1}, experimental values are generally in the range 20-25 g·m^{-2}·day^{-1} because current raceway design do not allow to optimise light utilisation [18]. It is accepted that the irradiance inside the cultures is negatively affected by culture depth and biomass concentration because of the known as self-shading or shadow effect of microalgae. Still, biomass productivity values were in line with those reported previously in the same region [16,19]. Different strategies have been used to increase biomass productivity in wastewater treatment processes. For example, a novel attached-growth photobioreactor equipped with cylindrical glass rods was designed to facilitate harvesting [20]. A batch experiment conducted using this reactor (3.5 L) allowed biomass concentration values of 0.25 and 0.77 g·L^{-1} when operated using wastewater with low and high nutrient content respectively. These were higher than the conventional suspended system that allowed reaching concentrations of of 0.14 and 0.29 g·L^{-1} respectively [20]. This system was further validated in other studies that allowed not only higher biomass concentrations but also higher lipid productivities [21].

The use of thin-layer cascade reactors has been also studied as a potential strategy to increase biomass productivity at pilot-scale. The low culture depth of these systems permits a high availability of light and therefore higher productivities, but the volume of water that can be processed per surface area is lower. The use of thin-layer reactors and membrane technologies has not yet been assessed, up to the best of the authors' knowledge, but this strategy shows potential for industrial implementation. Moreover, permeate rate did not affect F_v/F_m values, shown in Figure 3B. This parameter measures the performance of the photochemical processes in the PSII complex [22]. The optimal F_v/F_m value for any microalgal strain is 0.6-0.7, demonstrating that microalgae were grown without stress conditions. These values were in line with those previously reported for wastewater treatment processes [13].

Briefly, the utilisation of membranes, and increasing the permeate rate to 0.4 day^{-1}, led to a 129.3% increase in the daily wastewater volume processed per square meter. Biomass productivity increased by an average of 48.7%. Extrapolating this value to a hypothetical 1 ha facility, the use of membranes would allow to process 2.58 M m^3 of primary wastewater while producing 79.92 Tn of valuable biomass. Membranes are already being used in conventional wastewater treatment processes because they allow improving the performance of these systems. Their industrial interest led to the development and commercialisation of reliable and low-cost membranes. Their utilisation increases wastewater treatment costs, both in terms of investment and operation (energy consumption). The current study demonstrated the potential of membranes for increasing microalgae productivity. An economic analysis must be performed based on data from larger-scale facilities.

3.2. Nutrient removal

Besides producing valuable biomass and increasing the amount of wastewater that can be processed, the current paper aimed at maximising nutrient removal per surface area. In this sense, the inlet and outlet content of ammonia, nitrates, and phosphates were measured. The composition of wastewaters varies with sources [1]. In the current study, wastewater used for microalgal growth was especially rich in ammonia and phosphates although it also had lower concentrations of nitrates – Table 1. The composition of wastewater in the inlets varies because more than one week is needed to achieve a steady-state and not all the experiments were performed on the same day.

The content of ammonia in the inlet stream varied between 60 and 140 $mg \cdot L^{-1}$ for the different experiments. The ammonia content in the outlet was low for all the studied operational conditions ($p<0.05$). As shown in Figure 4A, ammonia removal was significantly affected by permeate rate, increasing from 34.2 ± 2.4 $mg \cdot L^{-1} \cdot day^{-1}$ to 76.1 ± 4.1 $mg \cdot L^{-1} \cdot day^{-1}$ when operating at permeate rates of 0.0 and 0.4 day^{-1}, respectively (representing a 122.5% increase). Results were in line with those reported previously for wastewater treatment of wastewater using *Scenedesmus* in laboratory-scale trials [23]. When operating with the membrane, the ammonia content of the outlet was below 1 $mg \cdot L^{-1}$. The observed removal increase can be attributed not to a higher removal efficiency but to a larger amount of wastewater processed in the latter. When operating at a permeate rate of 0.4 day^{-1}, ammonia removal was even higher than when using thin-layer cascade reactors (15.0-30.6 $mg \cdot L^{-1} \cdot day^{-1}$), which are more productive than raceways [13]. In addition, the nitrate content in the inlet and outlet of the raceway reactor is shown in Figure 4B. The nitrate content of the wastewater (inlet) was relatively low and was around 5 $mg \cdot L^{-1}$ for all the experimental runs. The nitrate content in the outlet was higher than in the inlet for all the studied conditions ($p<0.05$),

suggesting that ammonia was not only consumed by microalgae but also used by nitrifying bacteria to produce nitrates. The effect of processing on the content of nitrifying bacteria will be discussed in the following section.

