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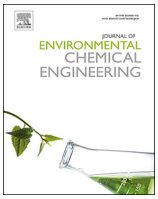

Optimising nutrient removal of a hybrid five-stage Bardenpho and moving bed biofilm reactor process using response surface methodology

Elham Ashrafi[a], Arjomand Mehrabani Zeinabad[a], Seyed Mehdi Borghei[b,*], Elena Torresi[c,d], Julian Muñoz Sierra[e,f]

[a] Chemical Engineering Department, Isfahan University of Technology, Isfahan, Iran
[b] Biochemical and Bioenvironmental Engineering Research Center (BBRC), Sharif University of Technology, Tehran, Iran
[c] DTU Environment, Technical University of Denmark, Bygningstorvet B115, 2800, Kongens Lyngby, Denmark
[d] Veolia Water Technologies AB, AnoxKaldnes, Klosterängsvägen 11A, SE-226 47, Lund, Sweden
[e] Section Sanitary Engineering, Department of Water Management, Delft University of Technology, Stevinweg 1, 2628CN, Delft, the Netherlands
[f] KWR Watercycle Research Institute, Groningenhaven 7, 3430 BB, Nieuwegein, the Netherlands

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ABSTRACT

Nutrient pollution has become a global environmental issue. Innovative biological nutrient removal (BNR) processes are needed to overcome the drawbacks of conventional technologies. This study evaluates the potential of a hybrid 5-stage Bardenpho - moving bed biofilm reactor (MBBR) process for organic carbon and nutrient removal from municipal wastewater at different hydraulic retention time (HRT) and nitrate recycle ratio (R). Response surface methodology (RSM) based on a central composite design (CCD) of thirteen experiments was applied to optimize the nitrogen and phosphorus conversion of the treatment system. High removal efficiencies of about 98.20%, 92.54%, 94.70% and 96.50% for total chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP) and ammonium, were achieved, respectively. The best performance was observed at HRT of 2, 4, 6, 2.67 and 1.07 h correspondingly in the anaerobic, first anoxic, first aerobic, second anoxic and second aerobic compartments, resulting in a total HRT of 15.74 h with a nitrate recycle ratio of 2. Biofilm nitrifying activity was four times higher than in suspended biomass. The hybrid 5-stage Bardenpho-MBBR process enhanced biological nutrient removal at comparatively short HRT and low R ratio due to biofilm contribution to the conversion.

1. Introduction

Wastewater streams with high nutrient content (nitrogen and phosphorus) can be the leading reason for several issues when discharged into the environment, such as oxygen consumption, eutrophication and toxicity [1]. Consequently, the nutrient pollution has turned out to be a global environmental threat [2–4]. In last decades, biological nutrient removal (BNR) processes have been comprehensively studied. BNR processes typically rely on an arrangement of different environmental redox conditions (anaerobic, anoxic and aerobic) into separate compartments [5]. Concisely, nitrogen (N) and phosphorus (P) are undertaken by heterotrophic organisms capable of denitrification and polyphosphate accumulation. A major drawback with conventional BNR systems is highlighted by massive reactor volumes requirement, which make them often unfeasible in terms of investment and space. Furthermore, the overall efficiency of the process is strictly depending on the solid-liquid separation accomplished in the clarifier, which may be deteriorated by poor biomass settling characteristics [6]. As a result over the last years, a number of studies have been carried out targeting innovative solutions with the aim of overcoming the main drawbacks of the conventional BNR configurations [7,8]. Among the novel technologies, membrane bioreactors (MBR), moving bed biofilm reactors (MBBR) and MBBR-based Integrated Fixed Film Activated Sludge (IFAS) reactors have captured attention due to their advantages compared to conventional processes [8]. Especially, MBBR systems using polymeric carrier elements for biofilm growth, are considered as a promising solution [9]. The advantage of MBBR-based processes is the presence of both suspended flocs and biofilm in the same reactor compartment [10,11]. Hence, application of attached growth in MBBR allows to have a compact reactor with higher biomass concentration compared to conventional BNR [12]. MBBR has been applied for synthetic [13,14], domestic [15] and industrial wastewater treatment

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[*] Corresponding author.
E-mail address: mborghei@sharif.edu (S.M. Borghei).

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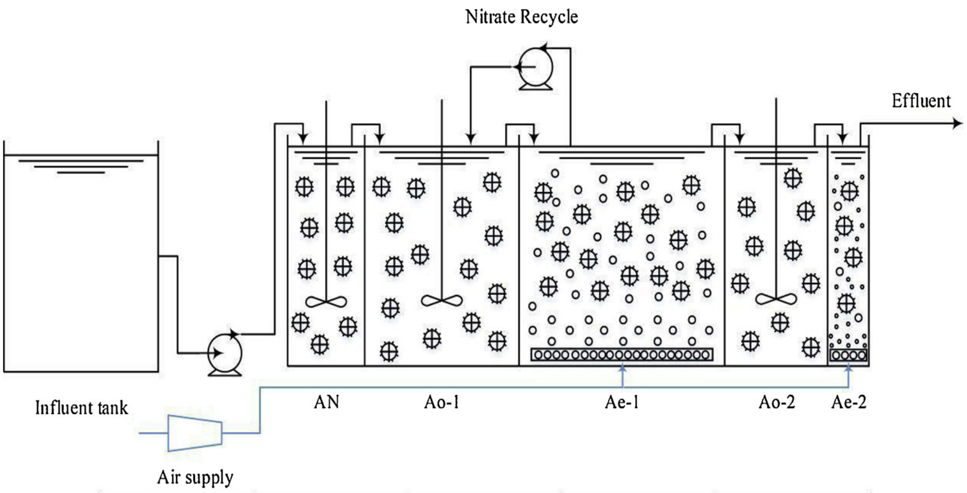

Anaerobic Compartment (AN)	First Anoxic Compartment (Ao-1)	First Aerobic Compartment (Ae-1)	Second Anoxic Compartment (Ao-2)	Second Aerobic Compartment (Ae-2)
Working volumes correlation: $V_{Ao\text{-}1} = 2V_{AN} = 1.5V_{Ao\text{-}2} = 3.75V_{Ae\text{-}2}$; $V_{Ae\text{-}1} = 54$ lit ; $HRT_{Ae\text{-}1} = 6$ h				

Fig. 1. Diagram of the lab-scale setup of the hybrid 5-stage Bardenpho-MBBR system.

