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Exploring the metabolic capabilities of purple phototrophic bacteria during piggery wastewater treatment

Cristian A. Sepúlveda-Muñoz [a,b], Andrés F. Torres-Franco [a,b], Ignacio de Godos [a,c], Raúl Muñoz [a,b,*]

[a] Institute of Sustainable Processes, Dr. Mergelina s/n, 47011 Valladolid, Spain
[b] Department of Chemical Engineering and Environmental Technology, School of Industrial Engineering, University of Valladolid, Dr. Mergelina s/n, 47011 Valladolid, Spain
[c] Department of Chemical Engineering and Environmental Technology, School of Forestry, Agronomic and Bioenergy Industry Engineering (EIFAB), University of Valladolid, Campus Duques de Soria, 42004, Soria, Spain

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ABSTRACT

Purple phototrophic bacteria (PPB) are receiving an increasing attention due to their extraordinary metabolic capabilities for nutrient and carbon recovery from wastewaters. Batch experiments were herein performed to assess the influence of the type of radiation (photosynthetic active radiation (PAR), near-infrared radiation (NIR) and PAR using an UV-VIS absorbing filter), temperature (13 °C and 30 °C), type of metabolism (photoheterotrophic and chemoheterotrophic), type of inoculum (mixed culture and pure strain *Rhodopseudomonas palustris*) and wastewater load (1:5 and 1:10 dilutions) during piggery wastewater (PWW) treatment by PPB. The use of UV-VIS filtered PAR supported both a high content of bacteriochlorophyll in PPB and the highest total organic carbon (TOC) and total nitrogen (TN) removal efficiency (RE) (74 % and 37 %, respectively), but at lower maximum removal rates. Interestingly, PPB exhibited similar TOC-REs and TN-REs (73 % and 37 %, respectively) at 13 °C than at 30 °C (71 % and 45 %, respectively), at similar removal rates. Mixed cultures of PPB achieved a higher nutrient assimilation rate than *R. palustris*, supporting a total assimilation of the volatile fatty acids present in 10 folds diluted PWW. In brief, mixed cultures of PPB were highly efficient during PWW treatment, regardless of the type of radiation and temperature under photoheterotrophic growth.

1. Introduction

Purple phototrophic bacteria (PPB) are a group of microorganisms composed of purple sulfur bacteria and purple non-sulfur bacteria, that exhibit highly versatile metabolic pathways, including different forms of energy production via oxidative phosphorylation and anoxygenic photosynthesis [1–3]. Depending on the presence or absence of oxygen and light, PPB can grow phototrophically or chemotrophically [1], absorbing energy from solar irradiation or from the degradation of macromolecules [2]. Organic molecules can be also degraded when used as electron donor under phototrophic conditions. Thus, PPB can grow using a broad portfolio of carbon and/or nitrogen sources. Furthermore, some PPB species can grow under extreme environments, under very acidic or alkaline conditions, with high concentrations of salts, and low or extremely high temperatures [4].

Photosynthesis is a fundamental process for energy production in photosynthetic microorganisms. Among them, PPB have been described as more efficient compared to microalgae in terms of photoconversion [5]. In PPB, bacteriochlorophylls and carotenoids are the main pigments responsible for light harvesting. These pigments absorb photons in wavelengths at the near-infrared and visible [1–3,6]. More specifically, PPB can absorb light in the near-infrared range (805 nm to 1035 nm) due to the presence of bacteriochlorophyll *a* and *b*, and also in the ultraviolet and visible spectrum (300–500 nm) due their ability to synthetize several carotenoids [1]. In addition to their function as accessory pigments in light absorption, carotenoids prevent photodegradation of bacteriochlorophyll during stressful environmental conditions [7]. Consequently, PPB hold a unique spectral niche of infrared light absorption, an advantage that maximizes their metabolism and facilitates their specific selection among photosynthetic microorganisms [5,8].

This extraordinary metabolic diversity and variety of mechanisms of light utilization enable multiple biotechnological applications of PPB for

* Corresponding author at: Institute of Sustainable Processes, Dr. Mergelina s/n, 47011 Valladolid, Spain.
E-mail address: mutora@iq.uva.es (R. Muñoz).

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the treatment of many types of wastewater, with efficient conversion of high pollutants loads [3]. Recent studies have demonstrated the high performance of PPB for the treatment of domestic wastewater [5,9,10], even at low temperatures of 10 °C [11] and 11 °C [12], which confirmed the high level of adaptation of PPB to ambient seasonal variations. The low concentrations of pollutants in domestic wastewaters facilitate PPB growth and therefore biological treatment [5,10,11]. However, the high content of organic matter and nutrients present in livestock wastewater, such as piggery wastewater (PWW), block light penetration and inhibit PPB growth as a result of NH_3 inhibition [13–15]. Despite recent works have reported high pollutant removal efficiencies during PPB-based treatment of diluted PWW [13,15], the impact of key parameters such as the radiation source, temperature, type of metabolisms and inoculum characteristics have not been yet systematically explored.

The influence of key environmental and operational parameters such as radiation source, temperature, inoculum characteristics and wastewater strength on carbon and nutrient removal, and biomass growth, was investigated during PWW treatment in batch photobioreactors.

2. Materials and methods

2.1. PWW and inoculum

PWW was obtained from a pig farm in Segovia (Spain) and maintained at 4 °C prior use. The PWW was centrifuged at 10,000 rpm for 10 min at 4 °C in a Sorvall Legend RT centrifuge (ThermoScientific, Germany) in order to remove most suspended solids. The chemical characteristics of the PWW are summarized in Table 1. The inoculum of the mixed PPB culture was prepared as described by Sepúlveda-Muñoz et al. [16]. *Rhodopseudomonas palustris* strain R1 (DSM 8283) was purchased from the culture collection DSMZ (Germany) and revitalized in autoclaved serum bottles (120 mL) containing Van Neil's yeast broth with a composition of 10 g L^{-1} of yeast extract, 1.0 g L^{-1} of K_2HPO_4 and 0.5 g L^{-1} of $MgSO_4$ (pH 7.0–7.2), under anaerobic conditions (completely filled bottles). This culture was crimp sealed with butyl septa and aluminium caps. The bottles were incubated under magnetic agitation at 300 rpm, constant temperature of 30 °C and continuous radiation of near-infrared light with an intensity of 50 W m^{-2} provided by light-emitting diodes SFH 4780S and SFH 4715AS (OSRAM, Germany) with a peak of intensity at 810 nm and 850 nm, respectively. After revitalization, *R. palustris* was cultured in 10 fold diluted, filtered (0.20 μm pore sized nylon filters) and autoclaved PWW to allow the acclimation of the model strain to the PWW.

