

Evaluation of the dynamics of microalgae population structure and process performance during piggery wastewater treatment in algal-bacterial photobioreactors

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Abstract

Microalgae cultivation in wastewater is crucial to the development of microalgae-based biorefineries for biofuel or biofertilizer production, whose viability depends on the supply of a biomass with a consistent year-round composition and characteristics. This work evaluated the dynamics of microalgae populations during piggery wastewater (PWW) treatment in 4 open photobioreactors inoculated with (R1) *Chlorella*, (R2) *Acutodesmus*, (R3) *Oscillatoria* and in the absence of inoculum (R4). In addition, TSS concentration and organic matter, nutrient, and heavy metal removals were assessed. The photobioreactors were fed with PWW diluted at 15% at a HRT of 27-days under 12h/12h light/dark irradiation cycles at $2800\mu\text{Em}^{-2}\cdot\text{s}^{-1}$. The pH was maintained at 8 via external CO_2 supplementation. *Chlorella* sp. and *Acutodesmus obliquus* were the dominant microalgae species regardless of the tested photobioreactor. The highest TSS concentration (3625 mg TSS/L) was recorded in R4 (the photobioreactor without initial microalgae addition) which showed the robustness of acclimation of native species to PWW. No significant differences were recorded among the 4 photobioreactors in terms of removal efficiencies of TOC (86-87%), IC (62-71%), TN (82-85%) and TP (90-92%). However, higher Zn removal efficiencies (47%) took place in the photobioreactors at higher biomass concentration.

Keywords: Algal-bacterial processes, Biomass production, Heavy metal removal, Microalgae dynamics, Piggery wastewater treatment.

1. Introduction

The current global energy and climate change crisis has triggered the quest for alternative green energy sources with a low carbon dioxide (CO_2) footprint (González-Fernández *et al.* 2012b). Microalgae have emerged as a promising renewable energy platform due to their ability to transform sunlight directly into gas biofuels or an organic

biomass feedstock that can be further bioconverted into multiple liquid and gas biofuels (Richmond 2004). Thus, microalgal biomass can be anaerobically digested yielding biogas and a nutrient rich digestate (González-Fernández *et al.* 2012a). Microalgae exhibit multiple advantages over conventional energy crops such as high areal productivities (50-100 tn/ha·y), cultivation in non-arable land (preventing competition with food) and high lipid or carbohydrate fractions depending on the cultivation conditions. In addition, microalgae can be cultivated in fresh, marine or wastewaters (Cheah *et al.* 2016). In this context, nutrient-rich wastewaters represent a valuable feedstock to reduce the costs of microalgae and cyanobacteria cultivation, which will ultimately increase the cost-competitiveness of microalgae-based biofuels. Algal-bacterial symbiosis can combine a low-cost mass production of biomass with the treatment of wastewater to levels required for discharge into natural water bodies. Domestic, industrial and livestock wastewaters have successfully supported microalgae cultivation (Muñoz *et al.* 2003). During microalgae-based wastewater treatment, both the organic carbon, nitrogen and phosphorous present in wastewater are assimilated into algal-bacterial biomass. Heavy metals and pathogens are also efficiently removed during microalgae growth as a result of adsorption and pH-mediated mechanisms. Despite microalgae cultivation in wastewater entails significant economic and environmental advantages over conventional carbon dioxide (CO_2)-supplemented mass production of microalgae in mineral salt media, controversy still exists in literature about the possibility of maintaining unialgal cultures during microalgae-based wastewater treatment. Hence, while most studies conducted under laboratory or outdoors conditions focused on the removal of key pollutants present in the wastewater, little attention has been paid to the monitoring of the dynamics of microalgae population. Pig production is a key economic sector in many countries in Europe, accounting for 148.7 million pigs heads and 44.3% of the

Table 1. Physical/chemical characterization of the 15% diluted swine manure and cultivation broth in the photobioreactors at steady state.

Parameter	PWW	R1	R2	R3	R4
Evaporation (%)	n.a	60	60	60	60
Temperature (°C)	n.a	30	30	30	30
Dissolved Oxygen (mg/L)	n.a	0.8	1.1	1.3	0.9
TOC (mg/L)	1375±121	459±31	452±31	482±27	490±37
IC (mg/L)	314±55	285±14	242±34	227±33	294±27
TN (mg/L)	393±26	174±11	166±15	165±12	149±10
Nitrite (mg/L)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
Nitrate (mg/L)	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
TP (mg/L)	9.4±0.4	2.4±0.3	2.1±0.2	1.9±0.5	1.8±0.3
Zinc (mg/L)	0.7±0.2	0.9±0.2	1.1±0.1	1.3±0.3	0.9±0.3
Copper (mg/L)	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6
Arsenic (mg/L)	< 0.6	< 0.6	< 0.6	< 0.6	< 0.6
TSS (mg/L)	1340±34	2610±191	2569±69	2445±222	3265±133