The phosphate content in the inlet and outlet of the reactors as well as the daily removal of phosphates are shown in Figure 4C. Metabolism of both microalgae and bacteria is dependent on phosphorus and phosphorylation [24]. Both phosphorus and nitrogen are the key nutrients of concern in eutrophication and are limiting factors in many growth scenarios [25]. The potential of microalgae from the *Scenedesmus* genus to remove not only nitrogen but also phosphorus from wastewaters has been demonstrated previously (Sánchez Zurano et al., 2020; Tomás-Almenar et al., 2018). Phosphate removal increased with increased permeate rate: when operating using a permeate rate of 0.4 day^{-1}, phosphorus removal increased by 294.6% when compared to the control without the membrane. This can be attributed to a larger volume of water processed but also to a higher nitrogen content in the inlet because previous reports demonstrated that phosphorus removal improved with increased nitrogen supply – while nitrogen removal was independent of phosphorus concentration [27]. Moreover, the COD was also determined at the reactors' inlet and outlet. Results, shown in Figure 4D, demonstrated a large variability in the COD content of the wastewaters used. Differences between the inlets and the outlets were significant, being lower than 80 $mg \cdot L^{-1}$ in all cases ($p<0.05$). Similar results were observed in a recent study where *Scenedesmus rubescens* was integrated into a wastewater treatment process achieving a decrease in COD from 400 $mg \cdot L^{-1}$ in the inlet to approximately 100 $mg \cdot L^{-1}$ after 8 days [28]. Operating at higher permeate rates led to a higher COD removal, mainly caused by a larger volume of processed wastewater. Results demonstrated a huge capacity of microalgae and bacteria to remove organic matter and depurate high

COD concentrations as those evaluated in the current study (300-900 $mg \cdot L^{-1}$). COD removal values shown in Figure 3D correspond to depuration efficiencies greater than 80%, which compared well with recent reports [13,29].

Overall, results demonstrated a high potential of the microalgae-bacteria consortium to remove nitrogen, phosphorus, and organic matter from primary wastewater. Nutrient removal was highly influenced by permeate rate, mainly caused by the larger wastewater volume processed. Results reported herein, obtained operating a pilot-scale reactor located outdoors are promising, as they suggest higher nitrogen and phosphorus removal rates than in previous reports conducted indoors under controlled conditions [30]. Up-scaling the reactor could potentially lead to nitrogen (ammonia plus nitrate) and phosphorus removals of 11.8 and 2.36 $kg \cdot year^{-1}$ respectively.

3.3. Taxonomic analysis

Microalgae-bacteria interactions are determined by multiple factors: (i) microalgal and bacterial strains (as the interactions are species-specific), (ii) composition of the microalgal cell wall, (iii) nutrient availability in the culture, and (iv) cultivation conditions such as dilution rate and HRT [31] . Besides, plenty of environmental fluctuations occur during the biological process. These include variations in light intensity, mixing, or temperature and these can affect not only the interactions between them but also the community structure or composition of the consortia [13,32]. To study the effect of the permeate rate on the composition of the microalgae-bacteria consortia, the composition of the biomass was studied using Illumina sequencing. The phylogenetic diversity was determined in the cultures after filtering for quality, trimming length, and assigning taxonomies. All the samples were classified according to their phylum, class, order, and family (Figure 5). The microbial community consisted of 16 phyla, 21

classes, and 31 orders – only the taxa with a relative abundance higher than 1.0% were considered. On the family level, only taxa with relative abundance higher than 0.1% were considered (52 families).

Results indicate that the most abundant phyla identified in all samples were Proteobacteria (51-54%), Bacteroidetes (12-20%), Actinobacteria (5-11%) and Verrucomicrobia (3-8%). Minoritarian phyla such as Planctomycetes (3-5%), Chloroflexi (3-5%), TM7 (1-6%), and Firmicutes (2-3%) were identified but at lower concentrations. Results agree with previous phylogenetic studies that described bacterial species associated with algal species. These species were distributed among six bacterial phyla, including Bacteroidetes, Proteobacteria, Firmicutes, Actinobacteria, Verrucomicrobia, and Planctomycetes [33]. Members of the phylum Bacteroidetes are associated with algal blooms because they are degraders of microalgal polysaccharides and are therefore key players in marine carbon cycling [34]. Ferro et al.(2020) found predominantly Proteobacteria and Bacteroidetes phyla for microalgae-based municipal wastewater treatment in open photobioreactors. Proteobacteria present a wide diversity of metabolic activities, acting in important environmental functions [35]. The classes Alpha- Beta-, Gamma- and Delta-Proteobacteria were identified in all samples, accounting for approximately more than 50% of total bacterial classes. Species of bacteria from Alpha and Gamma-Proteobacteria play an important role in the bioaccessibility of micronutrients for many species of microalgae. Specifically, some species produce siderophores, which binds Fe (III), making it available for microalgae. Consecutively, microalgae use micronutrients such as iron during photosynthesis. As a result, microalgae release dissolved oxygen and organic molecules (dissolved organic matter) that can be used for bacterial growth [36]. Within the phylum Bacteroidetes, bacteria belonging to the

