

# Removal of carbon and nutrients from wastewater in a moving bed membrane biofilm reactor: the influence of the sludge retention time

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## Abstract

A University of Cape Town (UCT) pilot plant combining both membrane bioreactor (MBR) and moving bed biofilm reactor (MBBR) technology was monitored. Three experimental Phases were carried out by varying the mixed liquor sludge retention time (SRT) (indefinite, 30 and 15 days, respectively). The system performance has been investigated during experiments in terms of: organic carbon, nitrogen and phosphorus removal, biokinetic/stoichiometric constants, membrane fouling tendency and sludge dewaterability.

The observed results showed that by decreasing the SRT the UCT pilot plant was able to maintain very high total COD removal efficiencies, whilst the biological COD removal efficiency showed a slight decrease. Nitrification was only slightly affected by the decrease of the mixed liquor SRT, showing high performance (as average). This result could be related to the presence of the biofilm able to sustain nitrification throughout experiments. Conversely, the average P removal efficiency was quite moderate, likely due to the increase of the ammonium loading rate that could promote an increased  $\text{NO}_3\text{-N}$  recycled from the anoxic to the anaerobic tank, interfering with phosphorus accumulating organisms (PAOs) activity inside the anaerobic tank. Membrane fouling increased at 30 days SRT likely due to a reduction of protective cake pre-filter effect. Moreover, it was noticed the increase of the resistance due to pore blocking and a general worsening of the membrane filtration properties.

## Keywords

UCT; MBBR; nutrients removal; MBR; membrane fouling.

## INTRODUCTION

Nowadays the increasing urbanization, coupled to industrial development, have caused a significant increase of consumption of water resources as well as their deterioration (Martín-Pascual et al., 2015), contributing to make water a global concern. Moreover, it is well known that nutrients (particularly, nitrogen and phosphorus compounds) may have adverse environmental impacts (e.g., eutrophication, toxicity towards the aquatic organisms, etc.) (Wang et al., 2006). Therefore, their removal from wastewater is an imperative requirement, especially when discharging in sensitive areas (Li et al., 2013). In the last years, several biological and physic-chemical methods have been developed to remove nutrients from wastewater. Among these methods, biological treatments are the most cost-effective methods (Chu and Wang, 2011). In the last years, biological nutrient removal (BNR) from domestic wastewater has been extensively investigated and developed and it is usually based on anaerobic, anoxic and aerobic reactors linked in-series (among others, Wanner et al., 1992; Lu et al., 2015). In BNR processes, N and P removal is accomplished, respectively, by heterotrophic denitrifying bacteria and polyphosphate-accumulating organisms (PAOs) which require carbon source (Naessens et al., 2012). In particular, 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 (Li et al., 2013). However, despite conventional activated sludge (CAS) processes are effective for removal of organic and nutrients compounds, the overall efficiency is strictly related to the performance of the solid-liquid separation into the final settler, which may suffer of separation problems (Wanner, 2002). In this context, membrane bioreactor (MBR) technology may represent a useful solution, since it enables to disconnect the efficiency of the biological processes from the biomass settling properties. Indeed, MBRs have attracted considerable interest due to various advantages compared

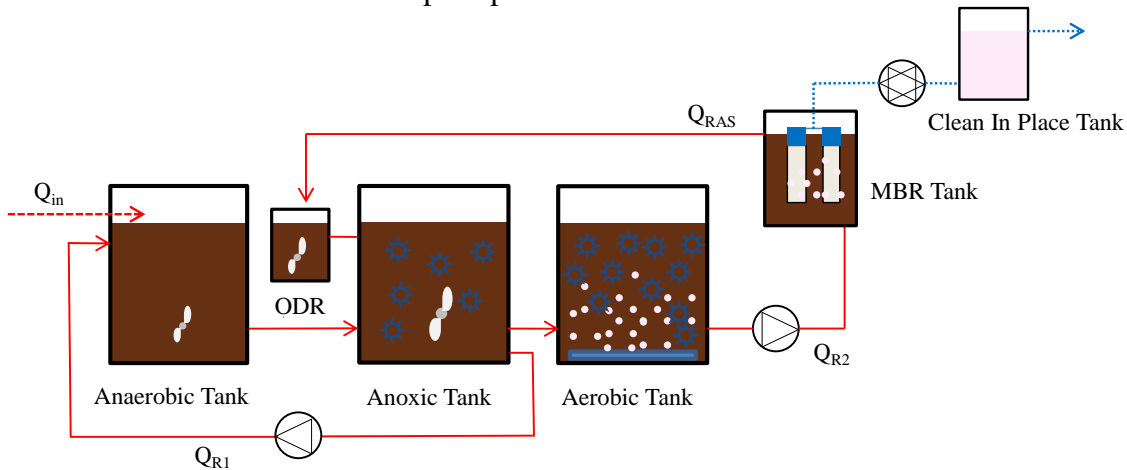
to conventional process that originate from the use of a membrane for solid–liquid separation (Fu et al., 2009). In particular, MBRs generally feature high quality effluent, small footprint and low sludge production rates compared to CAS systems (Stephenson et al., 2000). Therefore, in the last years the integration of BNR process with MBRs 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 (A2O) process (Hu et al., 2014). However, a major drawback of MBRs is still represented by fouling phenomena that may severely affect the filtration properties of the membrane modules (Judd and Judd, 2010). In particular, the mixed liquor suspended solid (MLSS) concentration has been recognized to play a significant effect on membrane fouling (Poyatos et al., 2008; Di Trapani et al., 2014). An alternative to manage this problem is to couple a MBR system with a moving bed biofilm reactor (MBBR) for the simultaneous growth of suspended biomass and biofilm into the system, realizing a so-called moving bed membrane bioreactor (MBMBR) (among others, Leyva-Díaz et al., 2013; Yang et al., 2014). MBMBR systems are amongst the new advanced wastewater treatments with the potential to utilize the best characteristics of biofilm processes and membrane separation (Leiknes and Ødegaard, 2007; Ivanovic and Leiknes, 2008). Briefly, MBBR technology relies on the use of small plastic carrier elements that are kept in constant motion throughout the entire volume of the reactor, for biofilm attachment and growth (Ødegaard, 2006). These systems are particularly useful when slowly growing organisms as nitrifiers have to be retained inside a wastewater treatment plant (WWTP) (Kermani et al., 2008). By using this technology, it is possible to reduce the MLSS concentration (while maintaining the same total biomass content) thus reducing the extent of membrane fouling. Nevertheless, MBMBRs are relatively new, especially when referring to system performance, biomass biokinetic activity and membrane fouling tendency. Very few studies have been reported so far for BNR systems adopting hybrid MBMBR processes (Yang et al., 2010). Therefore, to date there is a dearth of knowledge regarding the suitability of such systems as well as the influence that specific parameters may have on system performance. As an example, the sludge retention time (SRT) of the mixed liquor can exert a key role on the performance of a complex system conceived for nutrients removal and characterized by the simultaneous presence of suspended and attached biomass. Bearing in mind such considerations, the aim of the present research is to gain insights about the performance of a University of Cape Town (UCT) pilot plant, combining both MBR and MBBR technology (UCT-MBMBR), for the treatment of domestic wastewater and how the mixed liquor SRT influences physical (fouling) and biological performances. In particular, a UCT-MBMBR pilot plant was monitored for almost 150 days with the aim to investigate the system performance in terms of organic carbon and nutrient removal, biomass biokinetic behavior and membrane fouling tendency and sludge features.