[16–18]. Slow biofilm formation rate during start-up could be one of the drawbacks on the practical applications of MBBR [19]. Combination of MBBR and BNR systems have been reported, for example, Lai et al. [20] applied a modified Anaerobic/Anoxic/Oxic (A^2O) process based on a plastic-based biofilm support media to remove organics and nutrients from municipal wastewater. Compared to a traditional A^2O process the chemical oxygen demand (COD), total nitrogen (TN), and total phosphorus (TP) removal efficiencies increased from 91%, 48% and 56% to 98%, 73% and 71%, respectively. Mannina et al. [21,22] evaluated a hybrid MBBR-MBR-UCT (University of Cape Town) process and achieved removal efficiencies of about 98%, 60% and 77% for COD, TN and TP, respectively at a C/N ratio of 10. Leyva-Díaz et al. [1] evaluated a hybrid MBBR-MBR containing carriers in the anaerobic, anoxic and aerobic compartments and another with carriers only in the anaerobic and anoxic compartments. It was reported higher COD and TP removal efficiencies in the first system and the highest TN removal efficiency (61.39 ± 10.71%) in the latter. Recently, Ooi et al. [23] evaluated six moving bed biofilm reactors (MBBRs) in series targeting carbon, nutrients, and pharmaceutical/micropollutants removal. Interestingly, out of the 22 pharmaceutical/micropollutants studied, 17 compounds were removed higher than 20%.

Some studies were carried out focusing on the hydraulic retention time (HRT) influence in BNR systems. Bassin et al. [24] operated the MBBR system filled with different support media for simultaneous nitrification-denitrification (SND) under different organic loading rates and HRTs. They reported TN removal efficiencies up to 86% and 73% in MBBR filled with Kaldnes K_1 and Biochip carriers, respectively at HRT of 12 h. Zhang et al. [25] revealed that the HRT has a significant effect on the reliability and stability of BNR systems, meanwhile, HRT is a fundamental parameter for wastewater treatment plants (WWTPs) design and operation, which directly affect the infrastructure and operational cost. They found that HRT played a key role in enhancing denitrifying phosphorus removal and preventing secondary phosphorous release. Moreover, Akratos and Tsihrintzis [26], and Xu et al. [27] have investigated various HRTs for optimal nutrient removal in BNR systems. Another critical parameter for BNR performance is the nitrate recycle ratio (R). Larger R conveys a surplus of dissolved oxygen (DO) from the aerobic to anoxic compartments and as a result deteriorates the denitrification process, on the other hand, low R decreases the returned nitrate and reduces the TN removal [28,29]. Chen et al. [28,29] reported an operation of the Bardenpho system at different recycle ratios. However, the hybrid 5-stage Bardenpho-MBBR process is rather new and a few research studies reported the impact of operational parameters on system performance as well as the activity of suspended and attached biomass. Additionally, it is still unclear what would be an effective HRT in each compartment of a biofilm 5-stage Bardenpho system. Moreover, it has not been yet determined which R values are the most appropriate for enhancing the nutrient removal. Also, the assessment of biofilm capability for simultaneously nitrification-denitrification (SND) to improved nitrogen removal has not been reported. Therefore, this study aimed to evaluate and optimize the performance potential of a hybrid 5-stage Bardenpho-MBBR process on organic and nutrient removal efficiencies through response surface methodology based on the parameters HRT and the nitrate recycle ratio. Furthermore, biofilm contribution on nitrification activity was assessed. This study provides valuable knowledge for the application of the MBBR system in BNR process for municipal wastewater treatment to fulfil stringent discharge requirements.

2. Experimental

2.1. Experimental set-up

A lab-scale system was built-up according to the 5-stage Bardenpho scheme. The set-up consisted of an influent tank and five compartments (one anaerobic, two anoxic and two aerobic) in series. All compartments were made of transparent Plexiglas in a rectangular shape (Fig. 1). The effective volume of the first aerobic compartment (Ae-1) was regulated to maintain a constant HRT throughout the experiment. Mechanical stirrers with a rectangular paddle were installed in the anaerobic and anoxic zones to keep the sludge and carriers in suspension. Besides, air bubble diffusers were installed at the bottom of the aerobic zones to facilitate mixing. Total aeration rate was 0.18–0.20 m^3/h. Every compartment was packed with Kaldnes K_1 carriers with a 30%, 40% and 50% filling ratio in the anaerobic, anoxic, and aerobic compartments, respectively. The carriers' characteristics are presented in Table 1. The influent was pumped into the anaerobic compartment and the nitrate, product of the nitrification process in the aerobic compartment, was recycled into the anoxic compartment to be used as an electron acceptor for phosphorus removal and denitrification. The influent and nitrate recycle flow rates were controlled by a peristaltic pump (Longer pump, WT600-1 F, USA).

Table 1
Characteristics of Kaldnes K_1 carrier.

