Effect of N:P Ratio on Microalgae/Nitrifying Bacteria Community in Agro-Digestate Treatment

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Abstract** –The role of P content on the treatment and valorization of the liquid fraction of digestate, namely centrate, through microalgae-based technologies was evaluated in this study. The performance of four column photobioreactors, which were fed on diluted centrate with corrected (10 mg N/ mg P) and not modified (129 mg N/ mg P) N:P ratio, were monitored and compared. The results demonstrated that P shortage in the centrate affected neither the total nitrogen and COD removal rate nor the volumetric biomass productivity, suggesting that expensive addition of P salts is not necessary to maximize the efficiency of the process. On the contrary, the addition of P to the centrate promoted the ammonia oxidation process as higher nitrite production was observed in the photobioreactors with adjusted N:P ratio than in the ones fed with the non-adjusted N:P ratio. These findings were confirmed by fluorescence *in-situ* hybridization and quantitative PCR assays, which revealed a higher number of ammonia-oxidizing bacteria in the microalgal suspensions cultivated on centrate with P addition. In conclusion, the N:P ratio in the centrate seems to have a role in controlling the nitrification process rather than in the overall nutrient removal rate and biomass productivity of the microalgae-based system.*

***Keywords** – Ammonia oxidation; centrate; microalgae-based technology; nutrient removal; P content*

Nomenclature	
N	Nitrogen
P	Phosphorus
DM	Dry Matter
AD	Anaerobic Digestion
MAS	Microalgae-based technology
AOB	Ammonium Oxidising Bacteria
PBR	Photo-bioreactor
COD	Chemical oxygen demand
TSS	Total suspended solid
VSS	Volatile suspended solid
PCR	Polymerase chain reaction

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FISH	Fluorescence in situ identification	
P_v	Volumetric biomass productivity	mg TSS L^{-1} d^{-1}
rr_i	Volumetric removal rate for contaminant i	mg L^{-1} d^{-1}
η_i	Removal efficiency for contaminant i	–

1. INTRODUCTION

In Lombardy, which is the most populated region of Northern Italy, 69 % of the land is employed for agricultural purposes; more than 50 000 farms are present breeding ca. 1.5 million cows and 4 million pigs. Therefore, proper management of agricultural and livestock wastewaters is required to protect the environment and to comply with the European Nitrate Directive (91/676/CEE). To reduce treatment costs and recover energy, anaerobic digestion (AD) is largely applied, with around 500 operative biogas plants producing more than 350 MW. However, AD produces a nitrogen rich digestate with concentrations ranging from 44 to120 g N kg^{-1}DM [1].

Microalgae-based technology (MAS) is now under development since microalgae assimilate nutrients while growing, support bacteria activity via photo-oxygenation, and generate biomass that can be further used as a feedstock for the production of bioenergy and biofertilizers.

The production of bioenergy as biogas or biodiesel from photosynthetic organisms (cyanobacteria and microalgae) was suggested in the 1950s [2], but only recently this potential has risen again motivated by the search for renewable energy sources and effective circular solutions [3]. Indeed, methane yield from microalgae is reported to range from 166 mL gVS^{-1} in *Chlorella minutissima* to 380 mL gVS^{-1} in *Chlamydomonas* spp. [4]. The higher values are comparable with the yields from other crops typically used for biogas production. When applied to soil, microalgal biomass improves soil fertility and structure, and can produce plant growth hormones, polysaccharides, antimicrobial compounds and other metabolites to stimulate plant growth. The effects of cyanobacteria and green algae as biofertilizers in terms of improving fertility and quality of soil and promoting plant growth is going to be a new application in modern agriculture [5], [6]. Microalgal biomass is also a potential source for producing bioplastics, and can be used directly, as well as a feedstock for secondary processes [7].

