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Abstract. Two microalgae species (*Chlorella vulgaris* and *Chlorella protothecoides*) were tested at lab scale in order to select the optimal conditions for biomass productivity and the efficient remediation of effluents from poultry and pig industries. Both microalgae showed biomass productivities in the agro-industrial effluents that were comparable to the Chlorella synthetic medium used as control. *C. protothecoides* presented the higher productivities both for poultry effluents (46.13 and 41.75 $mg.L^{-1}.day^{-1}$ for raw and flocculated effluents) and for pig manure (95.86 $mg.L^{-1}.day^{-1}$). The supplementation of pig effluents with biomass ash increased by 50% the microalgae productivity with the highest results obtained for *C. protothecoides* and *C. vulgaris* at ash concentrations of 1.5 g/L and 3.0 g/L, respectively. The optical density of both effluents was efficiently reduced by both microalgae but particularly by *C. protothecoides* and in the presence of added ash, indicating that significant reductions of suspended solids and organic matter occurred. The results showed that poultry and pig effluents may be efficiently remediated with microalgae and the fortification with biomass ash benefits the process.

Keywords: microalgae, bioremediation, poultry industry, pig industry, *Chlorella vulgaris*, *Chlorella protothecoides*.

1 Introduction

Current technologies for the treatment of urban wastewaters and agro-industrial effluents represent complex and costly procedures but are necessary because those effluents contain a high organic load and can cause eutrophication in freshwater and marine ecosystems if discharged without treatment [1, 2].

The animal production industry (poultry, pig and cattle industries) is a source of various wastes with significant environmental impact, including manure, effluents from cleaning activities and wastewaters from dead animal processing. All organic residues from the slaughterhouses are homogenized, treated thermally and sent to a solid-liquid separator. The decanted solid wastes are used for pet food production while the aqueous phase with a high fat content is subject to a series of unitary operations to reduce its organic load and sent to the wastewater treatment plants. In traditional productions the mix of urine, faeces and wastewater were disposed on the ground as fertilizer, but with the increase in intensive farms this option is no longer usable [3].

Moreover the direct use of agro-industrial effluents as fertilizers has negative environmental impacts such as unpleasant odour emissions, contamination with pathogenic microorganisms and contamination of ground waters with components of those effluents.

Microalgae present very high biomass production rates (much higher than the vascular plants) and have their low cultivation demands enable their production on degraded lands, wastelands, deserts and even on off-shore structures, thereby not competing with the food sector [4].

In the case of agro-industrial effluents with high levels of nutrients but not contaminated with hazardous elements the produced algal biomass can be incorporated at different stages of the industrial process, in a circular economy strategy. Microalgae applications include feed supplement [5], bio-fertilizers for feed crops [6] and/or feedstock for biofuel production [7].

Microalgae can be used in food and feed due to their chemical composition, with a high protein content, but also because they are a source of almost all the essential vitamins (eg A, B1, B2, B6, B12 , C, E, biotin, folic acid, pantothenic acid, nicotinate) are rich in pigments such as chlorophylls, carotenoids and phycobilins [8, 9, 10].

This PhD program is focused in the optimisation of microalgae-based bioremediation processes for agro-industrial effluents and studying various applications of the produced microalgae biomass.

The different tasks to be implemented in order to achieve this main objective are:

a) Optimization of the growth of different microalgae (*Chlorella vulgaris*, *Chlorella protothecoides* and *Scenedesmus obliquus*) in effluents from poultry and piggery industries.
b) Mixing of animal production effluents with other agro-industrial effluents with nutrient limitations in order to achieve the remediation of both effluents.
c) Testing the use of biomass ashes as inorganic additives for microalgae growth as an alternative to the use of pure inorganic components of the culture medium.
d) Characterisation of the microalgae biomass as a supplement for animal feed.
e) Use of the produced microalgae biomass to increase the organic load of soils and as a fertilizer for fast growing vegetable species.
f) Application of the optimized methods in the construction and test of a pilot plant for the integrated bioremediation of mixed agro-industrial effluents.

In the present work aims it was studied the bioremediation of effluents from the poultry and pig industries using the microalgae *Chlorella vulgaris* and *Chlorella protothecoides*. Because these effluents have a strong organic load, the mixotrophic alga (*Chlorella protothecoides*) was used to evaluate the contribution of the heterotrophic metabolism to the microalgae productivity. Biomass ashes (raw or pre-digested) were evaluated as mineral supplements for the pig effluents, in order to test growth limitations due to inorganic factors.

2 Relationship to Cyber-Physical Systems

The production of microalgae in an industrial scale and their use in the remediation of industrial effluents requires as other industrial processes, the implementation of automated systems for process monitoring and control. In microalgae production the automated control of liquid and gaseous flows and the continuous measurement of parameters such as pH, conductivity, temperature and optical density is critical to achieve stable operation and apply the necessary correction actions whenever these parameters deviate from optimum values.