classes Saprospirae, Flavobacteriia, Cytophagia, and Sphingobacteria were detected in all samples. Some members of Saprospirae and Cytpophagia have been described as algicidal agents, capable to degrade algal cell wall macromolecules [37]. To take a closer look at the variability of the microbial community structure, the data was further analysed and compared at the family level. Rhodobacteraceae and Comamonadaceae, belonging to the Alpha-Proteobacteria and Beta-Proteobacteria class respectively, were the most abundant families determined. The family Rhodobacteraceae belongs to the Rhodobacterales group, which address a full range of metabolisms including aerobic respiration, anaerobic fermentation, sulphur oxidation, autotrophic carbon fixation, nitrogen fixation, and hydrogen production [38]. Within these groups, heterotrophic bacteria play an important role because have been associated with phytoplankton during algal blooms acting as consumers of organic carbon [39]. The family Comamonadaceae has been identified as the dominant family present in the *Scenedesmus* phycospheres [40], the areas around microalgal cells where bacteria feed on extracellular products of microalgae. Other main bacterial families observed were Xanthomonadaceae, Saprospiraceae, Verrucomicrobiaceae, Rhodocyclaceae, and Sphingomonadaceae. The family Rhodocyclaceae includes some known organisms such as polyphosphate-accumulating organisms (PAOs), present in full-scale wastewater treatments because they facilitate the removal of large amounts of phosphorus [41]. However, the most relevant family identified was Sphingomonadaceae. Members of this group have been associated with typical conditions found in microalgae-based systems: low concentrations of dissolved organic carbon (DOC) and high radiation because they exhibit minimal DNA damage and high levels of UV-B resistance [13,42,43].

Other less abundant bacterial families but with a fundamental role in the nitrogen cycle are nitrifying bacteria. Nitrification is the biological oxidation of ammonia to nitrite by ammonia-oxidizing bacteria (AOB), followed by the oxidation of nitrite to nitrate by nitrite-oxidizing bacteria (NOB). AOB and NOB performed this aerobic process and are known as nitrifying bacteria or nitrifiers [44]. In the samples, four families with AOB or NOB members were detected: The primary AOBs identified belonged to the families Chromatiaceae and Nitrosomonadacea. Furthermore, two families with common NOBs in wastewater treatment were detected in the microalgae-bacteria samples: Nitrospiraceae and Bradyrhizobiaceae. In the current study, nitrate production was affected by permeate rate as higher values led to larger water volumes processed and to higher nitrate production. In this sense, the amplification and sequencing of the microalgae-bacteria samples using the V3-V4 region of the 16S rRNA gene revealed a positive correlation between permeate rate and relative abundance of nitrifiers (p<0.05; R^2=0.865; Figure 4).

4. Conclusions

The use of ultrafiltration membranes in the large-scale microalgae-based bioremediation of wastewater shows great potential to maximise the volume of sewage that can be processed in raceway reactors. This technique could significantly improve biomass productivity. Results suggest that a raceway reactor with a total surface area of 1 ha would allow to process 2.58 M m^3 of wastewater while producing 79.92 tn of biomass. Nutrient removal was also significantly improved by increasing the permeate rate, although nitrification caused by nitrifying bacteria is a problem that still needs to

be overcome. Future studies will assess the effect of operational conditions on the growth of nitrifying bacteria and optimise nutrient removal from primary wastewater.

Acknowledgements

This work forms part of the SABANA Project, funded by the European Union's Horizon 2020 Research and Innovation Programme (Grant # 727874) and the PURASOL and AL4BIO projects, funded by the Spanish Ministry of Economy and Industry (CTQ2017-84006-C3-3-R; and RTI2018-099495-A-C22 respectively). A. Sanchez-Zurano is in receipt of a FPU predoctoral grant awarded by the Spanish Ministry of Education (FPU16/05996). T. Lafarga thanks the Spanish Ministry of Science, Innovation, and Universities (IJC2018-035287-I) and the BBVA Foundation for the Leonardo 2020 Grant for Researchers and Cultural Creators.

Declaration of competing interest

Authors declare that they have no conflict of interests.

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& Editing, Supervision, and Funding Acquisition; and **C.V. González-López**: Supervision and Funding Acquisition.

Statement of informed consent, human/animal rights

Not applicable.

Table 1. Composition of the culture medium and primary domestic wastewater used (inlet). Concentrations are expressed as mg·L^{-1}. Values correspond to the mean values of three independent determinations ± SD (n=3).