Several research groups are focusing on the feasibility of remediation and resource recovery from various agro-industrial wastewaters, including the liquid fraction of digestate [8]–[10]. In these bioremediation systems, complex consortia of microalgae and bacteria develop and nitrogen conversion is carried out by microalgae and nitrifying bacteria (ammonia which oxidize ammonia to nitrate via nitrite [10]–[12]. It is well known that microalgae satisfy the oxygen requirement of bacteria by generating oxygen through the photosynthesis [13], but the understanding of the interaction between these two types of microbes, as well as the role of oxidizing bacteria and nitrite oxidizing bacteria on the speciation of nitrogen in the system is still limited. Several factors, including light, temperature, and nutrient availability, seem to regulate the assembling of the microalgal-bacteria consortium, as well as the growth and biodegradation activity of each type of microbe [14].

It is generally assumed that phosphorus availability has a key role being the limiting factor for the growth of microalgae [15]. According to Redfield (1934), the optimal N:P molar ratio for the growth of algae is 16, though the microalgae are quite flexible and can adapt within a large range of N:P ratio [8], [16].

The aim of this work was to evaluate if P availability affects nitrogen removal efficiency and biomass productivity in a microalgae-based system by focusing on the effect of the N:P ratio on the activity and function of microalgae and ammonia oxidizing bacteria (AOB). As growth medium, the liquid fraction of an agro-industrial digestate, referred to as 'centrate' (i.e. obtained by digestate centrifuging), from a typical farm situated in Northern Italy was used.

2. MATERIALS AND METHODOLOGY

2.1. Photobioreactors set up and operation

Four parallel plexiglass columns (height 35 cm and diameter 10 cm, working volume 1.5 L) were set up as photobioreactors (PBRs) (Fig. 1).

Columns were mixed by magnetic stirrers and light was provided by fluorescent lamps (FLUORA model, OSRAM, Munich, Germany) providing a light intensity of 40 W m^{-2} and working according to 12/12 h dark/light periods. PBRs were operated at room temperature (15–32 °C, average = 27 ± 2 °C). Even if temperature can affect biological kinetics, in this case it was not set constant as it is not relevant to the specific purpose of the research; pH was not controlled.

The PBRs were inoculated with a consortium of microalgae dominated by *Chlorella* spp. e *Scenedesmus* spp. (500 mL with an $OD_{680} = 1.6$), which were previously grown spontaneously, without any selection, on the same centrate used for the experiments. The PBRs were fed on a diluted centrate collected from a full-scale biogas plants fed on the solid fraction of piggery wastewater, energy crops, and agricultural wastes (three anaerobic digesters of 3000 m^3 each, total hydraulic retention time HRT ≈ 30 days, T = 40 °C). The centrate was diluted (6.8 folds) with tap water in order to achieve the following characteristics: NH_4^+-N = 200 mg N L^{-1}, NO_3^--N = 2.3 mg N L^{-1}, PO_4^--P = 1.6 mg P L^{-1}, COD = 790 mg L^{-1}, TSS = 190 mg L^{-1}. Feeding was provided in a semi-continuous mode (twice per week) for 103 days, at a fixed average HRT of 10 days.

Two PBRs (NC1 and NC2) were fed on the unmodified diluted centrate (i.e. with a N/P ratio of 129 mg mg^{-1}, corresponding to a molar ratio of 58, while the other two PBRs (C1 and C2), were fed on P-supplemented diluted centrate, obtained by adding KH_2PO_4 to the diluted centrate to achieve a N/P ratio of 10 mg mg^{-1}. The final value corresponded to a molar ratio of 4.5, about four times lower than the Redfield ratio, and was chosen in order to maximize the effects of the change. Samples were collected twice a week for chemical-physical and microbiological analyses.
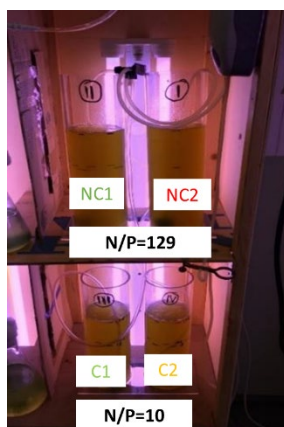
Fig. 1. The lab-scale photobioreactors used for the experiments.