Characteristics	Kaldnes K_1
Material	High-density polyethylene
Shape	cylinder
Nominal diameter (mm)	9.1
Nominal length/thickness (mm)	7.2
Apparent density (kg/m^3)	150
Specific surface area (m^2/m^3)	500

2.2. *Influent synthetic wastewater*

The system was inoculated with activated sludge taken from the Ekbatan Sewage Treatment Plant (Tehran, Iran). The initial total suspended solids (TSS) and volatile suspended solids (VSS) were 2.5 g/L and 1.2 g/L respectively. The synthetic wastewater was composed of a medium A consisting of: 0.8 gCOD/L (a mixture of glucose and sodium acetate with molar ratio of 1:1); 3.6 mM $MgSO_4.7H_2O$; 4.7 mM KCl, and medium B consisting of: 69 mM NH_4Cl; 4.2 mM K_2HPO_4; 2.1 mM KH_2PO_4; 15 mL milk; and 10 mL/L trace element solution, all according to Vishniac and Santer [30]. On every time 150 mL of both media was dosed to the reactor together with 1.2 L of tap water De Kreuk et al. [31].

2.3. *System operational conditions*

Temperature and pH were kept at 25–28 °C and 7.0–7.5 in all the reactors by using a water bath and pH probes (WTW, Germany). DO concentration was controlled at 3 mg/L in both aerobic compartments. Influent COD, TN, and TP, were in the range of 700–800, 35–40 and 7–8 mg/L, respectively. The flow rate was 216 L/d. The working volume of each compartment varied depending on the corresponding HRT for each run. However, the compartment Ae-1 had a fixed volume and HRT (6 h) throughout the experiments. Table 2 indicates HRT in all compartments with their corresponding operational conditions. The system was operated for 40 days as start-up period and 273 days along with thirteen experimental runs. Each run was carried for 21 days.

2.4. *Statistical design of experiments, data analysis, and optimization*

A two-factor central composite design (CCD) was performed using the statistical Design-Expert software, version 10.0.2.0 (Stat- Ease Inc., Minneapolis, MN, USA) to analyze the significance of experimental results [32]. Both experimental conditions and their responses (TN and TP removal efficiencies) are presented in Table 2, as well as the organic (OLR) and nitrogen loading rates (NLR). The range and the levels of the independent variables are presented in Table 3. The two independent factors (HRT_{AN} and R) were studied at five different levels. The HRT of the other compartments were calculated based on their volume correlation. Thereby, a central composite design for two independent variables at each of the five levels was used to fit the model with a total of 13 experimental runs required for this procedure. A multiple regression model was obtained to estimate the predicted value of the dependent variables [33]. A second order polynomial regression model was used to express the response Y as a function of the independent factors as follows:

Table 3
Experimental range and levels of the independent variables.

Variable	Low axial (-α= -4)	Low factorial (-1)	Center (0)	High factorial (+1)	High axial (+α= +4)
HRT_{AN}	0.5	2.0	2.5	3.0	4.5
R	0.0	1.5	2.0	2.5	4

$$Y = b_0 + \sum_{n=1}^{2} b_n X_n + \sum_{n=1}^{2} b_{mn} X_n^2 + \sum_{n<m}^{2} b_{nm} X_n X_m \tag{1}$$

where Y is the dependent variable i.e. TN removal efficiency; X is the independent variables such as HRT, R; b_0 is the constant value at central point; b_n, b_m and b_{nm} are the linear, quadratic and cross product coefficients.

2.5. *Biomass oxygen uptake and nitrification rates*

In order to assess the specific nitrifying activity of the both attached and suspended biomass and estimate the amount of ammonium oxidized by each biomass fraction in Ae-1 zone, batch tests were carried out according to the methods described by Bassin et al. [24]. Briefly, the reactor feeding was stopped, and a pulse of a concentrated stock solution of ammonium chloride was added to reach an initial ammonium concentration similar to that of the reactor influent. Samples were collected every 20 min for 6 h. The volumetric ammonium removal rate was calculated by linear regression of ammonium concentration over time. The biomass-specific ammonium removal rate was determined by dividing the volumetric ammonium removal rate by the total amount of biomass (i.e., volatile total solids (VTS) = volatile attached solids (VAS) + volatile suspended solids (VSS)). Subsequently, all the carriers were removed to evaluate the nitrification rate of the suspended solids. Respirometric batch experiments were carried out along with the procedure described by Mannina et al. [21]. Biomass samples were aerated until endogenous conditions were achieved, by measuring the specific oxygen uptake rate (SOUR) values.

Table 2
Operational conditions of the hybrid 5-stage Bardenpho-MBBR system.

Run	Period (day)	HRT_{AN} (h)	HRT_{Ao-1} (h)	HRT_{Ao-2} (h)	HRT_{Ae-2} (h)	HRT_{Total} (h)	NO_3 recycle ratio (R)	TN removal (%)	TP removal (%)	Volumetric OLR (kgCOD/m^3.d)	Surface OLR (gCOD/ m^2.d)	Volumetric NLR (kgNH_4^+-N/m^3.d)	Surface NLR (gNH_4^+-N /m^2.d)
1	40-61	0.5	1	0.67	0.27	8.44	2	84.3	83.2	2.13	4.26	0.1	0.2
2	62-82	2.5	5	3.33	1.33	18.16	2	92.8	94.6	0.99	1.98	0.05	0.1
3	83-103	2.5	5	3.33	1.33	18.16	2	93.5	98.1	0.99	1.98	0.05	0.1
4	104-124	4.5	9	6	2.4	27.9	2	82.3	84.6	0.64	1.29	0.03	0.06
5	125-145	2.5	5	3.33	1.33	18.16	0	79.5	82.3	0.99	1.98	0.05	0.1
6	146-166	2.5	5	3.33	1.33	18.16	2	94.9	96.3	0.99	1.98	0.05	0.1
7	167-187	2.5	5	3.33	1.33	18.16	2	95.2	97.2	0.99	1.98	0.05	0.1
8	188-208	2.5	5	3.33	1.33	18.16	2	96.1	97.8	0.99	1.98	0.05	0.1
9	209-229	2.5	5	3.33	1.33	18.16	4	84.5	98.3	0.99	1.98	0.05	0.1
10	230-250	2	4	2.67	1.07	15.74	1.5	90.5	90.3	1.14	2.29	0.055	0.11
11	251-271	2	4	2.67	1.07	15.74	2.5	89.8	96.5	1.14	2.29	0.055	0.11
12	272-292	3	6	4	1.6	20.6	2.5	87.1	93.2	0.87	1.75	0.04	0.08
13	293-313	3	6	4	1.6	20.6	1.5	89	90.2	0.87	1.75	0.04	0.08

