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A novel photoanaerobic process as a feasible alternative to the traditional aerobic treatment of refinery wastewater

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ABSTRACT

Refinery wastewater (RWW) treatment is outdated since new wastewater management and reuse challenges require more environmental-friendly and cheap alternatives. Conventional biological treatments focused on activated sludge are highly energy-intensive and resource-dissipating processes. However, anaerobic treatments are an excellent alternative to reduce costs derived from aeration and carbon footprint. This work proposes a novel strategy for the treatment of RWW involving a photoanaerobic membrane bioreactor (PAnMBR) with a mixed culture of purple phototrophic bacteria (PPB). PPB upcycles the organic matter, nitrogen, and phosphorus in an assimilative way, leading to a much higher biomass yield and nutrient removal than aerobic cultures. The enriched PPB culture was generated from the RWW as the sole substrate without specific PPB inoculation. The RWW (exempted from sufficient nutrients) was successfully treated with additional ammonium and phosphates provided by domestic wastewater (DWW). Preliminary batch tests determined the best DWW/RWW volumetric mixing ratio at 25:75. The PAnMBR was operated for 144 days under different specific loading rates (SLR) by modifying hydraulic and solid retention times. The maximum specific loading rate (SLR) for the efficient RWW/DWW mix treatment was 0.3 $mgCOD_{inlet}/mgCOD_{biomass} \cdot d$. The COD consumption was mainly mediated by *Rhodopseudomonas* sp. and *Rhodobacter* sp. PPB genera. The PPB-based photo-anaerobic membrane reactor was able to comply with regulated parameters for wastewater discharge for the more restrictive use of reclaimed water according to the European legislation in force.

1. Introduction

Proper industrial wastewater management is enforced in most developed countries due to its environmentally hazardous nature [1]. Traditional wastewater treatment facilities are high energy-demanding, inefficient, and solely focused on depuration. Because of this, industries are slowly shifting towards more sustainable and less energy-intensive alternatives [2]. Nevertheless, it has long since most industries relied on conventional, old-fashioned technologies such as activated sludge. This is the case, for instance, of the refinery and petrochemistry industries. Conventional refinery wastewater (RWW) treatment plants are based on the removal of high organic fractions and partially recovering non-soluble oils by several physicochemical units consisting of a Dissolved Air Flotation (DAF) process and gravity American Petroleum Institute (API) oil-water separators. Then, the RWW flows to aerobic activated sludge processes with efficiencies in organics removal above 90 % and ternary treatments that enable the production of reusable water for the oil petroleum plant. The sludge from primary and secondary treatments is usually managed by external disposal due to its hazardous substances composition [3,4].

However, the treatment of RWW through conventional activated sludge has several limitations. Firstly, the activated sludge is an energy-

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Abbreviations: ANOVA, Analysis of variance; API, American Petroleum Institute; CFU, Colony-forming unit; COD, Chemical oxygen demand; DAF, Dissolved air flotation; DNA, Desoxiribonucleic acid; DWW, Domestic wastewater; HRT, Hydraulic retention time; IUPAC, International Union of Pure and Applied Chemistry; MBR, Membrane Bioreacto; NIR, Near Infrared; NTU, Nephelometric Turbidity unit; OD, Optical density; OLR, Organic loading rate; PAnMBR, Photoanaerobic membrane bioreactor; PCA, Principal component analysis; PPB, Purple phototrophic bacteria; RDA, Redundance analysis; RNA, Ribonucleic acid; rDNA, Ribosomal DNA; rRNA, Ribosomal RNA; RWW, Refinery wastewater; SCOD, Soluble chemical oxygen demand; SCR, Specific consumption rate; SLR, Specific loading rate; SRT, Solids retention time; TCOD, Total chemical oxygen demand; TSS, Total suspended solids; UPW, Ultrapure Milli-Q water; VIS/UV, Visible/Ultraviolet; VSS, Volatile suspended solids; WWTP, Wastewater treatment plant; $Y_{x/s}$, Apparent biomass yield.

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intensive process due to its need for aeration. The average energetic demand of this process is 1.02 kWh/m^3, as compared to the 0.43 kWh/m^3 reported for anaerobic biological processes [5]. Thus, anaerobic technologies are considered potential alternatives for reducing energy consumption and carbon emissions [2,6]. However, anaerobic microorganisms, in general, cannot efficiently treat effluents with low organic concentrations (a minimum of 750 mgCOD/L is recommended) [7,8].

Purple phototrophic bacteria (PPB) are a kind of phototrophic facultative microorganisms gaining significant interest in the last years as a feasible alternative to conventional technologies. PPB have shown promising results (high carbon elimination up to over 90 % of chemical oxygen demand (COD)) for the treatment of different industrial wastewaters such as rubber sheet production [9], pharmaceutical [10], saline [11], sugar refinery [12], agro-industrial [13], and domestic wastewaters. In the latter case, ammonium and phosphates were also removed (up to 92.2 and 97.5 %, respectively), reaching values below the regulated discharge limits of nutrients and COD [14–16]. To the authors' knowledge, only a few works can be found in the literature concerning the treatment of refinery wastewater. Those works evidenced the growth and activity of PPB in the treatment of oil refinery wastewater. However, they studied the treatment of an oil refinery wastewater before the primary treatment under batch mode operation and using a culture enriched in a mixture of *Pseudomonas* (a genus of aerobic bacteria) and *Rhodopseudomonas* (a genus of PPB) [17]. Another work also demonstrated the bioremediation of oil-polluted wastewater with pure cultures of *Rhodopseudomonas sp* on seawater. [18].

According to previous works, PPB could replace conventional aerobic biological treatments [19]. Moreover, as other phototrophic microorganisms (cyanobacteria and microalgae), PPB upcycles the organic matter, nitrogen, and phosphorus in an assimilative way, leading to a much higher biomass yield than aerobic cultures (nearly 1 $mgCOD_{biomass}/mgCOD_{substrate}$ compared to 0.5 $mgCOD_{biomass}/mgCOD_{substrate}$) as well as a faster and higher nutrient assimilation rate [20]. Moreover, these phototrophic facultative microorganisms do not require a high organic loading and have shown a higher resistance to inhibition caused by potentially toxic compounds in the RWW than conventional (aerobic and anaerobic) technologies, even at peak concentrations [21]. However, the RWW is limited by the low nutrient content in the wastewater, and ammonia nitrogen and phosphates are needed for the assimilative growth-based microorganisms like PPB, requiring higher amounts than the non-photosynthetic microorganisms. Meanwhile, the addition of synthetic compounds to the medium is deprecated since wastewater treatment techniques tend to reduce chemicals spent under the circular economy paradigm [22]. A novel way of solving this issue is using a co-substrate as domestic wastewater (DWW) to provide the nutrients for the RWW treatment as well as the treatment of the two effluents. Taking into account the well-demonstrated ability of PPB to treat DWW, a synergy between both substrates may be expected.

This work proposes the co-treatment of RWW and DWW in a photo-anaerobic membrane bioreactor (PAnMBR) at lab-scale, producing high-quality water for an oil refinery plant. The start-up of the bioreactor was performed using the refinery wastewater as the only substrate without pre-inoculation. The study of the specific loading rate on the performance of the co-treatment of RWW and DWW followed a rational approach, varying the hydraulic and the solid retention times. The development of the microbial communities during the start-up and the subsequent co-treatment of the RWW was also studied. The outcomes of this work will serve as a basis to further scale up the process in an industrial scene.