Cyber-physical systems (CPS) are physical and engineered systems whose operations are monitored and controlled by a computing and communication core [11]. In the case of microalgae-base bioremediation systems there are two levels of parameter monitoring that should be implemented: process parameters such as temperature or pH that must be constant for ideal operation and culture parameters such as optical density or concentrations of specific elements that are being consumed during algae growth.

During microalgae growth automated systems should contemplate stirring of the culture medium and may include mechanical systems to adjust the position of the bioreactors in order to achieve a maximum exposure to solar light.

Mechanical systems for continuous cleaning of the internal surface of the bioreactor may also be implemented to ensure maximum light penetration in the culture medium. When the culture achieves a given value of optical density a fraction of the culture medium should be discharged to a decantation pond and a given volume of fresh medium should be added to the bioreactor in order to replenish the level of nutrients to sustain microalgae growth.

The association of continuous sensors, automated valves and computational control center are essential elements for the implementation of large scale microalgae bioremediation systems in which the culture achieves a steady state of biomass production.

3 Methods

Two microalgae (*Chlorella vulgaris* and *Chlorella protothecoides*) were grown in two different effluents from the poultry industry (raw effluent and flocculated effluent) and a pig manure effluent mainly composed by urine. For the control it was used a *Chlorella* culture synthetic medium [12].

Biomass ashes were added to the pig manure effluent at concentrations of 1.5 g/L and 3g/L and in two different formulations: as received or pre-digested with concentrated sulfuric acid. The pig manure effluent was boiled and diluted (1:2) with tap water, before use.

The experiments were conducted at room temperature (22 ºC ± 2 ºC), under artificial lighting (10000 lux) with cycles of 12 h light/12 h dark, using 500 mL of effluent agitated by air bubbling. Algal growth was followed by measuring the culture optical density at 540 nm and dry weight. The pH was also measured and controlled in order to remain in the range of 7 to 8. The parameters evaluated in the effluents before and after microalgae growth were: total and suspended solids, total nitrogen, ammonia nitrogen, nitrate, nitrite, total phosphorus, chemical oxygen demand and biochemical oxygen demand. The growth experiments were performed in duplicate and the trials ended when the optical density started to decrease.

4 Results and Discussion

The characteristics of the tested effluents and the culture control medium were analyzed and presented in Table 1.

It can be observed that nitrogen concentrations in the effluents are comparable or higher than in the culture medium therefore this is not a limiting nutrient in these agro-industrial waste streams. On the other hand, the presence of various organic components namely proteins and lipids, some with a limited solubility in water, explains why the agro-industrial effluents have an optical density considerably higher than the synthetic culture medium.

The flocculated poultry effluent had an optical density lower than the raw effluent because the flocculation/decantation process is mainly effective in the removal of fat and suspended solids but does not affect dissolved nitrogen and phosphorus species, whose concentration in both effluents depends on the mix of wastes being treated.

The raw effluent and the flocculated effluent used in this trial were supplied by the same poultry industry but correspond to different moments of collection so their mineral composition is not necessarily related. The high content of nitrogen of the pig effluent is expected since it is mainly composed of pig urine. On the other hand the poultry and pig effluents may have limitations of some inorganic components that are essential for the microalgae growth since total ash content is significantly lower than the synthetic culture media.

Table 1. Characterization of the effluents used in the trials.

Effluent	Total Nitrogen (mg N/L)	Total Phosphorus (mg P/L)	O.D. (540nm)	Ash Content (g/L)
Raw Effluent (poultry)	160.78 ± 6.5	37.3 ± 0.5	0.778	0.46 ± 0.03
Floccul. Effluent (poultry)	202.03 ± 2.6	105.4 ± 8.4	0.372	0.54 ± 0.04
Manure Effluent 1:2	313.60 ± 15.8	83.7 ± 0.5	1.561	1.66 ± 0.01
Synthetic Culture Medium	173.1	283.9	0.087	4.64

In a first set of experiments microalgae *C. vulgaris and C. protothecoides* were grown in the raw and flocculated poultry effluents and the productivities were comparable but inferior to those of the control medium (Fig 1), although C. *protothecoides* had a better performance both in the control medium and in the effluents.