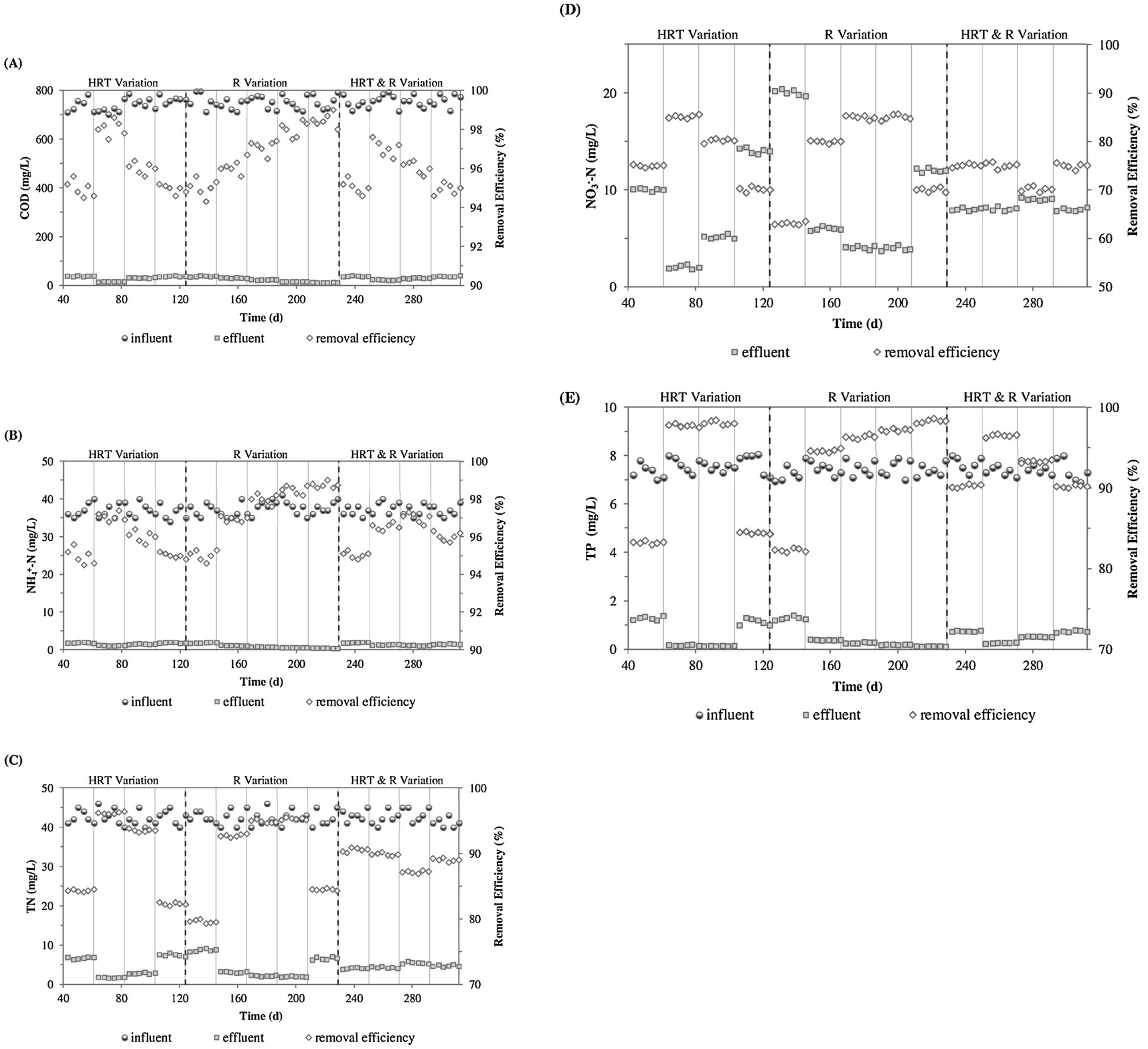

Fig. 2. Removal performance of the hybrid 5-stage Bardenpho-MBBR system, COD (A), NH4 + -N (B), TN (C), NO3–N (D) and TP (E), each run separated by vertical lines.

2.6. Analytical methods

Total suspended solids (TSS), volatile suspended solids (VSS) and biofilm density were calculated for suspended and attached biomass as described by De Kreuk et al. [34] and Bassin et al. [24], respectively. Biofilm thickness was determined by microscopy. Twice a week influent wastewater and mixed liquor of the distinctive compartments were collected and filtered through a 0.45 μm filter paper. The analysis of COD, ammonium (NH_4^+ – N), nitrite (NO_2^- –N), nitrate (NO_3^- – N) and orthophosphate (PO_4^{3-} – P) were carried out according to standard methods [35]. TN was determined by means of NH_4^+ – N, NO_2^- – N and NO_3^- – N concentrations addition.

