# Assessing the potential of purple phototrophic bacteria for the simultaneous treatment of piggery wastewater and upgrading of biogas

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## ABSTRACT

The potential of purple phototrophic bacteria (PPB) for the simultaneous treatment of piggery wastewater (PWW) and biogas upgrading was evaluated batchwise in gas-tight photobioreactors. PWW dilution was identified as a key parameter determining the efficiency of wastewater treatment and biomethane quality in PPB photobioreactors. Four times diluted

PWW supported the most efficient total organic carbon (TOC) and total nitrogen removals (78% and 13%, respectively), with $CH_4$ concentrations of 90.8%. The influence of phosphorous concentration (supplementation of 50 mg $L^{-1}$ of P-$PO_4^{3-}$) on PPB-based PWW treatment coupled to biogas upgrading was investigated. TOC removals of ≈60% and $CH_4$ concentrations of ≈90.0% were obtained regardless of phosphorus supplementation. Finally, the use of PPB and algal-bacterial consortia supported $CH_4$ concentrations in the upgraded biogas of 93.3% and 73.6%, respectively, which confirmed the potential PPB for biogas upgrading coupled to PWW treatment.

**Keywords:**

Biogas upgrading, Biomethane, Piggery wastewater treatment, Purple phototrophic bacteria

## 1. Introduction

The annual production of pig meat in the European Union (EU) accounted for 24.1 million tons in 2017, which ranked the EU as the second largest pig producer in the world. In this context, a total of 150.1 million pig heads were produced in the EU in 2017 (Statista, 2018). Unfortunately, this key economic sector annually generates in the EU between 217 and 434 million $m^3$ of piggery wastewater (PWW) containing high concentrations of organic matter, nitrogen and phosphorus (De Godos et al., 2009; García et al., 2017). The management of such high volumes of PWW represents nowadays an economic, environmental and technical challenge for the EU livestock industry. Anaerobic digestion and activated sludge processes are typically implemented on-site or in centralized facilities in order to fulfill with European wastewater discharge regulations (Andreoli and Von, 2007). In addition, alternative technologies based on the intensification of algal-bacterial symbiosis have been also tested both at lab and pilot scale in order to reduce the operating costs and enhance nutrient recovery during PWW treatment compared to conventional technologies (De Godos et al., 2009; García et al., 2018, 2017). Nevertheless, PWW treatment based on algal-bacterial symbiosis is limited by the high $NH_4^+$ concentrations of this type of wastewater and its poor performance at low temperatures, which requires the development of more resilient biotechnologies capable of cost-competitively recovering the carbon and nutrients from PWW.

In this context, purple phototrophic bacteria (PPB) have emerged as a promising technology platform for wastewater treatment based on their ability to assimilate a higher fraction of the carbon, nitrogen and phosphorous present in wastewater compared to their aerobic and anaerobic counterparts (Hiraishi et al., 1991; Khatipov et al., 1998; Takabatake et al., 2004). Compared to microalgae, PPB utilize infrared radiation (IR) as source of energy, which reduces the power required by photon emission and allows a deeper light penetration into the cultivation broth (thus reducing the footprint of the process) (Hülsen et al., 2014). In addition, the influence of temperature on the growth of PPB is low, which makes them ideal microorganisms to support wastewater treatment under multiple weather conditions. Literature studies have shown the promising potential of these microorganisms for municipal and PWW treatment. For instance, Kim et al. (2004) reported chemical oxygen demand (COD) and orthophosphate removals of 50% and 58%, respectively, under anaerobic conditions in a PPB photobioreactor. PPB have been also successfully applied for industrial wastewater treatment in membrane photobioreactors and sequencing batch stirred tank photobioreactors with COD removal efficiencies of 73-75% (Chitapornpan et al., 2012; Kaewsuk et al., 2010). The ability of PPB to simultaneously remove COD, nitrogen and phosphorus from domestic wastewater has been recently evaluated in photo-anaerobic batch tests and in a continuous membrane photobioreactor (Hülsen et al., 2016, 2014). A recent comparison between the use of PPB and microalgae for the recovery of carbon, nitrogen and phosphorous from pork, poultry, sugar, dairy and red meat wastewater was carried out by Hülsen et al. (2018), who confirmed that PPB are more efficient for organic and nutrient removal than microalgae.

On the other hand, biogas from the anaerobic digestion of wastewater or organic solid waste represents a renewable energy vector with potential to partially reduce the current world's dependence on fossil fuels (Andriani et al., 2014; Muñoz et al., 2015). In the EU, the contribution of biogas to the energy sector has increased by a factor of 3 concomitantly with an increase in the number of biogas plants from 6227 in 2009 to 17662 by the end of 2016 (European Biogas Association, 2017). Biogas upgrading to biomethane is required prior injection into gas grids or use as a vehicle fuel due to the large number and high concentrations of impurities: $CO_2$ (15-60%), $H_2S$ (0.005-2%), $O_2$ (0-1%), $N_2$ (0-2%), CO (<0.6%), $NH_3$ (<1%), volatile organic compounds (<0.6%) and siloxanes (0-02%) (Ryckebosch et al., 2011); while most international regulations require concentrations of $CH_4$ $\geq$ 95%, $CO_2 \leq$ 2-4%, $O_2 \leq$ 1% and negligible amounts of $H_2S$ (Muñoz et al., 2015). Algal-bacterial systems have been consistently investigated as a low cost and environmentally sustainable technology to simultaneously remove $CO_2$ and $H_2S$ from biogas. However, $O_2$ stripping from the cultivation broth to the biomethane as a result of the oxygenic nature of algal photosynthesis represents the main limitation of algal-bacterial systems in biogas upgrading (Marín et al., 2018; Posadas et al., 2017, 2015). In this sense, the versatile metabolism of PPB, capable of using $H_2S$ in biogas or the organic matter present in wastewater as electron donor to reduce $CO_2$ from biogas without $O_2$ generation, could eventually support a cost-effective biogas upgrading. Overall, there is a lack of comparative studies assessing the potential of PPB and algal-bacterial systems in order to determine the most cost-effective and environmentally friendly biotechnology for biogas upgrading.

This study aimed at evaluating, for the first time, the potential and limitations of using PPB for the simultaneous treatment of PWW and upgrading of biogas under IR in batch photobioreactors. The influence of PWW dilution and phosphorous concentration on PPB-based PWW treatment coupled to biogas upgrading were also investigated batchwise. The mechanisms and limiting factors underlying wastewater treatment and $CO_2$/$H_2S$ removal by PPB were investigated. A comparative evaluation of PPB-based biogas upgrading vs. algae-based photobioreactors was finally conducted batchwise. The use of batch photobioreactors allowed to systematically test multiple environmental conditions. This work constitutes, to the best of our knowledge, the first proof of concept of the biogas upgrading using PPB under IR.

