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Phycoremediation to Reduce the Pollution Load of Piggery Wastewater: Results of Semi-Continuous Lab Tests Using *Chlorella* and *Scenedesmus* Spp.

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This work concerns the possibility of using phycoremediation of piggery wastewater (PW) in order to remove nitrogen and to send the produced algal biomass to anaerobic digestion. A semi-continuous culturing test was performed in 150 mL glass test tubes (4.5 cm diameter, 20 cm high), in four replicates, and run for 90 days. Light was provided artificially, with 12 h dark/light periods, mixing was allowed by air bubbling and temperature was 20±2°C. Algae developed quickly with values over 1 g TSS L^{-1} after one week and maximum concentrations between 2.4 and 2.7 g TSS L^{-1}, and the community was made by *Chlorella* and *Scenedesmus* spp., in similar proportion all over the test. The average productivity was 0.26 to 0.40 g L^{-1} day^{-1}. In the absence of pH control, the intense photosynthesis raised pH, whose value was proportional to the TSS concentration. The pH raise caused the production and stripping of NH_3-N (26% of the inflow N), while the algal assimilation of N accounted for 45% and the oxidation for 25%. The overall ~~%~~ removal was about 96% for NH_4-N, 85% for P and 74% for COD. The maximum methane production was 267 NmL CH_4 g VS^{-1} (mean of the two replicates), higher than reported for pig and cow manure.

1.Introduction

Piggery wastewater (PW) consists on a mix of urine, manure and flushing wastewater, which is characterized for having high concentrations of nitrogen, phosphorus, chemical oxygen demand (COD) and total suspended solids (TSS). The high loads of ammonia nitrogen must be carefully managed to prevent environmental damages. In the past, PW was commonly spread in agricultural lands as fertilizer. Nowadays, it is well known that the nitrogen load applied to soil by such practice is not completely uptaken by crops and has an unbalanced N/P ratio with respect to the plant needs (Cai et al. 2013). Consequently, the accumulation of nitrogen in soil could contaminate groundwaters by leaching and surface waters by run-off. In this light, the current legislation strictly regulates the amount of nitrogen which can be applied to agricultural land, especially in nitrate sensitive areas, where the maximum amount of nitrogen that can be applied to soil is 170 kg N ha^{-1} y^{-1}. Therefore, it is important to find the most cost-effective, simple and environmentally friendly technology to treat PW. At present, after preliminary solid-liquid separation, PW treatment mostly follows two alternative ways. Primary sludge can be separated by primary settling or floatation and undergoes anaerobic digestion to produce biogas, while the liquid fraction, rich in nitrogen, is fed to a conventional biological treatment including denitrification/nitrification. Alternatively, PW as such can be treated directly by anaerobic digestion and, after that, by biological oxidation and denitrification/nitrification. This work concerns the possibility of using phycoremediation as a first step of treatment in order to remove nitrogen and to send the produced algal biomass to anaerobic digestion, as previously shown by different authors (among others Ward et al., 2014; Ramos Tercero et al., 2014; Ficara et al., 2014). The results of a first set of lab-scale tests are presented including both the treatment of piggery wastewater and the measurement of the biomethane potential of the microalgal biomass.

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2. Material and Methods

The wastewater came from a large piggery farm in Casaletto di Sopra (CR, Northern Italy). It was characterized as reported in Table 1 and used as such as substrate for algal culturing.

Table 1: Characterization of the piggery wastewater used as a feed for microalgae

Parameters	Mean± standard deviation
Conductivity (mS cm^{-1})	2±0.63
pH	7.7±0.26
Absorbance (680nm)	0.7±0.02
N-NH_4 (mg L^{-1})	424±34
N-NO_2(mg L^{-1})	0.03±0.03
N-NO_3(mg L^{-1})	3.29±0.74
P-PO_4(mg L^{-1})	30±7
COD (mg L^{-1})	2,280±390
Turbidity	732±36
TSS (g L^{-1})	0.76±0.03

