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Carbon and nutrient biological removal in a University of Cape Town membrane bioreactor: Analysis of a pilot plant operated under two different C/N ratios

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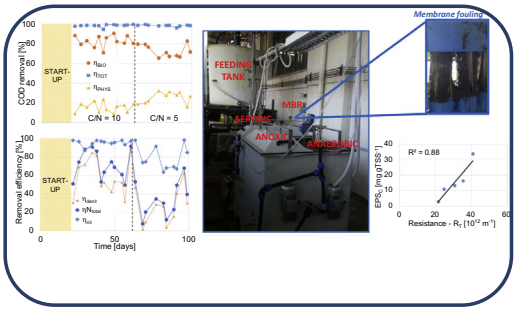
HIGHLIGHTS

- A UCT-MBR pilot plant subjected to a C/N variation was investigated.
- The biological performances were significantly affected by the C/N decrease.
- Competition between PAOs and denitrifying species decreased P removal dramatically.
- Respirometry showed that nitrification was significantly affected by C/N decrease.
- A reduction of membrane filtration performance due to the increase of EPS took place.

GRAPHICAL ABSTRACT



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ABSTRACT

The effect of the carbon-to-nitrogen (C/N) ratio variation in a University of Cape Town Membrane bioreactor (UCT-MBR) was investigated. The experimental campaign was divided into two phases, each characterized by a different C/N ratio (namely, 10 and 5, Phase I and Phase II, respectively). The UCT-MBR pilot plant was analysed in terms of carbon and nutrients removal, biomass respiratory activity, activated sludge features and membrane fouling. The results highlighted that the nutrients removal was significantly affected by the decrease of the C/N ratio during the Phase II. The biological carbon removal was also affected by the low C/N value during the Phase II. Indeed, the average biological COD removal efficiency was equal to 72.9% during the Phase II, while the average value was 82.8% in the Phase I. The respirometric batch test suggested that both heterotrophic and autotrophic species were severely affected by the lower C/N ratio in the Phase II. Moreover, a decrease of the membrane filtration properties was observed during the Phase II, mainly due to the worsening of the activated sludge features, which enhanced the increase of SMP production.

1. Introduction

Nitrogen (N) and phosphorus (P) play a crucial role in water eutrophication thus requiring their removal from wastewater especially when discharged in sensitive areas [1]. Biological nutrient removal (BNR) from domestic wastewater has been extensively investigated and developed in the last years and it is usually based on anaerobic, anoxic and aerobic reactors linked in-series (*among others,* Wanner et al. [2]; Cosenza et al. [3]; Lu et al. [4]). In BNR processes, N and P removal is accomplished, respectively, by heterotrophic denitrifying bacteria and polyphosphate-accumulating organisms (PAOs) which require carbon source [5]. In particular,

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the biological phosphorous removal is commonly conducted by exploiting the ability of PAOs to accumulate P and to store it as intracellular polyphosphate (poly-P) under alternating anaerobic/aerobic conditions [1]. Therefore, in BNR systems the sufficient amount of a carbon source is crucial for the proper removal of nitrogen and phosphorous [6]. However, when treating wastewater characterized by low carbon-to-nitrogen (C/N) ratio the rapid enrichment of PAOs could not be achieved and another group of microorganisms usually known as glycogen accumulating organisms (GAOs) might compete with PAOs for the available organic substrate without contributing to P removal [7]. Furthermore, at low C/N the NO_3-N in the return sludge (from anoxic, or aerobic, tank to the anaerobic one) can inhibit the phosphorus release in anaerobic zone where denitrifiers compete with PAOs for carbon source, consequently the phosphorus release does not occur until denitrification is completed [8].

Therefore, the C/N ratio represents a key parameter for nutrient removal from wastewater. Indeed, in terms of nitrogen removal C/N ratio might directly affect the activity of functional microorganism species, including autotrophic populations, such as ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), as well as heterotrophic denitrifying bacterial species [9]. Indeed, the decrease of the C/N ratio mainly related to increase of ammonia loading rates might significantly reduce the activity of specific nitrifying populations and can severely hamper the nitrification and/or nitrogen removal [10]. Among others, Choi et al. [11] investigated the effects of C/N ratios on an intermittently aerated membrane bioreactor (MBR) system. Choi and co-workers found that a C/N ratio over 7 is required for nitrogen removal; conversely, authors found that a C/N ratio of 4.5 promotes a decrease of nitrogen removal capacity. In terms of phosphors removal, studies on the influence of C/N ratio have been mainly performed on conventional activated sludge (CAS) systems: CAS combined with biological aerated filter (BAF) [12] and sequential batch reactors (SBR) [13]. Literature shows that C/N ratio has a significant effect on P removal in CAS systems or SBR. Specifically, at moderate C/N ratio (e.g., C/N = 6) P can be released inside the settling tank due to the occurrence of anaerobic condition and the availability of soluble carbon sources [12].

Recently, the integration of BNR process with membrane bioreactor (MBR) as an efficient combined process has been proposed for the wastewater treatment to treat the quality of the effluent, including such BNR processes as University of Cape Town (UCT) process, anoxic/oxic (A/O) process and anaerobic/anoxic/oxic (A^2O) process [14]. Indeed, MBRs have attracted considerable interest due to various advantages compared to conventional process that originate from the use of a membrane for solid–liquid separation [9]. In particular, MBRs generally feature high quality effluent, small footprint and low sludge production rates compared to CAS systems [15]. In terms of P removal, MBRs preserve from the release of P inside the settling tank in case of anaerobic conditions and carbon availability. Moreover, previous investigations highlighted that incorporating membranes into BNR activated sludge systems could have a profound difference not only in the design of the BNR system but also in the operation for the whole wastewater treatment plant (WWTP) [16].

However, few studies can be found in the technical literature on the role that the C/N variation play on the performance of a BNR system including MBR, referring in particular to the removal pollutant (i.e., carbon and nutrients) efficiencies, biomass biokinetic behaviours and membrane fouling. Recently, Xiang et al. [17] investigated a full scale modified A_2O–MBR plant combined with the step feed strategy and operated at low C/N ratio (3.8). Xiang and co-workers found that at low C/N the addition of external carbon source was required to improve TN and TP removal efficiencies. However, as authors are aware, no studies have yet been performed comparing the behaviour of an MBR in terms of COD removal and BNR for different values of influent C/N. Furthermore, although there are several studies carried out separately on membrane processes and BNR in conventional activated sludge processes, the combination of membrane and BNR processes needs further studies to optimize the full scale processes to be used.

Bearing in mind these considerations, the aim of the paper is to explore the effect of a C/N ratio variation on a BNR process integrated with a membrane for the solid–liquid separation phase. In detail, the objective was to assess the impact of C/N variation in terms of carbon, nitrogen and phosphorous removal, nitrification ability, biomass respiratory activity and membrane fouling. To accomplish such goal, an UCT-MBR pilot plant was built-up and fed with a mixture of real domestic and synthetic wastewater. The UCT-MBR pilot plant was started-up with a C/N ratio equal to 10 (Phase I) that was decreased to 5 (Phase II) by increasing the influent ammonia loading rate.