2.2. Experimental procedures

A total of 33 photobioreactors were used in batch tests treating 10 fold diluted PWW (unless otherwise specified). Experiments were conducted in triplicate. Each photobioreactor consisted of a 1.2 L bottle (Afora, Spain) with a working volume of 0.5 L and 50 mL of inoculum (pre-cultured at an absorbance at 808 nm of 13.5 ± 0.5). The photobioreactor headspace was flushed with helium (>99.9 %, Abello Linde, Spain) for 3 min before each trial and closed with butyl septa and plastic caps in order to maintain anaerobic conditions. The bottles were incubated inside a water bath Tectron-Bio (J.P. SELECTA, Spain) at a constant temperature of 30 °C and a cooling bath CC-K6 CC1 (Huber, Germany) for assays at 13 °C. Photosynthetic active radiation (PAR) and near-infrared radiation (NIR) were provided using a white light-emitting diodes (LED) panel Clearflood (Phillips, Spain) and a LED panel with diodes OSLUX® SFH 4780S and 4715AS (OSRAM, Germany), respectively. A white LED panel equipped with UV-VIS absorbing foil ND 1.2 299 [5] was also applied as a radiation source. The intensities of PAR and NIR were determined with a LI-250A light meter (LI-COR Biosciences, Germany) and PASPort PS-2148 IR sensor (PASCO, USA), respectively.

Liquid samples of the culture broths of 5 mL were periodically withdrawn to monitor culture absorbance, pH, concentration of total dissolved organic carbon (TOC), total dissolved carbon (TC), dissolved inorganic carbon (IC), total dissolved nitrogen (TN), volatile fatty acids (VFAs) and total suspended solids (TSS). Gas samples of the headspace with a total volume of 100 μL were also periodically withdrawn using a gastight syringe (Hamilton, USA) in order to monitor the concentrations of CO_2, H_2S and CH_4.

2.2.1. Test series I: influence of the light source on PWW treatment

The influence of the light source on PPB growth and assimilation of pollutants from PWW was investigated using 10 fold diluted PWW at constant temperature of 30 °C. Four different light source were assessed: PAR with a photon flux density of 1055 ± 8 μmol m^{-2} s^{-1} (equivalent to a NIR of 431 ± 9 W m^{-2}) (S-PAR), PAR filtered with an UV-VIS absorbing foil resulting in 123 ± 4 W m^{-2} (P-ABF), low NIR with an intensity of 126 ± 5 W m^{-2} (L-NIR) and high NIR at 443 ± 3 W m^{-2} (H-NIR). The selected PAR mimicked sunlight intensities during a summer day in Valladolid (Spain) [13,17]. All tests were performed under

Table 1
Composition of the 10 fold diluted PWW and final cultivation broths, and key performance indicators, in the tests devoted to assess the influence of type and intensity of illumination, temperature, type of inoculum and PWW load during PPB-based PWW treatment (values represent average ± standard deviation).

Parameters	PWW (10 fold diluted)	Test series I				Test series II			Test series III			
		S-PAR	P-ABF	L-NIR	H-NIR	T-13	T-30	D-30	MC-1:5	MC-1:10	RP-1:5	RP-1:10
TOC (g L^{-1})	0.86 ± 0.13	0.21 ± 0.02	0.20 ± 0.00	0.23 ± 0.01	0.24 ± 0.01	0.22 ± 0.00	0.24 ± 0.01	0.80 ± 0.01	1.09 ± 0.02	0.30 ± 0.01	1.53 ± 0.05	0.23 ± 0.01
TC (g L^{-1})	1.00 ± 0.20	0.41 ± 0.00	0.40 ± 0.00	0.41 ± 0.01	0.42 ± 0.01	0.43 ± 0.00	0.43 ± 0.01	0.93 ± 0.01	1.39 ± 0.01	0.56 ± 0.01	1.83 ± 0.04	0.50 ± 0.01
IC (g L^{-1})	0.11 ± 0.03	0.19 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.21 ± 0.00	0.19 ± 0.00	0.12 ± 0.00	0.53 ± 0.01	0.27 ± 0.00	0.29 ± 0.01	0.27 ± 0.01
TN (g L^{-1})	0.38 ± 0.08	0.17 ± 0.00	0.19 ± 0.01	0.18 ± 0.00	0.17 ± 0.00	0.23 ± 0.01	0.21 ± 0.00	0.37 ± 0.00	0.63 ± 0.01	0.29 ± 0.00	0.71 ± 0.02	0.26 ± 0.00
TSS (g L^{-1})	0.76 ± 0.09	1.92 ± 0.02	1.86 ± 0.04	1.91 ± 0.02	1.89 ± 0.02	1.86 ± 0.02	1.87 ± 0.03	0.67 ± 0.01	4.11 ± 0.16	2.27 ± 0.08	2.12 ± 0.12	2.07 ± 0.03
pH	7.73 ± 0.12	7.60 ± 0.03	7.57 ± 0.04	7.57 ± 0.04	7.54 ± 0.05	7.63 ± 0.03	7.65 ± 0.05	6.92 ± 0.04	7.64 ± 0.09	7.26 ± 0.01	7.16 ± 0.03	7.26 ± 0.12
Abs (500/808 nm)	–	1.65 ± 0.02	1.32 ± 0.02	1.49 ± 0.01	1.61 ± 0.02	1.43 ± 0.02	1.25 ± 0.02	–	1.11 ± 0.01	1.24 ± 0.00	1.31 ± 0.02	1.35 ± 0.03
Time (d)	–	24				14			40			
TOC-RE (%)	–	71 ± 2	74 ± 1	69 ± 1	69 ± 2	73 ± 0	71 ± 1	1 ± 1	52 ± 2	73 ± 1	29 ± 2	79 ± 1
VFA-RE*(%)	–	64 ± 4	59 ± 3	55 ± 1	56 ± 8	68 ± 7	63 ± 9	−4 ± 9	81 ± 2	99 ± 0	33 ± 2	99 ± 0
TN-RE (%)	–	45 ± 1	37 ± 2	42 ± 0	44 ± 0	37 ± 3	45 ± 1	1 ± 1	24 ± 2	37 ± 1	16 ± 1	42 ± 1

TOC, TC, IC, TN, TSS, TSS and pH correspond to the average values recorded from the three final samplings (n = 9).
* VFA-REs based on the total amount of carbon present in the VFA.

continuous illumination. The photobioreactors were supplied every three days with 25 mL of pure CO_2 (>99.9 %, Abello Linde, Spain) in order to avoid the inhibition of PPB growth by the increase in pH.