2. Materials and methods

2.1. Refinery and domestic wastewaters

The RWW was obtained from a refinery and petrochemistry complex in Spain. Samples were taken directly from the DAF unit before the biological treatment and preserved at 4 °C. The DWW was obtained after primary treatment from the Estiviel wastewater treatment plant (WWTP) in Toledo, Spain. These samples were also taken before the biological treatment and preserved at 4 °C. “Two different wastewater samples from effluents of RWW and DWW were used in this work due to the long operation time of the experimental work and the insufficient wastewater volume taken in the first collection campaign. Table 1 shows the physicochemical characterization of the two wastewater samples of both effluents. The characterization results were in the typical range of RWW and DWW characterization data [3,4,23]. As expected, a higher nutrients content of NH_4^+ and PO_4^{3-} was detected in the DWW. However, a relevant decrease of SCOD and NH_4^+ concentrations was noted in the second sample of DWW. These alterations can take place in the characterization parameters of real effluents since both refinery and domestic wastewaters are subject to variations over the time by operational production periods or seasonal fluctuations, respectively.

2.2. Batch tests

The DWW is proposed as a feedstock to provide nutrients to allow the PPB growth on the RWW. Batch tests were performed to find the best mixing ratio of the two RWW and DWW wastewaters regarding wastewater biodegradability and biomass growth. Five mixing ratios of DWW: RWW were tested: 0:100, 25:75, 50:50, 75:25 and 100:0. Three control experiments were carried out by using only RWW (0:100), DWW (100:0), or ultrapure MiliQ water (UPW). In all the control experiments, the medium was supplemented with acetic acid as a biodegradable carbon source and macro/micronutrients as previously described in literature for optimal growth of the purple phototrophic culture (further details in the Supplementary Information SI-1, Table S1). Each control experiment was used as a reference for PPB growth under each of the studied water matrices (RWW, DWW, and UPW) to assess the effect of the water matrix on the PPB growth.

The tests were performed in 160 mL glass serum bottles inoculated with a PPB biomass previously enriched in RWW during the start-up of the photoanaerobic MBR described below. The bottles were filled to a working volume of 100 mL, closed with rubber caps, and sealed with aluminum crimps. The headspace was flushed with argon for 10 min to assure an anaerobic and inert atmosphere since other gases like nitrogen or carbon dioxide could be fixed as additional nitrogen and carbon sources by PPB. In this way, all the biological activity can be related to the water phase composition. The pH naturally evolved during the experiment. Bottles remained at 25 °C in a temperature-controlled incubator shaker for 3 d, and illumination was provided by three 150 W infrared lamps (Philips, BR125 IR, Spain) to an average effective near-infrared (NIR) radiance around 20 W/m^2. The bottles were covered with a VIS/UV filter to prevent the growth of other photosynthetic organisms (ND 1.2299, Transformation Tubes, Banstead, UK).

Sampling was performed at the beginning and the end of each

Table 1

Physicochemical characterization of the refinery (RWW) and domestic (DWW) wastewater samples (errors are 95 % confidence intervals from triplicate measurements).

Sampling	Refinery wastewater (RWW)		Domestic wastewater (DWW)	
	I	II	I	II
TCOD (mg/L)	530 ± 20	310 ± 20	330 ± 20	300 ± 20
SCOD (mg/L)	340 ± 20	240 ± 20	240 ± 20	180 ± 40
NH_4^+ (mg/L)	8.1 ± 0.8	7.2 ± 1.5	52 ± 2	39 ± 2
PO_4^{3-} (mg/L)	0.13 ± 0.01	0.08 ± 0.01	11 ± 1	11 ± 2
SCOD/N/P	100/2.4/0.03	100/2.4/0.03	100/17.1/1.58	100/21.4/2.11
TSS (mg/L)	130 ± 30	90 ± 30	200 ± 10	160 ± 30
VSS (mg/L)	70 ± 20	40 ± 10	160 ± 30	130 ± 20
pH	7.0 ± 0.1	7.3 ± 0.1	7.4 ± 0.1	7.5 ± 0.1

experiment to measure total COD (TCOD), soluble COD (SCOD), ammonium, orthophosphates, VSS/TSS, and optical density (OD). SCOD, ammonium, orthophosphates, and OD were also monitored three times a day during the experiments. The duration of the experiments lasted 71 h until the biomass growth stopped.

2.3. *Continuous operation of photoanaerobic membrane bioreactor (PAnMBR)*

The co-treatment of RWW and DWW was performed in continuous mode in the PAnMBR previously described by de las Heras et al. [24]. Further details and experimental set-up of the PAnMBR can be found in the Supplementary Information SI-2 and Fig. S1. Initially, the PAnMBR was started-up with only the RWW. After that, it was operated following several stages at different specific loading rates (SLR). Table 2 summarizes the duration, operation conditions, and average concentration of the inlet wastewater in terms of SCOD, NH_4^+ and PO_4^{3-} of each stage. The PAnMBR reactor was maintained at RT (25.7 ± 0.5 °C) and the feedstocks at 4 °C to prevent substrate degradation. There was no pH control since it was not expected to reach inhibitory values ($pH > 9$; [25]). The presence of PPB on the PAnMBR biomass was checked daily by VIS/NIR absorbance spectra (400–950 nm). The macroscopic parameters such as SCOD, TCOD, NH_4^+, PO_4^{3-}, TSS, and VSS were daily measured to check the system's performance. Microbiological analysis was performed to analyze the microbial community through 16S rRNA amplification (described below) of DNA extracted from the biomass of the PAnMBR. This analysis was performed for single samples of selected operational days (0, 1, 5, 7, 11, 13, 19, and 21 of the start-up phase, and 25, 60, 96, 116 and 144 of the continuous co-treatment of RWW and DWW). The analysis of the inoculum used when starting the co-treatment was also performed.

2.3.1. *Start-up with RWW*

The start-up of the continuous treatment was performed for 21 days feeding the RWW as the sole substrate without PPB pre-inoculation. This stage served to develop an enriched PPB culture acclimatized to the RWW. The biomass developed after this stage was initially used as inoculum for the batch tests and thereafter for the continuous co-treatment of RWW and DWW. Prior to the continuous treatment, the inoculum was stored at 4 °C. Initial operational conditions of the start-up stage were selected following the recommendation of a previous work, where the PPB enrichment was developed in a similar experimental setup treating DWW [14].

2.3.2. *Co-treatment of the RWW and DWW*

The continuous co-treatment of RWW and DWW was focused on increasing the SLR. This fact was performed by modification of the hydraulic and solid retention times (HRT and SRT) to achieve a stable performance of the PAnMBR that assure values of COD, NH_4^+, and PO_4^{3-} below the legal discharge limits. The RWW:DWW mixing ratio was selected according to the results obtained from the batch tests. Table 2 shows the operating conditions of the PAnMBR, including the inlet COD, NH_4^+, and PO_4^{3-} concentrations. During the first acclimatization phase (stage I), the organic loading rate (OLR) was initially varied between 77 and 145 mg $COD \cdot L^{-1} \cdot d^{-1}$ with HRTs in the range of 75–40 h. Initially, the biomass was not purged, so the SRT was not controlled for 40 d to achieve a stable biomass concentration in the bioreactor. After this acclimation period, the SLR was increased following two different strategies. Firstly, the SLR was increased from stages II to VI by decreasing the HRT from 60 to 30 h, setting the SRT at 30 d by purging the biomass. Then, from stages VII to IX, the HRT was set at 25 h and the SRT was gradually decreased from 25 to 16 d to assess the limitation of the treatment when increasing the amount of purged biomass. Finally, the HRT and SRT were set to 25 h and 20 d to stabilize the treatment at the maximum SLR in stage X.