A semi-continuous culturing test was performed in 150 mL glass test tubes (4.5 cm diameter, 20 cm high), in four replicates, and run for 90 days. Light was provided by 6 fluorescent lamps (FLUORA model, OSRAM, Munich, Germany), 18 W each one, with 12 h dark/light periods. Air was sparged from the bottom of each flask through a fine bubble diffuser to keep continuous and homogeneous mixing. Temperature was 20±2°C. A mixed microalgal community dominated by *Chlorella* spp. and *Scenedesmus* spp., grown on agricultural digestate, was used (0.12 g of microalgae dry matter per vial) as inoculum. The analyses of nitrogen compounds (ammonia, nitrite and nitrate), COD, P-PO_4 (by Hach-Lange kits) and TSS, as well as the measurements of pH, turbidity, conductivity and optical density at 680 nm, were performed 3 times per week. TSS and optical density provided data about the growth and density of algal biomass in the suspensions. At the same time, direct algal counts were also carried out using a haemocytometer (Burker's chamber) and an optical microscope (Optika B-350, magnification 40 x).
Before sampling, distilled water was added to the four vials to compensate the evaporated water. If the concentration of ammonium was below 100 mg L^{-1}, a fraction of the algal suspension was replaced by PW in order to raise the N-NH_4 concentration in each vial till 150 - 200 mg L^{-1}. Consequently, the average hydraulic retention time (HRT) was not fixed but ranged between 5 and 9 days.
Biochemical methane potential (BMP) tests were performed in duplicate using the total algal biomass produced in the four replicates by an automated volumetric system (AMTPS, Bioprocess control, Sweden) at 35±0.5 °C with anaerobic sludge as inoculum. The inoculum to substrate ratio was between 0.7 and 1 g_{VS} g_{VS}^{-1}. Oligo-elements were supplied by dosing a mineral medium, as suggested by OECD (2006). Tests were continued until the daily methane production was less than the 1% of the accumulated methane during a minimum of 3 consecutive days. Data processing was performed by subtracting the methane production of the blank bottle and by referring this net methane production to the amount of volatile solids of the algal biomass.

3. Results and discussion

3.1 Microalgal growth

Algal population developed quickly with values over 1 g TSS L^{-1} after one week and maximum concentrations between 2.4 and 2.7 g TSS L^{-1} (Figure 1). The average productivity in the four replicates was 0.26 to 0.40 g L^{-1} day^{-1}. *Scenedesmus spp*. and *Chlorella spp*. remained in the same proportion all over the test, as shown in Figure 2, and no other strain developed. These data confirm the long term stability of the microalgal/bacteria consortium grown under semi-continuous conditions.
In the absence of pH control, the intense photosynthesis raised pH. The pH increase was proportional to the TSS concentration. Indeed, in the first period (0-60 days) the average values of TSS concentration and pH were 1.3 g L^{-1} and 9.3, while in the second one they were 2.0 g L^{-1} and 10.3, respectively.
Algae count by haemocytometer and TSS were found to be consistent with OD measurements, when performed on 72 little squares of the haemocytometer, while they were not when performed on a smaller number of little squares, as frequently reported in the literature (among others, Krediet et al., 2015).
At the beginning of the test, counts had been performed in just 9 little squares and the result was not consistent with OD.

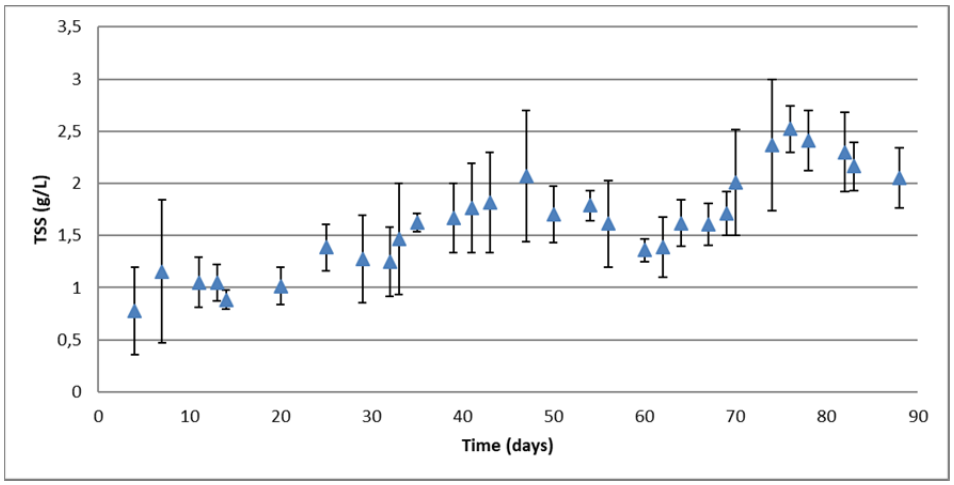

Figure 1: Average TSS concentration (g L^{-1}) in the four replicates along the whole test.