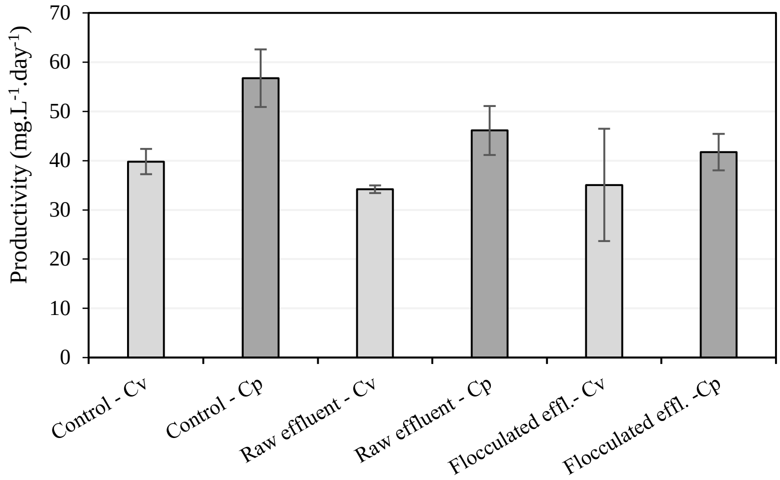

Fig. 1. Microalgae productivity in poultry effluents (mean values and standard deviation).

The lower microalgae productivity in both poultry effluents when compared with the control medium indicates that their lower mineral content and phosphorus concentrations are probably limiting microalgae growth.

The effect of algal growth in the optical density of the poultry effluents is presented in Fig 2.

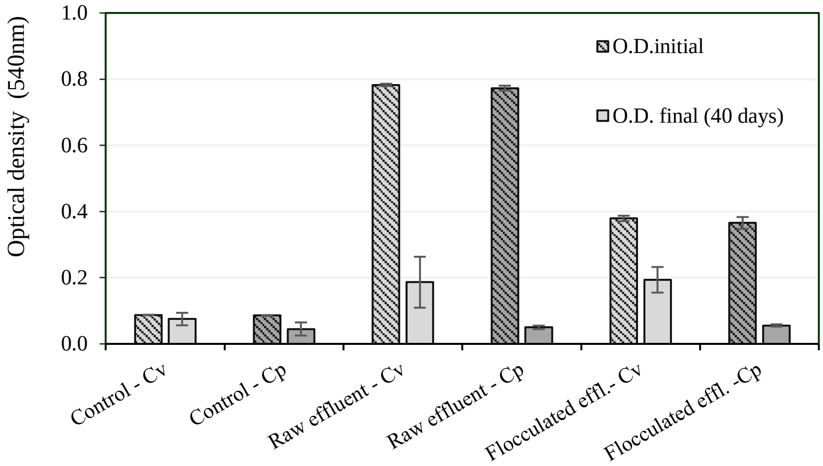

Fig. 2. Optical density of the poultry effluents measured before and after the microalgae growth experiments (mean values and standard deviation).

The microalgae growth caused an important reduction on the optical density of the effluents indicating that total solids and organic matter were used by the microalgae or aggregated to their cell wall. This effect was more pronounced for *C. protothecoides* that achieved final optical densities comparable to the control medium, probably due to its ability to metabolize organic compounds using heterotrophic pathways.

In a second set of experiments pig effluents were used as media to grow the same microalgae (Fig. 3). This effluent already has nitrogen and phosphorus contents higher than the poultry effluents so is expected to better fulfil the requirements of microalgae growth and the incorporation of biomass ashes as mineral supplements aims at testing the role of inorganic components as limiting nutrients. Effectively both *C. vulgaris* and *C. protothecoides* had better productivities in the pig effluent than in the poultry one achieving values comparable to the control medium (Fig. 3).

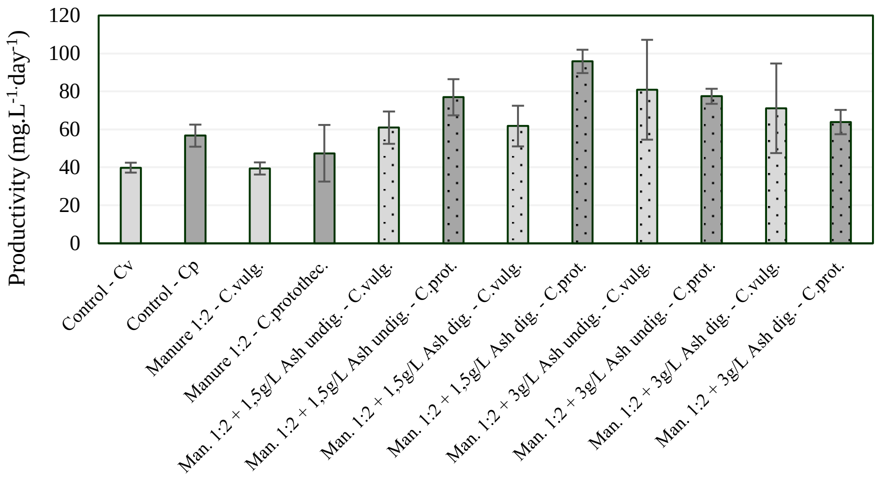

Fig. 3. Microalgae productivity in pig manure effluent, with and without ash addition (mean values and standard deviation).