Parameters	Primary domestic wastewater*	Arnon medium
pH	7.9 ± 0.2 - 8.2 ± 0.1	7.5 ± 0.2
COD	296 ± 23 - 858 ± 39	16.0 ± 1.2
Sulphate	98.1 ± 0.9 - 105.3 ± 1.2	6.3 ± 0.8
Nitrogen-Nitrate	1.5 ± 0.8 - 5.6 ± 1.1	140.0 ± 4.5
Chloride	410.6 ± 3.6 - 435.4 ± 4.2	78.9 ± 2.1
Sodium	222.5 ± 5.1 - 312.1 ± 9.2	276.1 ± 7.9
Potassium	8.4 ± 0.7 - 9.8 ± 0.6	325.1 ± 6.3
Calcium	31.1 ± 0.9 - 31.9 ± 0.7	364.9 ± 5.5
Magnesium	52.1 ± 2.3 - 65.7 ± 2.2	12.2 ± 0.6
Phosphorus-Phosphate	7.9 ± 1.0 - 27.7 ± 1.2	39.3 ± 3.1
Nitrogen-Ammonium	58.2 ± 1.2 - 136.9 ± 8.0	0.0 ± 0.1
Iron	0.20 ± 0.05 - 0.22 ± 0.02	5.0 ± 0.3
Copper	0.09 ± 0.01 - 0.12 ± 0.04	0.02 ± 0.01
Manganese	0.03 ± 0.02 - 0.04 ± 0.03	0.5 ± 0.0
Zinc	0.10 ± 0.06 - 0.18 ± 0.07	0.06 ± 0.01
Boron	0.36 ± 0.11 - 0.45 ± 0.09	0.41 ± 0.08

* Minimum and maximum values

Figure legends

Figure 1. Schematic representation of the membrane photobioreactor

Figure 2. Effect of permeate rate on (A) cellular and hydraulic retention times and (B) volume of water used to compensate evaporation, volume of permeate, volume of culture harvested, and total operating inlet volume per m^2. Values correspond to the mean values of three independent determinations ± SD (n=3).

Figure 3. Effect of permeate rate on (A) biomass productivity and (B) maximum quantum efficacy of the PSII photochemistry – F_v/F_m. Values correspond to the mean values of three independent determinations ± SD (n=3).

Figure 4. Effect of permeate rate on the removal/production of (A) NH_4^+, (B) NO_3^-, and (C) PO_4^{3-}. Values correspond to the mean values of three independent determinations ± SD (n=3).

Figure 5. Effect of permeate rate on the relative abundance of bacteria organised by (A) phylum, (B) class, (C) family, and (D) families of nitrifying bacteria. Values correspond to the mean values of three independent determinations ± SD (n=3).

