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Comparative evaluation of piggery wastewater treatment in algal-bacterial photobioreactors under indoor and outdoor conditions

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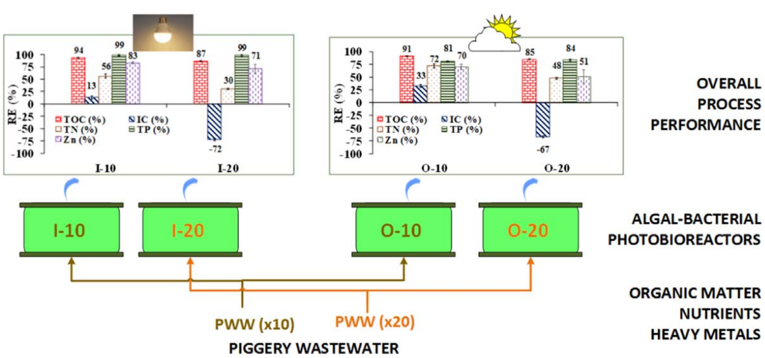
GRAPHICAL ABSTRACT



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ABSTRACT

This work evaluated the performance of four open algal-bacterial photobioreactors operated at ≈26 days of hydraulic retention time during the treatment of 10 (×10) and 20 (×20) times diluted piggery wastewater (PWW) under indoor (I) and outdoor (O) conditions for four months. The removal efficiencies (REs) of organic matter, nutrients and zinc from PWW, along with the dynamics of biomass concentration and structure of algal-bacterial population were assessed. The highest TOC-RE, TP-RE and Zn-RE (94 ± 1%, 100% and 83 ± 2%, respectively) were achieved indoors in ×10 PWW, while the highest TN-RE (72 ± 8%) was recorded outdoors in ×10 PWW. *Chlorella vulgaris* was the dominant species regardless of the ambient conditions and PWW dilution. Finally, DGGE-sequencing of the bacterial community revealed the occurrence of four phyla, *Proteobacteria* being the dominant phylum with 15 out of the 23 most intense bands.

1. Introduction

Europe, with an annual production of 23.5 million tn of pork meat, was the second largest pig producer in the world in 2015 (Statista, 2016). Europe's pig production accounted for 149 million heads, which represented approx. 44.3 % of the total European livestock in 2015 (EU, 2015; MAGRAMA, 2015). However, this relevant economic sector annually generates 217–434 million m^3 of piggery wastewater (PWW)

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(4–8 L/d·pig) containing high concentrations of organic matter, nutrients, solids and heavy metals (De Godos et al., 2009; Franchino et al., 2016). The treatment of such high strength wastewaters represents both a technical challenge and a severe economic burden for the livestock sector. In this context, next generation PWW treatment technologies should allow complying with European wastewater regulations (1999/31/EC) (Council Directive, 1999) while producing added-value bioproducts out of the organic matter and nutrients present in PWW (2008/98/EC) (European Commisssion, 2008).

Algal-bacterial symbiosis has emerged as a promising platform for resource recovery and recycling from PWW in rural areas (where space is often not limiting). Algal-bacterial symbiosis has been successfully applied in photobioreactors for the treatment of domestic wastewater (García et al., 2017a; Oswald et al., 1957), digestates (Anbalagan et al., 2016; Wang et al., 2013), livestock effluents (Tigini et al., 2016), parboiled rice wastewater (Bastos et al., 2009), olive oil mill wastewater and wastewater from the pulp and paper industry (Muñoz and Guieysse, 2006). The use of microalgae during PWW treatment can support a cost-effective removal of organic matter, nutrients, heavy metals, pathogens and emerging pollutants as a result of their dual autotrophic and heterotrophic metabolisms, photosynthetic O_2 release and ability to increase the pH of the cultivation broth (García et al., 2017a; Muñoz and Guieysse, 2006). The ability of microalgae to grow on both wastewater alkalinity and the carbon dioxide (CO_2) released during organic matter oxidation entails 2–3 folds larger productivities (compared to activated sludge systems) of a biomass that can be used as a feedstock for the production of biofertilizers or bioenergy. In addition, the lower energy demand of microalgae-based wastewater treatment, along with the CO_2 fixation ability of microalgae, significantly increase the environmental sustainability of this technology (Cheah et al., 2016; Dassey and Theegala, 2013). Despite the merits of algal-bacterial processes for PWW treatment and the intensive research conducted in this field in the past 10 years, very few studies have been carried out outdoors under the periodically fluctuating and high solar irradiations and temperatures (De Godos et al., 2009; García et al., 2017b; Posadas et al., 2017). In this context, the absence of comparative studies systematically assessing the representativeness of the results obtained indoors (under artificial irradiation and temperature controlled environments) compared to those supported by outdoors photobioreactors severely limits the use of most data available in literature for the design and operation of full-scale microalgae-based systems.