## **MATERIAL AND METHODS**

### **The pilot plant**

The UCT-MBMBR pilot plant was built at the Laboratory of Sanitary and Environmental Engineering of Palermo University (Figure 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 (Ekama et al., 1983). The solid-liquid separation phase was carried out by means of one ultrafiltration hollow fibre membrane module (PURON<sup>®</sup>). The membrane module was located inside an aerated tank (MBR tank) (36 L). An oxygen depletion reactor (ODR) allowed the oxygen stripping/consumption 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 anoxic and aerobic compartments were filled with suspended plastic carriers (carriers density = 0.95 g cm<sup>-3</sup>; carriers specific surface = 500 m<sup>2</sup> m<sup>-3</sup>), with a 15 and 40% filling ratio, corresponding to a net surface area of 75 and 200 m<sup>2</sup> m<sup>-3</sup> in the anoxic and aerobic reactor, respectively. In Figure 1 a

schematic view of the UCT-MBMBR pilot plant is shown.



**Figure 1.** Schematic lay-out of the UCT-MBMBR pilot plant

### The experimental campaign

The UCT-MBMBR pilot plant was operated according to three phases, each characterized by a different SRT value: i. Phase I, with  $SRT = \infty$ ; ii. Phase II, with  $SRT = 30$  days; iii. Phase III, with  $SRT = 15$  days. The extraction flow rate was set equal to  $20 \text{ L h}^{-1}$  ( $Q_{IN}$ ). During the pilot plant operations, a  $20 \text{ L h}^{-1}$  flow rate ( $Q_{R1}$ ) was continuously recycled from the anoxic to the anaerobic tank. Furthermore, a  $100 \text{ L h}^{-1}$  flow rate ( $Q_{R2}$ ) of mixed liquor was pumped from the aerobic to the MBR tank. A net permeate flow rate of  $20 \text{ L h}^{-1}$  was extracted ( $Q_{OUT}$ ) through the membrane module. The recycled activated sludge ( $Q_{RAS}$ ) from the MBR to the anoxic tank through the ODR compartment was equal to  $80 \text{ L h}^{-1}$ .

The UCT-MBMBR pilot plant was operated for almost 150 days and was fed with a mixture of real domestic and synthetic wastewater. Briefly, the synthetic wastewater represented almost 50% of the total wastewater, 30% of which was readily biodegradable COD (RBCOD) (dosed as sodium acetate), whilst the remaining 70% was more slowly biodegradable (dosed as glycerol). The synthetic wastewater was added to meet the design organic loading rate fed to the pilot plant.

Permeate flux was maintained equal to  $21 \text{ L m}^{-2} \text{ h}^{-1}$ , the hydraulic retention time was equal to 20 h with a permeate flow rate of  $20 \text{ L h}^{-1}$ .

Table 1 summarizes the average features of the inlet wastewater during experiments.

**Table 1.** Average features of the influent wastewater and operation conditions during the Phases I, II and III, respectively.

Parameter	Units	Phase I	Phase II	Phase III
		Value		
COD	$[\text{mg L}^{-1}]$	602	583	543
Total nitrogen (TN)	$[\text{mg L}^{-1}]$	55.46	76.91	105.00
Total phosphorus (TP)	$[\text{mg L}^{-1}]$	7.08	8.8	9.86
Permeate Flux	$[\text{L m}^{-2} \text{ h}^{-1}]$	21	21	21
Flow rate	$[\text{L h}^{-1}]$	20	20	20
SRT	[d]	$\infty$	30	15
HRT	[h]	20	20	20
Duration	[d]	0-66	67-95	96-115

### Analytical methods

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<sub>TOT</sub>), supernatant COD (COD<sub>SUP</sub>), ammonium nitrogen (NH<sub>4</sub>-N), nitrite nitrogen (NO<sub>2</sub>-N), nitrate nitrogen (NO<sub>3</sub>-N), total nitrogen (TN), phosphate (PO<sub>4</sub>-P), total phosphorus (TP). All analyses were carried out according to the Standard Methods (APHA, 2005); pH, dissolved oxygen (DO) and temperature were also monitored in each tank by using a multi-parameter probe. Referring to the COD removal, in order to distinguish the removal due to the biological processes from that one due to the filtration operated by the membrane, two different removal efficiencies have been calculated (Di Trapani et al., 2014): the biological removal efficiency and the total removal efficiency. The biological COD removal efficiency was calculated as the difference between the COD<sub>TOT</sub> value in the influent and the COD<sub>SUP</sub> 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<sub>TOT</sub>, respectively. Periodic test on carrier samples were carried out, in order to establish the biofilm growth on the carriers; briefly, a carriers sample was taken from the anoxic and aerobic reactors (10 and 15 carriers, respectively), dried in an oven for one night at 105°C and then weighted (W1). After biofilm was removed, the carriers were dried another night at 105°C and then weighted again (W2); thereafter, the amount of the attached biomass was then calculated as W1–W2. For further details, the reader is addressed to literature (Di Trapani et al., 2013-2014).

Respirometric batch tests were carried out by means of a “flowing gas/static-liquid” respirometer to evaluate the kinetic and stoichiometric parameters for both autotrophic and heterotrophic biomass (Di Trapani et al., 2015). Briefly, the suspended biomass samples were taken from the aerobic reactor and eventually diluted with permeate in order to obtain a VSS concentration in the range of 2.0–3.0 g L<sup>-1</sup>. The batch tests on biofilm were performed with carriers and permeate, by imposing in the respirometer the same filling fraction of the UCT-MBMBR pilot plant.

In the batch tests aimed at assessing the heterotrophic biokinetic parameters, the nitrifying biomass was inhibited by adding 10 mg L<sup>-1</sup> of Allylthiourea (ATU), whilst the exogenous oxygen uptake rate (OUR) was enhanced by the addition of a readily biodegradable organic substrate (sodium acetate in this case). The substrate biodegradation rate was then assumed proportional to the exogenous OUR. On the other hand, the estimation of the kinetic parameters for the autotrophic population was carried out with a very similar procedure. Nevertheless, no inhibiting substance like ATU was added and ammonium chloride (NH<sub>4</sub>Cl) was spiked to evaluate the biokinetic parameters. During the batch tests, the pH values were constantly monitored to avoid the process inhibition. Moreover, the evaluation of the nitrification as well as denitrification rate, ammonium utilization rate (AUR) and nitrate utilization rate (NUR) tests were performed by adopting a modified protocol derived by Kristensen et al. (1992).

The soluble EPSs or soluble microbial products (SMPs) were obtained by centrifugation at 5000 rpm for 5 min, whilst the bound EPSs (EPS<sub>Bound</sub>) were extracted by means of the thermal extraction method (among others et al., 2013b). The extracted EPS<sub>Bound</sub> and the SMP were then analysed for proteins by using the Folin method with bovine serum albumin as the standard (Lowry et al., 1951), whereas the carbohydrates were measured according to DuBois et al. (1956), which yields results as glucose equivalent. Moreover, the sum of proteins and carbohydrates was considered as the total EPSs (EPS<sub>T</sub>), according to the following expression:

$$EPS_T = \underbrace{EPS_P + EPS_C}_{EPS_{Bound}} + \underbrace{SMP_P + SMP_C}_{SMP} \quad (1)$$

where the subscripts “P” and “C” indicate the content of proteins and carbohydrates respectively in the EPS<sub>Bound</sub> and SMP, that typically constitute the main fractions.

Membrane fouling has been analysed by monitoring the total resistance (RT) to membrane filtration

which is calculated according to Equation 2, derived by the Darcy's law:

$$R_T = \frac{\text{TMP}}{\mu J} \quad (2)$$

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

$R_T$  can be expressed 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 Equation 3.

$$R_F = R_{PB} + R_{C,irr} + R_{C,rev} = R_T - R_m \quad (3)$$

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.