The incorporation of biomass ashes had a positive effect in the growth of both microalgae but *C. protothecoides* was again the microalga with higher specific growth rate. The maximum productivity for *C. protothecoides* (95.9 mg.L^{-1}.dia^{-1}) was attained in the experiment with 1.5 g/L of digested ashes while for *C. vulgaris* a maximum productivity of 80.9 mg.L^{-1}.dia^{-1} was obtained with incorporation of 3 g/L of undigested ashes.

The yield obtained for *C. vulgaris* grown in pig manure (39,4 mg.L^{-1}.day^{-1}) was higher than that obtained by Reda *et al.* (23 mg.L^{-1}.day^{-1}) which used biologically-treated piggery wastewater effluent [13]. Other authors used piggery wastewater (COD of 11g/L) autoclaved at 120ºC and diluted about 10 times with distilled water to grow the microalgae *Chlorella pyrenoidosa* and obtained a productivity of 6.3 mg.L^{-1}.day^{-1} [14]. The incorporation of biomass ash resulted in a 50% increase in growth indicating that this mineral waste could be thus valorised as an inorganic supplement and no significant toxic effects were detected.

The effect of the microalgae growth on the optical density of the pig effluent is presented in Fig. 4.

The optical density of the pig effluent used for microalgae growth with or without ash supplementation suffered a significant reduction achieving in some cases values comparable to the control medium (Fig. 4).

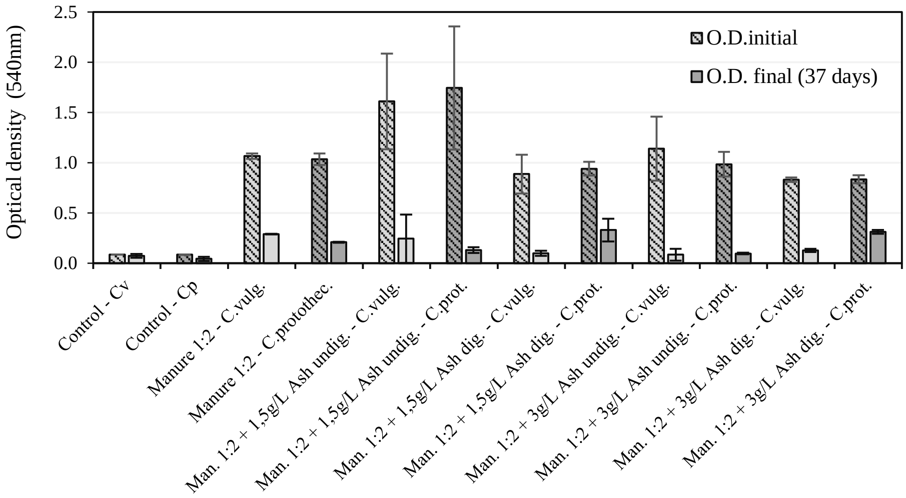

Fig. 4. Optical density of the pig effluents measured before and after the microalgae growth experiments (mean values and standard deviation).

The addition of biomass ashes contributed to an increase of the initial optical density of the pig effluent, especially for the 1.5 g/L dose in the undigested formulation. Nevertheless the final optical densities of the pig effluents used with added ash were generally lower than the pig effluents used without ash supplementation either because a higher microalgae productivity resulted in a better remediation of the effluent or because the biomass ashes induce the aggregation of the organic matter present in the effluent by altering their electrostatic status.

5 Conclusions

Microalgae can have an important role in the low cost remediation of complex effluents whose chemical and physical treatment methods are complex and expensive.

The results obtained in this series of experiments demonstrate the ability of *C. vulgaris* and *C. protothecoides* to bioremediate poultry and pig effluents, achieving a significant reduction in total solids and organic matter contents.

The incorporation of an inorganic waste (biomass ash) had a positive effect both in microalgae growth and effluent remediation. This approach constitutes an alternative use for these mineral wastes and reduces microalgae culture requirements of inorganic supplements, and therefore the production costs.

Future work comprehends the conception of a pilot scale microalgae remediation system applied to a small scale agroindustry to achieve an efficient and fast remediation of wastewaters and production of a biofertilizer. This system will comprise a cyber-physical monitoring system with automated data acquisition linked with alert systems to ensure maintenance of equilibrium conditions. Automatic replacement of culture medium with fresh effluent to achieve a steady state culture is another goal that could be attained by incorporating electronically actuated mechanical devices.

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