## 2. Materials and methods

### 2.1 Cultivation media

Fresh centrifuged PWW was collected from a nearby farm at Segovia (Spain) and stored at 4°C prior to use. The composition of the PWW was: total organic carbon (TOC) concentration of 10350 mg $L^{-1}$, inorganic carbon (IC) concentration of 215 mg $L^{-1}$, total nitrogen (TN) concentration of 2685 mg $L^{-1}$, $P\text{-}PO_4^{3-}$ concentration of 15 mg $L^{-1}$, total suspended solids (TSS) concentration of 5.9 g $L^{-1}$. Prior to each test, PWW was centrifuged at 10000 rpm for 20 min in order to separate the soluble from the solid phase. A mineral salt medium (MSM) consisting of distilled water with 1.00 g $(NH_4)_2SO_4$, 0.05 g $K_2HPO_4$, 0.02 g $MgSO_4$ and 2.00 g NaCl per liter was used in the control tests. A synthetic biogas mixture composed of $CO_2$ (29.5%), $H_2S$ (0.5%) and $CH_4$ (70%) was used as a raw biogas in the present study (Abello Linde; Spain).

## 2.2 Inocula

A set of duplicate glass bottles of 1.2 L was initially filled with 450 ml of centrifuged PWW under a helium headspace, while another set was filled with 440 ml of centrifuged PWW and 10 ml of activated sludge (Valladolid wastewater treatment plant) under a helium headspace. The pH of the cultivation broth was 7.3. The systems were incubated batchwise under magnetic agitation at 200 rpm, 30 °C and continuous IR of 50 W $m^{-2}$ (Oslon black series model SFH4780S with centroid wavelength of 850 nm, OSRAM GmbH, Germany) for 24 days in order to enrich PPB to a final concentration of 0.88 g TSS $L^{-1}$. A mixture of the enrichments from both sets of bottles was used as inoculum to conduct test series 1-3.

## 2.3 Piggery wastewater treatment coupled to biogas upgrading in purple phototrophic bacteria photobioreactors

### 2.3.1. Test series 1: Influence of piggery wastewater dilution on purple phototrophic bacteria-based piggery wastewater treatment coupled to biogas upgrading

The influence of PWW dilution on PPB-based treatment performance was evaluated in 1.2 L bottles under a biogas headspace. The bottles were filled with 360 ml of PWW (undiluted, 2 times diluted and 4 times diluted) and 40 ml of PPB inoculum, and incubated under magnetic agitation at 200 rpm, 30 °C and 50 W $m^{-2}$ of continuous IR for 25 days. A test with 2 times diluted PWW, prepared as above described and incubated in the darkness, was used as control to assess the influence of IR. An additional set of duplicate glass bottles was filled with 360 ml of MSM and 40 ml of inoculum under a biogas headspace to serve as biotic control. Finally, a set of glass bottles was prepared with 360 ml of MSM and 40 ml of inoculum under a biogas headspace and its pH adjusted to 2.0 (thus preventing biological activity) to serve as abiotic control. The assays were conducted in duplicate.

### 2.3.2 Test Series 2: Influence of phosphorous concentration on purple phototrophic bacteria-based piggery wastewater treatment coupled to biogas upgrading

A set of duplicate glass bottles of 1.2 L was filled with 360 ml of 4 times diluted PWW and 40 ml of inoculum under a biogas headspace. A second set of duplicate glass bottles was filled with 360 ml of 4 times diluted PWW, 40 ml of inoculum and supplemented with a P-$PO_4^{3-}$ concentration of 50 mg $L^{-1}$ under a biogas headspace. The systems were incubated under magnetic agitation at 200 rpm, 30 °C and IR at 50 W $m^{-2}$ for 22 days. The assays were conducted in duplicate.

### 2.3.3 Test Series 3: Comparative evaluation of the potential of purple phototrophic bacteria and algal-bacterial consortia for biogas upgrading

A set of duplicate glass bottles of 1.2 L was filled with 360 ml of 4 times diluted PWW and 40 ml of inoculum under a biogas headspace. A second set of bottles was prepared with 400 ml of MSM under a biogas headspace to serve as abiotic control. The systems were incubated under magnetic agitation at 200 rpm, 30 °C and an IR of 50 W $m^{-2}$ for 23 days.

At the same time, a set of duplicate glass bottles of 1.2 L was filled with 360 ml of MSM and 40 ml of algal-bacterial inoculum (obtained from an outdoor high rate algal pond treating biogas and centrate at the Valladolid University (Spain)) under a biogas headspace. The pH of the cultivation medium was adjusted to 7.0. A second set of duplicate bottles was prepared with 400 ml of MSM under a biogas headspace to serve as abiotic control. The bottles were incubated under magnetic agitation at 200 rpm, 30 °C and 200 µmol $m^{-2}$ $s^{-1}$ of photosynthetic active radiation provided by high intensity LED PCBs (Phillips SA, Spain) for 23 days. The assays were conducted in duplicate.

In all test series, the pH, headspace gas composition ($CH_4$, $CO_2$, $H_2S$, $O_2$, and $N_2$) and concentrations of dissolved TOC, IC, TN, N-$NO_3^-$, N-$NO_2^-$, P-$PO_4^{3-}$, $SO_4^{2-}$ and volatile fatty acids (VFAs) were periodically monitored. The initial and final biomass concentrations (measured as TSS) were also determined.

### 2.4 Analytical Procedures

Dissolved TOC, IC and TN concentrations were analyzed using a Shimadzu TOC-VCSH analyzer (Japan) equipped with a TNM-1 chemiluminescence unit. N-$NO_3^-$, N-$NO_2^-$, P-$PO_4^{3-}$ and $SO_4^{2-}$ concentrations were quantified by HPLC-IC according to Serejo et al. (2015). VFAs were analyzed in an Agilent 7820A GC-FID (Agilent Technologies, Santa Clara, USA) according to López et al. (2018). The pH was determined with an Eutech Cyberscan pH 510 (Eutech instruments, The Netherlands), while the determination of TSS concentration was performed according to Standard Methods (APHA, 2005). The concentration of $CH_4$, $CO_2$, $H_2S$, $O_2$, and $N_2$ in the headspace of the bottles was determined using a Varian CP-3800 GC-TCD (Palo Alto, USA) according to Posadas et al., (2015).

### 2.5 Statistical analysis

The results here presented were provided as the average values along with their standard deviation from replicate measurements. An analysis of variance (ANOVA) was performed to determine how changes in PWW dilution influenced the quality of the upgraded biogas.