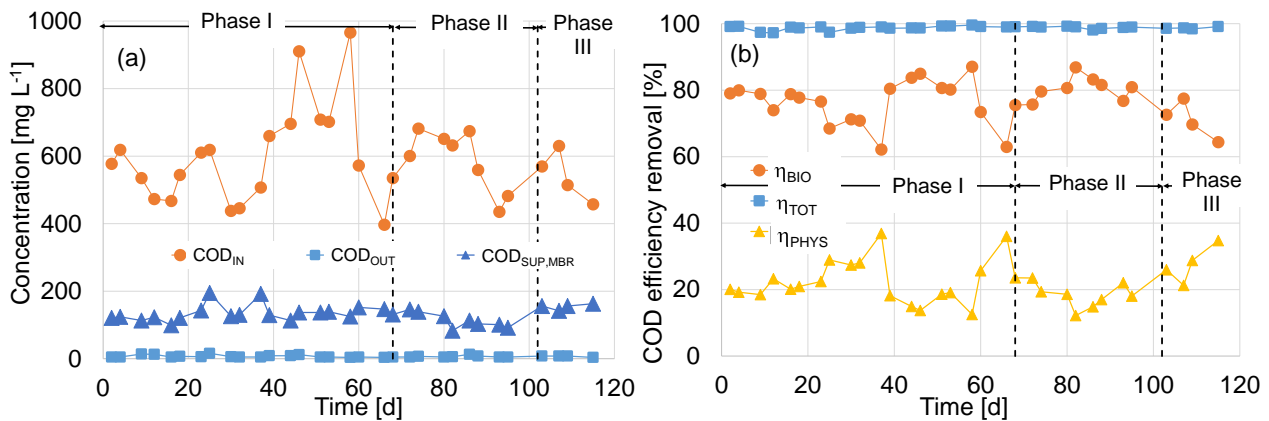
In order to analyse the specific fouling mechanisms the resistance-in-series (RIS) resistances method according to Di Trapani et al. (2014) has been applied.

The capillary suction time (CST) and the specific resistance to filtration (SRF) were measured in order to investigate the sludge dewaterability features (Vesilind, 1988; Peng et al., 2011). CST and SFR were measured in accordance with EN 14701-1 (2006) and EN 14701-2 (2006), by analyzing fresh samples collected from the anaerobic, anoxic, aerobic and MBR tanks. For further details on the adopted procedure, the reader is kindly referred to literature (Mannina et al., 2016).

## RESULTS AND DISCUSSION

### Pilot plant performance

Figure 2 depicts the pattern of influent ( $\text{COD}_{IN}$ ), MBR supernatant ( $\text{COD}_{\text{SUP,MBR}}$ ) and effluent COD ( $\text{COD}_{OUT}$ ) (Figure 2a) as well as the COD removal efficiencies, expressed as total ( $\eta_{TOT}$ ), biological ( $\eta_{BIO}$ ) and physical contribution due to membrane filtration ( $\eta_{PHYS}$ ) throughout experiments.



**Figure 2.** Pattern of influent, effluent and MBR supernatant COD (a); pattern of COD removal efficiencies expressed as biological ( $\eta_{BIO}$ ), physical ( $\eta_{PHYS}$ ) and total ( $\eta_{TOT}$ ) removal (b).

The results showed that, despite the fluctuations of the influent concentration, a quite high total COD removal efficiency was achieved during the three phases (Phase I: 97%; Phase II: 98%; Phase III: 99%) (Figure 2b). Referring to COD biological removal, it is worth noting that it was affected by the SRT variation. Indeed, when the SRT was decreased from indefinite to 30 days, the supernatant COD in the MBR compartment decreased from  $135 \text{ mg L}^{-1}$  to  $114 \text{ mg L}^{-1}$  (Figure 2a), highlighting an increase of the biological performance. This result might be due to the reduced

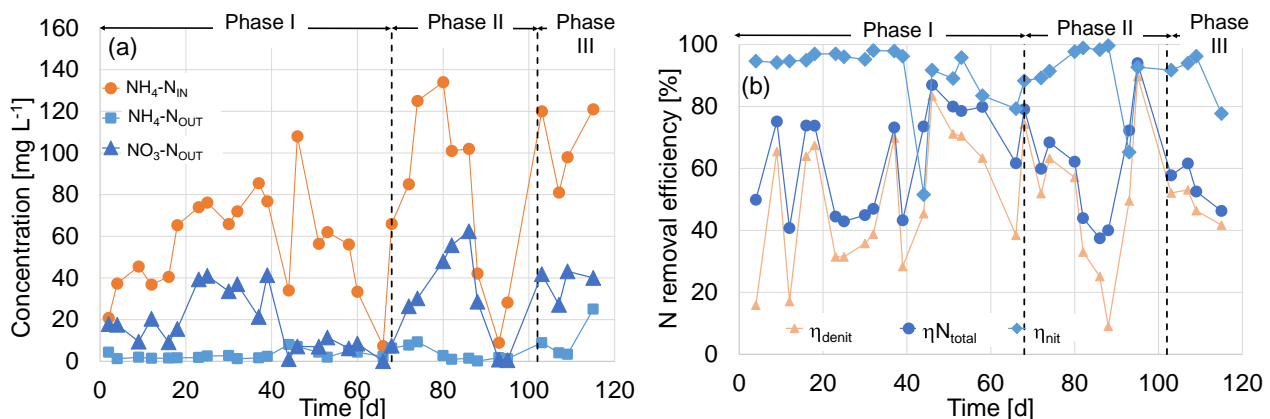
competition between the suspended biomass and the biofilm attached to the carriers as confirmed by the respirometric tests as well as to a “renewal” of the suspended biomass due to sludge withdrawals. Conversely, when the SRT was decreased down to 15 days, the biological COD removal slightly decreased (Figure 2b), with a minimum value equal to 70.4% during the Phase III. Nevertheless, it has to be stressed the effect of membrane filtration that contributed to retain inside the bioreactor the particulate COD as well as the portion of the soluble COD characterized by average size higher than membrane porosity (0.03  $\mu\text{m}$ ). The achieved results confirmed the robustness of MBR systems towards organic carbon removal.

In terms of nitrogen removal, Figure 3 shows the pattern of influent and effluent ammonia, effluent nitrate (Figure 3a) as well as the achieved performance in terms of nitrification ( $\mu_{\text{nit}}$ ), denitrification ( $\mu_{\text{denit}}$ ) and total nitrogen removal ( $\mu_{\text{N}_{\text{total}}}$ ) (Figure 3b). It is worth noting that the SRT did not influence significantly the nitrification performance of the system. Indeed, the ammonium removal was excellent, with efficiencies close to 97% for most of the experiments. Only rarely the nitrification efficiency was subjected to sharp decreases, mostly related to sudden decrease of the inlet ammonia concentrations, for the dilution effect of the inlet wastewater due to sporadic rainy events.

It has to be stressed that the UCT-MBMBR pilot plant was able to maintain high nitrification despite the high ammonia loading rate (inlet ammonia concentrations close to 100  $\text{mg NH}_4\text{-N L}^{-1}$ ). This result may be likely due to the presence of the biofilm in the aerobic compartment (mostly autotrophic biofilm) that was able to sustain a very high nitrification during experiments.