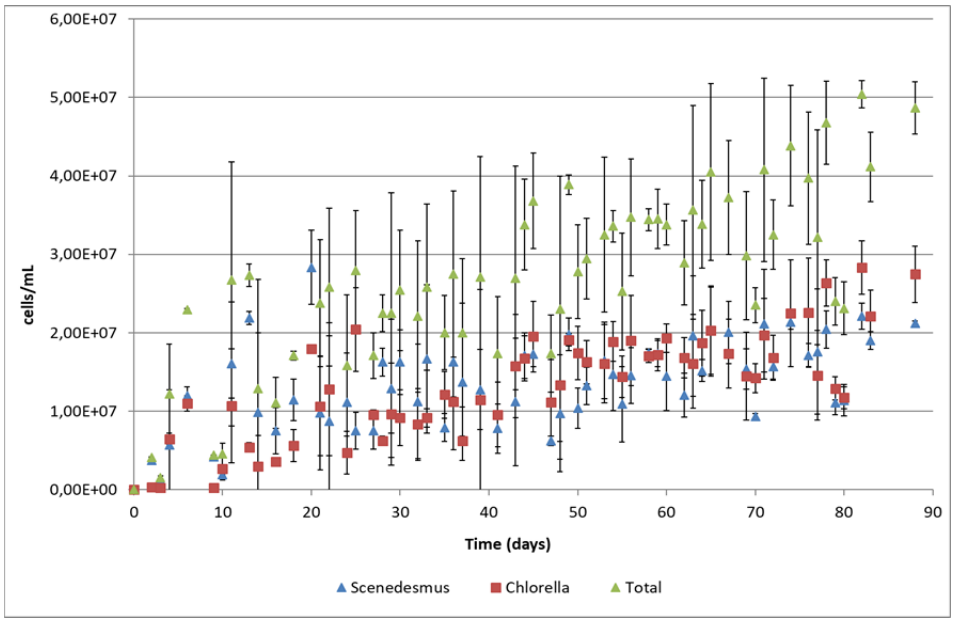

Figure 2: Total concentration (cells mL^{-1}) and distribution of the microalgae detected in the four replicates along the whole test.

3.2 Removal of nutrients and COD

The removal of nutrients and COD (Table 2) was due to the synergic action of microalgae and bacteria, as well as to chemico-physical processes. The repeatability of experimental results was satisfactory. The action of algae was surely prevailing for total nitrogen removal, while the removal of ammonia nitrogen was partially due to nitrifying bacteria, producing nitrate and nitrite.

Table 2: Percent Removal efficiencies of NH_4-N, total nitrogen, PO_4-P and COD from piggery wastewater by microalgae-bacterial based system (mean ± standard deviation)

Parameters	% Removal efficiency
NH_4-N	94 ± 3
Total N	73 ± 2
PO_4-P	85 ± 1
COD	74 ± 1

On the whole, the observed removal efficiencies are comparable to the ones reported in literature for lab-scale experiments (de Godos et al., 2011) and for pilot experiences (Garcia et al., 2017). The specific role of the different mechanisms involved in nitrogen removal was calculated. To this purpose, a mass balance was set based on the inflowing and outflowing concentrations of the different nitrogen forms and assuming that the N content in the algal biomass was 10% d.w. on the basis of previous analyses and within the range indicated by various Authors (among others, Reynolds, 2006; Gonzales-Lopez et al., 2010). The results are reported in Table 3. Again, the 4 replicates gave very similar results. Stripping was estimated by difference and resulted to account for the 25% of the overall nitrogen removal (Table 3).
An important fraction of nitrogen has been incorporated in algal biomass and a less important, but still relevant one, has been oxidized. The 25% of stripped nitrogen is quite high considering that it poses an environmental concern. Nonetheless, stripping was fostered by the air bubbling used to maintain well mixed conditions and by the high pH value. A reduced gas transfer coupled a pH control system could minimize the relevance of this process.

Table 3: Apportioning of nitrogen after microalgae-bacteria based treatment (mean ± standard deviation)

Parameters	% Apportioning
NH_4-N	4 ± 1
NOx-N	25 ± 4
Biomass N	45 ± 1
Stripped N	26 ± 4

Phosphorus removal was very high and can be attributed to algal assimilation, considering that algal cells may have highly variable P content (0.03 to 3% d.w., according to Reynolds, 2006). A partial role of precipitation, due to the high pH, should be also considered. However, as the influent N/P ratio was quite high, it is likely that algae growth has been the most relevant process contributing to P removal.
It is interesting to observe that COD removal was 73-75% and this is typically the result of the combined effect of bacterial oxidation, algal degradation and release from death cells. Many authors report effective mixotrophic growth of algae, such as various species of *Chlorella*, accounting for strong COD removal also in poorly degradable wastewaters, pointing out the double role of organic compounds as both carbon source and growth factors (among others Hemalatha et al, 2016; Kim et al., 2013; Kandimalla et al., 2016). Moreover, oxygen production from algal photosynthesis sustains bacterial aerobic metabolism which, anyway, was surely active due to air bubbling during the test. So, we cannot distinguish, in this stage of the work, the role of microalgae from the role of bacteria, but we can observe that such a high COD removal is likely to have been unaffected by the release from dead biomass.