2.4. *Analytical methods*

The optical density (OD) was measured at 665 nm with a JASCO V-630 UV-VIS (Madrid, Spain) spectrophotometer along with VIS/NIR absorbance spectra (450–950 nm) to check the typical peaks of carotenoids and bacteriochlorophyll of PPB. OD was also directly related to the VSS by linear calibration. OD/VSS calibration was performed for every trial to avoid differences in biomass composition that might affect the OD/VSS ratio. The pH was analyzed with a CRISON GLP 22 pHmeter (Barcelona, Spain). TCOD, SCOD, NH_4^+, PO_4^{3-}, VSS, and TSS were measured according to APHA/AWWA/WEF Standard Methods [26]. SCOD, NH_4^+, PO_4^{3-} were measured after filtration of the mixed liquor sample of the bioreactor with 0.45 μm glass fiber syringe filters. The inlet wastewaters and effluent after treatment samples was also analyzed by GC/MS (Gas Chromatography coupled to Mass Spectrometry) using a Stalbiwax-MS (30 m × 0.25 mm, 0.25 μm) column to identify potential toxic compounds and byproducts. The light intensity was measured at the surface of the bottles and the PAnMBR with a StellarNet Blue-Wave spectrometer (Tampa, FL, USA) with a fiber optic cable and a cosine receptor with a 10 % aperture.

Biomass samples for DNA extraction of the PAnMBR reactor were concentrated by centrifugation at 9000 rpm for 10 min. DNA extraction prior to microbiological analysis was made through HigherPurity™ Bacterial Genomic DNA Isolation Kit by Canvax Biotech (Córdoba, Spain). The extracted DNA samples were sent to FISABIO Sequencing and Bioinformatics (Valencia, Spain) for 16 s rRNA gene amplicon sequencing following the 16S rDNA gene Metagenomic Sequencing Library Preparation Illumina protocol (Cod. 15,044,223 Rev. A). Further details of the microbiological analysis method are summarized in Supplementary Information SI-3.

2.5. *Data handling and statistical treatment*

Apparent biomass yield ($Y_{x/s}$, in gVSS/gCOD) was calculated as the ratio between the total biomass growth measured as VSS and the total COD consumed. ANOVA was performed to check possible differences

Table 2
Stages and operational parameters of the continuous treatment of RWW and DWW on PAnMBR.

Stage	Start-up	I	II	III	IV	V	VI	VII	VIII	IX	X
Duration (d)	21	21	24	11	5	9	7	9	15	15	7
HRT (h)	7.5	[75–40]	60	45	30	35	30	25	25	25	25
OLR ($mgCOD_{inlet}/L \cdot d$)	1090 ± 90	[77–145]	95 ± 5	110 ± 20	134 ± 9	126 ± 6	140 ± 20	160 ± 20	172 ± 8	150 ± 7	140 ± 10
SLR ($mgCOD_{inlet}/mgCOD_{biomass} \cdot d$)	1.2 ± 0.2	[1.27–0.11]	0.11 ± 0.02	0.15 ± 0.02	0.18 ± 0.05	0.20 ± 0.02	0.21 ± 0.07	0.24 ± 0.02	0.36 ± 0.03	0.50 ± 0.08	0.49 ± 0.06
SRT (d)	2	[∞-40]	30	30	30	30	30	25	20	16	20
Inlet SCOD (mg/L)	340 ± 30	240 ± 10	240 ± 10	210 ± 40	170 ± 10	184 ± 9	180 ± 20	160 ± 20	179 ± 8	156 ± 7	150 ± 10
Inlet NH_4^+ (mg/L)	8.1 ± 0.8	22.2 ± 0.6	22 ± 1	17 ± 2	16.8 ± 0.3	18 ± 3	16.7 ± 0.7	16 ± 1	18 ± 1	14.8 ± 0.7	12 ± 2
Inlet PO_4^{3-}(mg/L)	0.11 ± 0.01	4.6 ± 0.7	3.9 ± 0.3	3.7 ± 0.4	3.4 ± 0.1	4.9 ± 0.7	4.6 ± 0.3	4.7 ± 0.5	4.8 ± 0.3	4.1 ± 0.4	2.6 ± 0.8

among the means of the $Y_{x/s}$ values. All provided errors corresponded to the 95 % confidence intervals. SLR was calculated as the ratio between OLR and the biomass concentration in the liquor mixture expressed in terms of COD. Specific consumption rate (SCR) was calculated as the ratio between consumed COD per day and the biomass concentration in the liquor mixture expressed in terms of COD.

Regarding the analysis of the microbiological communities in the phototrophic biomass during the bioreactor operation, taxonomic data obtained from 16 s rRNA gene amplificon sequencing were analyzed at the genus level. Genera with relative abundances below 1 % in all the samples were gathered in the same group as "others". Genera composition of microbial communities for biomass of different periods of the continuous operation of PAnMBR were compared using the principal component analysis (PCA) method. Two principal components (PC1 and PC2) were used, considering results within 50 % of the variance [27]. Genera composition of those samples was also compared with environmental and response variables of the process (COD, NH_4^+, and PO_4^{3-} consumptions and SLR) by redundancy analysis (RDA). The genera data used for both analyses was compared by relative abundance. PCA and RDA were performed in RStudio (version 2021.09.0) with the functions "prcomp" and "rda" of the vegan package (version 2.5–7) [28].

3. Results and discussion

3.1. Selection of volumetric RWW and DWW ratio

The DWW was used to provide and compensate for the scarce nutrients of the RWW. Fig. 1 shows the biomass yields of the batch experiments performed at different mixtures of DWW and RWW, including the initial 100COD/P/N ratio of each experiment. Remaining concentrations of SCOD, NH_4^+, and PO_4^{3-} at the end of the batch tests after 71 h can be found in Supplementary Information SI-4 (Table S2).

There was a significant increase in the biomass yield when the original COD/N/P ratio of the RWW (mixture DWW:RWW of 0:100) raised from 100/3.2/0.2 to 100/6.7/0.5 (mixture DWW:RWW of 25:75). Statistical significance of this increase was determined by Analysis of Variance (ANOVA) with a p-value < 0.05. However, no significant differences were observed in the batch tests performed with different DWW:RWW ratios (from 25:75 to 100:0, only the DWW) with p-value of ANOVA > 0.05. Likewise, the control experiments using only RWW, DWW, and UPW with additional carbon and nutrient sources for the PPB growth showed similar values. These results evidenced that the nutrient scarcity of the RWW as the sole substrate is responsible for the lower biomass yield. On the other hand, the biomass yields obtained near 1 (in terms of COD) when DWW was added to RWW are typical of non-inhibited PPB cultures [19]. Therefore, according to the biomass yield, the DWW:RWW ratio of 25:75 is enough for the PPB culture development.

The addition of the DWW as co-substrate to the RWW also allowed a higher reduction of the SCOD from the DWW:RWW of 25:75, keeping quite similar when the DWW:RWW was increased to 50:50 and 75:25. The DWW/RWW ratios above 25:75 up to 100:0 showed final SCOD values well below discharge limit (SI-4; Table S2), 125 mgTCOD/L according to the current EU legislation [29], whereas the experiment with only the RWW achieved a closer value (123 ± 9 mg/L). Nevertheless, note that this TCOD discharge limit is referred to final effluents after the biomass/water separation, and SCOD in this work is representative of the soluble fraction of COD in the liquid phase after separation with 0.45 μm syringe filters.