Figure 1

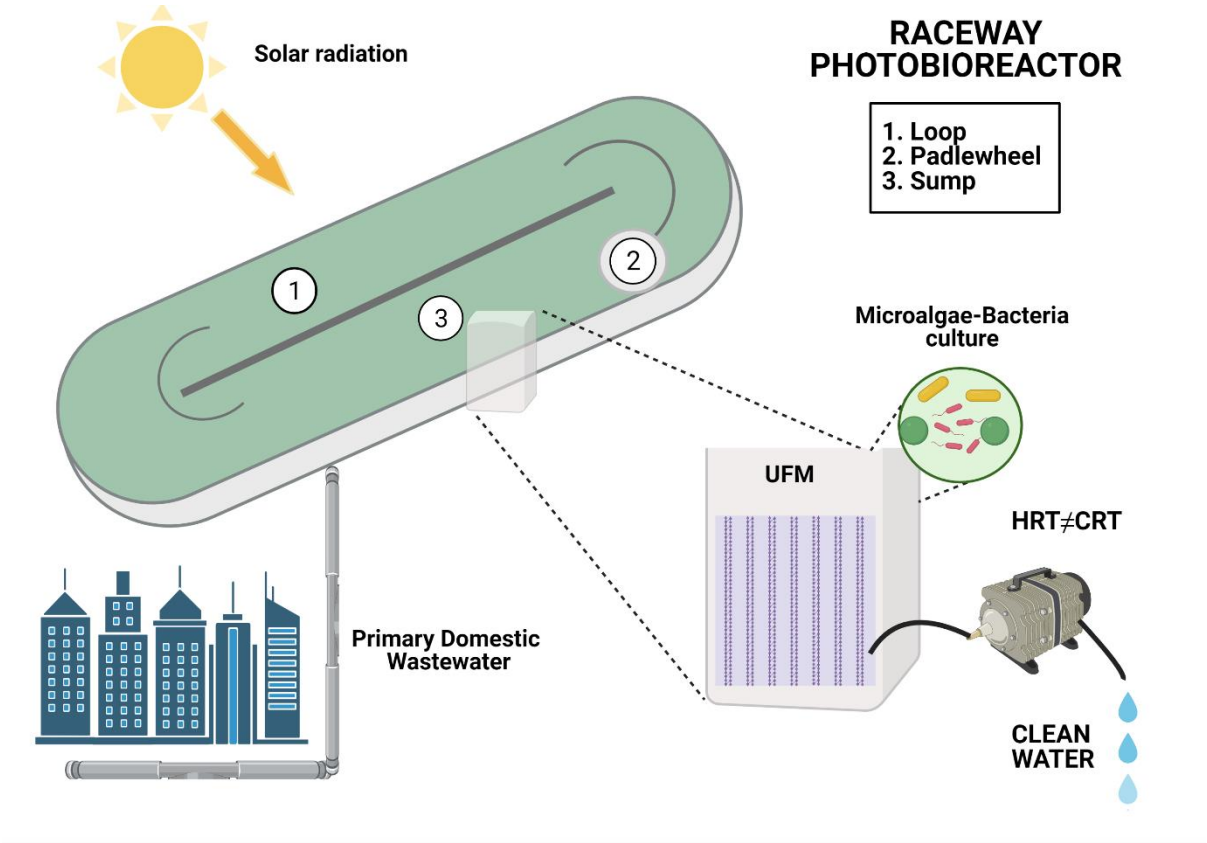

Solar radiation
RACEWAY PHOTOBIOREACTOR
1. Loop
2. Padlewheel
3. Sump
1
2
3
Microalgae-Bacteria culture
UFM
HRT≠CRT
Primary Domestic Wastewater
CLEAN WATER