2. Material and methods

2.1. Pilot plant and sampling campaign

An UCT-MBR pilot plant was built at the Laboratory of Sanitary and Environmental Engineering of Palermo University (Fig. 1). The pilot plant consisted of an anaerobic (volume 62 L), an anoxic (volume 102 L) and an aerobic (volume 211 L) tanks according to the UCT scheme [18]. The solid–liquid separation phase was carried out by means of an ultrafiltration hollow fibre membrane (PURON®). The membrane module was located inside an aerated tank (MBR tank) (36 L). An oxygen depletion reactor (ODR) allowed the oxygen stripping in the mixed liquor recycled from the MBR tank to the anoxic one (Q_{RAS}). The membrane was periodically backwashed (every 9 min for a period of 1 min) by pumping, from the Clean In Place (CIP) tank a volume of permeate back through the membrane module. The extraction flow rate was set equal to 20 L h^{-1} (Q_{in}). During the pilot plant operations, a 20 L h^{-1} flow rate (Q_{R1}) was continuously recycled from the anoxic to the anaerobic tank. Furthermore, a 100 L h^{-1} flow rate (Q_{R2}) of mixed liquor was pumped from the aerobic to the MBR tank. A net permeate flow rate of 20 L h^{-1} was extracted (Q_{OUT}) through the membrane module. Therefore, the recycled activated sludge (Q_{RAS}) from the MBR to the anoxic tank through the ODR tank was equal to 80 L h^{-1}.

The pilot plant was fed with municipal wastewater mixed with a synthetic wastewater characterized by Sodium Acetate (CH_3COONa), glycerol ($C_3H_8O_3$), dipotassium hydrogen phosphate (K_2HPO_4) and ammonium chloride (NH_4Cl). The synthetic wastewater was added in order to control the C/N ratio fed to the pilot plant. The UCT-MBR pilot plant was started up with sludge inoculum, withdrawn from the WWTP of Palermo, to obtain an initial total suspended solid (TSS) concentration of 3500 mg L^{-1}. After a 20 days start-up phase, the experimental campaign was divided in two phases each characterized by a different C/N value: (i) Phase I, with a C/N = 10 (duration: 41 days); (ii) Phase II, C/N = 5 (duration: 39 days). During both periods, the UCT-MBR pilot plant was operated with periodical sludge withdrawals with the aim to obtain an average weighted TSS concentration of 5 g L^{-1}. In Table 1 the main influent and operational features and Standard Deviations (SD) of both experimental phases are reported.

During pilot plant operations, the influent wastewater, the mixed liquor inside the anaerobic, anoxic, aerobic and MBR tank and the effluent permeate have been sampled and analysed for TSS, volatile suspended solids (VSS), total chemical oxygen demand (COD_{TOT}), supernatant COD (COD_{SUP}), ammonium nitrogen

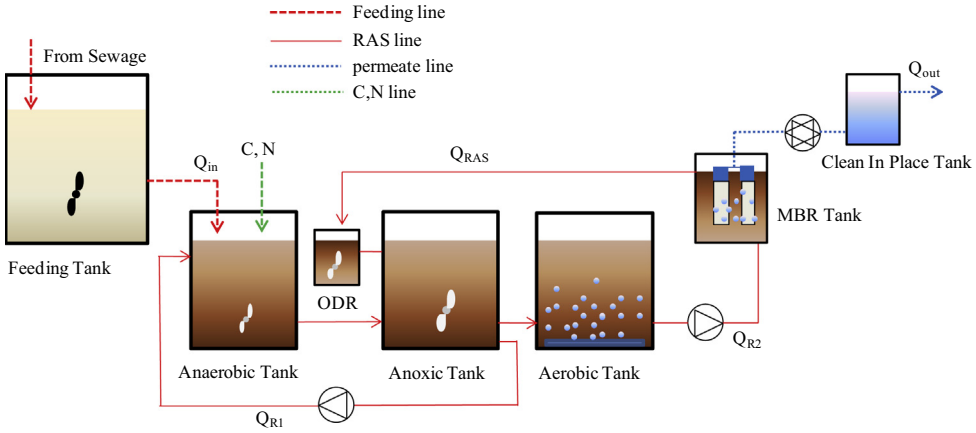

Fig. 1. Layout of the UCT-MBR pilot plant (where, Q_{in} = inlet flow rate Q_{R1} = flow rate recycled from the anoxic to the anaerobic tank Q_{R2} = flow rate pumped from the aerobic to the membrane tank Q_{RAS} = recycle activated sludge line ODR = oxygen depletion reactor).

Table 1
Average influent features and operation conditions during the Phases I and II, respectively; SD = Standard Deviation.

Parameter	Units	Phase I		Phase II	
		Average	SD	Average	SD
COD	[mg L^{-1}]	502	145	411	57
Total nitrogen (TN)	[mg L^{-1}]	52.6	13.5	99.2	29
Total phosphorus (TP)	[mg L^{-1}]	4.2	0.5	5.4	1.2
Permeate flux	[L m^{-2} h^{-1}]	21	2	21	2
Flow rate	[L h^{-1}]	20	1.5	20	0.8
C/N	[–]	10	–	5	–
HRT	[h]	20	–	20	–
Period	[d]	21–61	–	62–99	–

(NH_4-N), nitrite nitrogen (NO_2-N), nitrate nitrogen (NO_3-N), total nitrogen (TN), phosphate (PO_4-P), total phosphorus (TP). All analyses have been carried out according to the Standard Methods [19]; pH, dissolved oxygen (DO) and temperature were also monitored in each tank by using a multi-parameter probe.

The concentration of free ammonia (FA) was estimated according to Hansen et al. [20].

The nitrification (η_{nit}), denitrification (η_{denit}) and total nitrogen (ηN_{total}) removal efficiencies were evaluated as follows [43]:

$$\eta_{nit}\ (\%) = \frac{(NH_4^+\text{-}N_{in}) - (NH_4^+\text{-}N_{out}) - N_{assimilation}}{(NH_4^+\text{-}N_{in}) - N_{assimilation}} \quad (1)$$

$$\eta_{denit}\ (\%) = \frac{(NH_4^+\text{-}N_{in}) + (NO_X\text{-}N_{in}) - (NH_4^+\text{-}N_{out}) - N_{assimilation} - (NO_X\text{-}N_{out})}{(NH_4^+\text{-}N_{in}) + (NO_X\text{-}N_{in}) - N_{assimilation}} \quad (2)$$

$$\eta N_{total}\ (\%) = \frac{(NH_4^+\text{-}N_{in}) + (NO_X\text{-}N_{in}) - (NH_4^+\text{-}N_{out}) - (NO_X\text{-}N_{out})}{(NH_4^+\text{-}N_{in}) + (NO_X\text{-}N_{in})} \quad (3)$$

where NH_4^+-N_{in} = influent nitrogen ammonia concentration, NH_4^+-N_{out} = permeate nitrogen ammonia concentration, $N_{assimilation}$ = assimilated nitrogen (5% of the BOD removed), NO_x-N_{in} = influent nitrite and nitrate concentration, and NO_x-N_{out} = permeate nitrite and nitrate concentration.

Referring to the COD removal, in order to distinguish the removal due to the biological processes from that one due to the cake filtration operated by the membrane, two different removal efficiencies have been calculated [15]: the biological removal efficiency and the total removal efficiency. The biological COD removal efficiency was calculated as the difference between the COD_{TOT} value in the influent and the COD_{SUP} measured in the supernatant of mixed liquor samples (filtered at 0.45 μm) withdrawn from the MBR tank. Conversely, the total COD removal efficiency (including the removal contribution due to membrane filtration) was assessed as the difference between the inlet and the permeate COD_{TOT}, respectively.