2.2. Analytical methods

Nutrient removal efficiency and microalgal productivity were assessed based on the concentration of the different forms of N, of P, and of biomass. The determination of NH_4^+-N, NO_2^--N, NO_3^--N, PO_4^--P concentrations and soluble COD were carried out by spectrophotometric test kits (Hach-Lange, DR6000TM UV VIS Spectrophotometer, Hach Lange LT200 Dry thermostat) on filtered (0.45 µm) samples of the microalgal suspensions. Total suspended and volatile solids (TSS and VSS) were measured in duplicate according to Standard Methods for the Examination of Water and Wastewater (APHA). The elemental composition of the dried biomass was determined by a Perkin Elmer CHNS/O analyzer2400series II. Absorbance and turbidity were measured spectrophotometrically at 680 nm wavelength in 1 cm cuvette and 860 nm wavelength in 5 cm cuvette, respectively (DR6000TM UV VIS, Hach Lange, Germany). Temperature and pH were routinely checked using a multiparametric probe (XS PC 510 Eutech Instruments, Stevensville, USA).

2.3. Microalgal counting and identification

Microalgal populations were monitored for strain composition and cell counts over time. An adequate dilution of the microalgal suspension was placed in a hemocytometer (Marienfieled, Germany) and the microalgal cells were counted by an optical microscope with a 40x objective (B 350, Optika, Italy). The total cell number was calculated from the average of 6 squares (1 mm^2) readings. During the counting, each algal cell was identified according to its morphology.

2.4. Molecular methods

Aliquots of microalgal suspensions (1.5 mL), which were collected at days 44, 51, 85 and 99, were immediately frozen at –20 °C after sampling for quantitative PCR assays. Prior to DNA extraction, the thawed samples were centrifuged at 13 000 rpm for 5 min; the resulting pellets were washed twice with 1 mL of distilled sterile water. Then, the DNA extraction was carried out using Power Soil DNA isolation kit (MO BIO, USA) according to the manufacturer's instructions. In the final step, the DNA was eluted with 100 µL sterile water,

its quality and concentration were checked using a spectrophotometer (BioTek EON microplate spectrophotometer; BioTek, Italy). All DNA samples were then frozen at −20 °C until PCR analyses.

The number of total bacteria and ammonia oxidizing bacteria was evaluated by quantitative PCR (qPCR). Primers targeting the 16S ribosomal RNA (rRNA) genes of bacteria (primer set: 357-F (5′-CCTACGGGAGGCAGCAG 3') and 907-R (5'-CCGTCAATTCCTTTGAGT TT-3') [17], [18] and primers targeting the ammonia monooxygenase structural gene amoA of AOB (primer set: amoA-1F (5'-GGGG TTTCTACTGGTGGT) and amoA-2R (5'-CCCCTCKGSAAAGCCTTCTTC) [19] were used. In each PCR reaction (total volume 20 μL) 3 μL of DNA was added to a mix solution containing Power SYBR Green PCR Master Mix (Applied Biosystems, USA) and 100 nM of each primer. The PCR cycles were performed on AB 7300 Real-Time PCR System (Life Technologies, Italy) as described in Bellucci *et al*. (2015) and Marazzi *et al*. (2019) [20], [21]. The 16S rRNA gene copy numbers were then converted to equivalent cell numbers assuming that an average of 4.2 rRNA operon exists in each bacterial cell; whereas the number of AOB was estimated by considering 2 copies of amoA gene in each AOB cell [22], [23].

AOBs were also detected directly into the microalgal suspensions by combining Fluorescence In-Situ Identification (FISH) and fluorescent microscopy. Freshly collected samples were stored in ethanol (sample/ethanol ratio = 1:2 [v/v]) at –20 ºC prior to fixation, which was then performed using 4 % paraformaldehyde fixative solution (PFA) as described elsewhere [23], [24]. Hybridization assays were then performed as previously described [25] with a mixture of probes targeting the 16s rRNA of different populations of AOB (Nso1225, NEU, and 6a192) [23], [26]. The hybridized cells were then visualized with a 40x objective by Zeiss fluorescence microscope (Axio Scope HBO 50).