n.a : Not applicable

total European livestock (MAGRAMA 2015) in 2015. European pig farming generated 217-434 million m³/y of PWW containing high concentrations of organic matter and nutrients (De Godos *et al.* 2009). The estimated average organic matter and nutrient discharge in the EU amounted to 8.923.000 ton (COD)/y, 890.000 ton (N)/y and 223.000 ton (P)/y. In addition, piggery wastewaters can contain high concentrations of heavy metals such as Zinc and Copper, typically used as growth promoter in swine nutrition. The experimental work herein conducted evaluated the dynamics of microalgae populations during piggery wastewater treatment in 4 open photobioreactors inoculated with three green microalgae species and in the absence of inoculum. In addition, the influence of the microalgae inoculum on the steady state organic matter, nutrient and heavy metal removal was assessed.

2. Materials and methods

2.1. Microalgae and Piggery wastewater

Chlorella minutissima Fott and Nováková was obtained from an indoor high rate algal pond treating centrate at the Dept. of Chemical Engineering and Environmental Technology at Valladolid University (Spain). *Acutodesmus obliquus* and *Oscillatoria sp.* were kindly provided by the Department of Chemical Engineering at Almeria University (Spain). Fresh centrifuged PWW was collected from a nearby farm at Cantalejo (Spain) and stored at 4°C (Table 1).

2.2. Experimental set-up

The experimental set-up consisted of four 15.8 cm deep 3L open photobioreactors illuminated at 2800 µmol/m²·s for 12 h/d (08h00 to 20h00) by LED lamps arranged in a horizontal configuration 20 cm above the photobioreactor

surface. The photobioreactors were immersed in a water bath to prevent the high temperatures imposed by the LEDs irradiation. Immersion water pumps were used to mix the algal-bacterial cultivation broth in the reactors. The system was fed with PWW diluted at 15% using an auto control 205U7CA multi-channel cassette pump (Watson-Marlow, UK). The pH in the cultivation broth was automatically maintained at 8 via CO₂ addition (CARBUROS METALICOS-Barcelona, Spain) using a Crison multimeter 44 control unit (Crison Instruments, Spain).

2.3. Experimental design

Photobioreactors 1, 2 and 3 (namely R1, R2 and R3, respectively) were inoculated with *Chlorella minutissima* Fott and Nováková, *Acutodesmus obliquus* and *Oscillatoria sp.*, respectively, at an initial TSS concentration of 220 mg/L. Photobioreactor 4 (R4) was not inoculated and served as control. The photobioreactors were operated at a hydraulic retention time (HRT) of ≈ 27 days for 176 days. Liquid samples of 30 mL were weekly drawn from the influent PWW and effluents of R1, R2, R3 and R4 to determine the concentrations of TOC, IC, TN, TP, NO₃⁻, NO₂⁻, TSS, Zn, Cu and As. Likewise, the microalgae population structure was weekly assessed from biomass samples preserved with lugol acid at 5% and formaldehyde at 10%, and stored at 4 °C prior to analysis (only 8 samples were analyzed). The dissolved oxygen (D.O) and temperature (T) of the cultivation broths were measured twice per day, while the influent and effluent flowrates were daily recorded in all reactors to monitor evaporation losses. Finally, the C, N and P content of the algal bacterial biomass was measured under steady state condition at the end of the experiment. .