2.2. Respirometric, AUR and NUR batch test analyses

Respirometric batch experiments were carried out on a "flowing gas/static–liquid" respirometer, according to the procedure described in Di Trapani et al. [10]. Briefly, the biomass samples were transferred to the respirometer and diluted with permeate, if necessary, in order to obtain a mixed liquor volatile suspended solid (MLVSS) concentration in the range of 2.0–3.0 g VSS L^{-1}. Furthermore, before running the batch tests, the biomass samples were aerated until endogenous conditions were reached, by monitoring the oxygen uptake rate (OUR) values. Referring to the heterotrophic biokinetic parameters, the nitrifying biomass was inhibited by adding 10 mg L^{-1} of Allylthiourea (ATU), while the exogenous OUR was enhanced by spiking a readily biodegradable organic substrate (sodium acetate in this case). The estimation of the endogenous decay coefficient b_H and the heterotrophic active fraction were carried out according to the "single batch test" (among others Di Trapani et al. [15]; Mannina et al. [45–47]). The kinetic/stoichiometric parameters of the autotrophic species were measured with a similar procedure. Nevertheless, in this case no inhibiting substance like ATU was added and ammonium chloride (NH_4Cl) was spiked to evaluate the biokinetic parameters. Furthermore, in order to evaluate the nitrification as well as the denitrification rate, ammonium utilization rate (AUR) and nitrate utilization rate (NUR) tests were performed by adopting a modified protocol derived by Kristensen et al. [21].

2.3. Extracellular polymeric substance (EPS) measurement

The soluble EPSs or soluble microbial products (SMPs) were obtained by centrifugation at 5000 rpm for 5 min, while the bound EPSs (EPS_{Bound}) were extracted by means of the thermal extraction method (among others Cosenza et al. [22]). The extracted EPS_{Bound} and the SMP were then analysed for proteins by using the Folin method with bovine serum albumin as the standard [23], whereas the carbohydrates were measured according to DuBois et al. [24], which yields results as glucose equivalent. Moreover, the sum of proteins and carbohydrates was considered as the total EPSs (EPS_T), according to the following expression:

$$EPS_T = \underbrace{EPS_P + EPS_C}_{EPS_{Bound}} + \underbrace{SMP_P + SMP_C}_{SMP} \quad (4)$$

where the subscripts "P" and "C" indicate the content of proteins and carbohydrates respectively in the EPS_{Bound} and SMP, that typically constitute the main fractions.

2.4. Membrane fouling analysis

Membrane fouling has been analysed by monitoring the total resistance (R_T) to membrane filtration which is calculated according to Eq. (5):

$$R_T = \frac{TMP}{\mu J} \quad (5)$$

where TMP is the transmembrane pressure (Pa), μ the permeate viscosity (Pa s), and J the permeation flux (m s^{-1}).

R_T can be defined as the sum between the intrinsic resistance of membrane (R_m) and the resistance due to membrane fouling (R_F). This latter can be fractionated according to Eq. (6).

$$R_F = R_{PB} + R_{C,irr} + R_{C,rev} = R_T - R_m \quad (6)$$

where: R_{PB} is the irreversible resistance due to colloids and particles deposition into the membrane pore; $R_{C,irr}$ is the fouling resistance related to superficial cake deposition that can be only removed by physical cleanings (hydraulic/sponge scrubbing); $R_{C,rev}$ is the fouling resistance related to superficial cake deposition that can be removed by ordinary backwashing.

In order to analyse the specific fouling mechanisms the resistance in series resistances method according to Di Trapani et al. [15] has been applied. In order to limit the total resistance with respect to a fixed threshold value, physical membrane cleaning were performed according to literature [25]. According the in series resistances method during the physical membrane cleaning R_{PB}, $R_{C,irr}$ and $R_{C,rev}$ can be quantified (see for instance Di Trapani et al. [15]).

Furthermore, the 20 °C normalised membrane permeability (K_{20}) was calculated using a simple filtration model that takes into account the TMP and J monitored data (Eq. (7)) [26].

$$K_{20} = \frac{J \cdot f_T}{TMP} \quad (7)$$

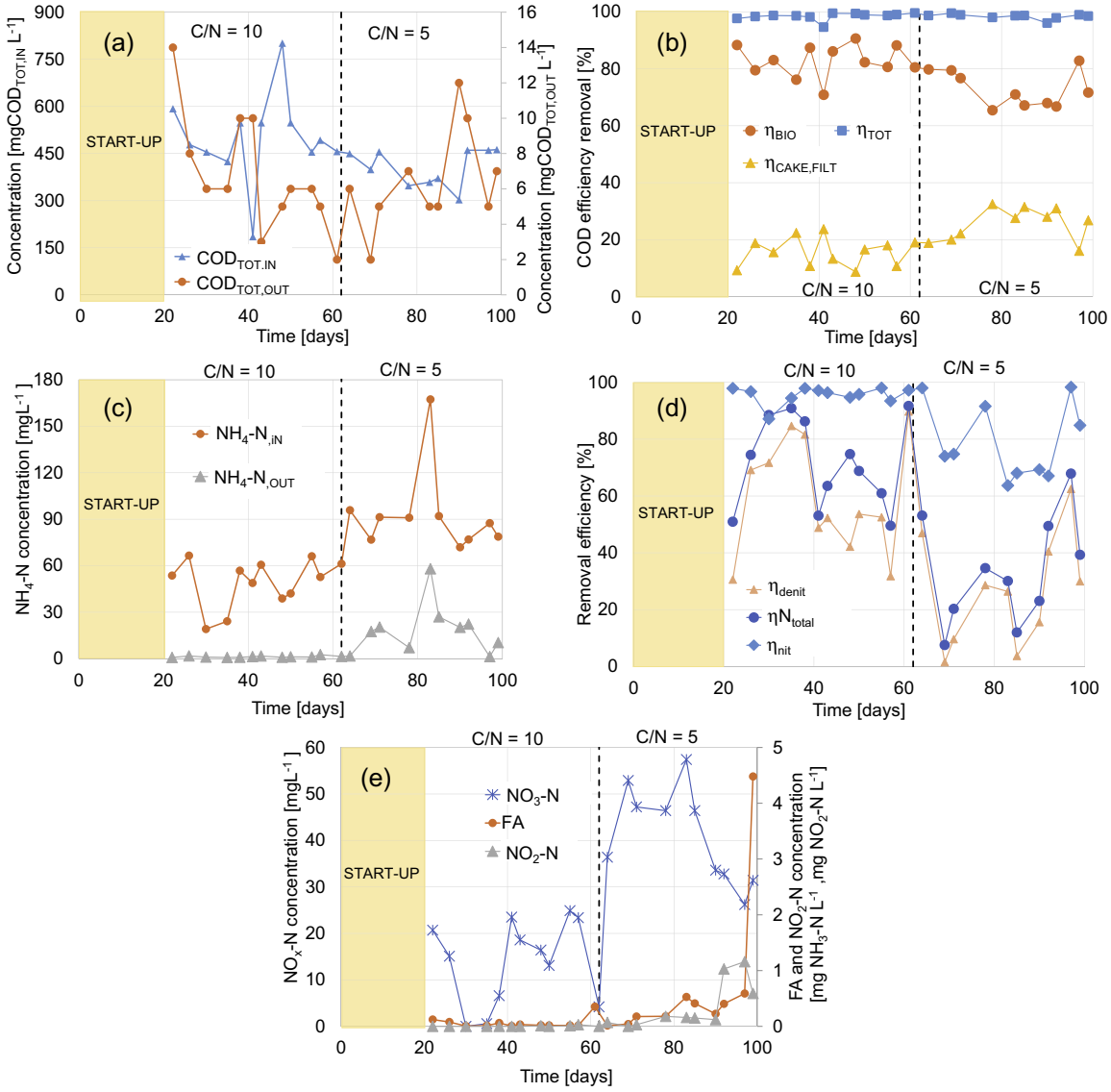

Fig. 2. Profile of $COD_{TOT,IN}$ and $COD_{TOT,OUT}$ (a); profiles of COD removal efficiencies as biological (η_{BIO}), cake filtration ($\eta_{CAKE,FILT}$) and total contribution (η_{TOT}) (b); profiles of NH_4-$N_{,IN}$ and NH_4-$N_{,OUT}$ (c); profiles of nitrification (η_{nit}), denitrification (η_{denit}) and total nitrogen removal efficiencies (ηN_{total}) (d); concentration profiles of NO_2-N, NO_3-N and FA inside the aerobic tank (e).