2.5. Data processing and statistical analyses

Performance parameters were computed from the concentrations of each relevant component either in the influent digestate (IN) or in the algal suspension (OUT) measured between two consecutive feedings (i.e. at time t_{i-1} and t_i) and considering the volume of the PBR (V_C) and the fraction of it that was withdrawn and substituted with fresh feed (V_{IN}).

As for the volumetric productivity (P_V), TSS values (in mg L^{-1}) were used in Eq. (1).

$$P_{V,t_i} = \frac{V_C \times TSS_{OUT,t_i} - \left[(V_C - V_{IN}) \times TSS_{OUT,t_{i-1}} + V_{IN} \times TSS_{IN} \right]}{V_C} \qquad (1)$$

As for volumetric removal rates (rr_j), the concentration of each relevant contaminant (C_j with j = COD, N-forms, P) were used in Eq. (2).

$$rr_{j,t_i} = \frac{\left[(V_C - V_{IN}) \times C_{j,OUT,t_{i-1}} + V_{IN} \times C_{j,IN} \right] - V_C \times C_{j,OUT,t_i}}{V_C} \qquad (2)$$

It should be noted that in the case of oxidized nitrogen forms (nitrite or nitrate), removal rates were negative, indicating that those components were produced by the microalgae-bacteria consortium. In this case, production rates (pr_{N-NOx}) are reported ($pr_{N-NOx} = - rr_{N-NOx}$).

Finally, removal efficiencies were computed for each relevant contaminant (C_j) in Eq. (3).

$$\eta_{j,ti}\left(\%\right) = \frac{C_{j,IN} - C_{j,OUT,ti}}{C_{j,IN}} \times 100 \tag{3}$$

The efficiency of the nitrogen transfer to the atmosphere (including NH_3 stripping or N_2 production by denitrification) was quantified as the difference between the influent ammoniacal nitrogen and the nitrogen leaving as residual ammonium, oxidized nitrogen and *assimilated nitrogen* (the later estimated by assuming a N content of 8 g N g TSS^{-1} as measured by elemental analyses) in Eq. (4).

$$\eta_{N,atm}\left(\%\right) = \left(1 - \frac{rr_{N\text{-}NH4} + pr_{N\text{-}NOx} + 0.08 \times P_v}{V_{IN} \times C_{N\text{-}NH4,IN}}\right) \times 100 \tag{4}$$

To compute the average performances of the system, these time series were averaged within operationally stable periods. In detail, the initially transient phase was disregarded (t_0-t_{15}) as well as a transient non-stationary phase between t_{60} and t_{80}, as it will be more thoroughly discussed later on.

Statistical analyses were conducted with the open source tool of R (3.6.0) Rstudio (Version 1.1.383) and the data analysis tool of Excel 2019. The statistical significance of the reproducibility (p-value > 0.05) of duplicate PBRs, as well as the differences (p-value < 0.05) between the two conditions (P addition or not), were assessed by pairwise t-paired tests. The significance of the influence of P addition on the performance and microbial population evolution in the PBRs was also confirmed by one-way analyses of variance (ANOVA).