3. Results

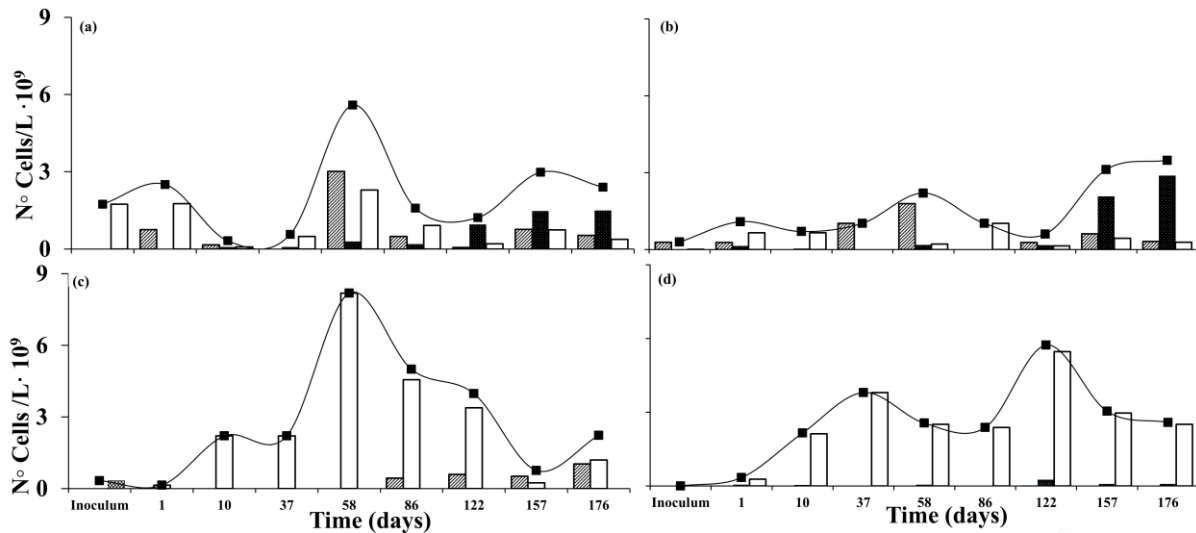


Figure 1. Microalgae dynamics and population structure in the photobioreactors during the entire operational period for (a) R1, (b) R2, (c) R3 and (d) R4. *Acutodesmus obliquus* ▨, *Aphanothece* sp. ■, *Chlorella* sp. □ and *Oscillatoria* ▩. Bold square are referred to the total number of cells (■).

Chlorella sp. was found through most of the experimental period in R1, being dominant the days 37 and 86 at concentrations of $0.5 \cdot 10^9$ and $0.9 \cdot 10^9$ N° cells/L, respectively. *Acutodesmus obliquus* was identified from the first operational days in R1 and it became the dominant species the day 176. Finally, *Aphanothece* sp. was detected the day 58 from the first time and since then its dominance increased to the end of the performance of R1 (Fig. 1a). Similar dynamics to *Chlorella* sp. in R1 was followed by *Acutodesmus obliquus* in R2 during the 176 operational days. In this regard, this inoculated microalga was identified during most of the days, with a significant dominance the days 37, 58 and 122 at cell concentrations of $1.3 \cdot 10^9$, $1.8 \cdot 10^9$ and $0.3 \cdot 10^9$ N° cells/L, respectively. *Chlorella* sp. was identified in R2 from the first operational days and it remained at similar cell concentrations through the complete study (from $0.3 \cdot 10^9$ to $0.7 \cdot 10^9$ N° cells/L). Finally, the dominance of *Aphanothece* sp. increased at the end of the performance in R2 with final cell concentrations of $2.9 \cdot 10^9$ N° cells/L (Fig. 1b). The increase in number of cells of *Aphanothece* sp. during the same operational days in R1 and R2 could be probably promoted by the characteristics of the received PWW. *Oscillatoria* was replaced in R3 from the first operational days by *Chlorella* sp. and *Acutodesmus obliquus*, *Chlorella* sp. being the dominant species through the entire performance with a maximum concentration of $8.2 \cdot 10^9$ N° cells/L the day 58 (Fig. 1c). The higher pollution-tolerance of *Chlorella* sp. to PWW or the combination of temperature/light could cause the replacement of *Oscillatoria* (Talbot *et al.* 1991). Despite R4 was not inoculated, *Chlorella* sp. and *Aphanothece* sp. appeared from the first days. *Chlorella* sp. was also the dominant species. The increase in number of cells of *Aphanothece* sp. in R4 during the last operational days corroborated the influence of the characteristics of the received PWW on microalgae population (Fig. 1d). These results were in agreement with those reported by (Posadas *et al.* 2015)

who identified different microalgae species depending on the characteristics of the received wastewater. The higher dominance of *Chlorella* sp. and *Acutodesmus obliquus* in the four photobioreactors through most of the research corroborated their high tolerance to the pollutants content in PWW (Kim *et al.* 2016). In this context, lower microalgae diversity was promoted at higher biomass concentrations (as it is below discussed) which was in agreement with (Park, Craggs, and Shilton 2011). Finally, the current morphological microalgae characterization revealed that the inoculation of a photobioreactor during PWW treatment with specific microalgae species did not assure their presence or dominance along the performance (Serejo *et al.* 2015). Biomass concentration in R1, R2, and R3 increased from 220 mg TSS/L to 530, 680, and 660 mg TSS/L, respectively, during the first 38 days and R4 increased from 45 to 200 mg TSS/L (Fig. 2). A significant biomass concentration increase took place in R1, R2 and R3 from the day 38 to the day 93 with TSS concentrations of 2440, 2140 and 2500 mg TSS/L, respectively. However, during these operational days biomass concentration in R4 exhibited slower growth and its concentration remained around 1200 mg TSS/L (Fig. 2). After day 93, biomass concentration in R2 and R3 remained quite constant, which resulted in final concentrations of 2569 and 2420 mg TSS/L, respectively. There was a biomass decrease in R1 the day 129 to 1740 mg TSS/L, followed by a subsequent biomass increase, which led in final values of 2610 mg TSS/L. Finally, in R4 biomass concentration increased exponentially from the day 93 to the end of the experimental research until values of 3265 mg TSS/L. Thus, algal-bacterial biomass concentration at steady state was the highest in R4. Despite the longer lag phase, these results showed the robustness of native species' acclimation to the operational conditions during PWW treatment (Fig. 2, Table 1) (Olguín *et al.* 2013). Similar performance in terms of TOC, IC, TN and TP-Removal Efficiencies (RE) took place regardless of the tested