## 3. Results and discussion

### 3.1 Influence of piggery wastewater dilution on purple phototrophic bacteria-based piggery wastewater treatment coupled to biogas upgrading

TOC concentration in biotic and abiotic control tests conducted with MSM remained constant at 134 ± 16 mg $L^{-1}$ and 69 ± 9 mg $L^{-1}$, respectively (Fig. 1a). On the other hand, TOC concentration in undiluted PWW and non-irradiated biotic control tests remained constant at 10318 ± 957 mg $L^{-1}$ and 3535 ± 236 mg $L^{-1}$, respectively (Fig. 1a). A significant TOC removal from 3977 ± 336 mg $L^{-1}$ to 1453 ± 134 mg $L^{-1}$ (TOC-removal efficiencies (REs) of 63%) in 2 times diluted PWW tests, and from 1989 ± 12 mg $L^{-1}$ to 436 ± 14 mg $L^{-1}$ (TOC-REs of 78%) in 4 times diluted PWW tests (Fig. 1a) was observed. The TOC-REs herein recorded were higher than those obtained by Hülsen et al. (2018), who reported removal efficiencies of COD of approximately 10% for the treatment of PWW in batch tests. At this point it should be highlighted that the TOC instrumental methodology exhibited an error lower than 2 %, while the error of the COD analytical methodology was < 10 %. These results confirmed the potential of PPB to anaerobically degrade organic matter at high concentrations in the presence of IR as energy source, although the high TN concentrations in undiluted PWW seems to inhibit PWW treatment. The pH in the undiluted, biotic control and non-irradiated biotic control tests initially decreased as a result of the $CO_2$/$H_2S$ acidification mediated by biogas, but remained stable at 6.9 ± 0.1, 6.7± 0.1 and 6.6 ± 0.1, respectively, from day 4 onwards. Meanwhile, the pH in the abiotic control remained constant at 2.1 ± 0.1. However, an increase from 6.6 ± 0.1 and 6.8 ± 0.1 (day 4) to 7.8 ± 0.1 and 8.0 ± 0.0 was observed by day 25 in 2 and 4 times diluted PWW tests likely mediated by the release of basic TOC biodegradation metabolites. The high TN concentration in PWW (mainly composed by 80-90% of $NH_4^+$ (Godos et al., 2010)) and relatively high pH represents a perfect combination for microbial toxicity by free ammonia (FA). Indeed, the main inhibitory mechanism of ammonium in anaerobic organisms is specifically the concentration of FA as a result of a high pH in anaerobic systems (Hansen et al., 1998). FA is a potent uncoupler of membrane transport in any microorganism, as is capable of destabilizing the proton gradient, thus preventing phosphorylation (Gallert et al., 1998; Rajagopal et al., 2013). In this context, only the presence of valinomycin, a potent antibiotic and a $K^{2+}$ transporter in membranes, can activate a similar effect over photophosphorylation in PPB (Fleischman and Clayton, 1968). PWW may contain other organic $Na^+$-$K^{2+}$ transporters that could boost the toxicity of FA upon proton motive force in PPB. Indeed, PWW usually contains a wide variety of emerging pollutants like antibiotics or animal health-care products that may act as FA transporters in membranes (Milić et al., 2013).

Similarly, in the assays conducted with undiluted PWW, the IC concentration remained approximately constant at 179 ± 21 mg $L^{-1}$. In 2 and 4 times diluted tests, IC concentrations increased from 105 ± 9 by day 7 to 336 ± 46 and 397 ± 15 mg $L^{-1}$, respectively, by day 20 (Fig 1b). This increase in IC concentration was mediated by the absorption of a fraction of the $CO_2$ present in the biogas. IC concentrations in the non-irradiated biotic control tests remained constant at 119 ± 19 mg $L^{-1}$, which confirm the absence of biological activity of PPB without IR radiation.

Finally, while TN concentration remained constant in the tests without biological activity (undiluted, abiotic, biotic and non-irradiated biotic control), TN concentration decreased from 1083 ± 75 to 811 ± 15 mg $L^{-1}$ and from 563 ± 5 to 488 ± 18 mg $L^{-1}$ in 2 and 4 times diluted PWW assays, respectively (Fig. 1c). This removal was likely due to nitrogen assimilation into PPB biomass, which amounted 1.5 ± 0.3 and 2.2 ± 0.2 g TSS $L^{-1}$ by the end of the tests conducted in 2 and 4 times diluted PWW, respectively. Neither $NO_2^-$ nor $NO_3^-$ were detected regardless of the TN concentration, which ruled out the occurrence of $NH_4^+$ nitrification (as expected from the reductive conditions prevailing in the tests).

PWW dilution and the presence of IR significantly impacted on the biogas upgrading performance. Thus, a decrease in $CO_2$ headspace concentrations from 28.7 ± 0.4% to 26.2 ± 0.2%, 23.1 ± 2.0% and 25.7 ± 0.9% mediated by $CO_2$ absorption in the cultivation broth was recorded in the assays containing undiluted, biotic and non-irradiated 2 times diluted PWW control tests, while in the abiotic test no significant variation in $CO_2$ concentration was observed (Fig. 1d). The largest reductions in $CO_2$ headspace concentrations, down to 9.6 ± 1.4% and 7.5 ± 0.1%, were recorded in 2 and 4 times diluted PWW tests (Fig. 1d). This removal of $CO_2$ from biogas was mediated by both an absorption into the cultivation broth (promoted by the above reported increase in pH) and a PPB-based $CO_2$ fixation using $H_2S$ from biogas and the biodegradable TOC as electron donor. Indeed, $H_2S$ in the headspace decreased from 0.40 to 0.24% and 0.04% in 2 and 4 times diluted PWW tests under reductive conditions, which suggests its biological utilization as electron donor (Fig. 1e). The unexpected increase in $H_2S$ concentration to 1.0% in undiluted PWW tests and 0.7% in the non-irradiated biotic control tests was likely induced by the use of dissolved sulphate as electron acceptor by sulfate-reducers in the mixed culture during the biodegradation of a minor fraction of biodegradable TOC. Finally, $H_2S$ concentration in the headspace initially decreased to 0.19% and 0.15% in the biotic and abiotic tests, respectively, as a result of $H_2S$ absorption in the MSM. On the other hand, $CH_4$ headspace concentrations of 88.7% and 90.8% were recorded at the end of the tests containing 2 and 4 times diluted PWW under IR radiation, which confirmed the technical feasibility of combining PWW treatment and biogas upgrading in PPB photobioreactors (Fig. 1f). In addition, a similar variation in $CH_4$ concentration in the headspace was recorded in the biotic and abiotic tests, increasing from 70 % up to 74.7% and 74.2%, respectively. The biomethane herein obtained in the test conducted with 2 and 4 times diluted PWW and irradiated PPB could be used as a vehicle fuel (a $CH_4$ content > 80% is required in some countries of the European Union) (Muñoz et al., 2015).

Finally, an ANOVA was carried out to elucidate how changes in PWW dilution influenced the quality of the upgraded biogas. Since the F values for $CH_4$ and $CO_2$ (5.6 and 5.2, respectively) were greater than the F critical value of 3.5, it can be concluded that the stated hypothesis was correct and therefore the quality of the upgraded biogas varied significantly with PWW dilution.

**<Figure 1>**

$CO_2$ capture in the Calvin cycle by PPB is possible only when the organic substrates present in the cultivation medium are in a reduced form, since PPB need $CO_2$ for maintaining the redox homeostasis (McKinlay and Harwood, 2010). This is crucial to achieve a net $CO_2$ capture, thus allowing biogas upgrading by using the biodegradable organic matter present in wastewater as electron donor. In order to confirm this hypothesis, the time course of VFAs in the experiments conducted with diluted PWW was recorded (Fig. 2). The initial characterization of the wastewater revealed that PWW contained highly reduced organics in the form of VFAs. The main VFAs detected were acetate (963 mg $L^{-1}$), propionate (230 mg $L^{-1}$), isobutyrate (126 mg $L^{-1}$), butyrate (109 mg $L^{-1}$), isovalerate (72 mg $L^{-1}$) and valerate (27 mg $L^{-1}$). The average oxidation state of the VFAs in the PWW herein used was calculated as -0.63 following (McKinlay and Harwood, 2010). These environmental conditions require PPB to use $CO_2$ to support microbial growth. Indeed, PPB consumed the VFAs concomitantly with biomass growth and $CO_2$ assimilation in the 2 and 4 times diluted tests (Fig. 2b and 2c, respectively), which confirmed the hypothesis proposed. Meanwhile VFAs remained constant in the undiluted and non-irradiated biotic control tests (Fig. 2a and 2d, respectively). Therefore, PPB use the excess of electrons from the VFAs to assimilate $CO_2$ in the Calvin cycle. The other major mechanism to achieve redox homeostasis is $H_2$ production, which was strongly inhibited by the high nitrogen concentration in these particular assays (Sweet and Burris, 1981).