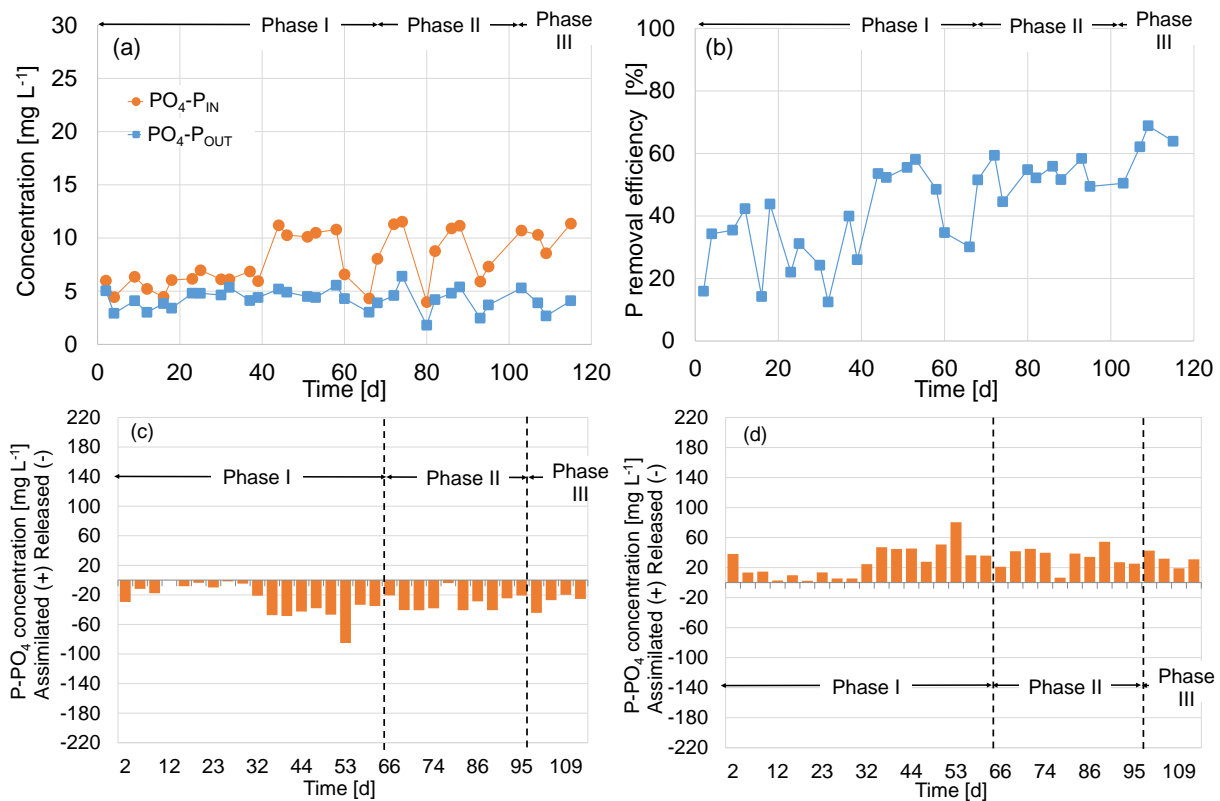
Compared to “conventional” technologies, the introduction of a moving bed biofilm process may promote an improvement of biomass performances, in good agreement with previous studies (Martín-Pascual et al., 2015).

On the other hand, the TN removal showed significant fluctuations during experiments, with average values of 62.92, 61 and 54.55% for Phase I, II, and III respectively. These results reflected the fluctuations of the denitrification efficiency observed during experiments, when low values of the C/N ratio were achieved, thus promoting a limiting effect of organic carbon for denitrification.



**Figure 3.** Profile of  $\text{NH}_4\text{-N}_{\text{IN}}$ ,  $\text{NH}_4\text{-N}_{\text{OUT}}$  and  $\text{NO}_3\text{-N}_{\text{OUT}}$  (a); performance of nitrification ( $\mu_{\text{nit}}$ ) denitrification ( $\mu_{\text{denit}}$ ) and total nitrogen removal ( $\mu_{\text{N}_{\text{total}}}$ ) during experiments (b).

In terms of phosphorus removal, Figure 4a reports the pattern of influent and effluent  $\text{PO}_4\text{-P}$  concentrations whilst Figure 4b shows the  $\text{PO}_4\text{-P}$  removal efficiency. During experiments, it was noticed a slight increase of bio-phosphorus removal with the decrease of the SRT. This result seems to confirm previous experimental studies highlighting that at high SRTs the competition for the available carbon source can hamper PAOs activity (Ge et al., 2015).



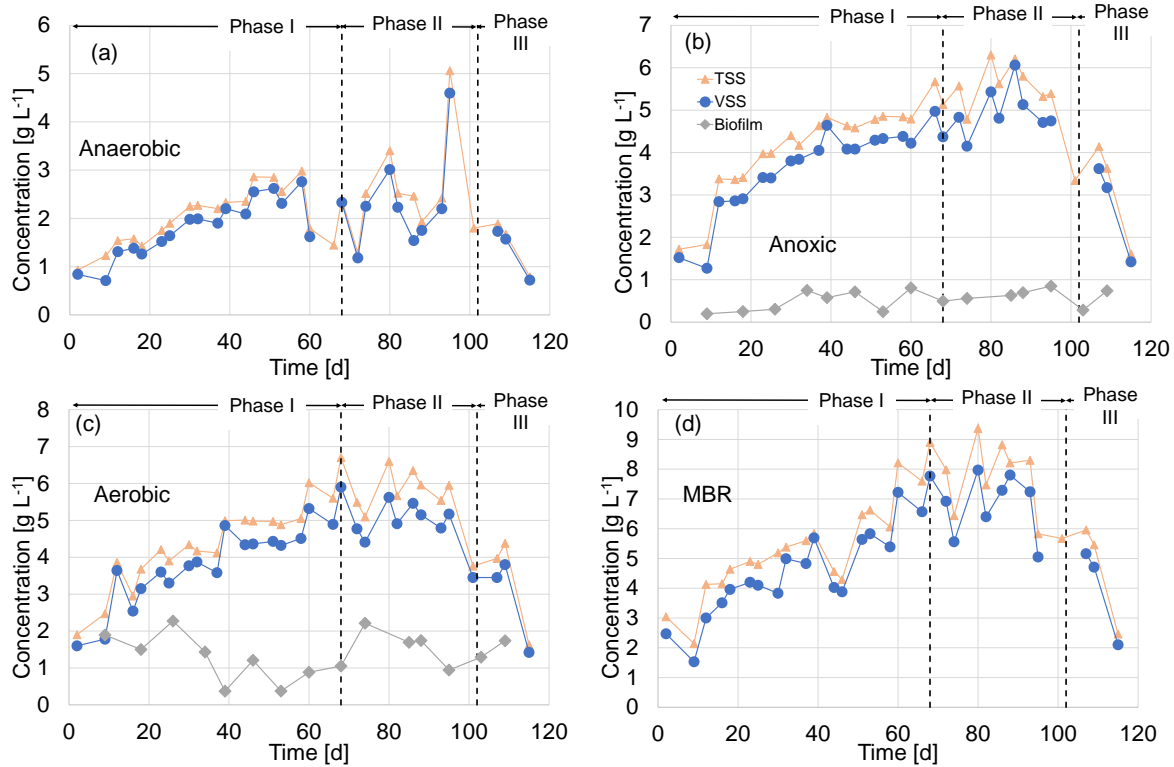
**Figure 4.** Profile of the influent and effluent PO<sub>4</sub>-P concentration (a); PO<sub>4</sub>-P removal efficiency (b); PO<sub>4</sub>-P concentration released or assimilated inside the anaerobic (c) and aerobic (d) tank.

Moreover, Figure 4 reports the assimilated (Figure 4c) or released (Figure 4d) PO<sub>4</sub>-P concentrations in the anaerobic and aerobic tanks, respectively. From the observation of Figure 4c-d one can observe that the mechanisms for the biological phosphorus removal developed properly, with PO<sub>4</sub>-P release in the anaerobic tank and PO<sub>4</sub>-P resuming in the aerobic compartment, respectively.