Figure 2

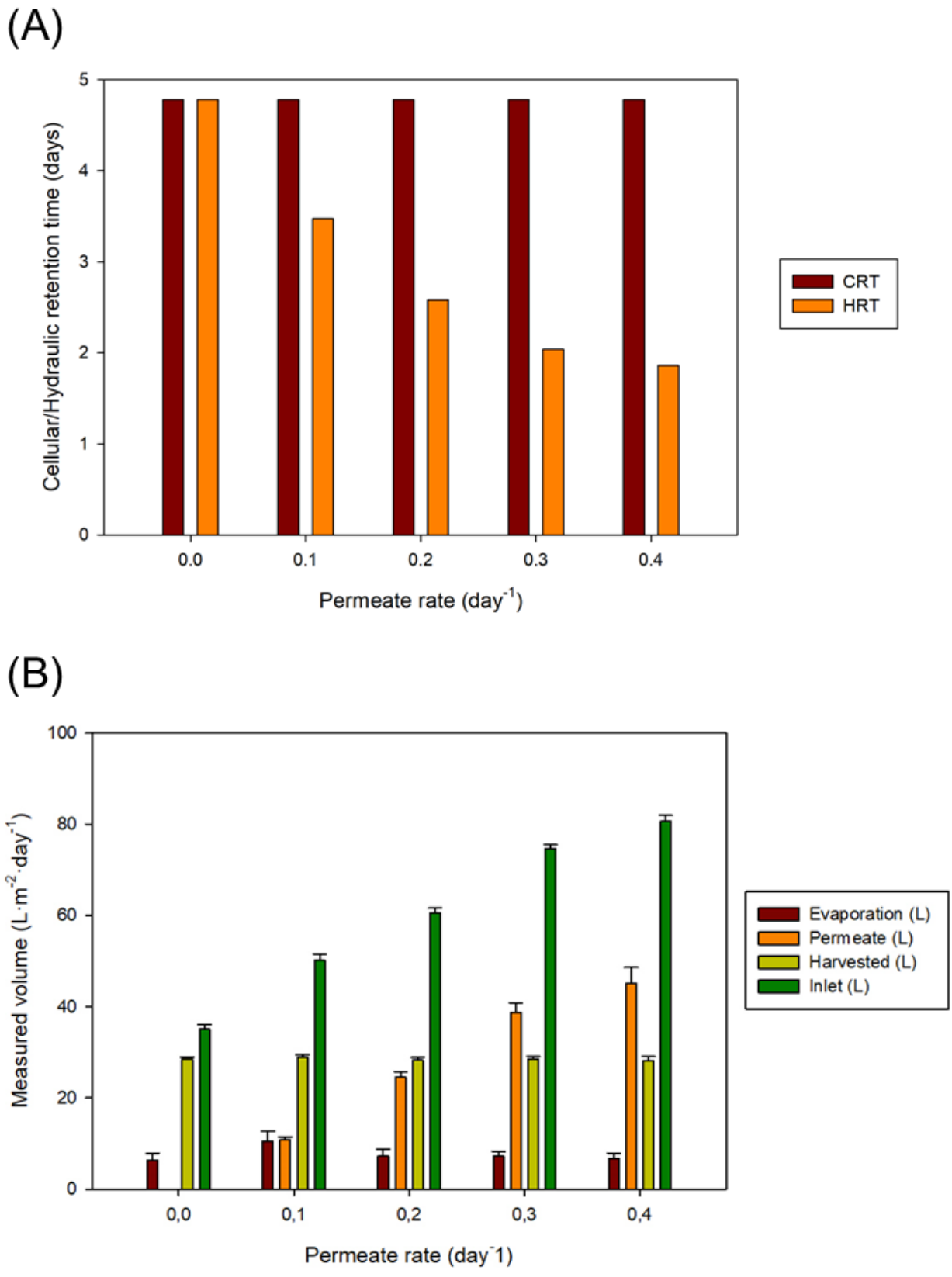

(A)
Cellular/Hydraulic retention time (days)
0
1
2
3
4
5
0.0
0.1
0.2
0.3
0.4
Permeate rate (day-1)
CRT
HRT
(B)
Measured volume (L·m-2·day-1)
0
20
40
60
80
100
0,0
0,1
0,2
0,3
0,4
Permeate rate (day-1)
Evaporation (L)
Permeate (L)
Harvested (L)
Inlet (L)

Figure 3

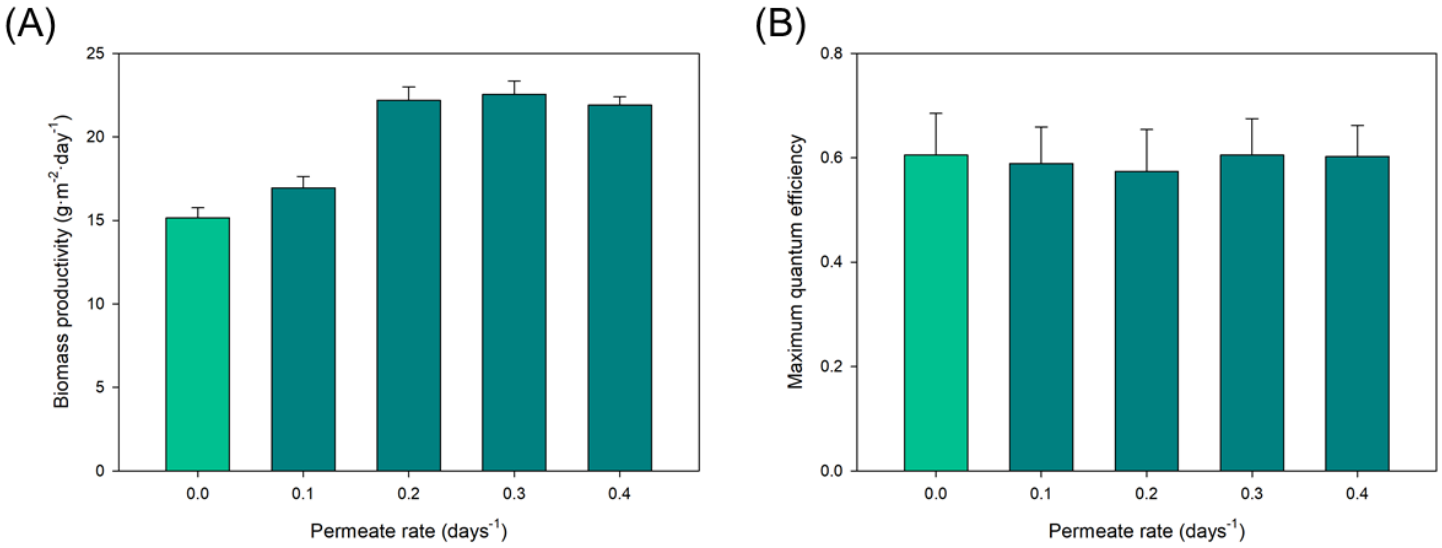

(A)
Biomass productivity (g·m-2·day-1)
25
20
15
10
5
0
0.0
0.1
0.2
0.3
0.4
Permeate rate (days-1)
(B)
Maximum quantum efficiency
0.8
0.6
0.4
0.2
0.0
0.0
0.1
0.2
0.3
0.4
Permeate rate (days-1)