3.3 Biomethane production

The results of BMP tests are reported in Figure 4 for two samples of microalgae. A typical exponential trend can be observed, as it is the case when hydrolysis is the limiting degradation phase.

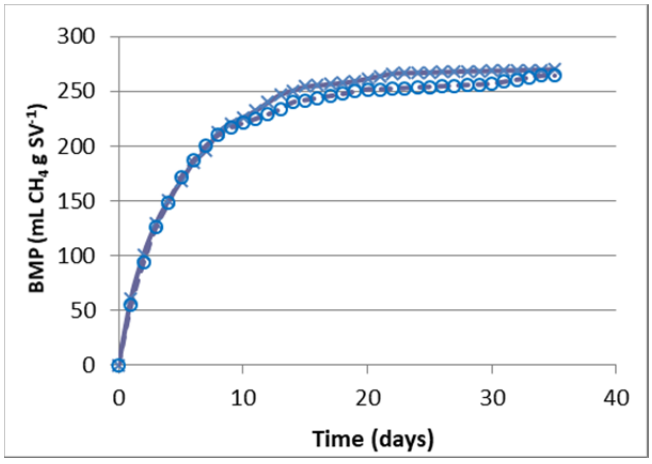

Figure 4: Cumulated specific Biomethane Production from two samples of microalgae grown on piggery wastewater.

Experimental data of cumulated biogas volumes (V_t) were fitted with an exponential model to assess the first order hydrolysis kinetic costant (K_h): $V_t = V_\infty \times [1-\exp(-K_h \times t)]$, where: V_∞ is the asymptotic biogas production. The minimum square error criterion was used to assess the optimal values of K_h and V_∞. The average values were: K_h = 0.2 d^{-1} with an asymptotic specific biogas production of 270 and 264 NmL CH_4 g VS^{-1}, higher than the values reported for raw pig and cow manure (Pham et al., 2013, Wang et al., 2017). The methane yield of the algal biomass is in agreement with literature values for *Scenedesmus spp.* and *Chlorella spp*.. Indeed, Mussgnug et al. (2010) and Ramos-Suárez & Carreras (2014) obtained 178 NmL CH_4 g VS^{-1} and NmL CH_4 g VS^{-1} for *Scenedesmus obliquus*. Similar or higher values were obtained on *Chlorella vulgaris* (191 NmL CH_4 g VS^{-1} by Mhady et al. (2014), up to 337 NmL CH_4 g VS^{-1} by Zhao et al. (2014)). Previous tests, carried out in the same laboratory, also confirmed that the cumulative BMP from microalgae (*Chlorella* and *Scenedesmus*) was higher than that of sewage sludge and animal manure, but only after 30 days (Marazzi et al., 2015; Marazzi et al., 2016).

4. Conclusions

The lab-test showed that a mixed population of microalgae, made of *Scenedesmus* and *Chlorella* spp., could easily grow on piggery wastewater and that the activity of the consortium microalgae/bacteria allowed an efficient removal of nutrients and COD. The obtained microalgal biomass was also a valuable substrate for anaerobic digestion, whose methane production was even higher than that from sewage sludge or manure. With respect to conventional treatment, phycoremedation would involve savings related to the release of oxygen by photosynthesis, with no need for external supply.
The removal of nitrogen could be optimized simpy buffering pH variations, as this would limit the formation of NH_3-N and its stripping, and prevent its possible toxicity on microalgae and/or bacteria.
These promising results are being confirmed in scale-up experiments, based on a HRAP pilot (approx. 4 m^2) to evaluate the possibility of modifying the current flow sheet of piggery wastewater treatment.