2.2.2. Test series II: influence of temperature and metabolisms on PWW treatment

The influence of temperature and light supply on the PPB growth and pollutant removal was evaluated in 10 fold diluted PWW inoculated with PPB mixed cultures at constant temperatures of 13 °C (T-13) and 30 °C (T-30). PAR filtered with an UV-VIS absorbing foil resulting in a NIR of 122 ± 3 W m^{-2} was constantly supplied. In addition, a test with no light supply (covering the bottles with aluminium foil) was also conducted at 30 °C to assess the potential of chemoheterotrophic metabolism of PPB to treat PWW (D-30). The photobioreactors were supplied every two days with 25 mL of pure CO_2.

2.2.3. Test series III: influence of inoculum and PWW load on PWW treatment

The performance of a mixed culture (MC) of PPB and a pure strain *R. palustris* (RP) was compared at 30 °C, using 5 and 10 fold diluted PWW, resulting in four conditions tested: namely MC-1:5, MC-1:10, RP-1:5 and RP-1:10, respectively. The volume of inoculum was adjusted based on the measurement of culture absorbance at 808 nm to provide the same concentration of PPB in all assays. All photobioreactors were constantly illuminated using PAR filtered with a UV–VIS absorbing foil at 124 ± 5 W m^{-2}. The photobioreactors were supplied every two days with 25 mL of pure CO_2.

2.3. Analytical procedures

A UV-2550 spectrophotometer (Shimadzu, Japan) was used to measure culture absorbance in samples diluted to achieve readings between 0.2 and 1.0. A 510 pH-meter (Cyberscan, Netherlands) was used to monitor the pH. TOC, TC, IC and TN concentrations were measured using a TOC-VCSH TOC analyser equipped with a TNM-1 unit (Shimadzu, Japan). A 7820A gas chromatograph (GC) equipped with an FID detector (Agilent, USA) was used to determine VFA concentrations according to López et al. [18]. TSS concentrations were determined according to the protocol described in Standard Methods [19]. Finally, the concentrations of CO_2, H_2S and CH_4 in the bottle headspace were determined using a 430 GC equipped with a TCD detector (Bruker, USA) according to Ángeles et al. [20].

2.4. Statistical methods

The average and standard deviations were calculated using the measurements of the triplicate bottles. Analysis of variance (one-way ANOVA) and pairwise comparison by Tukey test was used to assess the significance of the treatment effects, via Statgraphics centurion software (V.18). Comparisons were considered significant using values of $p < 0.05$. Data used for statistical analyses were obtained from the three final samplings (n = 9).

3. Results and discussion

3.1. Influence of the light source on PWW treatment

Light intensities and temperature were controlled at constant levels in all photobioreactors (Figs. S1A and S2A). All radiation sources supported PPB growth during PWW treatment via anoxygenic photosynthesis, resulting in a rapid increase of the pH from 7.62 ± 0.02 up to 9.42 ± 0.02, 8.75 ± 0.03, 9.24 ± 0.05 and 9.30 ± 0.04 in S-PAR, P-ABF, L-NIR and H-NIR, respectively, during the first three days of incubation (Fig. 1A). These increases in pH were attributed to the rapid VFAs assimilation by PPB, similar to previous experiments conducted to evaluate the performance of PPB for domestic wastewater treatment [5].

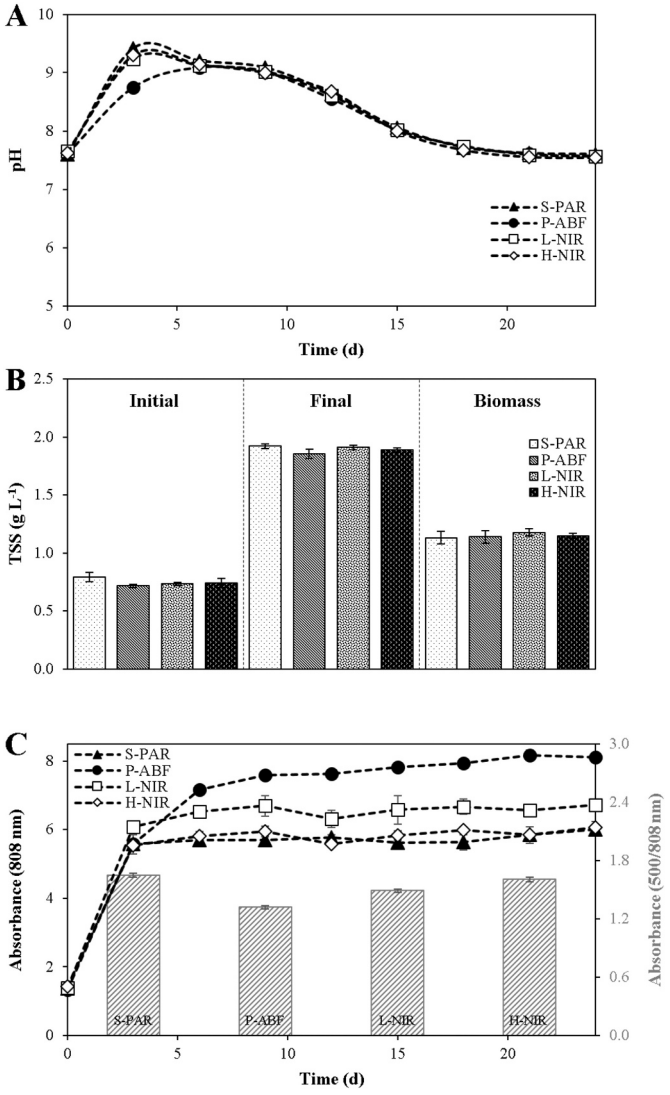

Fig. 1. Time course of the pH (A), final TSS concentration (B) and time course of the absorbance at 808 nm and carotenoid/bacteriochlorophyll ratio (secondary axis) (C) in test series I.

The gradual decreases of pH to values of 7.60 ± 0.03, 7.57 ± 0.04, 7.57 ± 0.04 and 7.54 ± 0.05 by the end of the experiment in S-PAR, P-ABF, L-NIR and H-NIR, respectively, were likely due to the constant addition and accumulation of CO_2 in the headspace, which contributed to avoid PPB inhibition [15]. Thus, these decreases in pH were concomitant with CO_2 accumulation in the headspace of all photobioreactors (Fig. S3A1) which evidenced the preference of PPB for the organic matter present in PWW over CO_2 even when PPB are capable of fixing CO_2 via Calvin-Benson-Bassham cycle [21]. Neither H_2S nor CH_4 were detected in the photobioreactor headspace during the 24 days of experimentation, confirming the absence of anaerobic fermentative processes (Fig. S3B1 and C1).