3.   RESULTS

3.1. Biomass productivity

In Fig. 2, the time trend of the optical density (OD_{680}), as a proxy parameter of the microalgae concentration in the PBR, is shown. The OD_{680} data series were similar in duplicate PBRs (p-value > 0.1, paired-t tests). After an initial colonization phase lasting approximately 2 weeks, the algae concentration stabilized until day 60. Fluctuations were however observed mostly due to variations in room temperature. The OD_{680} trend also evidences a significant decline of the microalgal biomass concentration between days 57 and 68, when a severe bloom of the filamentous cyanobacteria *Oscillatoria* sp. occurred, probably favoured by a decrease of the room temperature to 15 °C. The extent of the reduction in biomass concentration was similar in both NC and C PBRs, possibly suggesting that P availability did not significantly affect the cyanobacteria. Once the filaments of *Oscillatoria* sp. were manually removed, the microalgae density increased again, and a new steady state condition was reached from day 80 to the end of the experimentation.
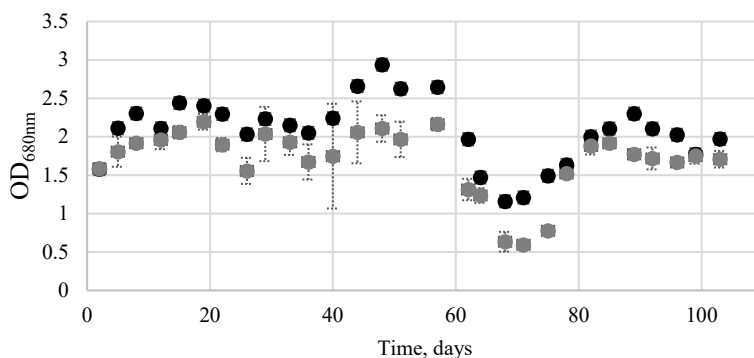
Fig. 2. Averaged OD_{680nm} detected in NC (black) and C (grey) over time. Error bars represent the standard deviation.

On the overall, these data suggest that microalgae could grow in all systems demonstrating that centrate is a suitable substrate for microalgal cultivation. A slightly higher OD_{680} was measured in NC reactors compared to C ones (p-value < 0.05; t-paired tests and ANOVA). From TSS data, the overall biomass productivity (P_v) was assessed, and average values of 64 ± 29 mg DW L^{-1} d^{-1} and 53 ± 30 mg DW L^{-1} d^{-1} in NC and C reactors, respectively, were computed. The slightly higher value of P_v in the NC PBRs was in fact non-significant (p-value > 0.31, ANOVA), suggesting that P_v is not affected by the N:P ratio in the feed.

3.2. Bioremediation performance

Table 1 summarizes the main bioremediation performances of the PBRs. All data series (COD, N-forms and P) were first checked for replicability by applying a paired-t test to series collected in replicate PBRs. Indeed, all time-series were similar in duplicate reactors (p-value > 0.05). Specifically, removal rates (rr_i) and removal efficiencies (η_i) were computed during the steady state performance of the PBRs.

As for COD, all PBRs were effective, removing approximately 65 % (45 ± 11 mg L^{-1} d^{-1}) the organic components in the digestate. The observed COD removal can be attributed to the activity of heterotrophic bacteria and/or the potential mixotrophic growth of microalgae. Available data do not allow distinguishing among these two removal mechanisms. Both average rr_{COD} and average η_i were similar in all PBRs (p-value = 0.95, ANOVA) regardless of the N:P ratio in the feed, suggesting that COD removal was not affected by the P availability.

As for P, average rr_P were similar in duplicate reactors (p-value > 0.1, t-paired tests), but higher in C reactors than in NC ones. The residual P concentrations in the effluents averaged 0.5 ± 0.4 mg L^{-1} and 11 ± 4 mg L^{-1} in NC and C PBRs, respectively and with a higher η_P for NC than for C reactors. However, considering the different P loading rate, C reactors removed from 6.2 times more P than NC reactors (see Table 1). By computing the ratio between rr_P and P_V, one can see the average P content was 0.2 % and 1.4 % of TSS for NC and C reactors, respectively.

TABLE 1. BIOREMEDIATION PERFORMANCES: REMOVAL OR PRODUCTION RATES (RR$_I$, PR$_I$ IN MG L^{-1} D^{-1}) COMPUTED FROM DATA OBTAINED DURING STEADY STATE CONDITIONS (MEAN ± STANDARD DEVIATION, N = 17). REMOVAL EFFICIENCIES (η_I) FOR THE PBRS ARE ALSO REPORTED.