Regarding the NH_4^+ and PO_4^{3-} nutrients, all experiments with a DWW:RWW ratio above 25:75 showed both NH_4^+ and PO_4^{3-} values far above the discharge limits (12.9 and 3.1 mg/L, respectively; see Supplementary Information SI-4; Table S2). However, it is noteworthy to mention that the higher DWW:RWW ratio (from 50:50 to 100:0), the more excess nutrients in the initial feedstock (initial 100COD/N/P ratios far above the reported average consumption for the PPB growth (100/5/

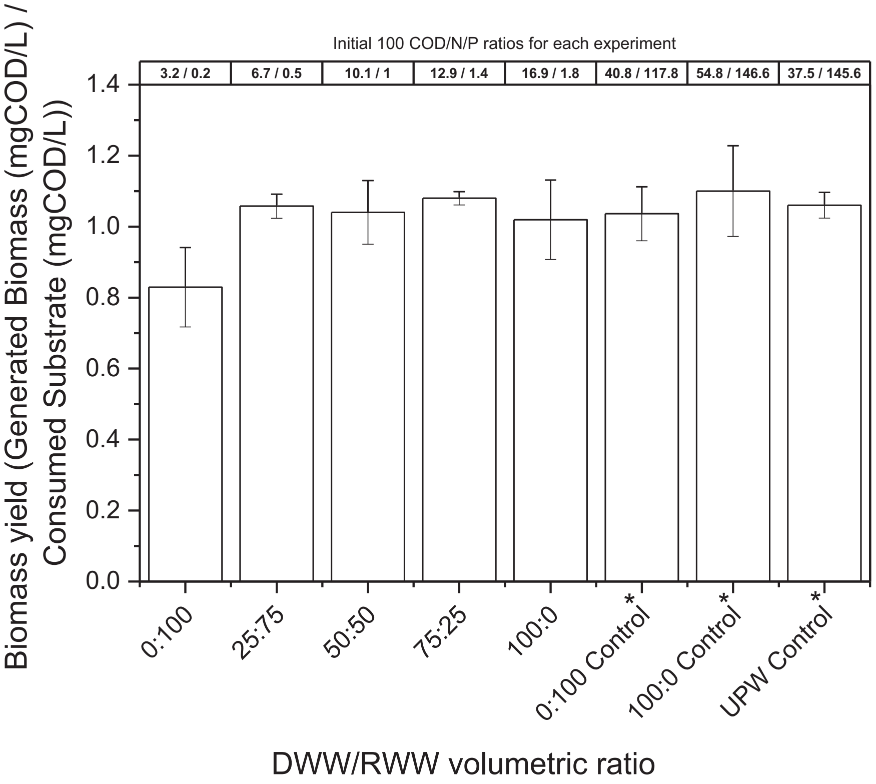

Fig. 1. Initial 100 soluble COD/N/P mass ratios (upper numbers) and biomass yield (bars) of batch tests performed at different volumetric DWW:RWW ratios as well as controls (0:100*, 100:0* and UPW*; *synthetic nutrient source added).

1) [19]). Consequently, microorganisms cannot uptake the nutrients′ excess upon carbon source depletion. There was little difference in the culture performance between 25:75 and 100:0 mix ratios, so the 25:75 mix ratio was selected as the best one since the NH_4^+ and PO_4^{3-} final values were closer and below the discharge limits. We also argued that some phosphorus fed from DWW must be ensured to properly develop and maintain the PPB mixed culture.

Although this study is based on the influence of the volumetric RWW/DWW ratio, it is important to point out that the organic and nutrients loadings of RWW and DWW can change over time and vary widely depending on their origin (different oil refinery plants and domestic wastewaters). Because of this, volumetric ratio could change depending on RWW and DWW compositions to aim for a specific 100COD/N/P ratio. In this work, a 100COD/N/P ratio of 100/6.5/0.5 (DWW:RWW of 25:75) obtained the best results in terms of biomass yield and COD and nutrients removal.

3.2. *Continuous co-treatment of RWW and DWW*

Fig. 2 shows the monitoring of the main variables (SCOD, NH_4^+, PO_4^{3-} and solid concentrations) as well as the SLR of the photoanaerobic membrane bioreactor (PAnMBR) during the start-up using only the RWW as the sole substrate and the following co-treatment of RWW and DWW through different SLR. Likewise, the microbial community dynamics of the reactor was studied along the different operative stages, and the performance of the biological process was assessed.

3.2.1. *Start-up of photoanaerobic membrane bioreactor*

As shown in Fig. 2, the COD consumption was stabilized on day 14 with an average of 139 mg/L, representing 44 % of the initial soluble COD. Regarding nutrients, NH_4^+ was fully consumed after 10 d, while the PO_4^{3-} remained below 0.15 mg/L from the beginning, as its concentration in the raw RWW was at a trace level. The low P concentration hindered the COD assimilation as COD consumptions above 80 % are typically achieved [19]. The concentration of VSS was stabilized at day 18 with an average concentration of 312 mg/L, evidencing a stable microbial community despite the scarcity of PO_4^{3-}. VIS/NIR absorbance spectra of mixed culture samples taken from the photoanaerobic membrane bioreactor showed typical peaks of carotenoids and bacteriochlorophyll from day 4 to the end of the start-up stage (see VIS/NIR absorbance spectra in Supplementary Information SI-5; Fig. S2), confirming the presence of PPB in the mixed culture [30]. Indeed, the mixed culture acquired a characteristic reddish-brown colour of PPB cultures within the first week.