No significant differences were recorded in the final biomass concentration among the different light sources tested, with values of 1.13 ± 0.05, 1.14 ± 0.05, 1.18 ± 0.03 and 1.15 ± 0.02 g L^{-1} under S-PAR, P-ABF, L-NIR and H-NIR, respectively (Fig. 1B). These similar TSS concentrations suggest that PPB were able to grow under all treatment conditions tested, supported by the anaerobic conditions and IR supply. Furthermore, no photoinhibition during PWW treatment was associated with high NIR levels. Indeed, the NIR intensities of 431 W m^{-2} in S-PAR and 443 W m^{-2} in H-NIR are among the highest intensities reported for PPB cultivation [3] and did not entail a severe photoinhibition.

However, such high light intensities did not support an enhanced PWW treatment performance. Previous investigations in PWW treatment under anaerobic conditions and IR supply revealed a high dominance of PPB under these conditions, with microbial abundances of 54–84 % as a result of the selective conditions favoring PPB growth [13,22,23].

Despite all treatments supported similar final biomass concentrations, PPB exhibited different trends in pigment accumulation as an adaptative response to light sources and intensities. Bacteriochlorophyll *a* accumulation was monitored via measurement of culture absorbance at 808 nm [15], which exhibited values of 8.1 ± 0.1 and 6.7 ± 0.1 under lower infrared radiations in P-ABF and L-NIR, respectively, and of 6.0 ± 0.0 and 6.1 ± 0.2 under higher infrared radiations in S-PAR and H-NIR, respectively. In this sense, lower NIR intensities likely promoted a higher accumulation of bacteriochlorophyll as a mechanism for enhancing light harvesting. In addition, the carotenoid/bacteriochlorophyll ratio (estimated as the ratio of absorbances at 500 and 808 nm) exhibited lower values in P-ABF (1.3 ± 0.0) and L-NIR (1.5 ± 0.0), than in S-PAR (1.6 ± 0.0) and H-NIR (1.6 ± 0.0), suggesting that less carotenoids compared to bacteriochlorophyll were produced in P-ABF and L-NIR. At this point, it must be stressed that carotenoids synthesis was typically attributed a photo-protective role in photosynthetic microorganisms exposed to high levels of radiation [24,25], which suggest lower stress in the photosynthetic apparatus of PPB growing in the absence of visible and ultraviolet radiation and low NIR intensities [26], and ultimately favoring a higher bacteriochlorophyll accumulation, mainly in P-ABF.

Similarly, a higher bacteriochlorophyll synthesis was observed by Suwan and co-workers in PPB cultures grown under radiation generated by tungsten lamps using UV-VIS absorbing foil compared to cultures grown under NIR LED [27], suggesting that this filter prevented the photodegradation of the photosynthetic apparatus. In our study, high infrared radiations resulted in lower absorbances at 808 nm and higher carotenoid/bacteriochlorophyll ratios, suggesting a reduced bacteriochlorophyll content and increased synthesis of carotenoids by PPB mediated by the above-mentioned photoprotective effect. In this context, it has been recently demonstrated that UV–VIS absorbing foils can be used under direct solar irradiation in outdoor large-scale PWW treatment systems [22]. This strategy represents the most economical source of NIR for PPB growth.

Interestingly, the type and intensity of the light source did not have a direct effect on TOC removal. A rapid carbon assimilation occurred in all photobioreactors during the first three days, resulting in final TOC concentrations of 0.21 ± 0.02, 0.20 ± 0.00, 0.23 ± 0.01 and 0.24 ± 0.01 g L^{-1} in S-PAR, P-ABF, L-NIR and H-NIR, respectively (Fig. 2A). This rapid assimilation of soluble carbon from the PWW was concomitant with the rapid increase in biomass concentrations. The final TOC removal efficiencies (TOC-RE) accounted for 71 ± 2 %, 74 ± 1 %, 69 ± 1 % and 69 ± 2 % in S-PAR, P-ABF, L-NIR and H-NIR, respectively, which were comparable to those observed by Sepúlveda-Muñoz and co-workers: 72 % TOC-RE in PPB cultures treating PWW supplemented with CO_2 under NIR of 50 W m^{-2} [15]. The metabolism of PPB herein used was efficient for carbon removal, as consistently described in the literature during the treatment of domestic wastewater (63 % COD-RE) [5], nitrogen-deficient wastewater (76 % COD-RE) [28], food industry wastewater (86 % COD-RE) [29] and poultry processing wastewater (90 % COD-RE) [30].

TOC removal was associated to a rapid VFAs consumption within the first days after inoculation (Fig. S4A, represented with the carbon contained in all VFAs). Short-chain VFAs (C2–C4) accounted for 95 % of the total VFAs mass in PWW, with concentrations of 0.86 g L^{-1}, 0.24 g L^{-1}, 0.12 g L^{-1} and 0.07 g L^{-1} for ethanoic, butanoic, propanoic and 2-methyl propanoic acids, respectively (Fig. 2B, black bars). Neither 4-methyl pentanoic nor hexanoic acids were detected in PWW. Similar concentrations of VFAs were reported in previous studies focused on the treatment of PWW with PPB coupled to biogas upgrading [14]. VFA concentrations rapidly decreased to average concentrations of 0.37 ± 0.02 g L^{-1} for ethanoic, 0.01 ± 0.00 g L^{-1} for propanoic, 0.00 ± 0.00 g L^{-1} for 2-methyl propanoic and 0.18 ± 0.03 g L^{-1} for butanoic acid by day 21 regardless of the radiation type and intensity (Fig. 2B, white bars). In addition, 3-methyl butanoic, pentanoic, and heptanoic acids were not detected at the end of the experiment. The VFA removal efficiencies (VFA-RE) achieved by day 21 accounted for 64 ± 4 %, 59 ± 3 %, 55 ± 1 % and 56 ± 8 % in S-PAR, P-ABF, L-NIR and H-NIR, respectively (Table 1). A slight increase in VFA concentration, probably due to cell lysis, was observed during the last day of the experiment in all photobioreactors (Fig. S4A). A high VFAs assimilation concomitant with high TOC removals during PWW treatment was previously described in experiments with CO_2 addition for pH control [15], resulting in a high PPB-based carbon removal efficiency (69 % and 92 % of TOC-RE and VFA-RE, respectively).