where: f_T represents the temperature correction to account for the dependence of permeate viscosity on temperature. Therefore, the term $J \cdot f_T$ represents the 20 °C normalized transmembrane flux.

2.5. Sludge dewaterability

The capillary suction time (CST) and the specific resistance to filtration (SRF) were measured in order to investigate the sludge dewaterability features [27,28]. CST and SFR were measured in accordance with EN 14701-1 (2006) [29] and EN 14701-2 (2006) [30], by analysing fresh samples collected from the anaerobic, anoxic, aerobic and MBR tanks. In details, the CST measurements were carried out by pouring a certain volume of sample into a sludge reservoir placed upon Whatman No. 17 filter paper. An electronic device recorded the time necessary for the filtrate to cover the space between two probes, which detected the advancement of the liquid front on the paper. The CST value was then assessed as the average value of three replicates. The SRF was evaluated in reduced pressure condition (−50 kPa). In details, the vacuum condition was achieved by means of a vacuum pump connected to a Buchner funnel where Whatman 41 (20 μm pore size) filter paper was placed. After pouring 100 of sample on the funnel the filtrate volumes (V) and the corresponding time (t) were recorded. The SRF was calculated in accordance with Eq. (8):

$$r = \frac{2 \cdot \Delta p \cdot A^2 \cdot b}{\mu \cdot C_0} \quad (8)$$

where Δp is the pressure drop across the filter; A is the filtration area; μ is the viscosity of filtrate at the temperature of the sludge; b is the slope of the linear part of the curve obtained by plotting t/V versus V; C_0 is the initial dry residue of the sludge.

The viscosity of filtrate was measured using a Brookfield rotational viscometer. In details, 16 mL of mixed liquor derived from the aerobic tank were put into a metallic cylindrical shaped vessel where the sludge temperature was controlled (20 °C ± 0.1 °C) by means of a thermostat. The rotor velocity was set equal to 60 rpm and the corresponding viscosity value was expressed in cP.

3. Results and discussion

3.1. Removal performance

3.1.1. Organic and nitrogen removal

In Fig. 2 the inlet ($COD_{TOT,IN}$) and permeate ($COD_{TOT,OUT}$) COD concentrations (Fig. 2a) as well as COD removal efficiencies (Fig. 2b) are reported. The influent and effluent ammonia profiles (NH_4-$N_{,IN}$ and NH_4-$N_{,OUT}$) are reported in Fig. 2c. Furthermore, in Fig. 2d the nitrification (η_{nit}), denitrification (η_{denit}) and total nitrogen (ηN_{total}), removal efficiencies are reported. The N-NO_3, N-NO_2 and FA concentration profiles inside the aerobic tank are shown in Fig. 2e.

In terms of COD removal, the results reported in Fig. 2 show that during the Phase I, characterized by a C/N ratio equal to 10, a very high total removal efficiency was obtained (average value of η_{TOT} = 98.4%; average value of η_{BIO} = 82.8%) (Fig. 2b). Conversely, the biological COD removal efficiency during the Phase II was affected by the lower value of the C/N ratio (equal to 5). Indeed, as depicted in Fig. 2b, the biological COD removal decreased to 72.9% as average value during the Phase II. However, the total COD removal efficiency remained still high with an average value of 98.36%. This result was mainly related to the biological/physical effect of the cake layer filtration, according to Mannina and Di Bella [41]. Indeed, although there was a worsening of the biomass activity in the Phase II likely due to the C/N variation (as better outlined

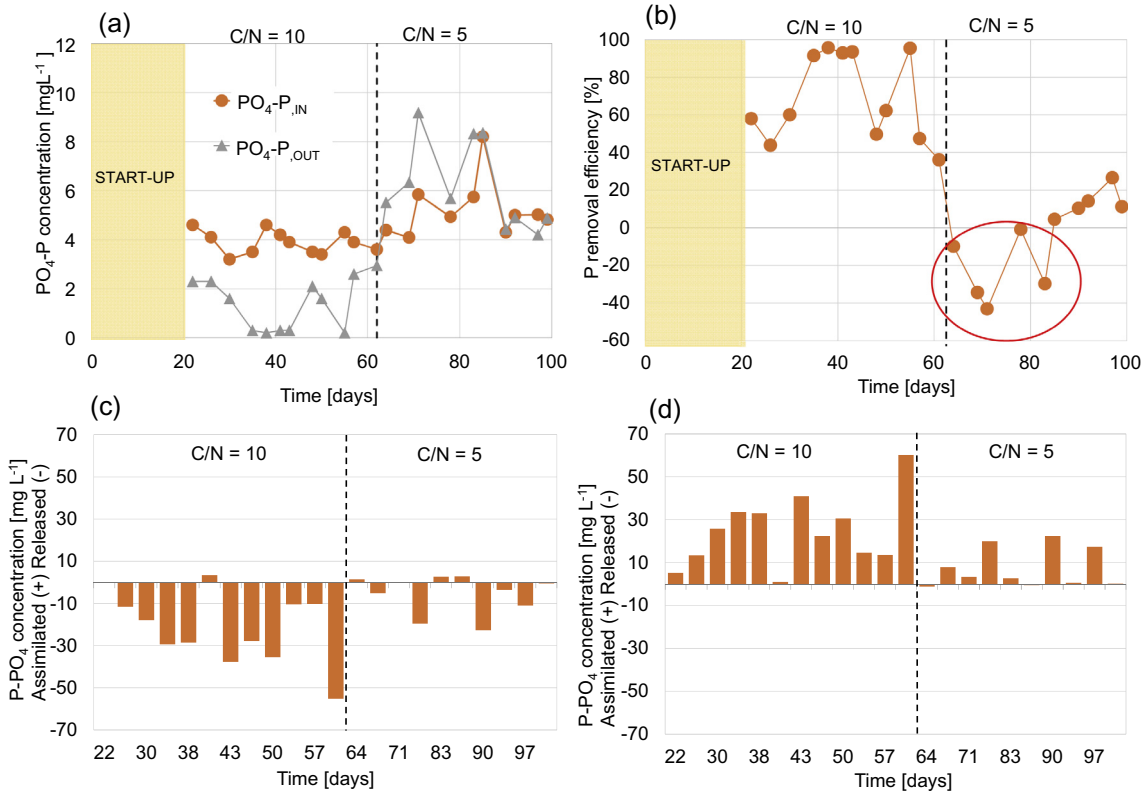

Fig. 3. Profile of the influent and effluent PO_4-P concentration (a); profile of the P removal efficiency (b); P concentration released or assimilated inside the anaerobic (c) and aerobic tank (d).

in the following Section 3.2.1), the membrane was able to retain/-trap inside the bioreactor the non-biodegraded particulate COD as well as a portion of the soluble COD (in the range of 0.04–0.45 μm, respectively membrane pores and porosity of filters used in the laboratory for soluble COD determination). Moreover, the gel and cake layer developed on the membrane surface may have contributed to further decrease the membrane porosity, increasing the overall COD removal efficiency. The retaining role of the membrane allowed to have a high total COD removal.