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References

Cai T., Park S.Y., Li, Y., 2013, Nutrient recovery from wastewater streams by microalgae: Status and prospects, Renewable and Sustainable Energy Reviews, 19, 360–369.
de Godos, I., Guzman, H.O., Soto, R., García-Encina, P.A., Becares, E., Muñoz, R., Vargas, V.A., 2011, Coagulation/flocculation-based removal of algal–bacterial biomass from piggery wastewater treatment, Bioresource Technology, 102, 923–927
Ficara E., Uslenghi A, Basilico D., Mezzanotte V., 2014, Growth of microalgal biomass on supernatant from biosolids dewatering, Water Science and Technology, 69, 896-902.
García D., Posadasa E., Grajeda C., Blanco S., Martínez-Páramoa S., Acién G., García-Encina P., Boladoa S., Muñoz R.,2017, Comparative evaluation of piggery wastewater treatment in algal-bacterial photobioreactors under indoor and outdoor conditions, Bioresource Technology, 245, 483-490
Girard M., Nikiema J., Brzezinski R., Buelna G., Heitz M., 2009, A review of the environmental pollution originating from the piggery industry and of the available mitigation technologies: towards the simultaneous biofiltration of swine slurry and methane, Canadian Journal of Civil Engineering, 36, 1946–1957.
González-Fernández C., Sialve B., Bernet N., Steyer J.P., 2013, Effect of organic loading rate on anaerobic digestion of thermally pretreated *Scenedesmus* sp. Biomass, Bioresource Technology, 129, 219–223.
Hemalatha M., Venkata Mohan S., 2016, Microalgae cultivation as tertiary unit operation for treatment of pharmaceutical wastewater associated with lipid production, Bioresource Technology, 215, 117–122.
Kandimalla P., Desi S., Vurimindi H., 2016, Mixotrophic cultivation of microalgae using industrial flue gases for biodiesel production, Environmental Science and Pollution Research, 23, 9345–9354.
Kim S., Park J., Cho Y., Hwang S., 2013, Growth rate, organic carbon and nutrient removal rates of *Chlorella sorokiniana* in autotrophic, heterotrophic and mixotrophic conditions, Bioresource Technology, 144, 8–13.
Krediet C.J., DeNofrio J.C., Caruso C., Burriesci M.S., Cella K., Pringle J.R., 2015, Rapid, Precise, and Accurate Counts of *Symbiodinium* Cells Using the Guava Flow Cytometer, and a Comparison to Other Methods, PlosONE, DOI:10.1371/journal.pone.0135725
Mahdy A., Mendez L., Ballesteros M. & González-Fernández C., 2014, Autohydrolysis and alkaline pretreatment effect on *Chlorella vulgaris* and *Scenedesmus* sp. methane production, Energy, 78, 48-52.
Marazzi F., Mezzanotte V., Scaglione D., Ficara E., 2016, Microalgal based treatment of digested agro-wastes - lab and pilot-scale experiments. IWA WWC, Brisbane, September 9-14, 2016.

Marazzi F., Martínez O., Mezzanotte V., Ficara E., 2015, Biogas production from microalgae grown on supernatant from biosolids dewatering, XIV World Congress on Anaerobic Digestion, Viña del Mar, Chile, 15-18th November 2015.

Mussgnug J. H., Klassen V., Schlüter A. & Kruse O., 2010, Microalgae as substrates for fermentative biogas production in a combined biorefinery concept, Journal of Biotechnology, 150, 51-56.

Pham C.H., Triolo J.M., CuT.T.T., Pedersen L., Sommer S.G., 2013, Validation and Recommendation of Methods to Measure Biogas Production Potential of Animal Manure. Asian-Australasian Journal of Animal Sciences, 26, 864–873.

Ramos-Suárez J. L. & Carreras N., 2014, Use of microalgae residues for biogas production, Chemical Engineering Journal, 242, 86-95.

Ramos Tercero E.A., Alibardi L., Cossu R., Bertucco A., 2014, Anaerobic digestion of microalgal residues to enhance the energetic profit of biocrude production, Chemical Engineering Transactions, 37, 793-798

Reynolds C., 2016, Ecology of Phytoplankton, Cambridge University Press, Cambridge (UK)

Tosta A. & Mendonça F., 2009, Technical analysis of the new Brazilian concentration limits for ammonia in effluents and bodies of water, Power, 27, 353–362.

Wang M., Lee E., Dilbeck M.P., Liebelt M., Zang Q., Ergas S.J., 2017, Thermal pretreatment of microalgae for biomethane production: experimental studies, kinetics and energy analysis, Journal of Chemical Technology and Biotechnology, 92, 399-407

Ward A.J., Lewis D.M., Green F.B., 2014. Anaerobic digestion of algae biomass: A review, Algal Research, 5, 204–214.

Zhao B., Ma J., Zhao Q., Laurens L., Jarvis E., Chen S. & Frear C. 2014, Efficient anaerobic digestion of whole microalgae and lipid-extracted microalgae residues for methane energy production, Bioresource Technology, 161, 423-430.