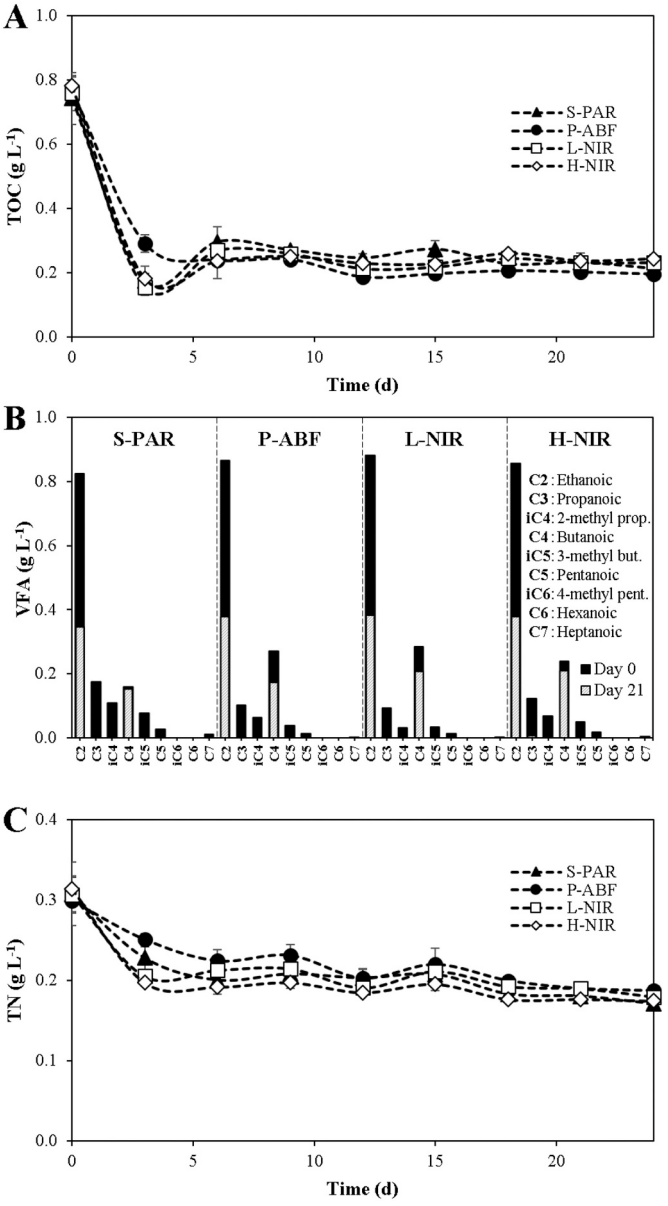

Fig. 2. Time course of TOC concentrations (A), initial (black bars) and final (gray bars) volatile fatty acids concentrations (B) and TN concentrations (C) in test series I.

Finally, TN concentrations of 0.17 ± 0.00, 0.19 ± 0.01, 0.18 ± 0.00 and 0.17 ± 0.00 g L^{-1} were recorded at the end of the experiment in S-PAR, P-ABF, L-NIR and H-NIR, respectively (Fig. 2C), resulting in TN removal efficiencies (TN-RE) of 45 ± 1 %, 37 ± 2 %, 42 ± 0 % and 44 ±

0 %, respectively (Table 1). PPB can assimilate all forms of nitrogen including N_2, NH_4^+, NO_3^-, NO_2^- and organic compounds containing nitrogen, such as amino acids and proteins [1,3]. Despite this efficient metabolism and the high nitrogen removal efficiencies recorded, the total depletion of nitrogen was hindered by carbon limitation, likely due to the recalcitrant nature of the remaining organic carbon (TOC concentration of 0.2 g L^{-1}, Fig. 2A), such as cellulose, starch and polymeric derivatives, which were not assimilated by PPB [7]. This carbon limitation is also evidenced by the 2.3:1 C:N recorded in the PWW herein used, which is lower than the typical C:N content of 5:1 described in PPB (*R. palustris* biomass) [13,16,31]. In addition, VFAs consumption suggested that PPB preferentially assimilated organic rather than inorganic carbon, revealing a predominant photoheterotrophic metabolism. This was also confirmed by the progressive accumulation of CO_2 in the headspace from day 18 onwards (Fig. S3A1) and the moderate pH values associated to CO_2 dissolution.

3.2. Influence of temperature and metabolism on PWW treatment

Light intensities below the UV–VIS absorbing foil remained at average values of 122 ± 3 W m^{-2} (Fig. S1B). Temperatures also remained constant during the experiment with average values of 13 °C at low temperature (T-13), 30 °C in the tests under optimal temperature (T-30) and 30 °C in the tests without light supply (D-30) (Fig. S2B). Previous studies have suggested 30 °C as the optimal value of temperature for PPB growth in wastewater [5,29,32,33]. However, cell growth and a satisfactory wastewater treatment performance have been observed at lower temperatures of 10–11 °C [11,12]. In our particular study a rapid pH increase from 7.70 ± 0.01 to 9.03 ± 0.04 and 9.07 ± 0.22 were measured in both T-13 and T-30 within the first 6 days of experiment. Afterwards, the pH progressively decreased to 7.63 ± 0.03 and 7.65 ± 0.05 in T-13 and T-30, respectively (Fig. 3A) as a result of the periodic addition of CO_2 in the bottles headspace. A slight decrease in pH was recorded in dark tests (D-30) by the end of the experiment due to the constant CO_2 supplementation (Fig. S3A2) and lack of biological activity. Despite the low PPB activity, no anaerobic metabolism was detected in D-30 since neither H_2S nor CH_4 were detected in the headspace of the photobioreactors (Fig. S3B2 and C2).

PPB biomass concentrations of 1.15 ± 0.05, 1.19 ± 0.04 and 0.01 ± 0.04 g L^{-1} were recorded in T-13, T-30 and D-30, respectively (Fig. 3B). Therefore, PPB biomass production did not experience any significant decrease at 13 °C compared to 30 °C. In this context, PPB growth at low temperatures such as 13 °C represents an advantage over the cultivation of other phototrophs such as microalgae, which typically exhibit reduced growth at low temperatures and ranges of optimal temperature between 15 and 30 °C [34]. No biomass production was detected in D-30 due to the absence of electron acceptors such as NO_3^-, O_2 or organic compounds [35] for energy production via oxidative phosphorylation, thus preventing chemotrophic growth [1–3]. In addition, low carbon compounds (mainly short-chain VFAs) in PWW provided insufficient energy for efficient chemotropic growth of PPB compared to other chemoheterotrophic microorganisms [36,37]. In this sense, anaerobic conditions and with sufficient NIR seemed to result in better conditions for PPB growth during wastewater treatment [36]. Moreover, the low temperature of 13 °C resulted in slight changes in pigment composition, without a significant effect over PWW treatment performance and total PPB biomass. The absorbance at 808 nm increased from 1.3 ± 0.0 to 6.2 ± 0.2 and 7.8 ± 0.1 in the assays T-13 and T-30, respectively (Fig. 3C), suggesting that growth at 13 °C decreased the concentration of bacteriochlorophyll present in PPB biomass without any significant impact in biomass production. Similarly, a slightly higher carotenoid/bacteriochlorophyll ratio of 1.4 ± 0.0 was measured in T-13, compared to 1.2 ± 0.0 in T-30, which suggested a higher carotenoids content at 13 °C.