Since 4 times diluted PWW (with a TN concentration of 600 mg $L^{-1}$) supported the most efficient TOC, TN, $CO_2$ and $H_2S$ removal, test series 2 and 3 were conducted under these experimental conditions.

**<Figure 2>**

### 3.2 Influence of phosphorous concentration on purple phototrophic bacteria-based piggery wastewater treatment coupled to biogas upgrading

A significant TOC removal from 1712 ± 143 to 803 ± 123 mg $L^{-1}$ (TOC-REs of 53%) was recorded in 4 times diluted PWW, while phosphorus supplementation supported a decrease in TOC concentration from 1625 ± 86 to 646 ± 110 mg $L^{-1}$ (TOC-REs of 60%) (Fig. 3a). The high TOC-REs recorded in test series 2 were mediated by the assimilation as biomass instead of by TOC oxidation to $CO_2$, and confirmed the potential of PPB to anaerobically degrade organic matter assisted by IR regardless of phosphorus supplementation. A pH increase from 6.7 ± 0.1 and 6.8 ± 0.0 (day 4) to 7.5 ± 0.1 and 7.7 ± 0.0 was observed by the end of day 22 in tests conducted without and with phosphorus supplementation to 4 times diluted PWW, respectively, due to PPB-based TOC biodegradation. IC concentration increased as a result of pollutant mineralization and $CO_2$ capture/fixation from 56 ± 3 to 369 ± 21 and 364 ± 9 mg $L^{-1}$ (day 19) without and with phosphorus addition, respectively (Fig 3b). TN concentration decreased from 620 ± 20 to 308 ± 39 mg $L^{-1}$ without phosphorous addition and from 611 ± 10 to 285 ± 33 mg $L^{-1}$ when phosphorus was supplemented (Fig. 3c). This removal was likely due nitrogen assimilation into PPB biomass, which averaged 1.8 ± 0.8 and 1.9 ± 0.1 g TSS $L^{-1}$ by the end of the tests without and with phosphorus supplementation, respectively. The TN-REs herein recorded were higher those reported by Hülsen et al. (2018), who achieved values of approximately 10% during the batch treatment of PWW by PPB. Neither $NO_2^-$ nor $NO_3^-$ were detected in these tests series. The results here recorded for TOC, IC and TN concentrations confirmed that phosphorous supplementation did not enhance significantly PPB-based PWW treatment under photo-anaerobic conditions. Finally, P-$PO_4^{3-}$ in the test supplemented with phosphorus was completely removed by day 13 mainly due to P assimilation into biomass, while P-$PO_4^{3-}$ in the non-supplemented test remained below the detection limit of the HPLC-IC (3 mg P $L^{-1}$).

The impact of phosphorus supplementation was more noticeable in the upgrading of biogas. A reduction in $CO_2$ concentration from 29.4% to 8.2% and from 29.0% to 5.2% was recorded with and without phosphorus supplementation, respectively (Fig. 3d). $H_2S$ concentration in the headspace decreased from 0.36% to 0.07% without phosphorus supplementation, while phosphorus supplementation supported a complete removal of $H_2S$. This suggested its biological utilization as electron donor to support $CO_2$ assimilation (Fig. 3e). Finally, an increase in $CH_4$ concentration from 70 % to 89.2% and 91.9% without and with phosphorus supplementation was recorded (Fig. 3f). The quality of the biomethane produced in P-supplemented tests complied with biomethane requirements for use as a vehicle fuel (Muñoz et al., 2015).

**<Figure 3>**

### 3.4 Comparative evaluation of the potential of purple phototrophic bacteria and algal-bacterial consortia for biogas upgrading

The ability of PPB and an algal-bacterial consortium to simultaneously treat PWW and upgrade biogas was comparatively assessed. A limited decrease in $CO_2$ headspace concentrations from 28.6% to 24.1% was recorded in the test inoculated with the algal-bacterial consortium, while $CO_2$ concentrations of 3.3% were obtained at the end of the experiment inoculated with PPB (Fig. 4a). The low pH in the cultivation broth of the algal-bacterial system (5.4 ± 0.7) imposed by the biogas headspace likely inhibited photosynthetic activity of microalgae. $H_2S$ concentration in the headspace of the PPB tests decreased from 0.35% to 0.10%, while in the algal-bacterial systems a $H_2S$ concentration of 0.47% was recorded by day 23 (Fig. 4b). $CH_4$ headspace concentration reached values of 93.3% and

73.6% in the tests with PPB and algal-bacterial consortium, respectively (Fig. 4c). Therefore, an enhanced biogas upgrading capacity was observed for PPB compared with the algal-bacterial consortium.

Finally, the TOC concentration in the algal-bacterial tests remained constant at 38 ± 7 mg $L^{-1}$, and gradually decreased from 2498 ± 0 to 1483 ± 7 mg $L^{-1}$ (TOC-RE of 41%) in PPB tests (Fig. 4d). On the other hand, the IC concentration in the algal-bacterial tests remained constant at 14 ± 4 mg $L^{-1}$ and increased in the PPB tests from 108 ± 0 to 459 ± 40 mg $L^{-1}$ by day 12 (Fig. 4e). Finally, no significant variation in the TN concentration was observed regardless of the consortia (624 ± 33 mg $L^{-1}$ in PPB tests and 200 ± 13 mg $L^{-1}$ in algal-bacterial tests) (Fig. 4f).

**<Figure 4>**

## 4. Conclusions

PPB represent an innovative biological platform for the simultaneous treatment of PWW and upgrading of biogas under photo-anaerobic conditions. PWW with TN concentrations of 600 mg $L^{-1}$ provided the best conditions for wastewater treatment and biogas upgrading. The presence of VFA in PWW supported $CO_2$ fixation in the Calvin cycle, thus allowing biogas upgrading. The low phosphorous concentrations inherent to PWW did not significantly impact on wastewater treatment performance but slightly improved biomethane quality. $CH_4$ concentrations of 93.3% can be achieved using PPB, which complied with most international regulations for biogas use as a vehicle fuel.

E-supplementary data of this work can be found in online version of the paper.


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## FIGURE CAPTIONS

**Figure 1.** Time course of (a) total organic carbon, (b) inorganic carbon, (c) total nitrogen, (d) $CO_2$, (e) $H_2S$ and (f) $CH_4$ concentrations during the biodegradation of undiluted (■), 2 times diluted (○), and 4 times diluted (▲) PWW coupled to biogas upgrading. Inoculated IR-deprived control test with 2 times diluted PWW (◊). Biotic control test with MSM (◆) and abiotic control test with MSM at pH 2.0 (□).