PBR	rr_{Ntot}	rr_P	rr_{COD}	rr_{N-NH4}	pr_{N-NO3}	pr_{N-NO2}	$\eta_{N,atm}$	η_N	η_P
NC1	6.2 ± 7.8	0.05 ± 0.19	48 ± 11	8.8 ± 8.1	0.4 ± 0.6	2.2 ± 3.5	19	34	85
NC2	8.7 ± 4.7	0.07 ± 0.14	47 ± 11	10.9 ± 3.8	0.4 ± 0.6	1.5 ± 4.7	19	38	92
C1	9.6 ± 7.1	0.79 ± 1.04	48 ± 12	16.5 ± 4.5	0.5 ± 0.7	5.4 ± 8.6	29	47	46
C2	10 ± 7	0.95 ± 1.08	45 ±13	17.5 ± 2.6	0.6 ± 0.7	6.9 ± 6.7	25	43	45
p-values	0.37	0.00058	0.84	<0.000	0.79	0.037			
Influence of P	No	Yes	No	Yes	No	Yes			

As to nitrogen, a detailed analysis of the N compounds in the PBRs effluent (Fig. 3 NC and Fig. 3 C) as well as the removal rates of the different N-forms (Table 1) revealed some interesting differences. Although the rr_{Not} (where $N_{tot} = N-NH_4^+ + N-NO_2^- + N-NO_3^-$) was similar in all PBRs (*p*-values > 0.05, t-paired tests and ANOVA), rr_{N-NH4} was higher in C (17 ± 3.7 mg L^{-1}d^{-1}) than in NC (9.8 ± 6.4 mg N L^{-1}d^{-1}) reactors (*p*-values = 0.005 ANOVA) Similarly, pr_{N-NO2} in C (6.2 ± 7.6 mg N L^{-1}d^{-1}) was higher than in NC (1.8 ± 4 mg N L^{-1}d^{-1}), (*p*-values = 0.037 ANOVA), while pr_{N-NO3} was practically negligible in all reactors (0.5 ± 0.6 mg N L^{-1}d^{-1}). Even the contribution of N transfer to the atmosphere was found to be different since it accounted for the 19 ± 0.1 % and 27 ± 3 % of the total NH$_4^+$-N removal in NC and C, respectively.
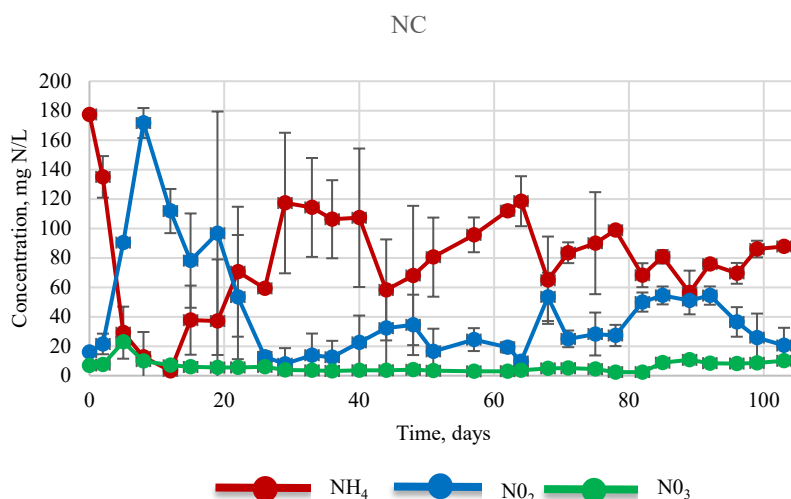