3. Results and discussion

3.1. Process performance

COD, NH_4^+ – N, NO_3^- – N, TN, and TP concentrations in the effluent and corresponding achieved removal efficiencies are depicted in Fig. 2. The NO_2^-- N and N_{org} -N in the effluent were neglected due to their minimum concentration compared to other compounds (< 0.2 mg/L). The COD results (Fig. 2. A) indicated that despite the gradual increase of the total HRT during runs 1 to 4, the COD removal within this period was roughly stable and higher than 95%. Notably, the high COD removal efficiency at run 2 (98%) was related to the improvement in nitrate removal efficiency (Fig. 2.D), since the more denitrification took place, the more COD was consumed. Such as high COD removal could be attributed to the biofilm contribution when compared with values obtained by Huang et al. [36] from the conventional activated sludge 5-stage Bardenpho system. The achieved NH_4^+ – N removal in the whole process was about 95% (Fig. 2.B). The growth of biofilm on the carriers in the aerobic compartment enhanced the nitrification process likely due to the high retention time of biofilm on the carriers, which is in agreement with previous studies [8,22]. According to the achieved COD and $NH4^+$-N removal efficiencies (see Fig. 2.A, B) of about 95% in the whole process, it can be inferred that HRT variation did not have a significant effect on them when compared with TN, nitrate and TP removal efficiencies (Fig. 2C, D and E).

A larger nitrate recycle ratio induced a higher NO_3^--N concentration and DO recirculation in Ao-1, and therefore a higher COD removal.

DO recirculation not only induced the aerobic oxidation of COD but also it promoted the aerobic oxidation of ammonium in the Ao-1 compartment [29]. Thereby, an increase in removal efficiencies was observed during runs 6 to 9, while from run 8 to 9 a minimum increase at COD degradation pointed out that the system was being operated under the maximum denitrification rate, suggesting that a R ≥ 4 will not be recommended from the technical-economical point of view.

TN removal improved from run 1 to run 2 (up to 96.1%) due to the higher $HRT_{Ao\text{-}1}$ which increased the time feasible for denitrification. However, removal decreased to 83% in runs 3 and 4 (Fig. 2.C). Longer $HRT_{Ao\text{-}1}$ (more than 5 h) resulted in low carbon concentration for denitrifiers in Ao-1 compartment since most of the organic carbon was consumed by the Phosphate Accumulating Organisms (PAOs), which compete with denitrifiers on nutrient sources [37]. Consequently, denitrification and TN efficiency declined during runs 3 and 4.

TN removal efficiency declined to 80% in run 5 due to nitrate recycle elimination. The reason behind is likely related to the decline of denitrification in run 5 (Fig. 2.D) because of NO_3^--N shortage. When R increased from 0 to 2, the higher amount of nitrate delivered to the Ao-1 compartment resulted in an enhanced TN removal efficiency trend during run 6 to 8, up to 93% (Fig. 2.C). At run 9, a higher R brought an excess DO from Ae-1 to the Ao-1 compartment. As a result, the accessible organic carbon for denitrification was degraded, and TN removal efficiency was deteriorated to 84% (Fig. 2.C). A remarkable TN removal was achieved compared to previous studies in which a biofilm reactor was not used with 72%, 75% and 58% of TN removal efficiency, respectively [38–40]. This is likely due to the simultaneous nitrification and denitrification (SND) process occurred in the biofilm, despite the aerobic conditions in the aerobic compartment (DO ranging from 2 to 3 mg/L). The thick biofilm (average thickness of 1.2 ± 0.1 mm) attached to the carrier may have resulted in oxygen mass transport limitation and thus, anoxic conditions in the inner zones of the biofilm [24].

TP removal efficiency improved from 83.2% in run 1 to 98.1% in run 3. However, it deteriorated to 84.2% in run 4 (Fig. 2.E). The increase of HRT_{AN} promoted the anaerobic phosphorus release and eventually increased the overall phosphorus removal. The substantial phosphorus uptake observed in run 3 might be attributed to denitrifying phosphorus removal (P-uptake by Denitrifying Phosphate Accumulating Organisms (DPAOs), [13,41–43]. On the other hand, lower TP removal at run 4 (82%) may indicate secondary phosphorous release. This phenomenon occurs when PAOs release phosphorus without storing polymerized volatile fatty acids (VFAs). Consequently, a lack of energy to uptake all of the released phosphorus would occur in the anaerobic compartment and the released phosphorous may be not uptaken by the PAOs in the anoxic or aerobic compartments [37]. Secondary phosphorus release also may occur at long HRT of the main anoxic compartment ($HRT_{Ao\text{-}1}$), when nitrates are consumed before the end of the HRT, due to the fact that DNPAOs, which utilize nitrate as an electron acceptor to uptake the released phosphate, do not have adequate nitrate concentration for denitrifying phosphorus removal [44]. Also, Wang and Park [45] showed that long HRT_{AN} (> 3 h) decreased the polyhydroxybutyrate (PHB) in the biomass which is crucial for phosphate uptake. A high R from run 6 to 9 (R = 2), conveyed more nitrate to the Ao-1 compartment, which led to denitrifying P removal. The DNPAOs would not compete with the ordinary heterotrophic organisms for nitrate and could use the excess nitrate for denitrifying phosphorus removal, provided that the nitrate load was adequately high to exceed the denitrification potential of ordinary heterotrophic organisms [29]. This also explains the declined TP removal efficiency at run 5 (P-uptake only occurred in the Ae-1 compartment).