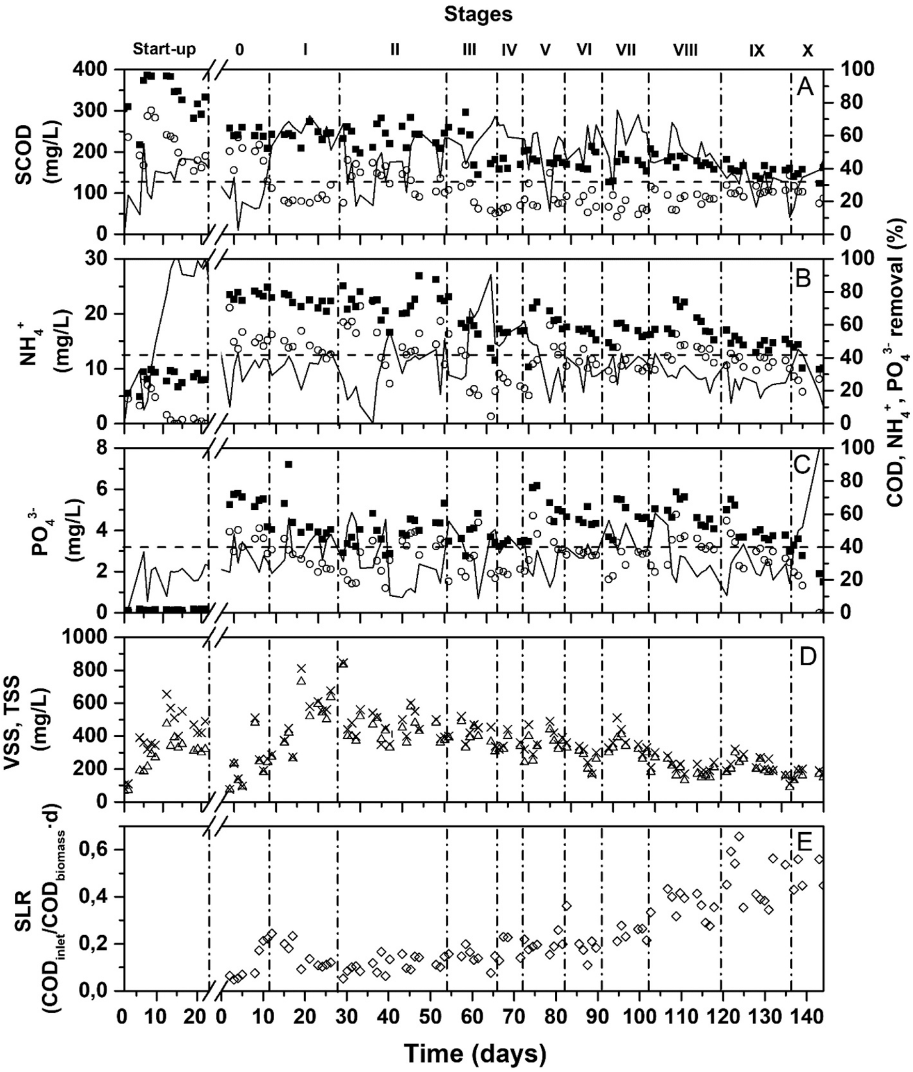

Fig. 2. Assessment of the macroscopic parameters during the PAnMBR operation: Soluble COD (A), NH_4^+ (B), PO_4^{3-} (C), VSS and TSS (D) and SLR (E). Symbols meaning: inlet concentration (■), outlet concentration (○), TSS concentration (X), VSS concentration (△), SLR values (◇). Units are in axis titles. Continuous lines represent removal efficiencies (%), dashed lines represent discharge limit concentration. Dash-dot vertical lines divide the graph into the operative stages.

The microbial community composition was also studied at selected samples taken on days 0, 1, 5, 7, 11, 13, 19, and 21. Relative abundance of the different taxa at the genus level during the start-up phase is shown in the Supplementary Information SI-5 (Fig. S3). Results on day 0 displayed a small community of PPB in the raw RWW (mainly photoorganoheterophs), thus acting as the initial seed for the subsequent PPB enrichment. Among the numerous PPB taxa, the most relevant genera or families (for non-identified genera) found in raw RWW correspond to *Rhodobacter* sp., *Thiobaca* sp., *Rhodoferax* sp., *Thiophaeococcus* sp., Comamonadaceae gen., Xantobacteraceae gen., *Bradyrhizobium* sp. and *Rhodopseudomonas* sp. with a notable prevalence of the last three. The PPB presence in the RWW may be due to the sunlight exposure of the raw RWW in certain phases before the biological treatment. The combined relative abundance of these PPB genera/families went from ca. 9 % on day 0 to 45 % on day 21, which unequivocally indicates the PPB enrichment in the mixed culture.

At the end of the start-up phase, *Rhodopseudomonas* sp. and *Rhodobacter* sp. were the most representative genera. Despite most of the mentioned microorganisms found in oil-polluted environments, *Rhodopseudomonas* sp. and *Rhodobacter* sp. seem to have a better adaptation in the refinery wastewater matrix. These results agree with previous studies that use these two genera in oil-polluted wastewater bioremediation for their enhanced oil-degrading capability [31–34].

Most of the other microorganisms found in the samples are typical in wastewater due to their hydrolytic and fermentative nature. Strict aerobic bacteria such as *Sphingomonas* sp. quickly decayed due to the lack of oxygen in the culture medium. Interestingly, *Acidovorax* sp. shows a significant and continuous presence during the start-up stage. This genus has been reported as a prominent actor in oil-polluted wastewater [35] and RWW [36]. It is known for its capability to degrade polymers and complex molecules [37–39]. Therefore, the competition between *Acidovorax* sp. and PPB species is not undesired since both are facultative bacteria capable of degrading complex hydrocarbons. In addition, anaerobic fermentative bacteria like *Proteinoclasticum* sp., *Youngiibacter* sp., and *Acetobacterium* sp. were also found, especially during the first days of the experiment.

According to the COD and ammonium removal data (Fig. 2), there is a close relationship between their increase and the rise of most representative genera of PPB, suggesting that these microorganisms lead to the consumption of ammonium and organic matter. However, the PPB growth is hindered by phosphorus scarcity, so higher enrichment and removal percentages might even be expected without this limitation, as has been previously demonstrated with other types of wastewaters [40].

3.2.2. Continuous co-treatment of RWW and DWW

a) Performance of PAnMBR

During the first stage of the continuous co-treatment, the PAnMBR was operated to increase the concentration of VSS using the inoculum generated during the previous start-up phase. The SRT was not controlled for 40 d in this stage, whereas the HRT varied from 75 h to 40 h. Under these operation conditions, the VSS increased up to 700 mg/L, and the SLR varied from 1.3 to ca. 0.11 $mgCOD_{inlet}/mgCOD_{biomass}$·d. At the end of this stage I, the PAnMBR achieved a stable COD (60–70 %) and NH_4^+ and PO_4^{3-} removals (ca. 40 %). Once the bioreactor was stabilized, the increase of the SLR from 0.11 ± 0.02 $mgCOD_{inlet}/mgCOD_{biomass}$·d at stage II to 0.21 ± 0.07 $mgCOD_{inlet}/mgCOD_{biomass}$·d at stage VI by the reduction of the HRT (from 60 to 30 h) at constant SRT (30 d) allowed to remain SCOD and NH_4^+ and PO_4^{3-} under the discharge limits. In this sequence of stages (II-VI), a sudden drop of the nutrients and SCOD in the inlet stream was observed (stage III), likely due to the renewal of RWW and DWW batches used as feedstock for the bioreactor. This fact derived in a significant VSS drop to ca. 400 mg/L at the end of stage VI, despite the increase of the OLR.

The increase of the SLR from 0.24 ± 0.02 to 0.50 ± 0.08 $mgCOD_{inlet}/mgCOD_{biomass}$·d during the stages VII to IX (keeping constant the HRT at 25 h and reducing SRT from 30 to 16 d) produced a loss of VSS with a consequent decrease of the bioreactor performance, leading to values of SCOD in the outlet stream above the discharge limits (stage IX). Thus, a clear destabilization of the bioreactor was evident with the increase of the SLR. Finally, during stage X, the system could be stabilized by increasing the SRT to 20 d, maintaining the concentration of VSS around 170 mg/L and a moderate performance in terms of SCOD and nutrients removals. Maximum COD removal was 75 % of the initial SCOD at stage VII, which is in the average range reported for an urban wastewater treatment [19]. At operation conditions of stage VII (25 h HRT and 25 d SRT; SLR of 0.24 ± 0.02 $mgCOD_{inlet}/mgCOD_{biomass}$·d), concentrations of COD, NH_4^+ and PO_4^{3-} were consistently below the regulated discharge limits (125 mg/L, 12.9 mg/L and 3.1 mg/L, respectively).