Regarding the nitrogen removal, by analyzing Fig. 2c it can be observed that during the Phase I (C/N = 10) a total NH_4-N nitrification occurred as demonstrated by the negligible value of NH_4-$N_{,OUT}$. Indeed, a quite high nitrogen removal efficiency was achieved. Specifically, the η_{nit}, ηN_{total} and η_{denit} were equal to 95.7%, 58.1% and 69.8%, respectively (Fig. 2d). These findings showed that a C/N ratio of 10 enabled a quite high TN removal likely due to the presence of denitrifying PAOs which could have enough carbon source for denitrification [31]. However, the lower C/N value during the Phase II played a relevant role in the biological nitrogen utilization process, thus leading to a significant stress effect on the autotrophic biomass species. Indeed, due to the higher nitrogen loading rate the nitrification performance during the Phase II (C/N = 5) was lower compared to that of the Phase I (average η_{nit} during the Phase I and Phase II was equal to 95.7% and 76.8%, respectively) (Fig. 2c and d). Therefore, despite the infinite SRT value would have supported the growth of nitrifiers, a decrease of both ηN_{total} and η_{denit} occurred (average values equal to 31.5% and 24.2%, respectively). This result was mainly attributed to an inhibition effect due to the lower C/N (corresponding to a higher $TN_{,IN}$ loading rate) in the Phase II. In detail, both high pH values (average value in Phase II equal to 8.1) and high ammonium nitrogen concentration (up to 93 mg NH_4-N L^{-1}) resulted within the reactors during the second experimental phase as a consequence of the decrease of C:N ratio. In such conditions the FA concentration increased in the mixed liquor (up to 4.5 mg NH_3-N L^{-1} at the end of the Phase II) (Fig. 2e). The increase of FA value might have caused a stress effect on the growth of nitrifying species. In particular, the NOB species are more sensitive to FA compared to AOB. Indeed, literature demonstrates that the growth of NOB species on NO_2-N can be inhibited at low FA concentration (0.1–1.0 mg NH_3-N L^{-1}) [10,32]. Conversely, the growth of AOB on NH_4-N (first nitrification step) is inhibited at higher FA values, in the range of 10–150 mg NH_3-N L^{-1} [32].

The inhibition effect due to the high FA value on NOB was confirmed by the accumulation of NO_2-N (around 1.2 mg NO_2-N L^{-1} during the last days of the Phase II) inside the aerobic tank as shown in Fig. 2e.

Therefore, the pilot plant operation at low C/N value led to a significant reduction of the nitrogen removal efficiency.

3.1.2. Phosphorus removal

In Fig. 3, the influent and effluent PO_4-P profiles (PO_4-$P_{,IN}$ and PO_4-$P_{,OUT}$, respectively) (Fig. 3a) as well and the P removal efficiency profile are reported (Fig. 3b). Furthermore, the assimilated or released PO_4-P concentration inside the anaerobic (Fig. 3c) and aerobic (Fig. 3d) tanks, respectively, is reported.

The P removal efficiency during the Phase I was satisfactory with an average value of 70% (Fig. 3a and b). Indeed, the average concentration of the total effluent phosphorus was around 1.30 mg L^{-1} which is lower than the Italian and European effluent limit (namely, 2 mg L^{-1}). However, the low C/N ratio during the Phase II had a significant influence on the system performances in terms of P removal (Fig. 3a and b). Particularly, during the Phase II it was observed a lower phosphorus removal efficiency. Indeed, during the Phase II the average effluent P concentration was higher than the influent (6.2 mg L^{-1} and 5.4 mg L^{-1}, respectively) and thus no net P removal took place (see negative efficiencies in Fig. 3b). This was mainly due to the fact that the higher $TN_{,in}$ loading rates during the Phase II promoted an increased production of NO_3-N (inside the aerobic tank) which was poorly denitrified as reported in Fig. 2d. Therefore, a great amount of NO_3-N was pumped back from the anoxic to the anaerobic tank according to the UCT-MBR scheme (Fig. 1). As a consequence, the NO_3-N concentrations within the anaerobic tank were higher than 1 mg L^{-1} becoming detrimental for the phosphorus removal mechanism. Indeed, high NO_3-N concentration causes a limitation to ensure a good PAO activity in the anaerobic tank [3,33]. Specifically, as a result of the high concentration of NO_3-N in the anaerobic tank (17 mg L^{-1}, as average), anoxic conditions were established in the anaerobic tank. Therefore, PAOs often grew in anoxic conditions imitating the amount of stored substrate [44]. Consequently, the availability of the stored substrate required for the PO_4-P uptake, during the aerobic conditions, was very limited. Therefore, the amount of up-taken PO_4-P during the aerobic conditions was reduced (Fig. 3c and d). The poor P removal performance during the Phase II is also supported by the amount of anaerobic PO_4-P release and the aerobic PO_4-P uptake (Fig. 3c and d). More specifically, with an influent C/N ratio of 10 the amount of anaerobic PO_4-P release was equal to 22.5 mg L^{-1} (as average) which was almost 5 times higher compared to the influent PO_4-P (Fig. 3c). This value is in agreement with literature results referred to the absence of limitation or inhibition effect for anaerobic PO_4-P releasing from PAOs [33]. During the Phase II, the amount of PO_4-P released during the anaerobic conditions decreased considerably, with an average value of 5.5 mg L^{-1} (Fig. 3c). This result is mainly due to the high NO_3-N concentration inside the anaerobic tank which limited the anaerobic PO_4-P release and consequently the aerobic uptake due to the limited amount of stored substrate [33,44]. As shown in Fig. 3c and d, the aerobic P uptake mechanism showed also a different behavior during the two experimental phases. Indeed, the amount of PO_4-P assimilated during the aerobic conditions in the Phase II was lower than that of the Phase I.

3.2. Biomass properties

3.2.1. Biomass activity and biokinetic/stoichiometric parameters

As aforementioned, the respirometric batch tests enabled to achieve the main parameters characterizing the biomass activity level. In general, the observed respirogram charts featured the typical exogenous/endogenous phases after substrate (sodium acetate or ammonium chloride) spiking. Table 2 summarizes the values of the main kinetic/stoichiometric parameters (average values) referring to both heterotrophic and autotrophic species. The data reported in Table 2 highlight that the C/N variation exerted a significant effect on biomass activity. Concerning the heterotrophic species, it was observed a significant decrease of the maximum growth rate ($\mu_{max,H}$) as well as of the specific OUR (SOUR) rates, as depicted in Fig. 4a and b.