This work aimed at systematically evaluating the potential of open algal-bacterial photobioreactors for the treatment of PWW under indoor and outdoor conditions. The removal of carbon, nitrogen, phosphorus and heavy metals was assessed at two PWW dilutions under solar and artificial illumination. Finally, the influence of both PWW dilution and environmental conditions on the structure of the microalgae and bacteria communities was investigated.

2. Materials and Methods

2.1. Algal-bacterial inoculum and piggery wastewater

An acclimated *Chlorella vulgaris* culture, obtained from an indoor open algal-bacterial photobioreactor treating 15% diluted PWW at the Department of Chemical Engineering and Environmental Technology at Valladolid University (Spain), was used as inoculum. Fresh PWW was collected from a nearby farm at Cantalejo (Spain) and stored at 4 °C. The PWW was centrifuged for 10 min at 10,000 rpm before dilution to reduce the concentration of suspended solids. The average composition of the 10 and 20 folds diluted PWW is shown in Table 1.

2.2. Experimental system

The indoors experimental set-up consisted of two 3 L open photobioreactors (15.8 cm depth, 15.5 cm internal diameter) illuminated at 1417 ± 82 µmol/m^2·s for 12 h a day (08h00–20h00) by LED lamps arranged in a horizontal configuration 60 cm above the photobioreactor surface under indoor conditions (Fig. 1, Table 1). Likewise, two similar open photobioreactors were located outdoors at the Department of Chemical Engineering and Environmental Technology at Valladolid University (Spain). The average photosynthetic active radiation (PAR) in these systems at 11h00 was 1394 ± 171 µmol/m^2·s (Fig. 1, Table 1). This value was comparable to the daily average PARs provided by the official AEMET meteorological station located at the University of Valladolid during the experimental period (1210 ± 126 µmol/m^2·s). The temperature of the indoor and outdoor photobioreactors was partially controlled using a water bath to prevent the high temperatures induced by both LEDs and solar irradiation. The algal-bacterial cultivation broth in the photobioreactors was gently mixed via water immersion pumps. The indoors and outdoors photobioreactors were fed with both 10 and 20 times diluted PWW using an auto control 205U7CA multi-channel cassette pump (Watson-Marlow, UK). PWW dilutions were selected based on previous investigations carried out with this kind of wastewater and aiming to avoid microbial inhibition as a consequence of PWW toxicity (De Godos et al., 2009; García et al., 2017b; González et al., 2008). Pure CO_2 was added to the cultivation broth of the phobioreactors to automatically maintain the pH at 8.0 using a Crison multimeter M44 control unit (Crison Instruments, Spain).

2.3. Experimental design and sampling procedure

The indoors photobioreactors fed with 10 and 20 times diluted PWW (namely I-10 and I-20, respectively) and the outdoors photobioreactors fed with 10 and 20 times diluted PWW (namely O-10 and O-20, respectively) were inoculated with a fresh *Chlorella vulgaris* culture at an initial TSS concentration of ≈680 mg/L (corresponding to an initial microalgae cell concentration of ≈$1.06{\cdot}10^9$ cells/L, respectively). The photobioreactors, which were initially filled with tap water, were operated at an average hydraulic retention time (HRT) of ≈26 days for 120 days (from May-2016 to Sept-2016). A higher HRT than in conventional HRAPs (3–10 days) was chosen in this research to guarantee an effective carbon and nutrients removal, and to prevent toxicity effects on microbial population due the high loads of organic matter and nutrients of the PWW treated in this study (Aguirre et al., 2011; De Godos et al., 2009). The effluent from the photobioreactors overflowed separately as a function of the evaporation rates. Liquid samples from the influent PWWs and effluents of the photobioreactors were taken weekly to determine the concentration of total organic carbon (TOC), inorganic carbon (IC), total nitrogen (TN), nitrate (NO_3^-), nitrite (NO_2^-), total phosphorus (TP), zinc (Zn) and total suspended solid (TSS). Likewise, the structure of the microalgae population in the photobioreactors was periodically assessed from biomass samples preserved with lugol acid at 5% and formaldehyde at 10%, and stored at 4 °C prior to analysis. A cultivation broth sample from the photobioreactors was also collected under steady state (day 120) and immediately stored at −20 °C to evaluate the richness and composition of the bacterial communities (Alcántara et al., 2015). Dissolved oxygen (DO) concentration and temperature in the photobioreactors were measured twice per day (11h00 and 17h00), while the influents and effluents flowrates were daily recorded to monitor water evaporation losses (Table 1). Finally, the C, N and P content of the algal-bacterial biomass present in the photobioreactors was measured under steady state.

The removal efficiencies of C, N, P and Zn were calculated according to Eq. (1):

$$RE\,(\%) = \frac{(C_{feed} \times Q_{feed}) - (C_{eff} \times Q_{eff})}{C_{feed} \times Q_{feed}} \times 100 \quad (1)$$

where C_{feed} and C_{eff} represent the dissolved concentrations of TOC, IC, TN, TP and Zn in the influent PWWs and photobioreactors effluents,