Figure 4

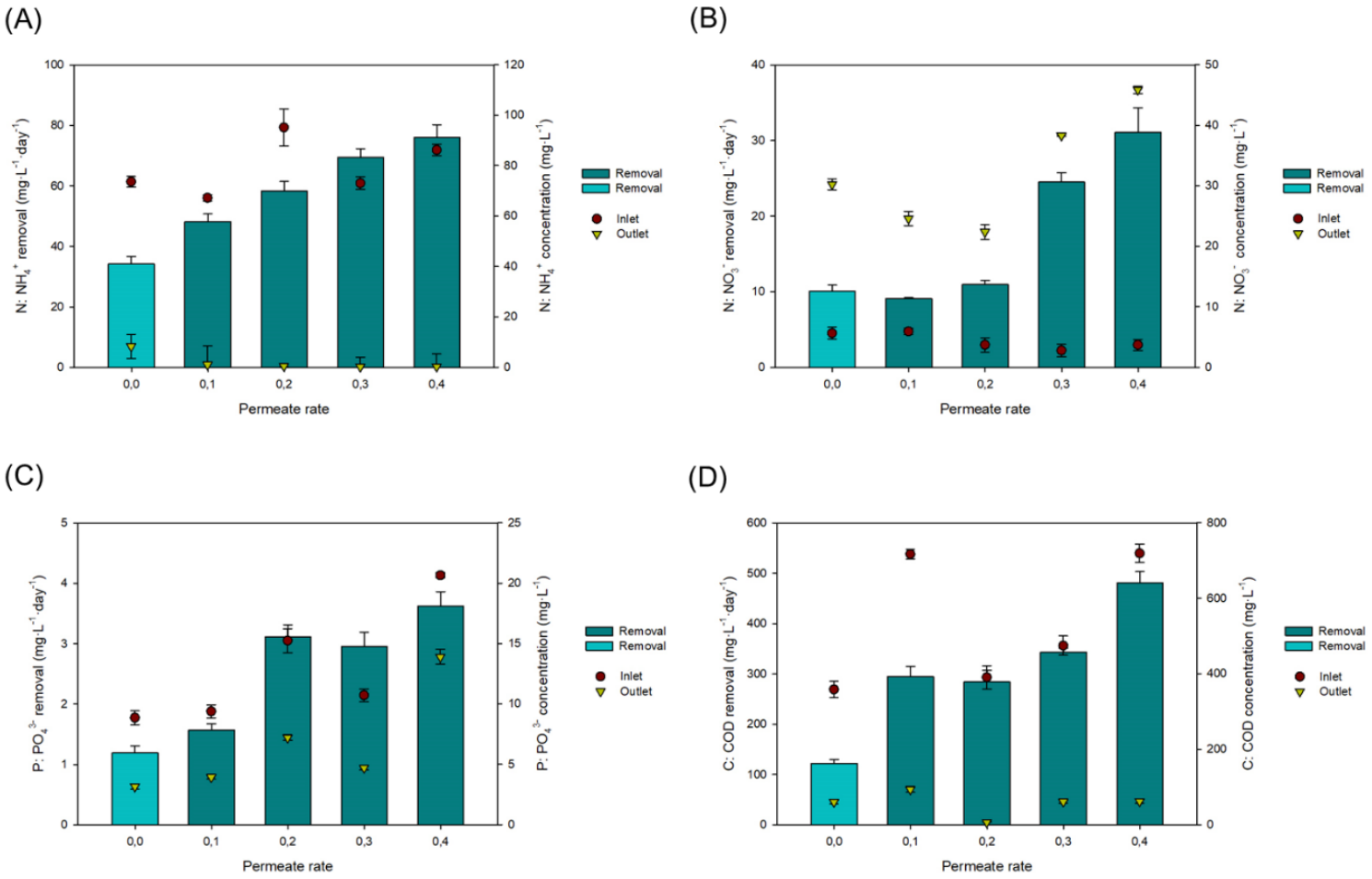
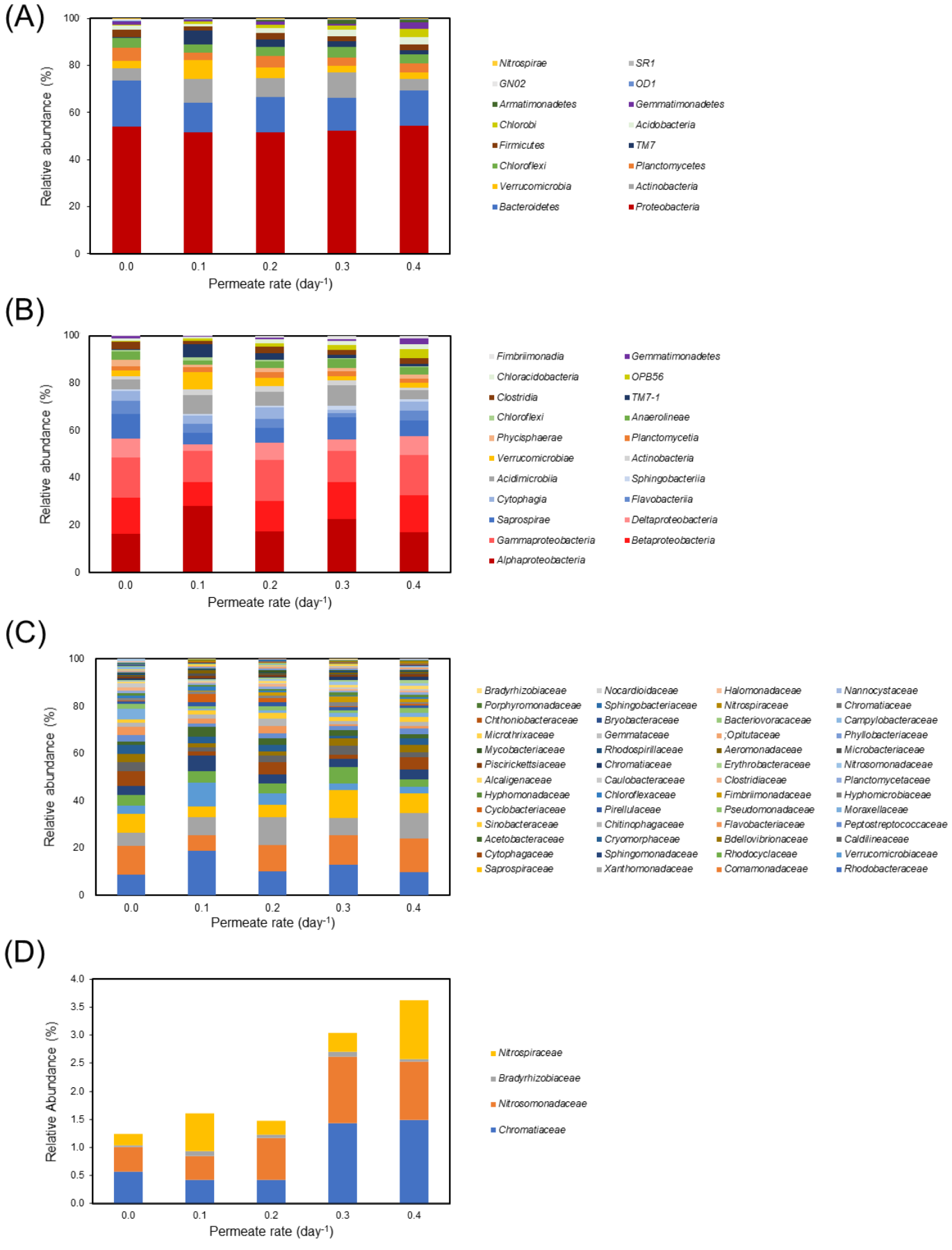