In particular, the SOUR values after sodium acetate addition provided a good indication of the heterotrophic activity inside the pilot plant, highlighting a different behaviour of biomass activity in the two experimental phases. The SOUR trend was also confirmed by the decay rate b_H, which also showed a decreasing trend (Fig. 4b), thus confirming its usefulness as direct indicator of biomass viability [34]. Moreover, it was observed a storage phenomenon, likely due to dynamic conditions related to the alternation of anaerobic, anoxic and aerobic conditions, typical of UCT systems. These peculiar conditions likely enhanced the growth of bacterial groups able to rapidly convert the organic substrate into storage products. The storage yield coefficient (Y_{STO}) was evaluated according to the procedure proposed by Karahan-Gül et al. [35].

Table 2
Summary of the kinetic/stoichiometric parameters measured during experiments (in brackets the SD values).

	C/N = 10	C/N = 5	Typical	Reference
Heterotrophic				
Y_H [mg COD mg^{-1} COD]	0.61 (±0.04)	0.62 (±0.01)	0.67	Hauduc et al. (2011)
Y_{STO} [mg COD mg^{-1} COD]	0.76 (±0.03)	0.76 (±0.03)	0.78	Karahan-Gül et al. (2002)
$\mu_{H,max}$ [d^{-1}]	5.41 (±0.63)	2.22 (±0.65)	6	Hauduc et al. (2011)
K_S [mg COD L^{-1}]	20.05 (±18.61)	3.65 (±1.56)	20	Hauduc et al. (2011)
b_H [d^{-1}]	0.24 (±0.03)	0.18 (±0.03)	0.20	Henze et al. (1987)
AF [%]	3.30 (±0.86)	4.43 (±0.29)	–	–
$SOUR_{max}$ [mg O_2 g^{-1} TSS h^{-1}]	13.07 (±4.13)	7.97 (±1.70)	–	–
Autotrophic				
Y_A [mg COD mg^{-1} N]	0.22 (±0.02)	0.29 (±0.04)	0.24	Henze et al. (1987)
$\mu_{A,max}$ [d^{-1}]	0.39 (±0.02)	0.24 (±0.05)	0.8	Hauduc et al. (2011)
K_{NH} [mg NH_4–N L^{-1}]	3.50 (±0.52)	1.16 (±0.23)	1.00	Hauduc et al. (2011)
Nitrif. rate [mg NH_4–N L^{-1} h^{-1}]	4.12 (±0.71)	2.04 (±0.83)	2.30	Di Trapani et al. (2011)

Y_H = heterotrophic yield coefficient, Y_{STO} = storage yield coefficient, $\mu_{H,max}$ = maximum heterotrophic growth rate, K_S = heterotrophic half-saturation coefficient, $SOUR_{max}$ = specific respiration rate, Y_A = autotrophic yield coefficient, $\mu_{A,max}$ = maximum autotrophic growth rate, K_{NH} = autotrophic half-saturation coefficient.

Concerning the autotrophic species, during the Phase I (C/N = 10) it was observed a good level of nitrification ability, with the biokinetic/stoichiometric parameters in good agreement with what reported in the technical literature [36–38].

Conversely, during the Phase II (C/N = 5) it was noticed a significant decrease of the system nitrification ability (Fig. 4c and d). This result could be likely related to the increased ammonia loading rate that could stress the activity of autotrophic populations. This result was confirmed by the AUR batch test performed on biomass samples withdrawn from the aerobic compartment (Fig. 5a and b). In particular, during the Phase I, the biomass showed a good nitrification activity with no nitrite accumulation during the test (ammonia uptake rate: 2.02 mg NH_4-N g^{-1} VSS h^{-1}) (Fig. 5a). On the contrary, during the Phase II it was noticed a slight decrease of the nitrification ability with ammonia uptake rate down to 0.505 mg NH_4-N g^{-1} VSS h^{-1} (Fig. 5b). However, at the end of experiments the ammonia uptake rate increased up to 1.362 mg NH_4-N g^{-1} VSS h^{-1}, suggesting an acclimation of autotrophic species to the low C/N value.

Concerning the denitrification activity, the NUR tests showed a good biomass behaviour with nitrate uptake rate equal to 3.20 mg NO_3-N g^{-1} VSS h^{-1} (Fig. 5c). During the Phase II it was observed a decrease of the denitrification activity, with nitrate uptake rates down to 0.299 mg NO_3-N g^{-1} VSS h^{-1} (Fig. 5d). This result was likely related to the decrease of the inlet carbon source compared to the available nitrogen, leading to the decrease of the denitrification ability and poorer assimilation of organic matter. This behaviour was in good agreement with previous results reported in the technical literature [8]. Nevertheless, at the end of experiments, the denitrification ability was restored, with an increase

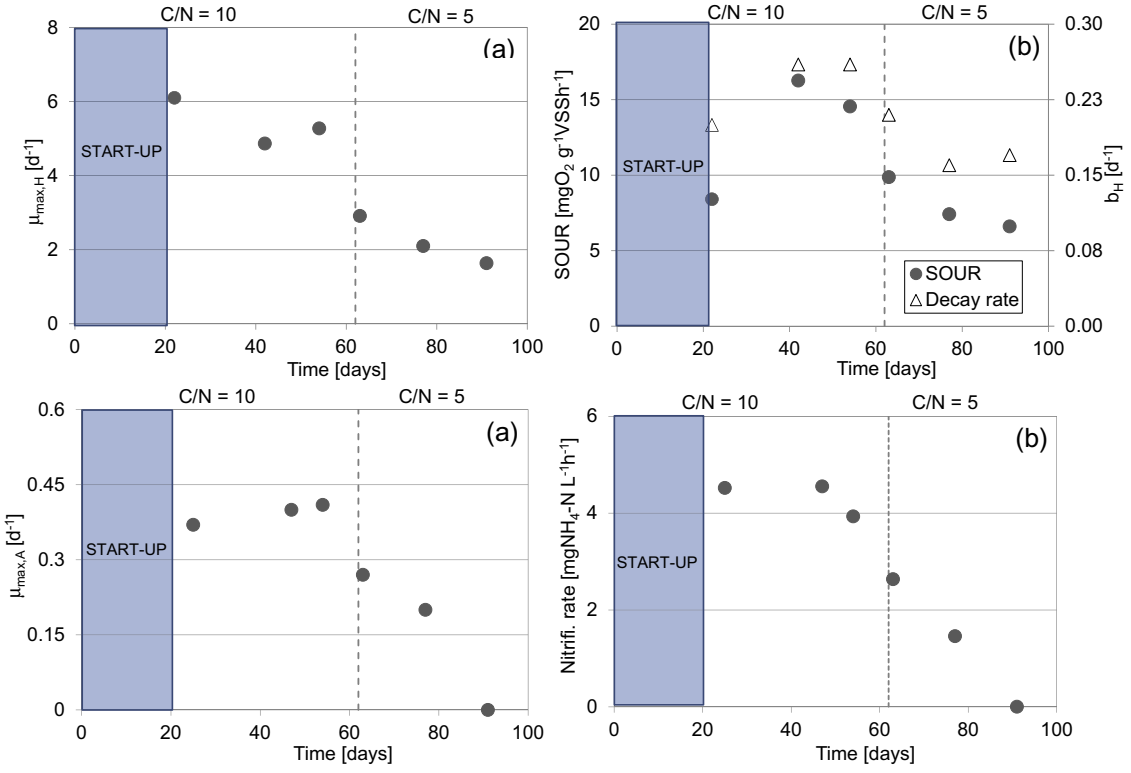

Fig. 4. Trend of heterotrophic maximum growth rate (a), SOUR and decay rate (b), autotrophic maximum growth rate (c) and nitrification rate (d) throughout experiments.