Fig. 3 NC. Averaged concentrations of ammonia (red circles), nitrite (blue diamonds) and nitrate (green triangles) measured over time in NC PBRs measured over time. Error bars represent the standard deviation.
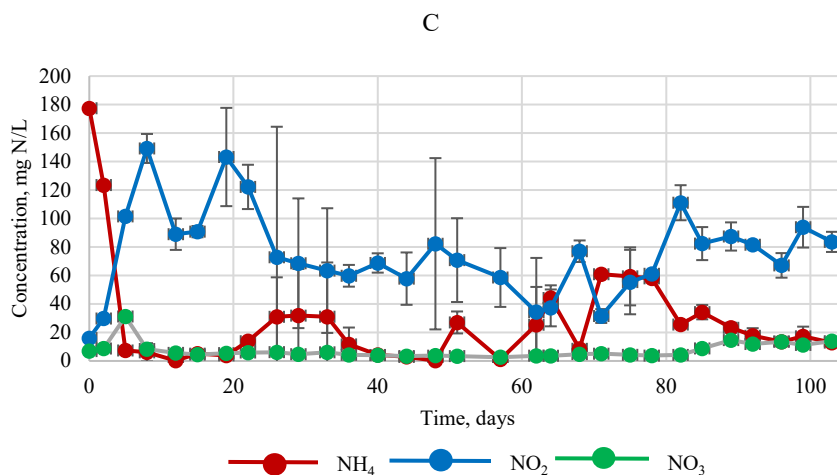
C

Fig. 3 C. Averaged concentrations of ammonia (red circles), nitrite (blue diamonds) and nitrate (green triangles) measured over time in C PBRs measured over time. Error bars represent the standard deviation.

As stated before, pH was not controlled and was on average 8.9 ± 0.7 in NC reactors and 7.3 ± 1.5 in C reactors. The lower pH in C than in NC was probably due to the higher ammonia oxidation activity in C.

3.3. Microbial community structure

Microalgae counts ranged between 2.8×10^6 and 3.3×10^7 cells mL^{-1}. In all PBRs, the microalgal community was dominated by *Chlorella* spp. (>90 % of the total community) most of the time, while *Scenedesmu*s spp., ranging between 7.5×10^5 and 5.7×10^6 cells mL^{-1}, was detected only during the second part of the experimentation, i.e. after the recovery from the cyanobacterial bloom.

FISH assays were performed before the cyanobacterial bloom and at the end of the experiment (Fig. 4).
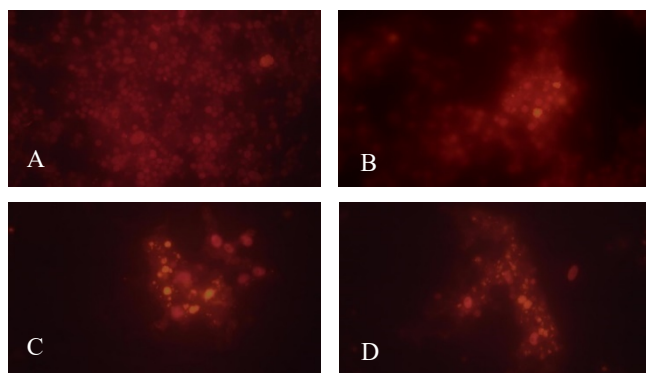

Fig. 4. AOB colonies (bright red) detected in microalgal aggregates in NC (A and B) and C (C and D) PBRs at days 99 by FISH assay.

It can be observed that the N:P correction promoted the growth and activity of AOB as they were visibly more abundant in C than in NC. Quantitative analyses (Fig. 5) confirmed that the number of AOB in the C reactors were almost 1 log higher than in NC (p-value < 0.05) demonstrating that AOB were favoured by the larger P availability. On the contrary, the total number of bacteria which ranged between 7.9×10^6 and 9.7×10^6 cells mL^{-1} was not influenced by the N:P ratio.
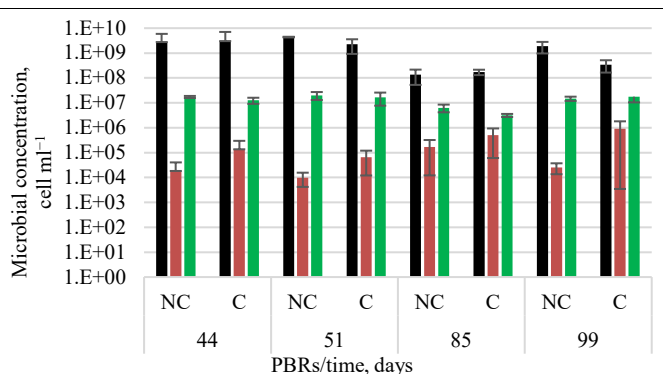

Fig. 5. Cell counts for total bacteria (black), AOB (red) and microalgae (green) in NC and C photobioreactors detected before the cyanobacterial bloom (days 44 and 51) and at the end of the trial (days 85 and 95).