**Figure 2.** Time course of VFA concentration during the biodegradation of (a) undiluted, (b) 2 times diluted and (c) 4 times diluted PWW coupled to biogas upgrading. (d) Inoculated IR-deprived control test with 2 times diluted PWW.

**Figure 3.** Time course of (a) total organic carbon, (b) inorganic carbon, (c) total nitrogen, (d) $CO_2$, (e) $H_2S$ and (f) $CH_4$ concentrations during the treatment of 4 times diluted PWW (□) and 4 times diluted PWW supplemented with 50 mg P-$PO_4^{3-}$ $L^{-1}$ (▲).

**Figure 4.** Time course of (a) $CO_2$, (b) $H_2S$, (c) $CH_4$ (d) total organic carbon, (e) inorganic carbon and (f) total nitrogen concentration during biogas upgrading with a PPB consortium treating PWW (□) and an algal-bacterial consortium (●).

**Figure 1.** Time course of (a) total organic carbon, (b) inorganic carbon, (c) total nitrogen, (d) $CO_2$, (e) $H_2S$ and (f) $CH_4$ concentrations during the biodegradation of undiluted (■), 2 times diluted (○), and 4 times diluted (▲) PWW coupled to biogas upgrading. Inoculated IR-deprived control test with 2 times diluted PWW (◊). Biotic control test with MSM (◆) and abiotic control test with MSM at pH 2.0 (□).

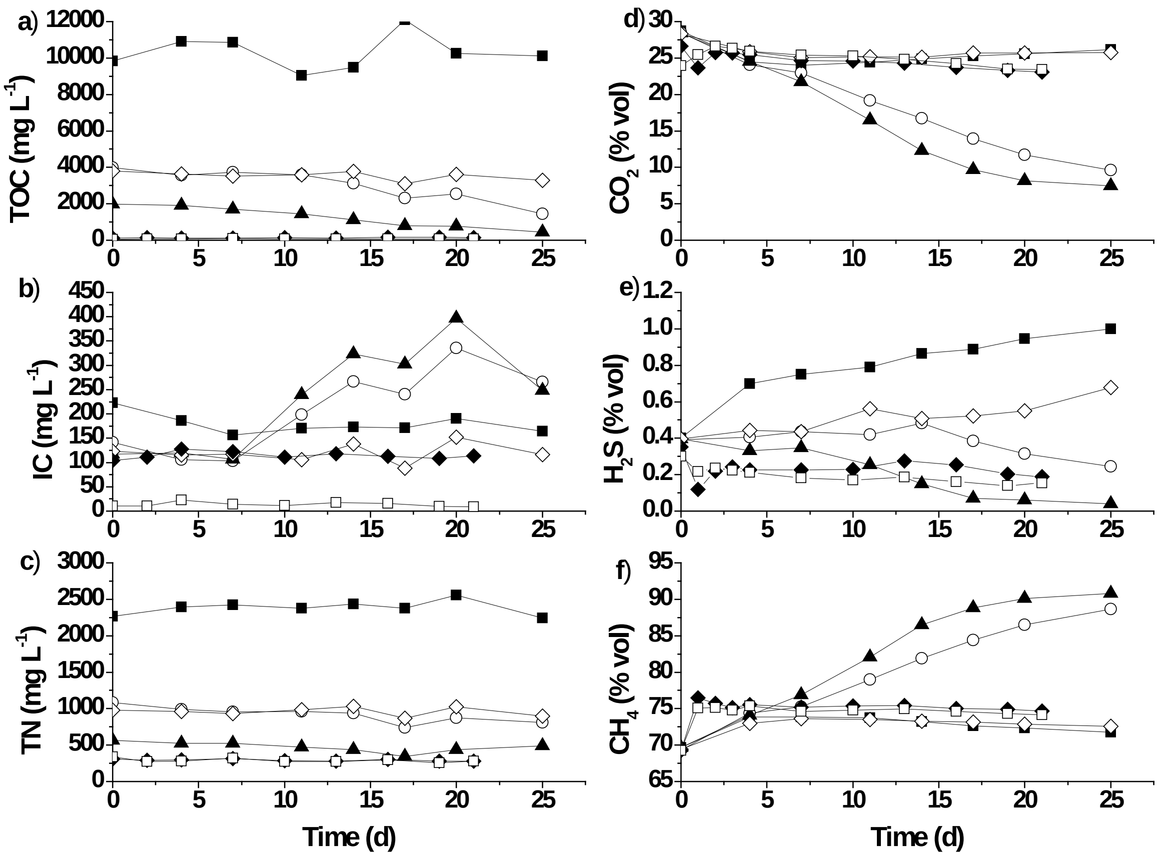


**Figure 2.** Time course of VFA concentration during the biodegradation of (a) undiluted, (b) 2 times diluted and (c) 4 times diluted PWW coupled to biogas upgrading. (d) Inoculated IR-deprived control test with 2 times diluted PWW.