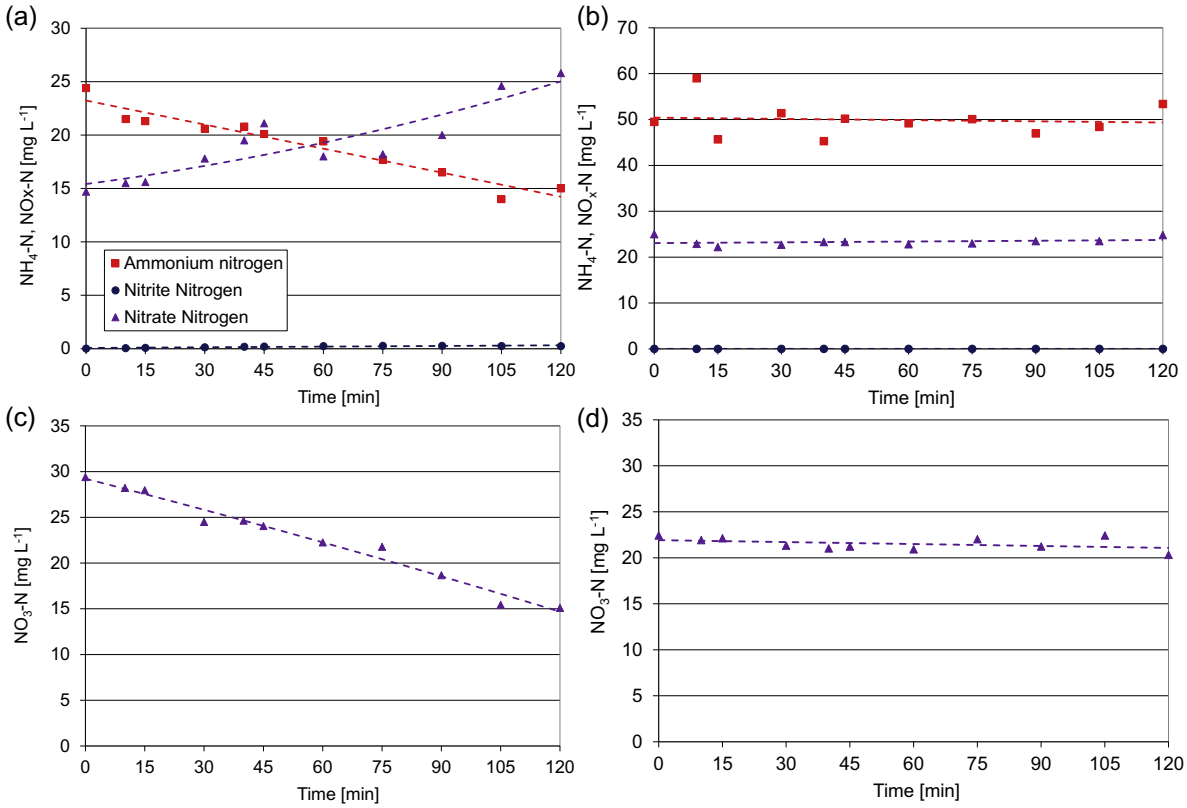

Fig. 5. Examples of AUR (a and b) and NUR (c and d) test during the Phase I and II, respectively.

Table 3
Summary of the specific EPS fraction for each tank.

Phase	Days	Anaerobic				Anoxic				Aerobic			
		EPS_P mg/gTSS	EPS_C mg/gTSS	SMP_P mg/gTSS	SMP_C mg/gTSS	EPS_P mg/gTSS	EPS_C mg/gTSS	SMP_P mg/gTSS	SMP_C mg/gTSS	EPS_P mg/gTSS	EPS_C mg/gTSS	SMP_P mg/gTSS	SMP_C mg/gTSS
I	22	173.7	19.8	0.0	0.0	257.9	28.4	0.0	0.4	172.2	21.7	0.0	2.4
	30	193.7	14.8	0.0	0.0	189.4	15.5	0.0	0.0	181.0	78.5	0.0	0.0
	35	100.4	8.3	0.0	0.0	207.4	28.0	0.0	0.0	151.7	22.6	0.0	0.0
	43	87.0	24.1	0.0	0.0	242.4	39.2	0.0	0.0	117.6	25.4	0.0	0.0
	50	182.6	13.3	3.1	0.0	294.2	29.2	0.0	0.0	164.9	18.0	0.0	0.0
	57	130.5	8.9	0.0	0.0	202.1	8.4	0.0	0.0	128.8	7.3	0.0	0.0
II	64	161.8	16.3	0.0	0.0	223.0	211.5	0.0	2.2	129.8	17.5	0.0	4.1
	71	272.2	52.5	0.0	0.7	127.9	28.4	0.0	6.0	80.9	22.0	0.0	7.7
	78	98.5	2.8	0.0	0.0	124.2	12.6	0.0	0.0	75.2	7.2	0.3	0.3
	85	65.9	11.0	0.0	0.4	129.2	20.1	0.0	0.3	94.8	8.5	0.4	0.4
	92	94.0	14.1	3.0	3.6	189.1	33.5	0.9	4.6	170.8	38.6	4.1	4.7

of the nitrate uptake rate up to 5 mg NO_3-N g^{-1} VSS h^{-1}, highlighting a sort of acclimation of the heterotrophic biomass to the low C/N value.

3.2.2. Extracellular polymeric substances

Table 3 reports the specific EPSs value for each fraction and tank. By analyzing Table 3 one can observe that during both experimental Phases the predominant fraction of the total EPS is represented by the EPS_{Bound}. However, a relevant decrease of EPS_{Bound} occurred during the Phase II. This result suggested a worsening of the sludge flocs stability (deflocculation) as also corroborated by the increase of SMP values during the Phase II. The sludge deflocculation could be due to a negative effect on the floc structure when C/N was varied from 10 to 5, likely due to loosely-bound EPS (LB-EPS) variation. This result is in agreement with previous literature findings [42].

Table 4
Mean values of CST, SRF and sludge viscosity measured during both experimental phases.

Parameter	Units	Phase	Anaerobic	Anoxic	Aerobic	MBR
CST	s L g^{-1}	I	4.58	3.34	2.76	3.05
		II	5.55	4.09	4.32	3.66
SRF	10^{12} m kg^{-1}	I	3.03	2.99	3.17	2.8
		II	2.91	3.28	3.54	3.76
Viscosity	cP	I	2.27	4.07	4.20	5.07
		II	2.28	2.85	2.40	3.55

3.2.3. Sludge dewaterability

Table 4 reports the average values of specific CST, SRF and sludge viscosity for each tank acquired during the two experimental phases. By analysing data of SRF one can observe that the C/N