(A)
(B)
(C)
(D)
Permeate rate
Removal
Removal
Inlet
Outlet
0,0
0,1
0,2
0,3
0,4

Figure 5


(A)
Relative abundance (%)
Permeate rate (day-1)
Nitrospirae
SR1
GN02
OD1
Armatimonadetes
Gemmatimonadetes
Chlorobi
Acidobacteria
Firmicutes
TM7
Chloroflexi
Planctomycetes
Verrucomicrobia
Actinobacteria
Bacteroidetes
Proteobacteria
(B)
Relative abundance (%)
Permeate rate (day-1)
Fimbriimonadia
Gemmatimonadetes
Chloracidobacteria
OPB56
Clostridia
TM7-1
Chloroflexi
Anaerolineae
Phycisphaerae
Planctomycetia
Verrucomicrobiae
Actinobacteria
Acidimicrobiia
Sphingobacteriia
Cytophagia
Flavobacteriia
Saprospirae
Deltaproteobacteria
Gammaproteobacteria
Betaproteobacteria
Alphaproteobacteria
(C)
Relative abundance (%)
Permeate rate (day-1)
Bradyrhizobiaceae
Nocardioidaceae
Halomonadaceae
Nannocystaceae
Porphyromonadaceae
Sphingobacteriaceae
Nitrospiraceae
Chromatiaceae
Chthoniobacteraceae
Bryobacteraceae
Bacteriovoracaceae
Campylobacteraceae
Microthrixaceae
Gemmataceae
;Opitutaceae
Phyllobacteriaceae
Mycobacteriaceae
Rhodospirillaceae
Aeromonadaceae
Microbacteriaceae
Piscirickettsiaceae
Chromatiaceae
Erythrobacteraceae
Nitrosomonadaceae
Alcaligenaceae
Caulobacteraceae
Clostridiaceae
Planctomycetaceae
Hyphomonadaceae
Chloroflexaceae
Fimbriimonadaceae
Hyphomicrobiaceae
Cyclobacteriaceae
Pirellulaceae
Pseudomonadaceae
Moraxellaceae
Sinobacteraceae
Chitinophagaceae
Flavobacteriaceae
Peptostreptococcaceae
Acetobacteraceae
Cryomorphaceae
Bdellovibrionaceae
Caldilineaceae
Cytophagaceae
Sphingomonadaceae
Rhodocyclaceae
Verrucomicrobiaceae
Saprospiraceae
Xanthomonadaceae
Comamonadaceae
Rhodobacteraceae
(D)
Relative Abundance (%)
Permeate rate (day-1)
Nitrospiraceae
Bradyrhizobiaceae
Nitrosomonadaceae
Chromatiaceae

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