### Suspended biomass development and biofilm growth

Figure 5 reports the profiles of suspended and attached biomass in the different compartments throughout experiments (Figure 5a-d). From the observation of Figure 5, it is possible to notice a general increase of the suspended biomass concentration in the different compartments during the Phase I, related to the absence of sludge withdrawals. In the Phase II the suspended biomass maintained quite constant values in the different compartments, whereas in the experimental Phase III it was noticed a sensible decrease of MLSS concentrations, due to the increased sludge withdrawals that would lead in the long term to a new steady state conditions.

Referring to biofilm, the achieved data highlighted a moderate development, either in the anoxic or aerobic compartment. This result could be likely due to the competition with the suspended biomass for the availability of the different substrates. In particular, the biofilm concentration showed fluctuations, likely due to detachment phenomena occurring in both compartments, with biofilm concentrations down to 0.4 and 0.2 g TS L<sup>-1</sup> in the aerobic and anoxic compartment, respectively. This behaviour could be related to a stress effect on the biofilm caused by the specific environmental conditions also contributing to increase the membrane fouling of the system, due to the significant hydrophobicity of the detached biofilm, in good agreement with previous experiences (Yang et al., 2014). However, in the last operational days, it was noticed a slight increase of biofilm concentration in both compartments, likely due to the simultaneous decrease of the suspended biomass (as a consequence of the increased sludge withdrawals) that contributed to reduce the competition for the availability of the substrates.



**Figure 5.** Biomass pattern during experiments, referring to the anaerobic (a), anoxic (b), aerobic (c) and MBR (d) compartment, respectively.

### Biomass respiratory activity and biokinetic parameter evaluation

Respirometric batch tests were carried out for measuring the biomass activity during the entire experimental campaign by evaluating the main kinetic and stoichiometric parameters of either suspended or attached biomass. Table 2 reports the average values achieved throughout experiments.

**Table 2.** Average values of kinetic and stoichiometric parameters in the overall experimental campaign for both biomasses

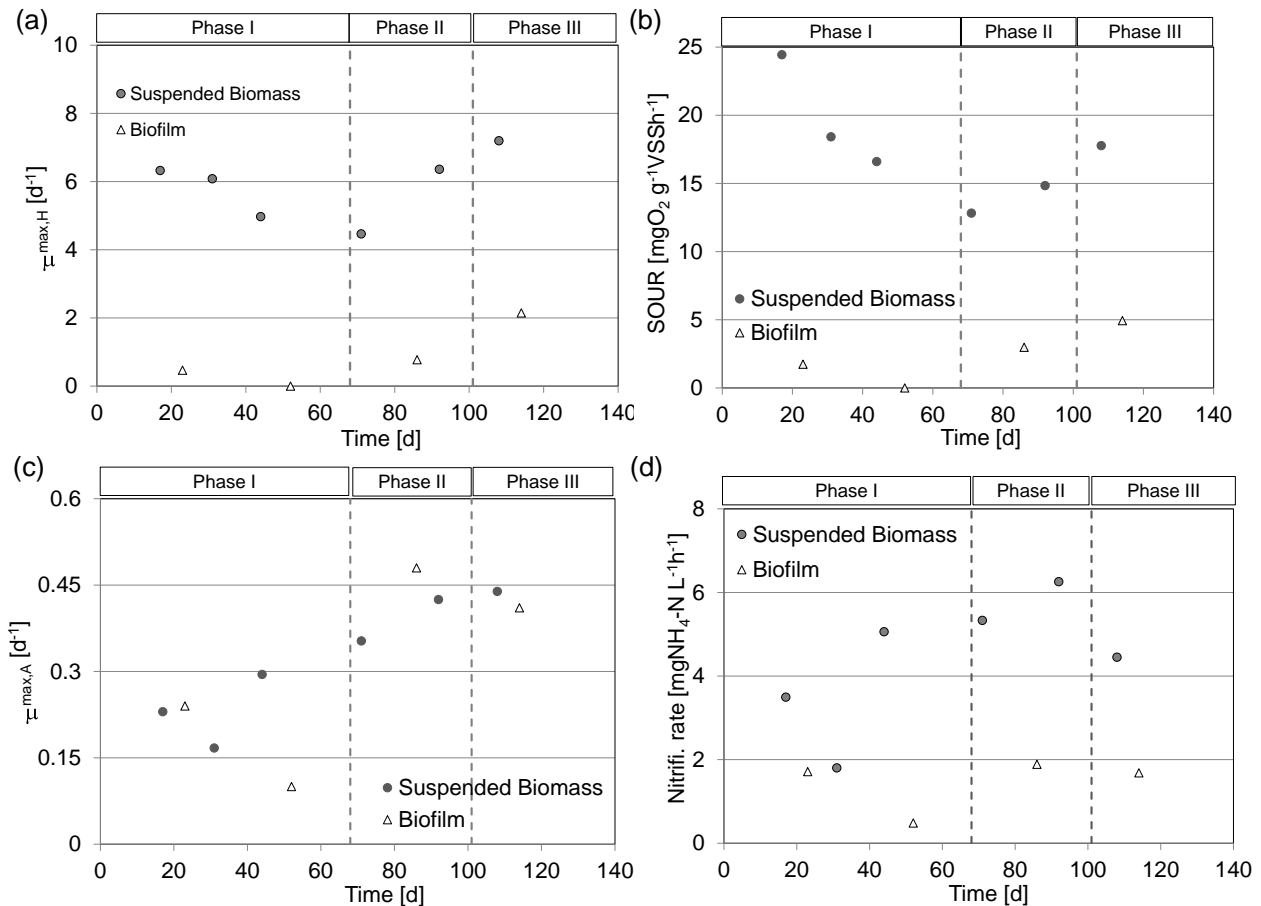
	Phase I (SRT indefinite)		Phase II (SRT 30 d)		Phase III (SRT 15 d)	
	Suspended	Attached	Suspended	Attached	Suspended	Attached
<i>Heterotrophic</i>						
$Y_H$ [mgCOD mg <sup>-1</sup> COD]	0.60	0.65	0.54	0.77	0.48	0.71
$Y_{STO}$ [mgCOD mg <sup>-1</sup> COD]	0.71	0.78	0.73	0.79	0.65	0.79
$\mu_{H,max}$ [d <sup>-1</sup> ]	5.79	0.47	5.41	0.77	7.20	2.15
$K_S$ [mgCOD L <sup>-1</sup> ]	5.43	5.05	6.19	3.81	4.00	3.00
$b_H$ [d <sup>-1</sup> ]	0.14	-	0.23	-	0.46	0.22
$SOUR_{max}$ [mgO <sub>2</sub> g <sup>-1</sup> VSSh <sup>-1</sup> ]	19.82	1.73	13.83	2.98	17.77	4.93
<i>Autotrophic</i>						
$Y_A$ [mgVSS mg <sup>-1</sup> N]	0.17	0.45	0.21	0.47	0.32	0.46
$\mu_{A,max}$ [d <sup>-1</sup> ]	0.23	0.24	0.39	0.48	0.44	0.41
$K_{NH}$ [mgNH <sub>4</sub> -N L <sup>-1</sup> ]	1.00	0.29	1.01	0.88	1.74	1.00
Nitrif. Rate [mgNH <sub>4</sub> L <sup>-1</sup> h <sup>-1</sup> ]	3.45	1.71	5.79	1.89	4.45	1.68

In general, from the observation of Table 2 it is possible to notice a higher activity of suspended biomass compared to biofilm, likely due to the moderate biofilm growth during experiments. Moreover, it was noticed a sort of “specialization” of the two biomasses, with the suspended showing higher affinity towards the organic carbon removal, while the biofilm more competitive in the nitrification process.