During run 10 and 11, TN removal slightly decreased from 91% to 89%, while TP removal exhibited a significant improvement due to increase of R value. During run 11 and 13, TP gradually decreased from 96% to 90% possibly due to secondary phosphorus release (from run 11 to 12) and nitrate shortage because of the decreased R from 2.5 to 1.5 in run 13. The results regarding the TP removal in this study were higher than the range reported by recent literature, for instance, Huang et al. [36], who employed the 5-stage Bardenpho system without biofilm, achieved 90% in TP removal efficiency. The abundance of the PAOs in the anaerobic tank (possibly present on the biofilm) might have contributed to the higher phosphorus removal efficiency in this study. In addition, Yang et al. [46] obtained the TP removal of 85.05 ± 8.02% in a membrane-coupled MBBR, Luo et al. [47] estimated around 89% of PO_4^{-3}-P removal in an MBBR system under 24 h of total HRT and 20% of filling fraction in the bioreactor. Moreover, Kermani et al. [48,49] evaluated the TP removal efficiency in a lab-scale MBBR system, which showed a performance of 87.92% and 89.73% on average, respectively.

TN removal efficiency indicated a decline during run 11 to 12 (from 89% to 87%) and an improvement at run 13 (89%). Lack of carbon source for denitrifiers in Ao-1 compartment limited the nitrogen reduction during run 11 to 12 and the decrease of R from 2.5 to 1.5, induced the improvement in run 13.

3.2. COD and nutrients removal at each compartment

The best process performance was obtained at a combined HRT_{AN} of 2.5 h and an R of 2, at run 2 (day 62 to 82). The major proportion (76%) of COD within the hybrid 5-stage Bardenpho-MBBR system was removed in the anaerobic compartment (AN) (Fig. 3). Simultaneously, 13% and 6% COD was consumed in the Ao-1 and Ae-1 compartments, respectively. The hybrid Bardenpho-MBBR system displayed a proper performance on organic carbon removal with a total COD removal efficiency of about 98%. NH_4^+-N, which was the main fraction of TN, decreased to 25, 8, 3.5, 1.3 and 1 mg/L in AN, Ao-1, Ae-1, Ao-2 and Ae-2 compartments, respectively. The sharp decrease in ammonium concentration at Ao-1 was due to the oxygen utilization, which was taken from Ae-1 to Ao-1 through internal recycle. The change of TN was similar to ammonium, and its removal efficiency was 92.5%. TP increased to 14 mg/L in AN compartment due to phosphorus release. After it was gradually reduced in further compartments as Ao-1 (8 mg/L), Ae-1 (2 mg/L), Ao-2 (1.2 mg/L) and eventually Ae-2 (0.5 mg/L). Based on the results in each compartment and in the overall system, the hybrid 5-stage Bardenpho-MBBR system can be taken into consideration as a potential technology for wastewater treatment as exhibited a superior COD, ammonium, TN and TP removal efficiencies.

3.3. Statistical data analysis and optimization

3.3.1. Fitting model for TN and TP removal

The analysis of variance (ANOVA) of the results was carried out to obtain the best possible response surface regression model [50] (Table 4, see also Tables S1 and S2.). The F-values of 10.84 and 20.22

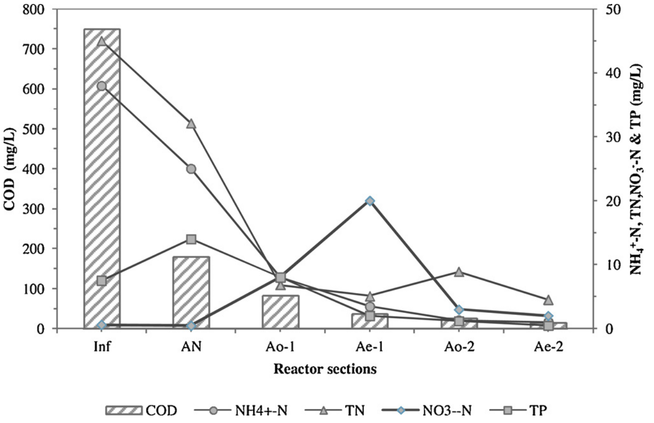

Fig. 3. COD and nutrients concentration within the hybrid 5-stage Bardenpho-MBBR system at HRTAN = 2.5 h and R = 2 (day 62–82).

Table 4
Analysis of variance (ANOVA) for the quadratic models.

		Sum of Squares	df	Mean Square	F Value	p-Value	Lack of Fit	PRESS	R-Squared
TN removal efficiency	**Model**	*288.61*	*4*	*72.15*	*10.84*	*0.0026*	*0.0486*	*444.77*	*0.8442*
	Residual	*53.24*	*8*	*6.66*					
TP removal efficiency	**Model**	*376.37*	*4*	*94.09*	*20.22*	*0.0003*	*0.1171*	*444.77*	*0.8442*
	Residual	*37.22*	*8*	*4.65*					

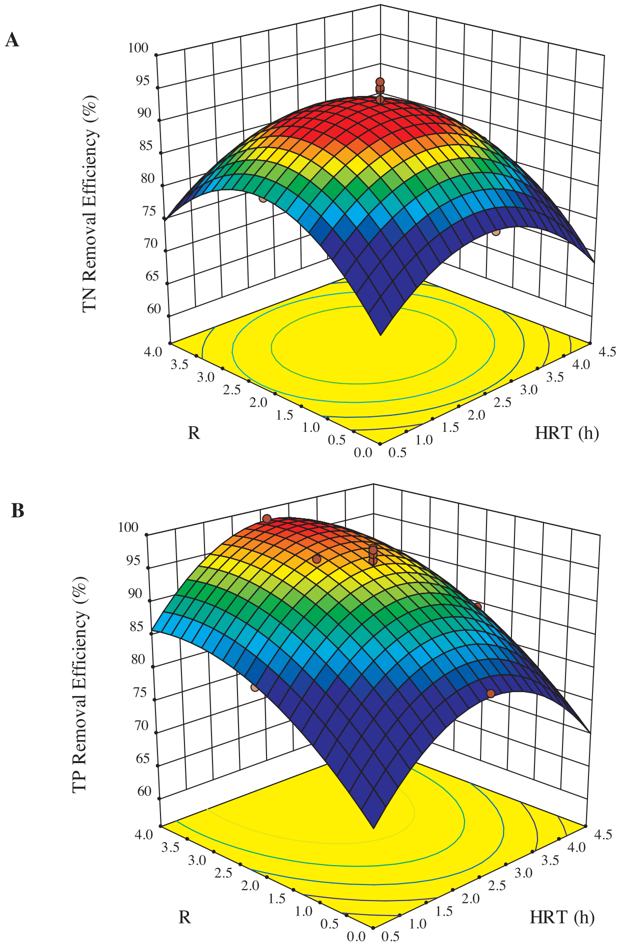

Fig. 4. Response surfaces for A. TN removal efficiency and B. TP removal efficiency. HRT refers to the anaerobic compartment (HRT_{AN}).