TOC was mainly assimilated during the first 6 days of experiment, resulting in an earlier TOC assimilation (Fig. 4A) in T-30 compared to T-13. However, PPB in T-13 achieved similar TOC concentrations after 6

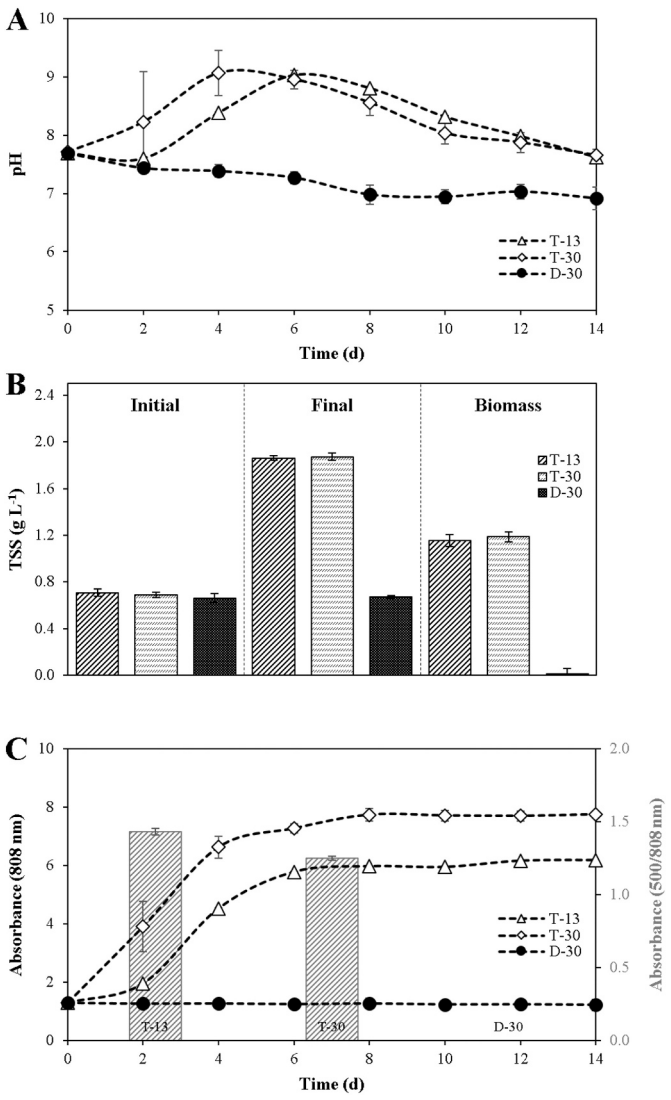

Fig. 3. Time course of the pH (A), final TSS concentration (B) and time course of the absorbance at 808 nm and carotenoid/bacteriochlorophyll ratio (secondary axis) (C) in test series II.

days with 0.22 ± 0.00 and 0.24 ± 0.01 g L^{-1} in T-13 and T-30, respectively. In D-30 no significant decrease in the initial TOC concentrations were recorded, with initial and final values of 0.81 ± 0.01 and 0.80 ± 0.01 g L^{-1}, respectively. Indeed, TOC-REs accounted for 73 ± 0 %, 71 ± 1 % and 1 ± 1 % in T-13, T-30 and D-30, respectively. These results confirm the ability of PPB to effectively support carbon removal during PWW treatment at low temperatures, similarly to previously reported by Hülsen et al. [11] during PPB-based domestic wastewater treatment, where COD-REs above 73 % at low temperatures were recorded, and by Dalaei et al. [12], who reported COD-REs of 93 % at 11 °C.

The concentration of VFAs exhibited a rapid decrease during the first days of experiment (Fig. S4B), with final VFA-REs of 68 ± 7 %, 63 ± 9 % and − 4 ± 9 % in T-13, T-30 and D-30, respectively. Both ethanoic and butanoic acid concentrations in T-13 and T-30 remained almost constant, while propanoic and 2-methyl propanoic acid concentrations were almost completely consumed. Similarly, an almost complete assimilation of the VFA in the C5-C7 range (3-methyl butanoic, pentanoic, 4-methyl pentanoic, hexanoic and heptanoic acids) was recorded in both T-13 and T-30 (Fig. 4B). VFA assimilation by PPB were not influenced at low temperature. Interestingly, the concentration of ethanoic acid increased in D-30, which suggested the growth and occurrence of an