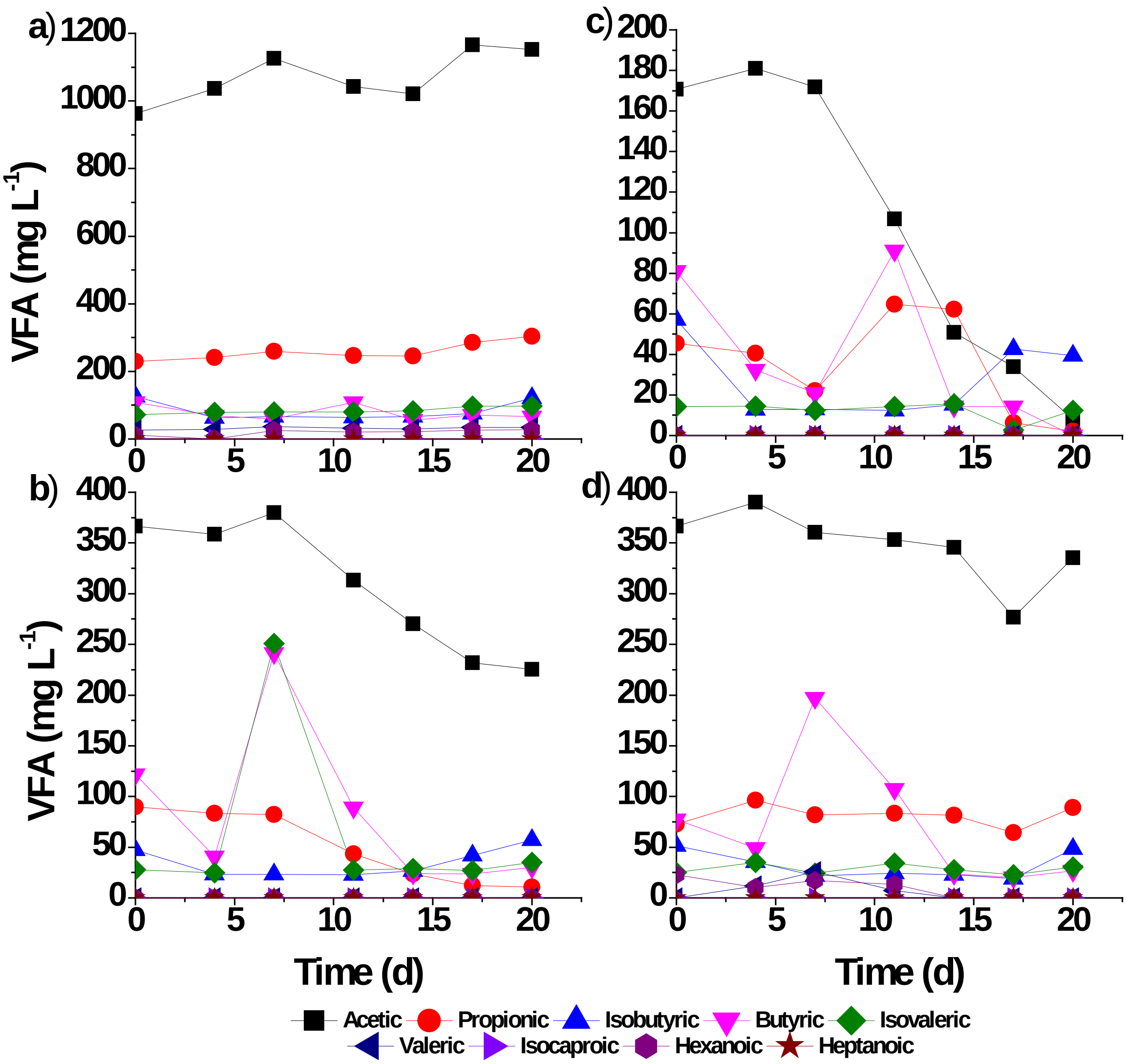


**Figure 3.** Time course of (a) total organic carbon, (b) inorganic carbon, (c) total nitrogen, (d) $CO_2$, (e) $H_2S$ and (f) $CH_4$ concentrations during the treatment of 4 times diluted PWW (□) and 4 times diluted PWW supplemented with 50 mg P-$PO_4^{3-}$ $L^{-1}$ (▲).

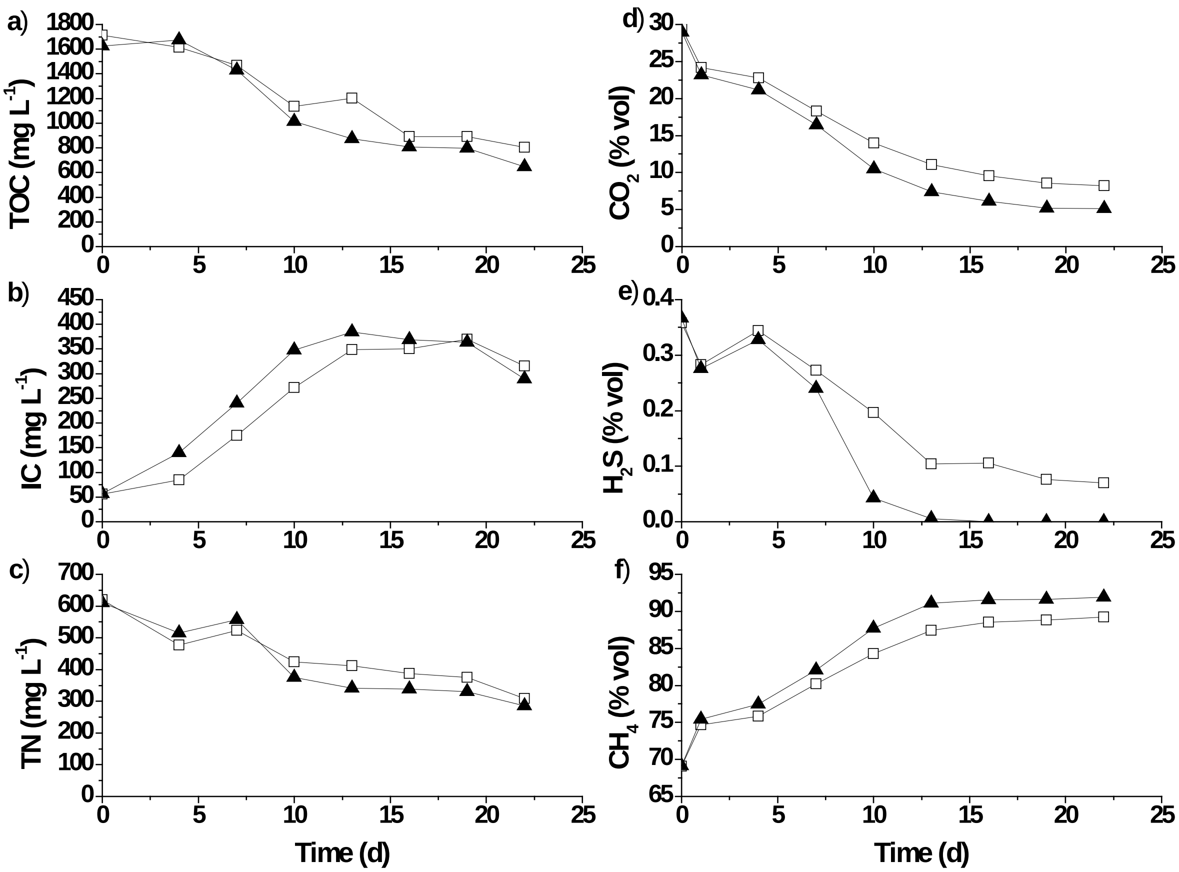
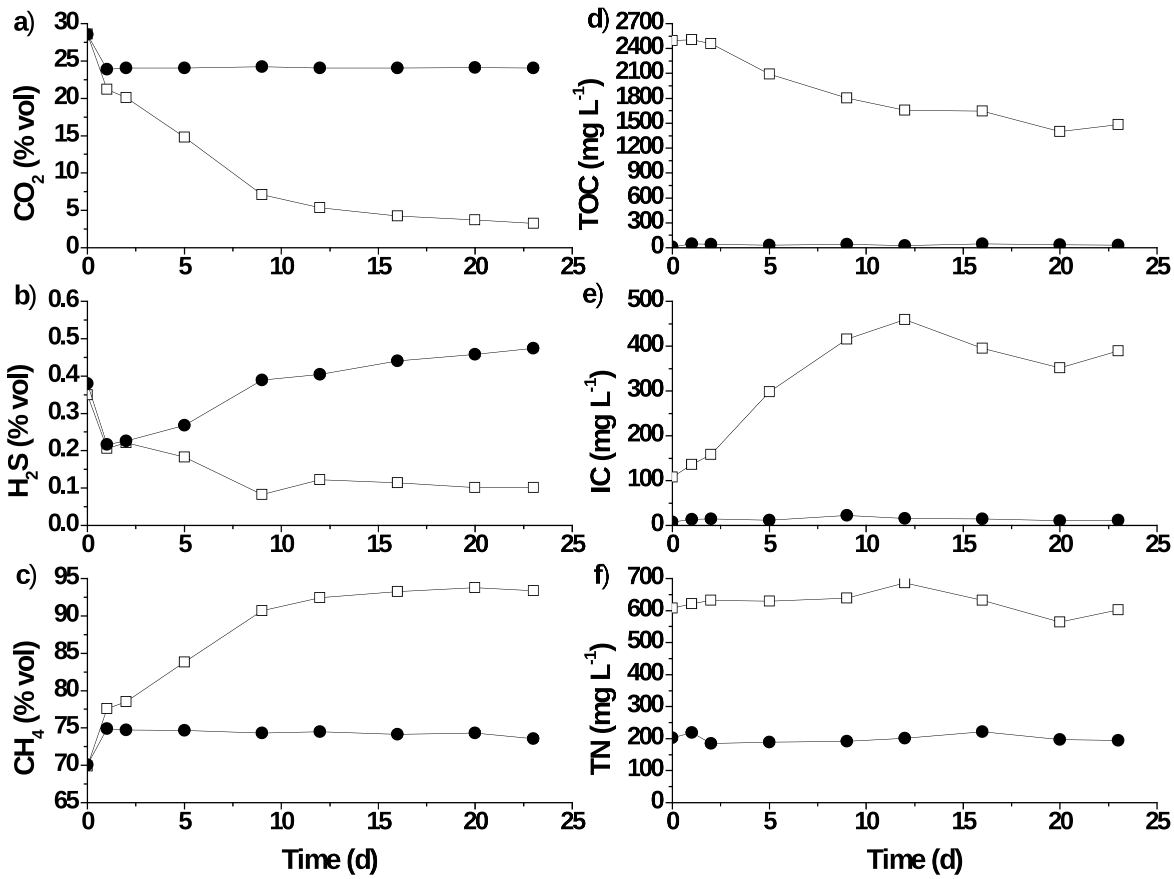