for nitrogen and phosphorus removal efficiencies, respectively inferred that the models were significant at less than a 0.05% level (i.e., 95% confidence interval). Equations (1) and (2) describe the models containing the significant effects. The factors with a value of "Prob > F" higher than 0.050 were excluded from the models since their effect was not significant. It was found that a quadratic response surface model could fit the best the experimental data. Adequate precision of the models were 8.156 and 12.160 for nitrogen and phosphorus removal. A ratio greater than 4 is desirable [51]. Since these values represent the model's differentiation power [52], it is expected that the models can be used properly to describe the responses under widespread conditions.

$$R_1 = 92.82 - 0.34 \times A + 0.48 \times B - 0.61 \times A^2 - 0.69 \times B^2 \tag{2}$$

$$R_2 = 95.49 + 0.061 \times A + 2.03 \times B - 0.74 \times A^2 - .034 \times B^2 \tag{3}$$

where R_1, R_2, A, and B refer to TN and TP removal efficiencies, HRT_{AN} and R (nitrate recycle ratio), respectively.

Fig S_1 confirmed the models robustness by exhibiting the predicted vs. actual values of TN and TP removal. Data points clustered next to the diagonal indicated a satisfactory correlation between the experimental and the predicted values.

Fig. 4 depicts the influence of HRT_{AN} and R on TN and TP removal (%) through three-dimensional response surfaces. At both high and low values of HRT_{AN} and R, TN removal efficiency was minimum. At a particular R-value, TN removal efficiency improved when HRT_{AN} increased to 2.5 h, however, decreased at longer HRT_{AN}. Moreover, at a specific HRT_{AN}, TN removal efficiency enhanced when R risen from 0 to 2 and reduced by applying a higher R. HRT_{AN} in the range of 2.1–2.9 h and R values in the range of 1.6–2.4 were the conditions for satisfactory TN removal (Fig. 4. A)

At low values of R with both low and high values of HRT_{AN}, TP removal efficiency was minimum. TP removal showed a positive correlation with increase of R. HRT_{AN} in the range of 1.7–3.4 h and R higher than 2 were the satisfactory values for reasonable TP removal efficiency. The maximum TP removal efficiency was approximately 98.3% when HRT_{AN} and R were 2.5 h and 4, respectively (Fig. 4. B).

3.3.2. Optimization of HRT_{AN} and R

Optimization was executed through Design-Expert software. The solutions for the optimization of several scenarios to fulfill the maximum TN and TP removal efficiencies or minimum HRT_{AN} and R are presented in Fig S2 – S5. For some existing wastewater treatment plants (WWTP), in which there is a shortage of construction site, a minimum HRT_{AN} is the crucial parameter to take into consideration [53,54]. In some other scenarios, such as low COD/N ratios, the key parameter to optimize the performance of the system is the nitrate recycle ratio. Under this circumstance the minimum value of R (≤ 3) is recommended [28,29]. Some municipal wastewater treatment plants must fulfill highly rigorous standards for discharge and in such cases, the maximum TP and TN removal efficiencies are required. According to our study, in the hybrid 5-stage Bardenpho-MBBR system an HRT_{AN} of 2 h and nitrate recycle ratio R of 2 are the most suitable values in which the TN and TP removal efficiencies were maximized to 92.5% and 94.7%, respectively. It must be pointed out that TN and TP removal efficiencies at R = 0 in this study were superior to those attained by Wang and Chen [40], who eliminated the internal recycle in a full-scale anoxic-oxic process, and by Chen et al. [28,29], who determined an R of 3 for an A^2O-biological aerated filter system.

3.4. Biomass oxygen uptake and nitrification rates

The specific oxygen uptake rate (SOUR) values revealed the heterotrophic activity (mixed ordinary heterotrophs and PAOs) and exhibited a significant decline from about 58 to 30 mg $O_2\,g^{-1}$ VSS·h^{-1} for suspended solids during runs 1–4 due to decrease in organic loading rate (Fig. 5.A). However, there was a roughly stable behavior for the heterotrophic activity of the suspended solids up to the end of the experiment about 35 mg $O_2\,g^{-1}$ VSS·h^{-1}. Fluctuations were observed in the attached biofilm, with lower values compared to the suspended biomass ($\leq$10 mg $O_2\,g^{-1}$ VSS·h^{-1}). Nitrification rates exhibited a declining tendency (from about 4 to 0.5 mg NH_4^+-N g^{-1} VTS·h^{-1}) in suspended biomass due to HRT and R variations and washout of autotrophic populations (mixed ammonia oxidized bacteria (AOB) and nitrite oxidized bacteria (NOB), Fig. 5.B). On the other hand, the autotrophic activity of the attached biofilm decreased only when organic loading rate (OLR) decreased. The autotrophic activity values of