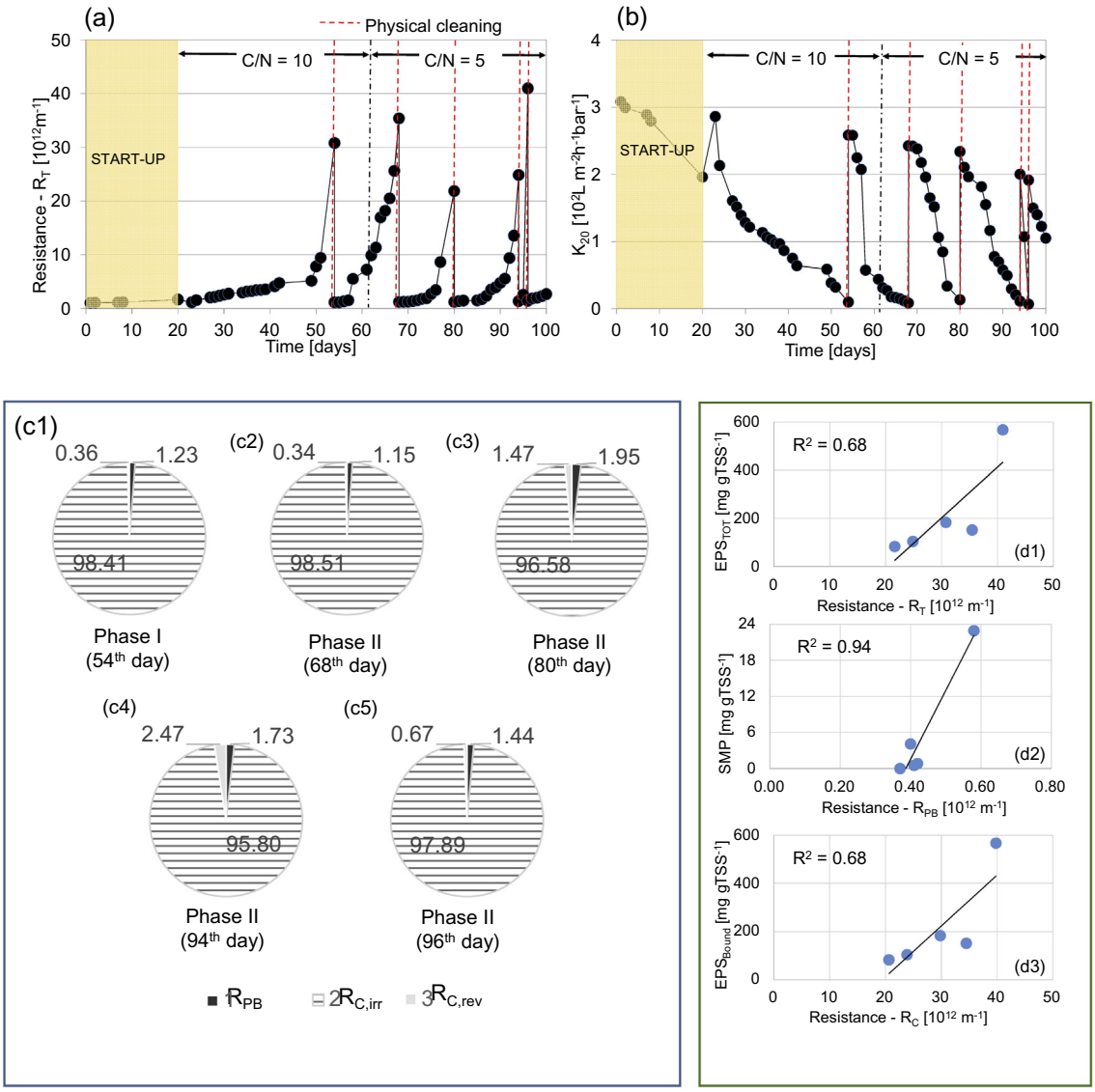

Fig. 6. Total membrane resistance (a), membrane permeability (K_{20}) (b), results of the in series resistance model (c1–c5) and correlations between EPS and resistance to filtration (either total or specific) (d1–d3).

did not provide a significant influence on the sludge dewaterability features.

Indeed, the average values of SRF do not differ significantly between Phase I and II (Table 4). Conversely, the specific CST highlighted a worsening of sludge dewaterability during the second experimental phase. Indeed specific CST values achieved in the second experimental phase resulted constantly higher than data acquired during the Phase I. Moreover, the sludge derived from anaerobic reactor was characterized by the highest specific CST value in both experimental phases. Such result is likely due to the lower sludge concentration in the anaerobic reactor. Furthermore, despite the EPS content is considered as the most important parameter affecting the sludge dewaterability [39], in the present study it was not possible to highlight a clear correspondence between EPS and sludge dewaterability. Such a result was likely affected by the quite total absence of SMP in both experimental phases.

Sludge dewaterability resulted mainly influenced by the TSS concentration; indeed, R^2 values achieved by interpolating the SRF values with the TSS concentration were equal to 0.47, 0.51, 0.57 and 0.68 for anaerobic, anoxic, MBR and aerobic reactor, respectively.

The higher influence exerted by the TSS concentration on sludge dewaterability is likely due to the relative short experimental period. Indeed, it is possible that the biological phenomena of EPS production and thus its influence in sludge dewaterability requires a longer time compared to the effect exerted by the TSS concentration. The sludge viscosity showed a decrease from the Phase I through the Phase II. This result is in accordance with TSS concentration measured in each reactor that showed a decrease from C/N = 10 to C/N = 5.

3.3. Membrane fouling

Fig. 6 reports the total resistance trend (R_T) (Fig. 6a) as well as the membrane permeability (K_{20}) (Fig. 6b) throughout experiments. Further, Fig. 6c1–c5 and d1–d3 report the resistance decomposition results and the correlation between EPS and resistances, respectively. The results reported in Fig. 6d1–d3 refer to the EPS measured in the aerobic tank.

As shown in Fig. 6a, over the entire experimental period five physical membrane cleanings were carried out, in order to avoid the TMP exceeding the critical values defined by the membrane

manufacturer (namely, 0.5–0.6 bar). By observing Fig. 6b, the membrane cleanings provided a partial recovery of membrane filtration in terms of K_0. Indeed, the maximum value of K_0 ranged between 3 and 2 10^2 L m^2 h^{-1} bar^{-1} at the beginning of the experimental period and at the day 96th, respectively (Fig. 6b). Up to operational day 50th, no significant fouling was observed and the R_T values remained very close to what achieved at the beginning of the experimental period. This behaviour indicated that throughout this operating period, R_T was mainly related to $R_{C,rev}$. However, by reducing the C/N value at 5 (Phase II) a decrease of the membrane filtration properties took place despite the fouling properties remained stable. With this regard, Fig. 6a shows that four membrane cleanings were required during the Phase II over around 30 days. Conversely, only one membrane cleaning was required during the Phase I over 55 days (Fig. 6a). This result should suggest a modification of fouling properties from the Phase I to the Phase II, e.g. a notable increasing of R_{PB} would be expected [40]. However, the results of the resistance decomposition show that the greatest fraction of the R_T was, over the entire experimental period, always the $R_{C,irr}$ (Fig. 6c1–c5). Therefore, the decrease of the membrane filtration properties are likely debited to the worsening of the activated sludge properties in terms of floc structure which led to the SMP production. Therefore, the EPS variation had a key role on the membrane fouling. As shown in Fig. 6d1–d3, a significant correlation was found between the EPS and total/specific resistance to filtration.

4. Conclusions

The findings of the present study highlighted the relevant role played by the C/N on nitrogen and phosphorus removal in a UCT-MBR plant. Indeed, while the removal efficiencies of organic carbon and nutrients were very high during the Phase I (C/N ratio equal to 10), the decrease of the C/N ratio down to 5 in the Phase II caused the decrease of TN removal, due to the simultaneous reduction of nitrification and denitrification. Moreover, the decrease of the C/N caused a dramatic reduction of P removal, due to the competition of PAOs with denitrifiers in the anaerobic compartment. Therefore, when operating with low C/N other strategies, such as chemical phosphorus removal, addition of an external carbon source and/or nitrate recycling adjustment, might be complemented to enhance BNR process.

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