b) Nature of organic pollutants

The nature of the organic compounds involved in the COD consumption was also studied for a better understanding of the performance of PAnMBR. Soluble organic compounds were detected by GC–MS/MS in the inlet and outlet streams for samples taken at the end of stage X (see Supplementary Information SI-5; Fig. S4). Typical short-chain carboxylic acids of domestic wastewater, such as acetic acid, were identified in the influent stream. Additionally, other characteristic compounds of refinery wastewaters like long-chain carboxylic acid derivatives (undecanoates and dodecanoates), alcohols (butanediol), aromatics (pyrroles), and nitrogen-containing compounds (amines and amides) were also identified [41]. Interestingly, the majority of the identified compounds (formamide, *N*,*N*-dimethyl; acetic acid; 1,3-butanediol; undecanoic acid, methyl ester; cyclohexanamine, N-cyclohexyl; 2 pirrolidinone, 1-methyl and 7 dodecanoic acid, methyl ester) were removed during the treatment. Due to the high metabolic versatility of the PPB, the fate of these compounds seems to be related to microbial assimilation and degradation. However, a soluble recalcitrant compound, methoxy-phenyl-oxime (codified as 8), remained in the outlet effluent after the treatment, which was likely affecting the decrease of the COD removal. The presence of this pollutant was confirmed by the urban wastewater treatment plant that provided the DWW as a recurrent pollutant used as a fungicide in nearby croplands. In any case, it is noteworthy that the mixed culture of the PAnMBR could deal with the aromatics and amines compounds that represent a potential hazard for conventional biological treatments [21]. Microbial degradation pathways are discussed more insightfully when describing the microbial communities' dynamics below.

c) Specific loading rate assessment

In order to assess the maximum capacity of the photoanaerobic treatment, the specific loading rate (SLR) was compared to the specific consumption rate (SCR), both in terms of the inlet and consumed COD (Fig. 3). For SLR values below ca. 0.3 $mgCOD_{inlet}/mgCOD_{biomass}$·d, there was a clear positive and stable linear trend between SCR and SLR. Note that this value is comparable to the typical SLR of conventional activated sludge treatments [23]. For example, the WWTP of a medium size refinery in Portugal based on activated sludge has a SLR of 0.35 $mgCOD_{inlet}/mgCOD_{biomass}$·d [4]. Additionally, ten case scenarios from different domestic WWTP based on activated sludge systems in Europe showed SLR values between 0.1 and 0.25 $mgCOD_{inlet}/mgCOD_{biomass}$·d [42]. On the other hand, the process became unstable for SLR above ca. 0.3 $mgCOD_{inlet}/mgCOD_{biomass}$·d evidencing that the biomass reached maximum values of the specific assimilation rate of the organic carbon. This fact is consistent with the lower consumption of COD and nutrients, started at stage VIII and confirmed at stage IX with SLR values of 0.36 ± 0.03 and 0.5 ± 0.08 $mgCOD_{inlet}/mgCOD_{biomass}$·d, respectively.

d) Analysis of dynamic microbiological populations

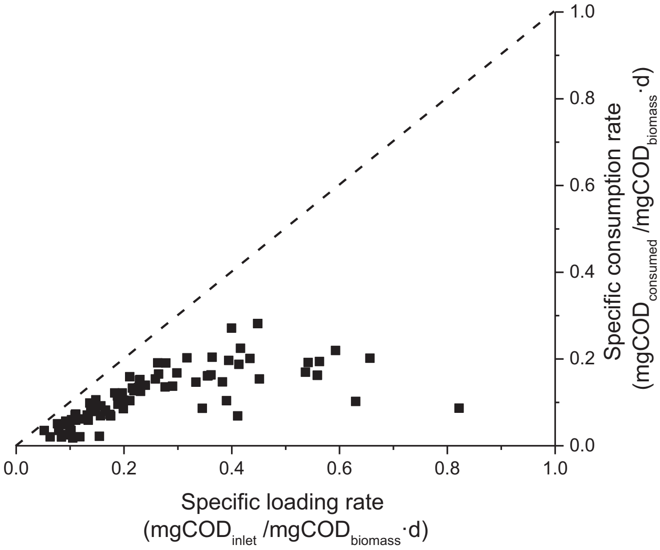

Fig. 3. Specific loading rate (SLR) versus specific consumption rate (SCR) (■). The dashed line with a slope of 1 represents the full theoretical consumption of the substrate.

Fig. 4 displays the results of the microbiological analysis corresponding to the samples of the beginning of the continuous phase (Inoculum), the end of the acclimatization phase (stage I; day 25), and relevant operation periods (stages III, VII, VIII, and X; days 60, 96, 116 and 144, respectively).

The most abundant PPB genera at the beginning of the continuous co-treatment of RWW and DWW were *Rhodopseudomonas* sp. and *Rhodobacter* sp., which is consistent with the final microbial characterization of the inoculum from the previous start-up phase (see Supplementary Information SI-5; Fig. S3). The relative abundance of PPB genera during the different stages of the co-treatment ranged from 30 to 60 %, evidencing an enriched PPB mixed culture during the whole operation treatment. Likewise, *Rhodopseudomonas* sp. and *Rhodobacter* sp. seemed to be the best-adapted genera for treating the RWW/DWW mixture for the different operational conditions. Other works involving the treatment of domestic and agro-industrial wastewaters also showed these genera as predominant in PPB enriched cultures [13,14]. Moreover, it was also observed that *Rhodobacter* sp. generally prevailed over *Rhodopseudomonas* sp. in this study, which has also been previously pointed out in other works [43,44]. This fact responds to the low OLR of the treatment that favors a K-strategist like *Rhodobacter* sp. instead of a r-strategist like *Rhodopseudomonas* sp. that is able to grow better at higher OLRs [45]. *Rhodocista* sp. and *Rhodoferax* sp. also proliferated among other PPB genera during the acclimation stage I that was operated under longer SRT and HRT, which promotes slowly growing bacteria like those PPB genera [43,46,47]. Other microorganisms that take advantage of these conditions were sulfate-reducing bacteria such as *Arcobacter* sp., which also have low growth rates [48].

Additionally, fermentative bacteria commonly found in anaerobic wastewater treatments, such as *Acetobacterium* sp., *Lentimicrobium* sp., *Paludibacter* sp., and bacteria from the family Rikenellaceae were also identified in the microbial community. *Lentimicrobium* sp. were found at stages III and VIII, corresponding to lower values of the PPB abundance. Although it is known for its low growth rates [49], *Lentimicrobium* sp. has been reported as a majoritarian taxon during the anaerobic digestion with a much higher OLR (1000 mgCOD/L·d) than the values used in this work [50]. Thus, the *Lentimicrobium* sp. presence is probably more related to PPB decay.

On the other hand, the highest relative abundance of PPB was observed at stage VII, corresponding to the beginning of the SLR rise (average SLR of 0.24 ± 0.02 $\text{mgCOD}_{\text{inlet}}/\text{mgCOD}_{\text{biomass}}\cdot\text{d}$). The system achieved the highest COD consumption in this stage. Thus, as in the start-up phase, the PPB seems to be the primary consumers of organic matter. However, the abundance of PPB was strongly reduced at the following stage VIII when the SLR was increased, favoring the appearance of other microorganisms like the abovementioned *Lentimicrobium* sp. and strict aerobes like *Acinetobacter*. This stage VIII also included extended maintenance and cleaning activities in which the MBR was exposed to air. This fact may explain the presence of these microorganisms. Even more, members of the Patescibacteria class, *Candidatus Falkowbacteria* sp., and Kryptoniales gen. proliferated in these periods, revealing an association between them and *Lentimicrobium* sp. *Acidovorax* sp. also appeared in high proportion, but its proliferation only increased during the last stabilization phase, contrasting with the start-up phase. *Acidovorax* sp. has a fast growth rate, which benefits from high