Figure 6 reports the pattern of  $\mu_{max,H}$  (Figure 6a), specific OUR (SOUR) (Figure 6b),  $\mu_{max,A}$  (Figure



6c) and nitrification rates (Figure 6d) for both suspended and attached biomass during experiments.



**Figure 6.** Pattern of maximum heterotrophic growth (a), specific OUR (b), maximum autotrophic growth (c) and nitrification rate (d) throughout experiments

It was noticed a significant influence of the MLSS SRT on the activity of the heterotrophic species. As noticeable from Figure 6a, the maximum growth rate of the suspended biomass showed a slight decrease in the Phase I, characterized by no sludge withdrawals (indefinite SRT). Conversely, it was observed a significant increase of  $\mu_{\max,H}$  in the Phases II and III, when the SRT was reduced at 30 and 15 days, with a maximum value equal to  $7.2 \text{ d}^{-1}$  reached at  $\text{SRT}=15$ . Indeed, when the UCT-MBMBR pilot plant was operated without sludge withdrawals a sort of suspended biomass “ageing” occurred, while the sludge withdrawals promoted a “renewal” of biomass, thus increasing its growth rate. Indeed, it was observed an increase of the NUR values of the suspended biomass from  $3.79$  to  $7.13 \text{ mgNO}_3\text{-N g}^{-1}\text{VSS h}^{-1}$  in the Phases I and II, respectively.

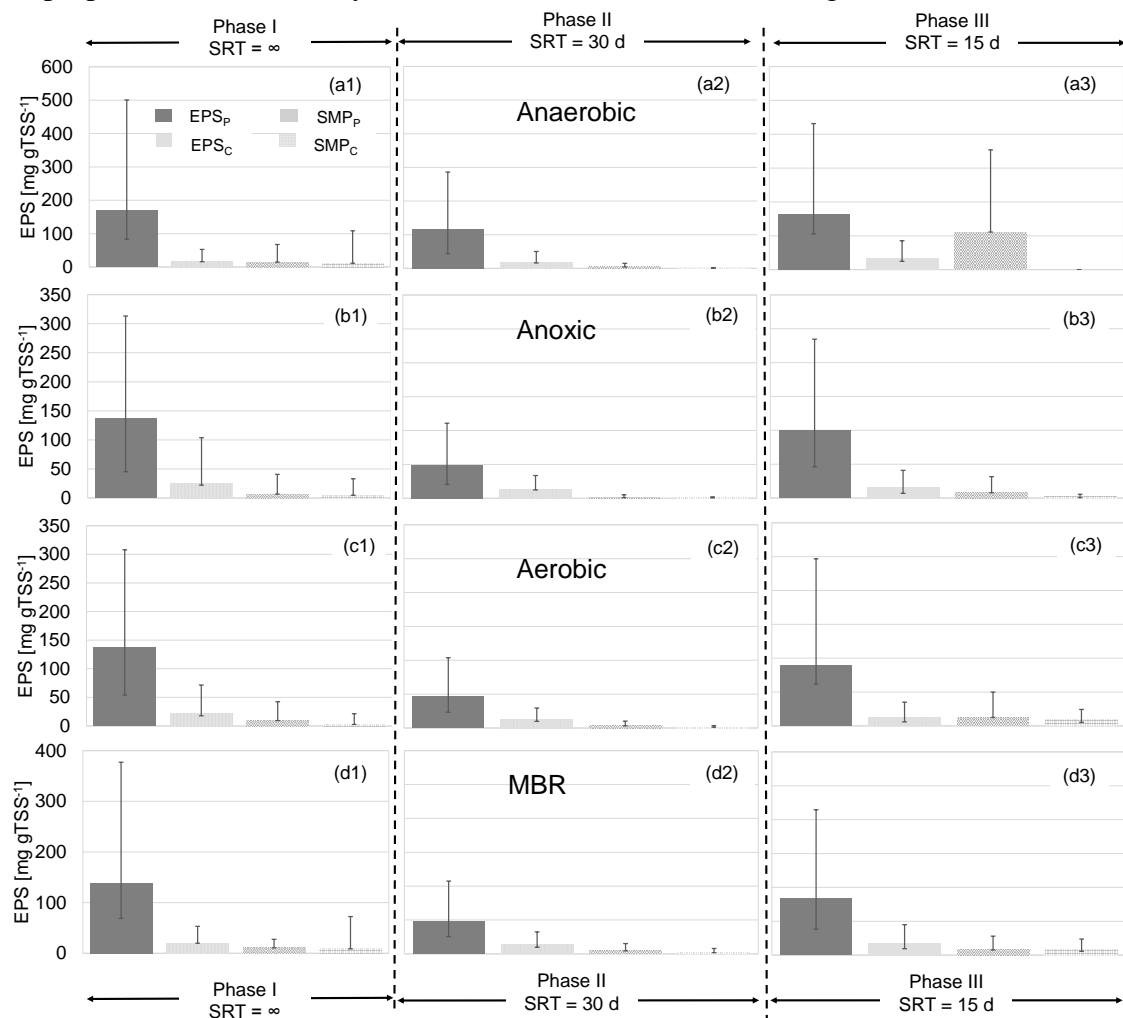
On the other hand, the maximum growth rate for the biofilm assumed much lower values compared to the suspended biomass. This result can be likely related to the aforementioned specialization of the two biomasses as well as to detachment biofilm phenomena occurring inside the bioreactor.

The respirometric batch tests carried out on nitrifying species revealed that the autotrophic activity was more pronounced in the attached biomass, thus confirming the “specialization” of the two biomasses (suspended and attached) within a hybrid configuration, with the biofilm more affine towards the nitrification of the influent ammonia loading rate. Nevertheless, also the suspended biomass of the UCT-MBMBR pilot plant showed good nitrification activity, thus suggesting the occurrence of the “seeding” effect of nitrifiers from the biofilm to the mixed liquor, as highlighted in previous experiences (Di Trapani et al., 2013). This result seems to be strengthened by an interesting aspect that was observed during experiments (Figure 6c-d). Indeed, the maximum growth rate of the suspended biomass showed an increasing trend when decreasing the MLSS SRT

from indefinite (no sludge withdrawals) to 30 and 15 days, respectively (Figure 6c). This result, apparently surprising for a pure activated sludge reactor working under the same operational conditions, is likely related to the aforementioned “seeding” effect due to the detached biofilm as well as the simultaneous growth of the biofilm during experiments that was able to support nitrification even in the mixed liquor. This result was confirmed by the increase of the AUR values of the suspended biomass from 1.33 to 2.34 mg NH<sub>4</sub>-N g<sup>-1</sup>VSS h<sup>-1</sup>, in the Phases I and II, respectively.

### EPS production

Figure 7 reports the average values of specific EPS concentration (i.e., referred to MLSS concentration) during experiments, expressed as carbohydrates and proteins in microbial flocs (EPS<sub>Bound</sub>) and dissolved in the bulk liquid (SMP). From the observation of Figure 7, it is possible to appreciate that SMP were significantly lower compared to EPS<sub>Bound</sub>, excepting some experimental days, at the beginning of the Phase I and during the Phase III. On the other hand, the protein fraction of EPS<sub>Bound</sub> was predominant and showed a general decrease from Phase I to Phase II, while it increased again during the Phase III. The observed values were higher compared to what achieved in previous experiences with UCT-MBR systems (Cosenza et al., 2013). This result could be likely due to biofilm detachment that might have promoted the increase of the mixed liquor hydrophobicity, contributing to worsen the membrane filtration properties compromising the filtration properties of the cake layer, as better outlined in the following section.