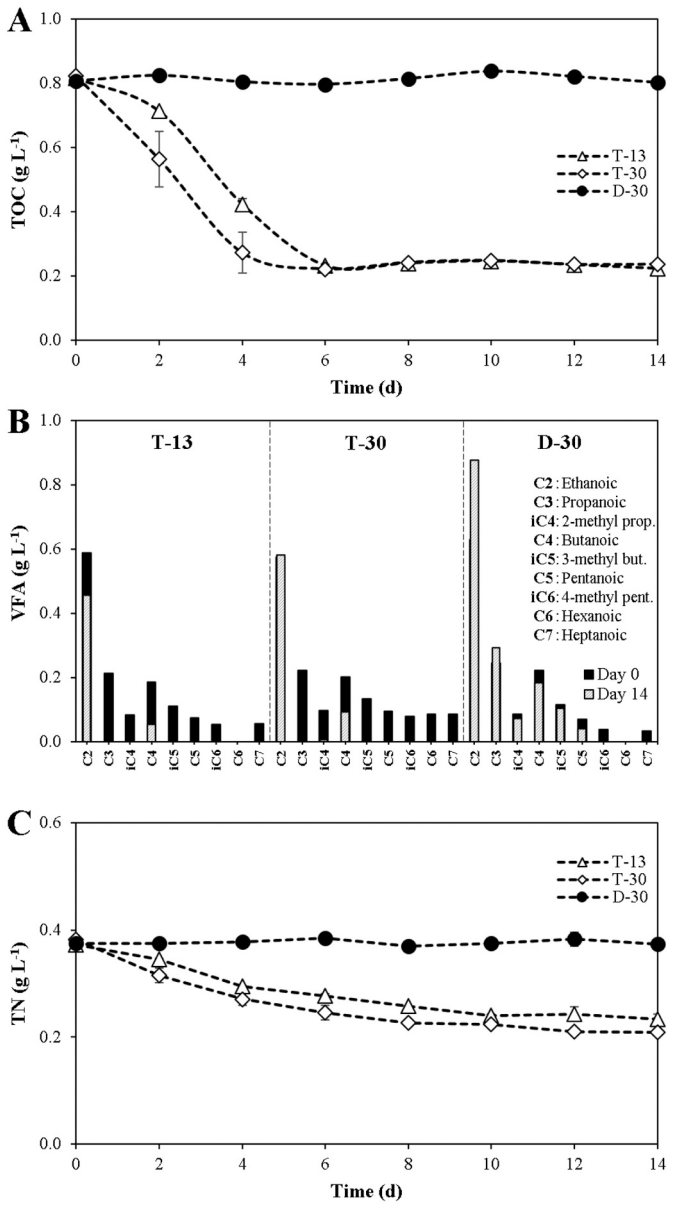

Fig. 4. Time course of TOC concentrations (A), initial (black bars) and final (gray bars) volatile fatty acids concentrations (B) and TN concentrations (C) in test series II.

intense metabolic activity of the acidogenic bacteria present in pig faeces [38], which is able to grow at temperatures of 10–30 °C.

Finally, TN concentrations of 0.23 ± 0.01, 0.21 ± 0.00 and 0.37 ± 0.00 g L^{-1} were recorded at the end of the assay in T-13, T-30 and D-30, respectively (Fig. 4C), resulting in TN removal efficiencies (TN-RE) of 37 ± 3 %, 45 ± 1 % and 1 ± 1 %, respectively. Unlike carbon removal, PPB treatment at 13 °C resulted in lower nitrogen removal efficiencies. Conversely, no significant differences were found by Hülsen et al. [11] in nitrogen removal (28 % and 29 %, respectively) at temperatures of 10 °C and 22 °C. The external carbon source supplemented by Hülsen et al. [11] to domestic wastewater to improve the C:N ratio may explain the recorded better performance at low temperatures (TN-REs >80 %) compared to the assays herein conducted with PWW and no additional organic carbon source.

3.3. Influence of inoculum and PWW load on PWW treatment

The initial average pH of 7.88 ± 0.03 increased by day 6 up to 8.17, 7.95 and 8.20 in MC-1:5, RP-1:5 and RP-1:10, respectively. On the other hand, a higher pH increase up to 8.73 was recorded in MC-1:10 likely due to the enhanced NIR penetration at this higher PWW dilution [15] and higher photosynthetic activity of the mixed culture of PPB compared to *R. palustris* (Fig. 5A). The supplementation of CO_2 contributed to decrease the pH to a range of 7.16–7.64 by the end of experiment, thus favoring the growth of mixed cultures of PPB and *R. palustris*, even at the high PWW loads associated to the 1:5 fold dilution, by preventing metabolic inhibition of PPB at high pH values. Interestingly, the production of H_2S (Fig. S3B3) was detected in RP-1:5 likely due to the growth of sulphate reducing bacteria as previously described [15]. Methane production (Fig. S3C3) was also detected in tests provided with the highest PWW load (1:5 fold diluted) due to the extended assay time and growth of indigenous methanogenic archaea present in PWW [15,38].

Final TSS concentrations of 2.31 ± 0.14 and 1.39 ± 0.11 g L^{-1} were observed in MC-1:5 and MC-1:10, whereas final TSS concentration of 0.44 ± 0.20 and 1.26 ± 0.04 g L^{-1} were recorded in RP-1:5 and RP-1:10, respectively (Fig. 5B). The mixed culture of PPB resulted in higher biomass concentrations at 1:5 folds dilution, whereas *R. palustris* produced more biomass in 1:10 diluted assays. These results were likely

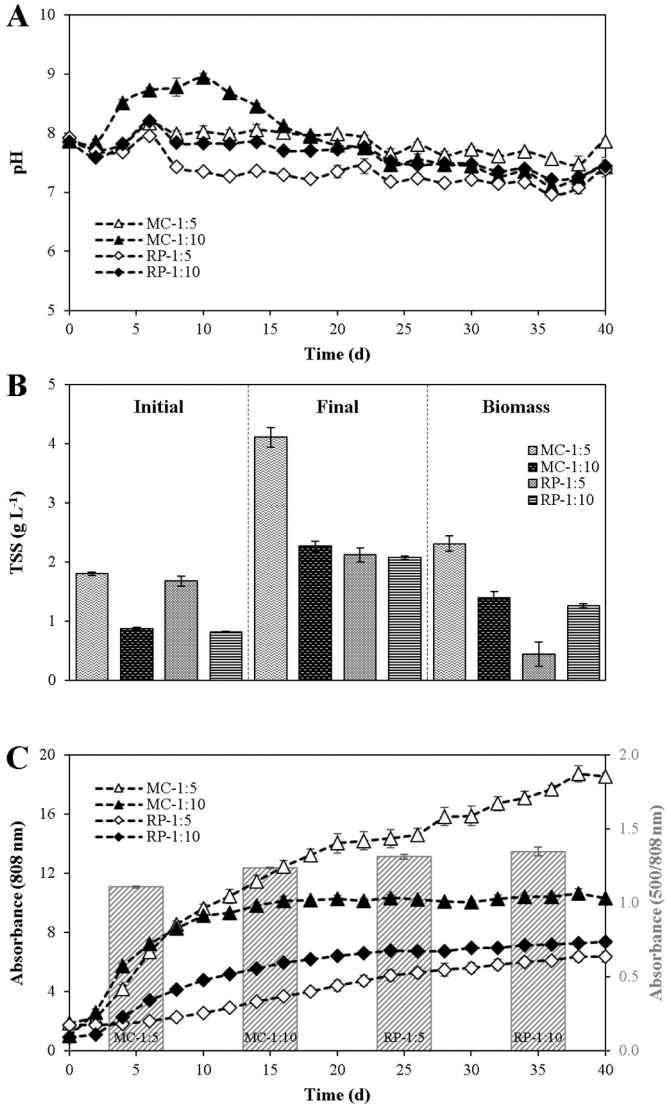

Fig. 5. Time course of the pH (A), final TSS concentration (B) and time course of absorbance at 808 nm and carotenoid/bacteriochlorophyll ratio (secondary axis) (C) in test series III.

associated to differences in the inhibitory effects in the two types of inocula. Thus, while the mixed culture of PPB generated more biomass due to a higher nutrient availability, *R. palustris* growth was reduced at the highest PWW loads due to pollutant inhibition. Pigment composition in mixed cultures of PPB exhibited a higher bacteriochlorophyll content compared to *R. palustris*, with values of absorbance at 808 nm up to 18.5 ± 0.2 and 10.3 ± 0.1 in MC-1:5 and MC-1:10, whereas values of 6.4 ± 0.3 and 7.4 ± 0.3 were recorded in RP-1:5 and RP-1:10, respectively (Fig. 5C). Interestingly, mixed cultures of PPB synthesized a higher content of bacteriochlorophyll under high PWW load (1:5 fold diluted), exhibiting a lower carotenoid/bacteriochlorophyll ratio of 1.11 ± 0.01, which suggested more favourable growth conditions compared with *R. palustris* (Fig. 5C).