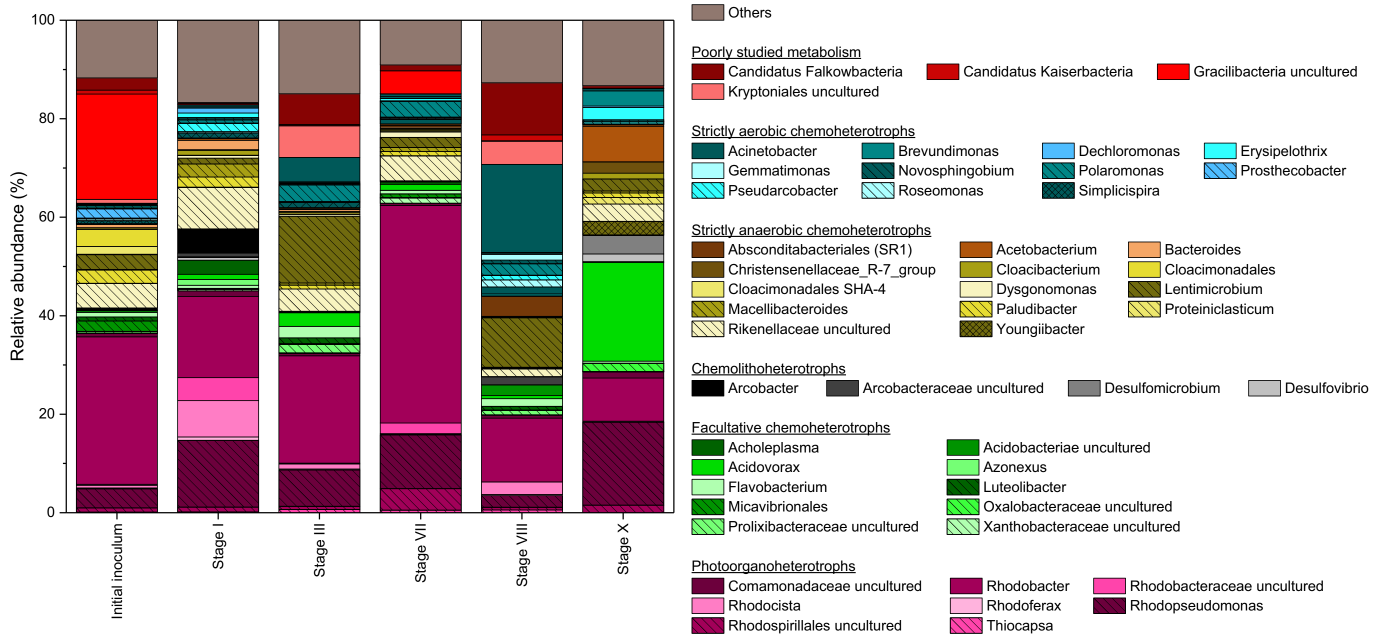

Fig. 4. Relative abundance of microorganisms at the genera level of a PPB culture developed using RWW and DWW as co-substrate in selected operation days.

OLR values like those obtained at stage X [51,52].

In terms of the compounds found in the inlet wastewater stream and the presence of the analyzed microorganisms, some links can be established. Formamide, a known protein denaturing agent, can be degraded to formic acid and ammonium by the action of enzymes as amidases [53]. Both formic acid and ammonium are beneficial chemicals for PPB metabolism, so formamide degradation is likely to occur. Regarding N-cyclohexyl cyclohexanamine, there are no reports of microbial degradation of this compound to the authors' knowledge. Nevertheless, several PPB species [54] and most sulfur-reducing bacteria [55] can assimilate cyclic compounds such as benzene and cyclohexane. The same is valid for 1 methyl,2-pyrrolydine. However, another cyclic compound such as methoxy-phenyl oxime seems to be resistant to the metabolic activity of the mixed PPB culture. Although, there is no information about its specific toxicity mechanism, this chemical has been reported as an antibacterial agent [58]. Additionally, other oximes were found as inhibitors of bacterial metabolism [56,57]. Thus, its potentially toxic nature may explain why the culture cannot degrade it.

PCA results shown in Fig. 5(A) combined microorganisms' abundance tendencies of biomass samples in two principal components strongly influenced by the variance of the data of the most abundant PPB genera, members of the phylum Patescibacteria, and fermentative bacteria. Principal component 1 (PC1) is mainly swayed by the presence of most PPB genera (*Rhodobacter* sp. and *Rhodopseudomonas* sp.) and *Acidovorax* sp. On the other hand, principal component 2 (PC2) is influenced by Gracilibacteria, *Acidovorax* sp., and fermentative bacteria such as *Acetobacterium* sp. and *Lentimicrobium* sp. The points shown in Fig. 5(A) correspond to the samples of the study of microbial community dynamics. The initial acclimatization stage (I) significantly affected the community structure of the initial inoculum. An antagonism between PPB and *Acidovorax* sp. also appeared, suggesting *Acidovorax* sp. as a potential opportunistic competitor of PPB. However, since this observation is only based on statistical correlation the relationship should be carefully addressed. PCA analysis also confirms that the destabilization of the system (stage IX) is related to the PPB community's decay. The relationship between *Lentimicrobium* sp. and *Candidatus Falkowbacteria* sp. and *Kryptoniales* sp. is also evident and opposed to the presence of Gracilibacteria. This finding may suggest a competitive behaviour between different Patescibacteria, which is not unlikely due to their common parasitic nature.

The RDA results in Fig. 5(B) combined microorganisms' abundance tendencies of the biomass and response variables of the PAnMBR in two axes. RDA1 is mainly related to SCOD and phosphate consumptions as well as the SLR (x axis) and RDA2 to the ammonium consumption (y axis). These results show a strong relationship between COD consumption and the presence of *Rhodobacter* sp., strongly supporting the assumption that PPB are the primary microorganisms responsible for the COD consumption. However, the increase of the SLR seems to harm this genus, probably due to a better adaptation of *Rhodopseudomonas* sp. to higher organic loadings and the increase of *Acidovorax* sp. The ammonium consumption seems to be related to *Lentimicrobium* sp. and their associated parasites, suggesting that these microorganisms could be the primary ammonium consumers in the absence of PPB. However, these results should be addressed carefully, as RDA 2 only explains 16.6 % of the variance [58].

3.3. Implications and prospects of the photoanaerobic treatment for RWW

The specific loading rate is a specific and critical parameter of each type of wastewater treatment, which is tightly related to the specific consumption rate and biodegradability of the wastewater. In this sense, operational parameters of the photoanaerobic treatment in this work have been compared with PPB-based treatments for different wastewaters and conventional activated sludge as the current biological process for refinery wastewater treatment. Generally, PPB can cope with low SRT (1–3 d) when the wastewater is easily biodegradable [19,43]. The continuous treatment of DWW by PPB accomplished removal yields over 90 % for both COD and nutrients using a similar PAnMBR under HRTs between 11 and 22 h and SRTs between 2 and 3 d [14]. In that study, the specific loading and consumption rates remained very closely (1.22–2.78 $\text{mgCOD}_{\text{inlet}}/\text{mgCOD}_{\text{biomass}}\cdot\text{d}$ and 1.18–2.67 $\text{mgCOD}_{\text{consumed}}/\text{mgCOD}_{\text{biomass}}\cdot\text{d}$, respectively) due to the highly biodegradable nature of DWW, which enhances the performance of the process by an increased phototrophic biomass yield [19]. However, low biodegradable wastewaters require higher SRTs to ensure a stable active biomass concentration. For example, the treatment of a high saline alimentary industry