Nevertheless, a reduction of the total bacterial abundance was observed after the cyanobacterial bloom (p-value < 0.04 ANOVA). These findings suggest that the microalgae produced enough oxygen to sustain the growth, not only of the AOB, but also of the heterotrophic bacteria. Yet, the growth of the heterotrophs was not limited by P shortage as similar and constant COD removal rate was observed in all PBRs. Nevertheless, a decline of the total bacterial number was observed after the bloom of *Oscillatoria* sp, suggesting that this cyanobacterium might release some antibacterial compounds [27].

4. DISCUSSION AND CONCLUSIONS

The objective of this study is to assess the role of P content on the performance of microalgal-based technologies treating agricultural and livestock wastewaters with unbalance N:P ratio, such as the centrates. To this purpose, the microalgal growth and evolution, COD and nutrient removal rates, as well the nitrogen removal pathways of column PBRs fed on diluted centrate with and without the supplementing of P salts were monitored and compared.

The above presented results clearly demonstrate that the microalgal productivity and total nitrogen removal were independent of the concentration of P in the centrate. An averaged P_V of 59 mg TSS L^{-1} d^{-1} of microalgal biomass was measured, while the nitrogen removal rate was ca. 10 mg N L^{-1} d^{-1} in all PBRs. The calculated P content in the biomass suggests that the microalgae-bacteria biomass could adapt to P shortage without detrimental effects on the biomass productivity. Indeed, the ability of the algae to change their internal N:P ratio according to the environmental conditions was already reported [16].

These results are in agreement with laboratory and pilot scale studies in which centrate with diverse N:P ratios were used as microalgae cultivating medium. Data of this experimentation

therefore confirm that P was not the limiting factor in microalgae growth as it was not in other experimentations testing centrate both in indoor and outdoor conditions [10], [15], [28] being light availability the typical limiting factor. Therefore, the practice of adding expensive P salts to optimize the process could be avoided without hampering biomass productivity.

Similarly, P availability did not affect the presence of heterotrophic bacteria. On the contrary, the N:P ratio did affect the nitrogen removal pathways. The addition of P in the centrate boosted the ammonia oxidation process as shown by the higher nitrite production in C than in NC reactors, and confirmed by results of the molecular assays. Nitrification, especially when stopped at the level of nitrite, is undesired in many microalgal cultivation technologies aiming to produce microalgal biomass, since AOB compete for nutrients and CO_2 with microalgae, and also they produce nitrite that can inhibit microalgal growth [29]. On the other hand, in wastewater treatment the synergy between microalgae and nitrifiers can be considered an advantage to reduce aeration costs. The role of P in controlling nitrification in microalgae-bacteria consortia was already reported in batch cultivation tests using centrate with diverse N:P ratio (3–79 gN gP^{-1}) [15]. The present study confirms that AOB are more sensitive to P shortage than microalgae also in PBRs operated in semi-continuous mode for long time. Although P shortage did not result in the washing out of AOB, nonetheless their concentration was depressed in NC PBRs. Indeed, a different affinity for P of the two types of microbes (microalgae and AOB) can be used as selective pressure to manipulate the microbial community assembly and function.

The following main conclusions can be finally be drawn:

1. Centrate can be used as a source of nutrients to grow a stable microalgae-bacteria consortium and interesting removal efficiencies as for N, P and COD were achieved; the N:P ratio (up to 129 mg/mg) is not a key factor to control the efficiency of microalgae-based technology, therefore expensive addition of P salts could be safely avoided;
2. P availability did not influence the activity of heterotrophic bacteria while it promoted the growth of AOB;
3. The bloom of *Oscillatoria sp.* inhibited the growth of heterotrophic bacteria and microalgae, while AOB were not affected.

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