TOC was assimilated in the first 26 days at low PWW loads. In MC-1:10 a faster carbon assimilation was recorded compared to *R. palustris*, removing the same TOC concentration at the end of the experiment (Fig. 6A). However, TOC assimilation occurred at low rates in 1:5 fold diluted PWW regardless of the inoculum. The final TOC concentrations were 1.09 ± 0.02 and 0.30 ± 0.01 g L^{-1} in tests inoculated with mixed PPB cultures and 1.53 ± 0.05 and 0.23 ± 0.01 g L^{-1} in tests inoculated with *R. palustris*, in 1:5 and 1:10 fold diluted PWW, respectively (Fig. 6A). TOC-REs of 52 ± 2 % and 73 ± 1 % were recorded in mixed PPB cultures and 29 ± 2 % and 79 ± 1 % in *R. palustris* in 1:5 and 1:10 fold diluted PWW, respectively, which showed higher TOC removals by PPB at high PWW dilutions.

VFAs concentrations decreased rapidly during the first days of incubation (Fig. S4C), with final VFA-REs of 81 ± 2 % and 99 ± 0 % in MC-1:5 and MC-1:10 and 33 ± 2 % and 99 ± 0 % with RP-1:5 and RP-1:10, respectively. The long duration on the experiment resulted in a complete assimilation of the C2–C7 VFAs initially present in the PWW at high dilutions regardless of the inoculum (Fig. 6B). Likewise, a complete VFA assimilation was also achieved in previous works when HCl or CO_2 supplementation was applied as pH control strategy in PPB-based PWW treatment [15]. In this context, the presence of multiplex VFAs promoted a better growth rate in PPB compared to the availability of only one VFA as carbon source, assimilated mainly via TCA cycle [32,39].

Final TN concentrations of 0.63 ± 0.01 g L^{-1} (TN-RE of 24 ± 2 %) and 0.29 ± 0.00 g L^{-1} (TN-RE of 37 ± 1 %) were recorded in MC-1:5 and MC-1:10, and 0.71 ± 0.02 g L^{-1} (TN-RE of 16 ± 1 %) and 0.26 ± 0.00 g L^{-1} (TN-RE of 42 ± 1 %) were observed in RP-1:5 and RP-1:10, respectively (Fig. 6C). These results confirmed the limited nitrogen assimilation potential of PPB in C:N unbalance effluents, which can be enhanced by coupling PPB treatment to a sequential microalgae-bacteria treatment [23]. The ammonium concentrations in 5 and 10 fold diluted PWW remained below the inhibitory levels reported by Puyol et al. [40].

4. Conclusion

PPB were able to effectively grow under PAR, PAR filtered with UV-VIS absorbing foil and NIR without significant differences under photoheterotrophic metabolism. TOC-REs of 74 % and TN-RE of 37 % were recorded under UV-VIS filtered PAR, representing the best and economical source of NIR specific for PPB growth. Interestingly, PPB were able to growth at 13 °C and support similar TOC-RE and TN-RE to 30 °C. Finally, mixed cultures of PPB were more efficient than *R. palustris* in terms of TOC and TN removal. This work confirmed the promising metabolic capabilities of PPB for carbon and nutrient recovery from PWW.

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.

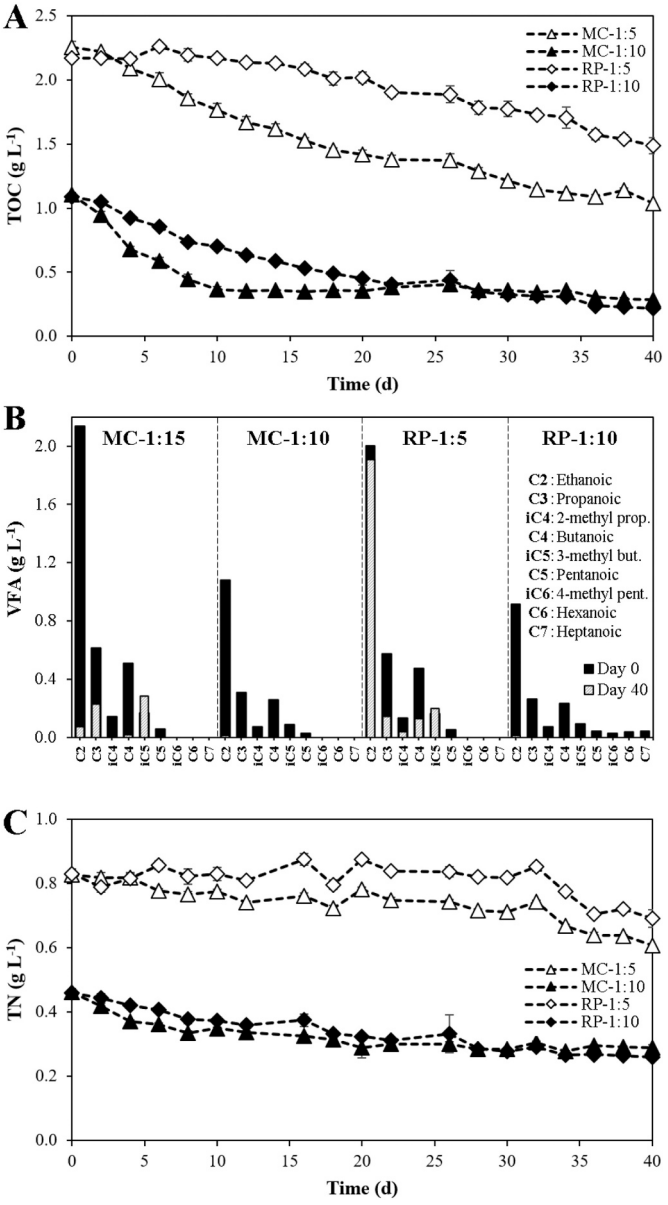

Fig. 6. Time course of TOC concentrations (A), initial (black bars) and final (gray bars) volatile fatty acids concentrations (B) and TN concentrations (C) in test series III.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jwpe.2022.103317.

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