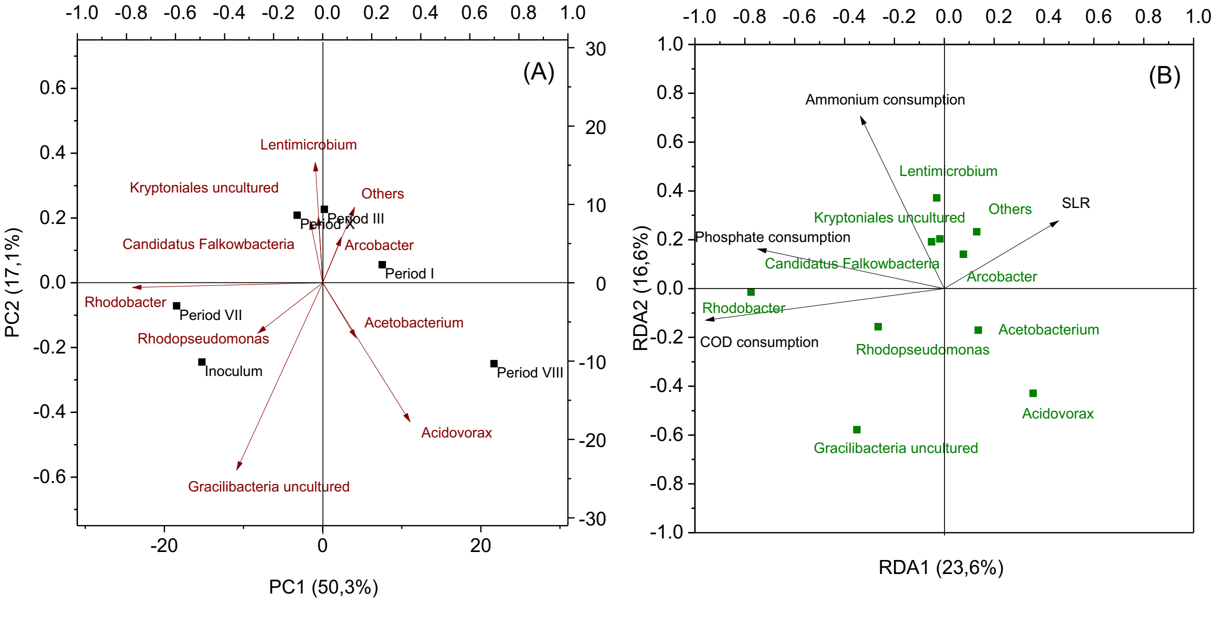

Fig. 5. Principal Component Analysis (PCA; A) and Redundance Analysis (RDA; B). PCA performed with two principal components which explain 67.4 % of the variance. Arrows represent species dependence on each principal component (only majority names are shown). Squares represents sample position among species. RDA performed with two constrained variables. Arrows represent response variables. Squares represent majoritarian genera's dependence on each principal component.

wastewater achieved 80 % of SCOD removal with a HRT of 48 h and SRT of 30 d [59]. The optimal specific loading rate for this wastewater was around 0.3 $mgCOD_{inlet}/mgCOD_{biomass}\cdot d$, which is quite similar the value determined in this work for the RWW. The specific consumption rate of high saline alimentary wastewater was near 0.24 $mgCOD_{consumed}/mgCOD_{biomass}\cdot d$, revealing that this effluent is slightly more biodegradable than the RWW by PPB but way less than the DWW. Regarding the conventional activated sludge, the treatment of RWW requires HRTs of 24–48 h and SRTs of 30–40 d [60,61] with specific loading and consumption rates of 0.56–0.71 $mgCOD_{inlet}/mgCOD_{biomass}\cdot d$ and 0.42–0.60 $mgCOD_{consumed}/mgCOD_{biomass}\cdot d$. Thereby, even far from the typical values for the photoanaerobic treatment, the final operational parameters of this work (stage X; 25 h HRT; 20 d SRT; SLR of 0.49 ± 0.06 $mgCOD_{inlet}/mgCOD_{biomass}\cdot d$) can compete with the conventional technology of activated sludge for the treatment of RWW with the benefit of reducing CO_2 emissions.

Additionally, the water stream collected in the final operational conditions complied with regulated concentration limits of COD, ammonium, and phosphates. Moreover, the separation of the suspended solids by the ultrafiltration-type membrane implemented in the photoanaerobic reactor allows fulfilling parameters regulated for reclaimed water, such as pathogens and specific macroscopic parameters [62,63]. The outlet stream falls into the highest quality category of residential use (<1 intestinal nematode egg/L, 0 CFU of *E. coli*/L, 2 NTU, 100 UFC of *Legionella* sp./L, 10 mg/L of TSS, and 10 $mgBOD_5/L$). Characterization results of inlet and outlet streams of PAnMBR are summarized in Supplementary Information SI-5 (Table S3).

Water reuse has an undeniable positive impact on every industry but usually comes with a high cost in the form of expensive tertiary treatments. There is no need for such tertiary treatment with the proposed technology since a high-quality effluent is achieved at the biomass-water separation stage. Reclaimed water of this quality may be used as process water, including boiling and refrigeration activities for any refinery unit. Additionally, it can be used for non-process-related issues such as cleaning activities, irrigation of green areas, and fire-fighting systems, depending on the regulations of each country. Besides the already commented economic benefits, reclaimed wastewater obtained in this way means a vast environmental improvement by saving water and avoiding the by-products obtained when using conventional tertiary treatments such as tetrachloromethanes coming from water chlorination [64].

Regarding prospects, the present work is key in the potential implementation of the proposed strategy at pilot or full scale. Apart from the high-quality final effluent, the treatment showed the maximum COD and nutrient elimination with a biomass concentration near 300 mg/L. Additionally, even with low VSS concentrations, the system remained highly stable until the final periods and performed accordingly with discharge regulations. Interestingly, the treatment of a synthetic wastewater with a similar reactor setup sustained a proper treatment with a similar VSS concentration [40]. Thereby, this behaviour is not inherent to the mixture of RWW and DWW, and promising results can be expected for other kinds of wastewaters. Finally, although RWW has been treated before with PPB [17], the present work answers different questions. The continuous mode was assessed, including the influence of SLR, which provides an early approach for the scaling-up of the technology. The effluent treated in this work is the wastewater produced after primary treatment, so the treatment does not need to deal with the excess oils, solids, and other immiscible materials. The mixed PPB culture developed in this work from the own RWW is much more diverse and realistic, making the process more feasible for potential implementation. In summary, the present work successfully stretched the gap between the use of PPB to treat RWW and its implementation at an industrial scale.

4. Conclusions

The RWW can be successfully treated with PPB-based cultures in a photo-anaerobic membrane bioreactor. However, due to the low nutrient content of RWW, the process can be improved by adding a 25 % volume of DWW as an additional nitrogen and phosphorus source, reaching a COD/N/P ratio of 100/6.5/0.5. The mixed culture enriched in PPB for this treatment can be obtained from the RWW without previous inoculation. Regarding the operational performance of the process, this work reports an optimum specific loading rate of 0.3 $mgCOD_{inlet}/mgCOD_{biomass}\cdot d$ to avoid the system destabilization. Within those conditions, *Rhodopseudomonas* sp. and *Rhodobacter* sp. were the most abundant bacteria, and they seem responsible for the highest eliminations of carbon and nutrients. The effluent obtained under such conditions complies with discharging regulations and falls under the maximum quality grade for its reuse as process water or domestic uses. This could make oil refinery industries (and others) benefit since the proposed technology can achieve reclaimed water without a tertiary treatment.

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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary Information

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jwpe.2022.103352.

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