**Figure 4.** Time course of (a) $CO_2$, (b) $H_2S$, (c) $CH_4$, (d) total organic carbon, (e) inorganic carbon and (f) total nitrogen concentration during biogas upgrading with a PPB consortium treating PWW (□) and an algal-bacterial consortium (●).



# Assessing the potential of purple phototrophic bacteria for the simultaneous treatment of piggery wastewater and upgrading of biogas

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## Inoculum enrichment

**Figure S1.** Inoculum enrichment set-up

**pH values**

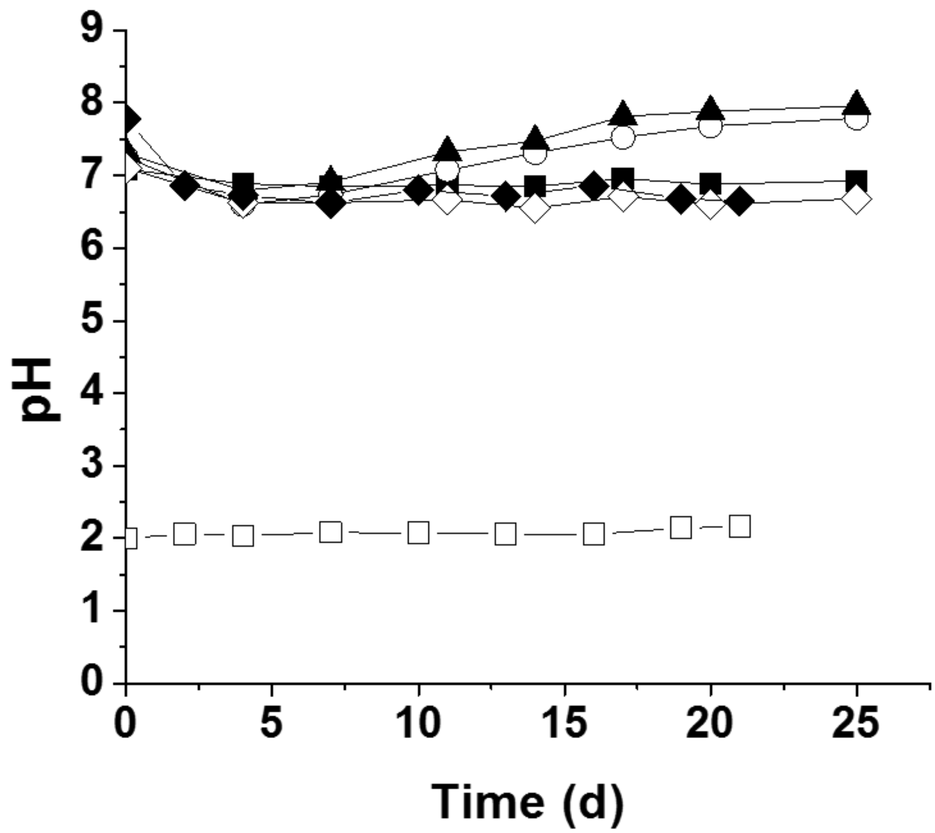


**Figure S2.** Time course of pH during the biodegradation of undiluted (■), 2 times diluted (○), and 4 times diluted (▲) PWW coupled to biogas upgrading. Inoculated IR-deprived control test with 2 times diluted PWW (◊), biotic control test with MSM (◆), abiotic control test with MSM at pH 2.0 (□).

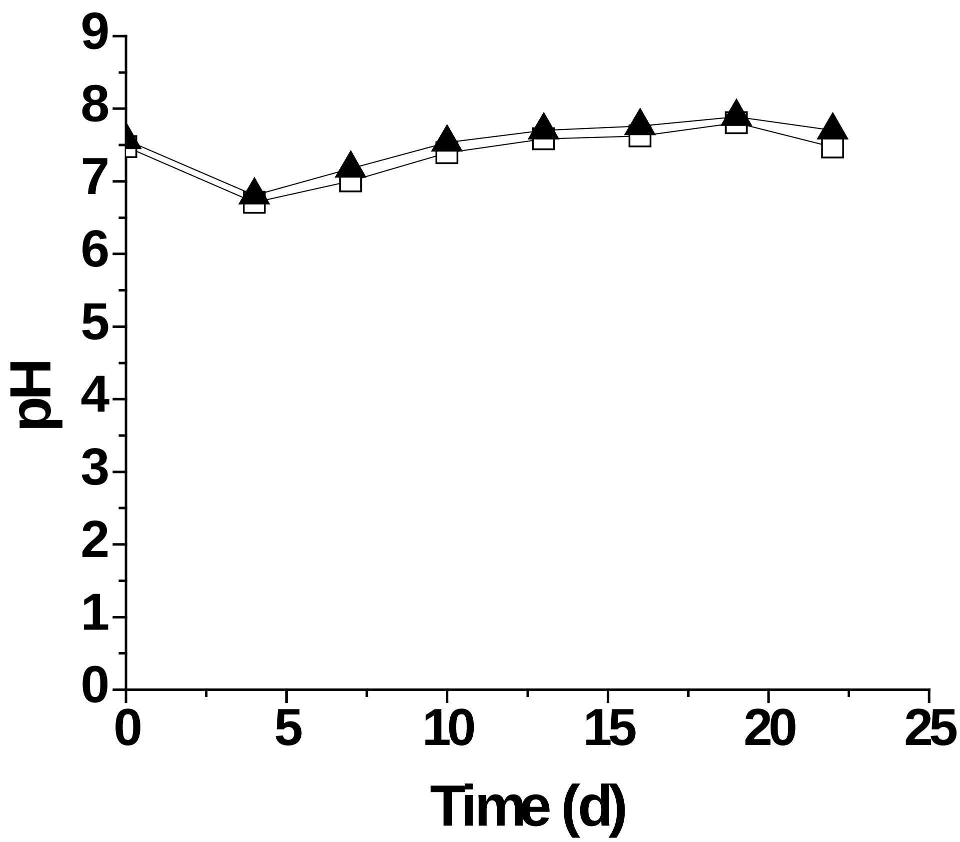


**Figure S3.** Time course of pH in test series 2. Four times diluted PWW without (□) and with P-$PO_4^{3-}$ supplementation (▲).