**Figure 7.** Average values of specific EPS<sub>Bound</sub> and SMP inside the anaerobic (a1-a3), anoxic (b1-b3), aerobic (c1-c3) and MBR (d1-d3) compartment, respectively.

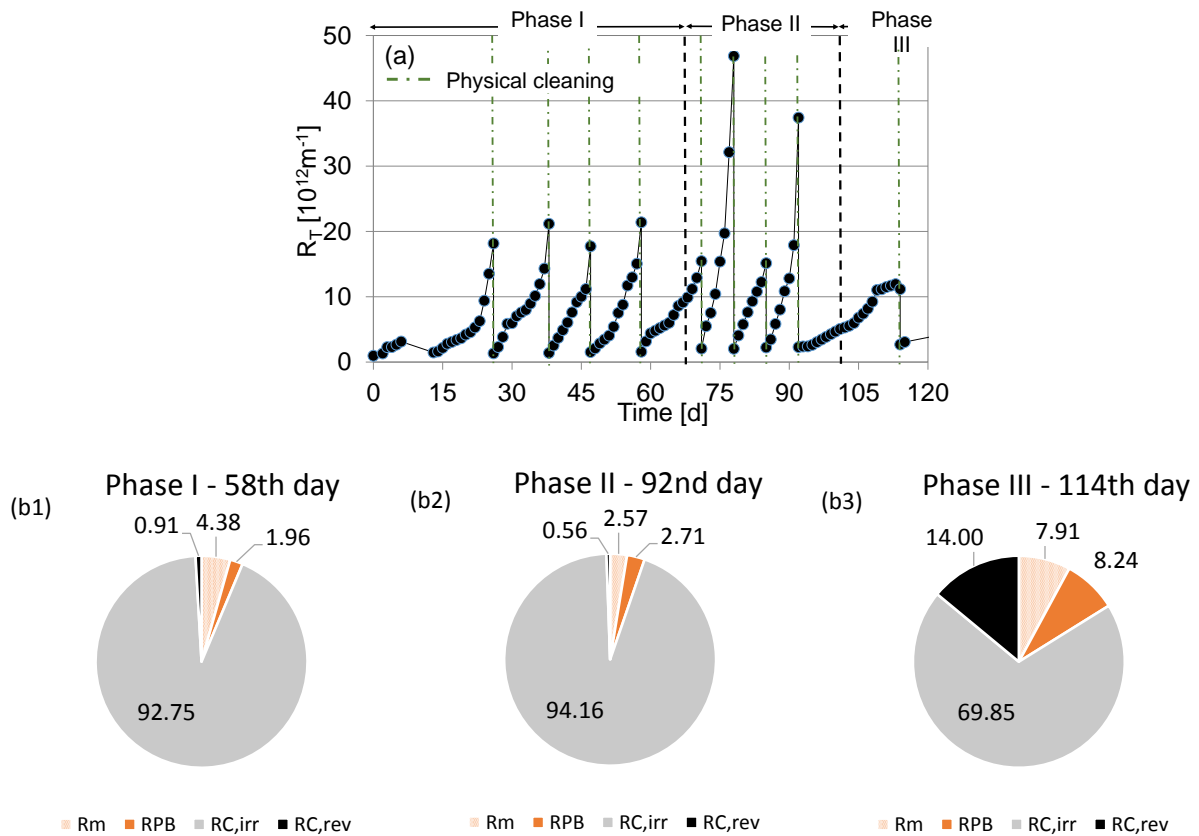
### Sludge dewaterability

The achieved results highlighted that the UCT-MBMBR pilot plant showed a good sludge dewaterability. The CST values were almost constant and slightly affected by the MLSS concentration, with average values of 15.27, 17.27, 15.07 and 18.93 s for the anaerobic, anoxic, aerobic and MBR compartment, respectively.

Furthermore, also the low SRF values confirmed the good sludge filtration properties, with average values for the different compartment close to  $4 \cdot 10^{12} \text{ m kg}^{-1}$ , significantly lower compared to what obtained by the same authors in previous experiences, when treating saline wastewater contaminated by hydrocarbons (Mannina et al., 2016). Moreover, the activated sludge filterability was mostly influenced by the specific  $\text{EPS}_{\text{Bound}}$  concentration (i.e., referred to MLSS concentration).

### Membrane filtration properties

Figure 8 reports the profile of  $R_T$  during the experimental campaign (Figure 8a) as well as the specific resistance contributions at day 58, 92 and 114 (Figure 8b1-b3) evaluated dividing each resistance, derived by applying the aforementioned RIS model, by the  $R_T$ .



**Figure 8.** Pattern of total resistance to filtration  $R_T$  (a) as well as average values of specific resistances (b1-b3) during experiments.

As noticeable from Figure 8a, nine extraordinary physical cleanings were carried out during experiments that were necessary in order to prevent the TMP exceeding the critical values defined by the membrane manufacturer (0.5–0.6 bar). As depicted in Figure 8b, the irreversible resistance due to superficial cake deposition ( $R_{C,irr}$ ) was the mechanism that mostly affected the membrane filtration properties. Moreover, it was noticed the increase of the resistance due to pore blocking ( $R_{PB}$ ) and a general worsening of the membrane filtration properties. This result could be due to the increase of the  $\text{EPS}_{\text{Bound}}$  fraction in the Phase III that could be enhanced by biofilm detachment phenomena occurred during experiments.

## CONCLUSIONS

The current study explored the influence of SRT in a UCT-MB-MBR pilot plant fed with a mixture of synthetic and real domestic wastewater. In the light of the results obtained during experiments, the following conclusions can be drawn:

- the UCT-MBMBR pilot plant provided very high total COD removal efficiencies throughout experiments: therefore, the SRT did not produce a significant effect, despite a slight reduction in the biological COD removal was observed;
- the nitrification efficiency was maintained even for the lowest SRT, thanks to the presence of the attached biomass, naturally characterized by high retention times. Moreover, thanks to the “seeding” effect of nitrifiers from the biofilm to the mixed liquor, the suspended biomass showed good nitrification ability;
- the reduction of the SRT suggested an increase of the PAOs activity, since the competition for the carbon source availability was reduced, increasing the biological phosphorus removal of the system, while maintaining high nitrification efficiency.
- the respirometric batch tests highlighted a sort of specialization of the two biomasses, with the suspended one more affine towards the carbon removal, whilst the biofilm towards the ammonia oxidation.

In view of the achieved results, the hybrid MB-MBR system highlight higher potentiality and process flexibility for nutrients biological removal, since it is possible to operate the system at lower SRTs, while maintaining high performance. Moreover, thanks to the biofilm, it is possible to operate the system at lower MLSS concentrations, thus enhancing the reduction of energy demand as well as fouling mitigation.