photobioreactor. In this context, the dominance of different microalgae species did not influence on the efficiency of the process performance. Likewise, the high light irradiances and the optimum temperature for microorganism's activity aimed the successful treatment of the PWW. Thus, despite the low DO concentrations in the broth (≤ 1.3 mg/L), TOC-REs accounted for 86, 87, 86 and 86 % in R1, R2, R3 and R4, respectively. These results herein obtained corroborated the consistent removal of organic matter from PWW by algal-bacterial processes and were in agreement with (De Godos *et al.* 2009) who reported removal efficiencies for COD of $76 \pm 11\%$ in a 464 L HRAP during the treatment of 20 and 10 folds diluted PWW. Similarly, IC-REs of 63, 69, 71 and 62 % were recorded at the end of the process in R1, R2, R3 and R4, respectively. C removal by stripping was the main mechanism since only 37, 38, 36 and 48 % for R1, R2, R3 and R4, respectively, of the total carbon removed was recovered in the harvest biomass. TN-REs of 82, 83, 83 and 85 % were recorded at the end of the process in R1, R2, R3 and R4, respectively. These TN-REs were similar than those reported by (De Godos *et al.* 2009) with average Total Kjeldahl nitrogen (TKN) RE of $86 \pm 6\%$ during PWW treatment. N removal by stripping was the main mechanism since only 26, 26, 23 and 31 % for R1, R2, R3 and R4, respectively; of the total nitrogen removed was recovered in the harvest biomass. TP-REs of 90, 91, 92 and 92 % were recorded at the end of the process in R1, R2, R3 and R4, respectively. The TP-REs herein reported were in agreement with Franchino *et al.* (2016). Phosphorous assimilation into algal-bacterial biomass was the main removal mechanism in the photobioreactors based on the pH values during all experiment (pH=8), which did not allow phosphate precipitation (García *et al.* 2017).

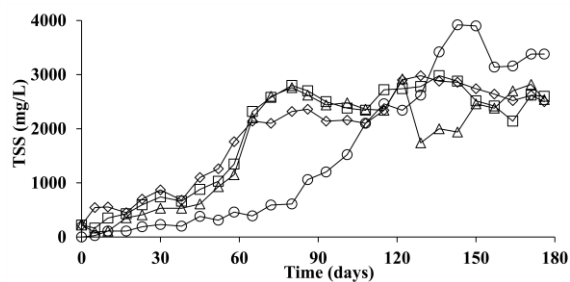


Figure 2. Time course of TSS concentration in R1 (Δ), R2 (\diamond), R3 (\square) and R4 (\circ).

Finally, the overall steady state Zn-REs in R1, R2, R3 and R4 accounted for 49, 37, 26 and 49 %, respectively. These values were similar to those reported by Abe *et al.* (2008) during PWW treatment in wetlands which resulted in Zn-REs of 37% (Abe, Komada, and Ookuma 2008). These removals of Zn showed the high tolerance of species such as *Chlorella* sp. to heavy metal contamination. The determination of copper and arsenic removal efficiencies were not possible to carry out due to their concentrations always remained below the detection limit of the equipment (< 0.6 mg/L).

4. Conclusions

This research work showed the difficulty to maintain unialgal cultures and constant microalgae population during PWW treatment in open photobioreactors. The dominance of *Chlorella* sp. and *Acutodesmus obliquus* in most of the photobioreactors corroborated their high tolerance to the pollutants content in PWW. The acclimation of native species to the characteristics of the PWW and operational conditions resulted in higher algal-bacterial biomass concentrations. Efficient PWW treatment took place regardless of the microalgae species found in the growth medium, which corroborated the robustness of microalgae-based processes applied for carbon and nutrients removal from wastewaters. Finally, process performance presented a successful biosorption capacity to remove heavy metals from PWW.

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