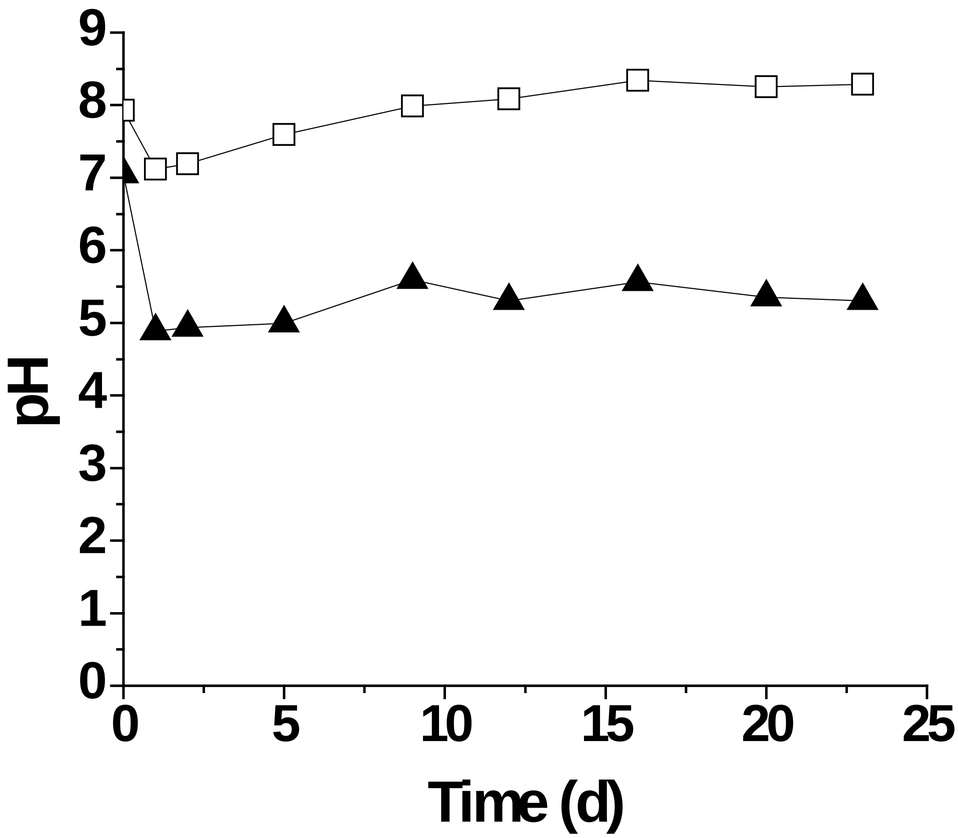


**Figure S4.** Time course of pH in test series 3. Purple phototrophic bacteria treating 4 times diluted PWW under a biogas atmosphere (□), algal-bacterial consortium in MSM under a biogas atmosphere (▲).

**P-$PO_4^{3-}$ concentration in test series 3**

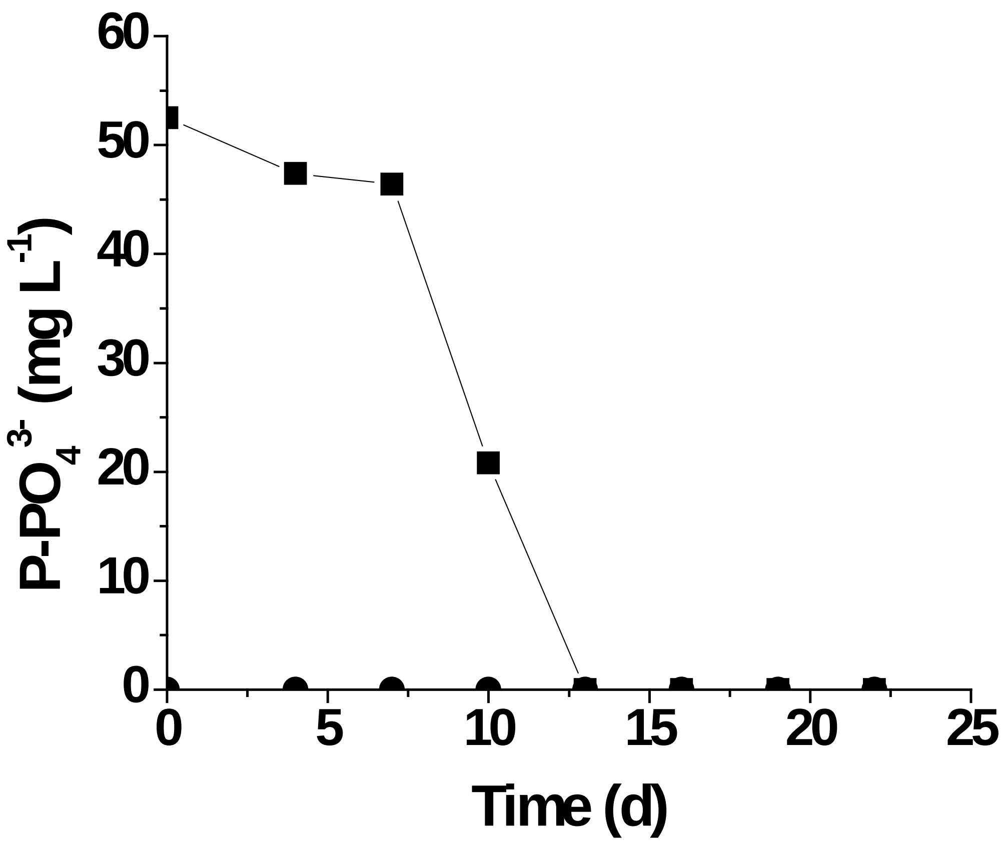


**Figure S5.** Time course of P-$PO_4^{3-}$ concentration in the cultivation broth of the assays conducted with 4 times diluted PWW (●) and 4 times diluted PWW supplemented with $PO_4^{-3}$ (■) in test series 3.

**Table S.1.** Analysis of variance

| | Sum of squares | Degrees of freedom | Mean square | F value | F critical |
|---|---|---|---|---|---|
| $CH_4$ | 410.8 | 2 | 205.4 | 5.6 | 3.5 |
| Error | 775.1 | 21 | 36.9 | | |
| $CO_2$ | 380.6 | 2 | 190.3 | 5.2 | 3.5 |
| Error | 763.9 | 21 | 36.4 | | |
| $H_2S$ | 1.4 | 2 | 0.7 | 35.4 | 3.5 |
| Error | 0.4 | 21 | 0.1 | | |