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## REFERENCES

- APHA, 2005. Standard Methods for the Examination of Water and Wastewater. APHA, AWWA and WPCF, Washington DC, USA.
- Chu, L., Wang, J., 2011. Comparison of polyurethane foam and biodegradable polymer as carriers in moving bed biofilm reactor for treating wastewater with a low C/N ratio. *Chemosph.* 83, 63–68.
- Cosenza, A., Di Bella, G., Mannina, G., Torregrossa, 2013. The role of EPS in fouling and foaming phenomena for a membrane bioreactor. *Bioresour. Technol.* 147, 184–192.
- Di Trapani, D., Christensson, M., Torregrossa, M., Viviani, G., Ødegaard, H., 2013. Performance of a hybrid activated sludge/biofilm process for wastewater treatment in a cold climate region: influence of operating conditions. *Biochem. Eng. J.* 77, 214–219.
- Di Trapani, D., Di Bella, G., Mannina, G., Torregrossa, M., Viviani, G., 2014. Comparison between moving bed-membrane bioreactor (MB-MBR) and membrane bioreactor (MBR) systems: Influence of wastewater salinity variation. *Bioresour. Technol.* 162, 60–69.
- Di Trapani, D., Di Bella, G., Mannina, G., Torregrossa, M., Viviani, G., 2015. Effect of C/N shock variation on the performances of a moving bed membrane bioreactor. *Bioresour. Technol.* 189, 250–257
- DuBois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., Smith, F., 1956. Colorimetric method for determination of sugars and related substances. *Anal. Chem.* 28, 350–356.
- EN 14701-1. European Standard. Characterization of sludges - Filtration properties – Part 1:

- Capillary Suction Time (CST). European Committee for Standardization. March 2006.
- EN 14701-2. European Standard. Characterization of sludges - Filtration properties – Part 2: Determination of the specific resistance to filtration. European Committee for Standardization. March 2006.
- Ekama, G.A., Siebritz, I.P., Marais, G.R., 1983. Considerations in the process design of nutrient removal activated sludge processes. *Wat. Sci. Tech.* 15 (3-4), 283 – 318.
- Fu, Z., Yang, F., Zhou, F., Xue, Y., 2009. Control of COD/N ratio for nutrient removal in a modified membrane bioreactor (MBR) treating high strength wastewater. *Bioresour. Technol.* 100, 136–141.
- Ge, H., Batstone, D.J., Keller, J., 2015. Biological phosphorus removal from abattoir wastewater at very short sludge ages mediated by novel PAO clade Comamonadaceae. *Water Res.* 69, 173-182.
- Hauduc, H., Rieger, L., Ohtsuki, T., Shaw, A., Takács, I., Winkler, S., Héduit, A., Vanrolleghem, P.-A., Gillot, S., 2011. Activated sludge modelling: development and potential use of a practical applications database. *Water Sci. Technol.* 63, 2164–2182.
- Hu, X., Xie, L., Shim, H., Zhang, S. and Yang, D. 2014. Biological Nutrient Removal in a Full Scale Anoxic/Anaerobic/Aerobic/ Pre-anoxic-MBR Plant for Low C/N Ratio Municipal Wastewater Treatment. *Chinese J. Chem. Eng.*, 22(4) 447—454.
- Ivanovic, I., and Leiknes, T. 2008. Impact of aeration rates on particle colloidal fraction in the biofilm membrane bioreactor (BF-MBR). *Desalin.* 231(1–3), 182–190.
- Jenkins, D., Richard, M.G., Daigger, G.T., 2003. *Manual on the Causes and Control of Activated Sludge Bulking, Foaming and Other Solids Separation Problems.* IWA Publishing, London.
- Judd, S.J., Judd, C., 2010. *Principles and Applications of Membrane Bioreactors in Water and Wastewater Treatment*, second ed. Elsevier, London, UK.
- Kermani, M., Bina, B., Movahedian, H., Ami, M.M., Nikaein, M., 2008. Application of moving bed biofilm process for biological organics and nutrients removal from municipal wastewater. *Am J. Environ. Sci.* 4(6), 675–682.
- Kristensen, G.H., Jørgensen, P.E., Henze, M., 1992. Characterization of functional microorganism groups and substrate in activated sludge and wastewater by AUR, NUR and OUR. *Water Sci. Technol.* 25, 43–57.
- Leiknes, T. Ødegaard H. 2007. The development of a biofilm membrane bioreactor, *Desalin.*, 202 (2007), 135–143.
- Leyva-Díaz, J.C., Calderón, K., Rodríguez, F.A., González-López, J., Hontoria, E., Poyatos, J.M., 2013. Comparative kinetic study between moving bed biofilm reactor-membrane bioreactor and membrane bioreactor systems and their influence on organic matter and nutrients removal. *Biochem. Eng. J.* 77, 28–40.
- Li, C., Wang, T., Zheng, N., Zhang, J., Ngo, H.H., Guo, W., Liang, S. 2013. Influence of organic shock loads on the production of N<sub>2</sub>O in denitrifying phosphorus removal process. *Bioresour. Technol.* 141, 160–166.
- Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265–275.
- Lu, Q., Wu, H., Li, H., Yang, D., 2015. Enhanced biological nutrient removal in modified carbon source division anaerobic anoxic oxic process with return activated sludge preconcentration. *Chinese J. Chem. Eng.* 23, 1027–1034.
- Majone, M., Dircks, K., Beun, J.J., 1999. Aerobic storage under dynamic conditions in activated sludge processes. The state of the art. *Water Sci. Technol.* 39, 61–73.
- Mannina, G., Cosenza, A. Di Trapani, D., Capodici, M., Viviani, G. 2016. Membrane bioreactors for treatment of saline wastewater contaminated by hydrocarbons (diesel fuel): An experimental pilot plant case study, *Chem. Eng. J.*, 291(1), 269–278.
- Martín-Pascual, J., Reboleiro-Rivas, P., López- López, C., Leyva-Díaz, J.C., Jover, M., Muñoz, M.M., González-López, J., Poyatos, J.M., 2015. Effect of the Filling Ratio, MLSS, Hydraulic

Retention Time, and Temperature on the behavior of the Hybrid Moving Bed Membrane Bioreactor plant to treat urban wastewater. *J. Env. Eng. (ASCE)* 141(7).

Naessens, W., Maere, T., Nopens, I., 2012. Critical review of membrane bioreactor models – Part 1: biokinetic and filtration models. *Bioresour. Technol.* 122, 95–106.

Ødegaard, H., 2006. Innovations in wastewater treatment: the moving bed biofilm process. *Water Sci. Technol.* 53, 17–33.

Peng, G., Ye, F., Li, Y., 2011. Comparative investigation of parameters for determining the dewaterability of activated sludge. *Water Environ. Res.* 83(7), 667-671.

Poyatos, J. M., Molina-Muñoz, M. A., Delgado, F., González-López, J. and Hontoria, E. 2008. Flux influence on membrane fouling in a membrane bioreactor system under real conditions with urban wastewater. *J. Environ. Sci. Health A.*, 43(14), 1685–1691.

Stephenson, T., Judd, S.J., Jefferson, B., Brindle, K., 2000. *Membrane Bioreactors for Wastewater Treatment*. IWA Publishing, London, UK.

Veselind P.A., 1988. Capillary suction time as a fundamental measure of sludge dewaterability. *J. Water Pollut. Control Fed.* 60, 215-220.

Wang, X.J., Xia, S.Q., Chen, L., Zhao, J.F., Renault, N.J., Chovelon, J.M., 2006. Nutrients removal from municipal wastewater by chemical precipitation in a moving bed biofilm reactor, *Process Biochem.* 41(4), 824–828.

Wanner, J., Cech, J.S., Kos, M., 1992. New process design for biological nutrient removal, *Water Sci. Technol.* 25(4-5), 445–448.

Wanner, J. 2002. Control of filamentous bulking in activated sludge. In: *Encyclopedia of Environmental Microbiology*, Bitton G Ed., 1306-1315. John Wiley & Sons Inc. New York USA.

Yang, S., Yang, F., Fu, Z., Wang, T., Lei, R. 2010. Simultaneous nitrogen and phosphorus removal by a novel sequencing batch moving bed membrane bioreactor for wastewater treatment. *J. Hazard. Mat.* 175 551–557.

Yang, W., Syed, W., Zhou, H., 2014. Comparative study on membrane fouling between membrane-coupled moving bed biofilm reactor and conventional membrane bioreactor for municipal wastewater treatment. *Water Sci. Technol.* 69, 1021–1027.