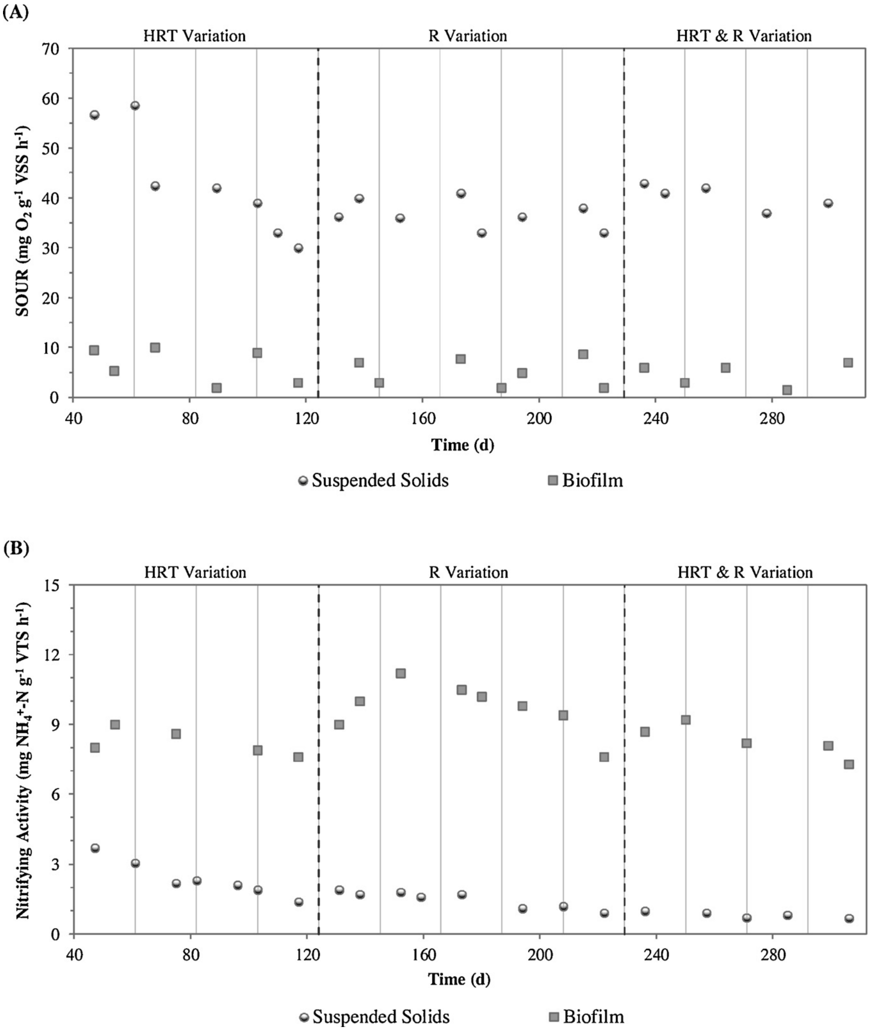

Fig. 5. Heterotrophic and autotrophic activities of suspended and attached biomass through experiment, SOUR (A) and nitrifying activity (B).

attached biofilm were significantly higher compared to the suspended biomass (between 7 to 11 mg NH_4^+-N g^{-1} VTS·h^{-1}). On average, the mean nitrifying activity of biofilm was 4 times superior to the nitrifying activity of suspended biomass. Roughly 80% of nitrification was accomplished by the attached biofilm and 20% by suspended biomass. The results suggested that suspended biomass exhibited better COD removal. On the other hand, the attached biomass displayed a higher nitrification capability likely due to the biomass retention time of the biofilm on the carriers which is decoupled from the HRT.

3.5. Biofilm formation

The attached biofilm thickness at Ae-1 compartment is shown in Fig. 6. Within 40 days (start-up period), most of the carriers were fully covered by biofilm (determined by microscopy). Biofilm is a matrix of metabolic activity of cells and extracellular compounds [55,56]. Factors such as material and shape of carriers, pH, nutrient levels, iron, oxygen, temperature, microbial activity, extracellular polymeric substances (EPS) concentration, etc. play important roles in formation rate and microbial community composition of biofilm [57]. The estimated averaged thickness of the biofilm, at Ae-1 compartment, was 1.2 ± 0.1 mm and the density was 32 gVSS/L. During day 40–61, biofilm and floc concentrations increased in all compartments (Fig. 7) due to higher total organic loading rate, enhancement of organic loading rate can accelerate the proliferation of biomass in biocarriers [58], while, from day 62 to 125, there was a decrease in both suspended and biofilm biomass concentrations owing to the increase of the total HRT. Similarly, Muda et al. [59] found that higher total HRT resulted in the reduction of both suspended and biofilm biomass concentrations. Through days 147–231, there was a substantial decrease in biofilm concentration in Ao-1 compartment due to R increase which promoted turbulence in the compartment. Shear force is an important factor in biomass accumulation, and microorganisms detach easily to the bulk

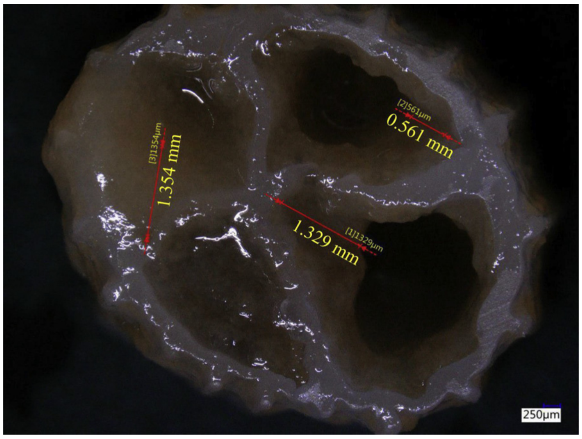

Fig. 6. The attached biofilm development and thickness on the carriers in Ae-1 compartment.

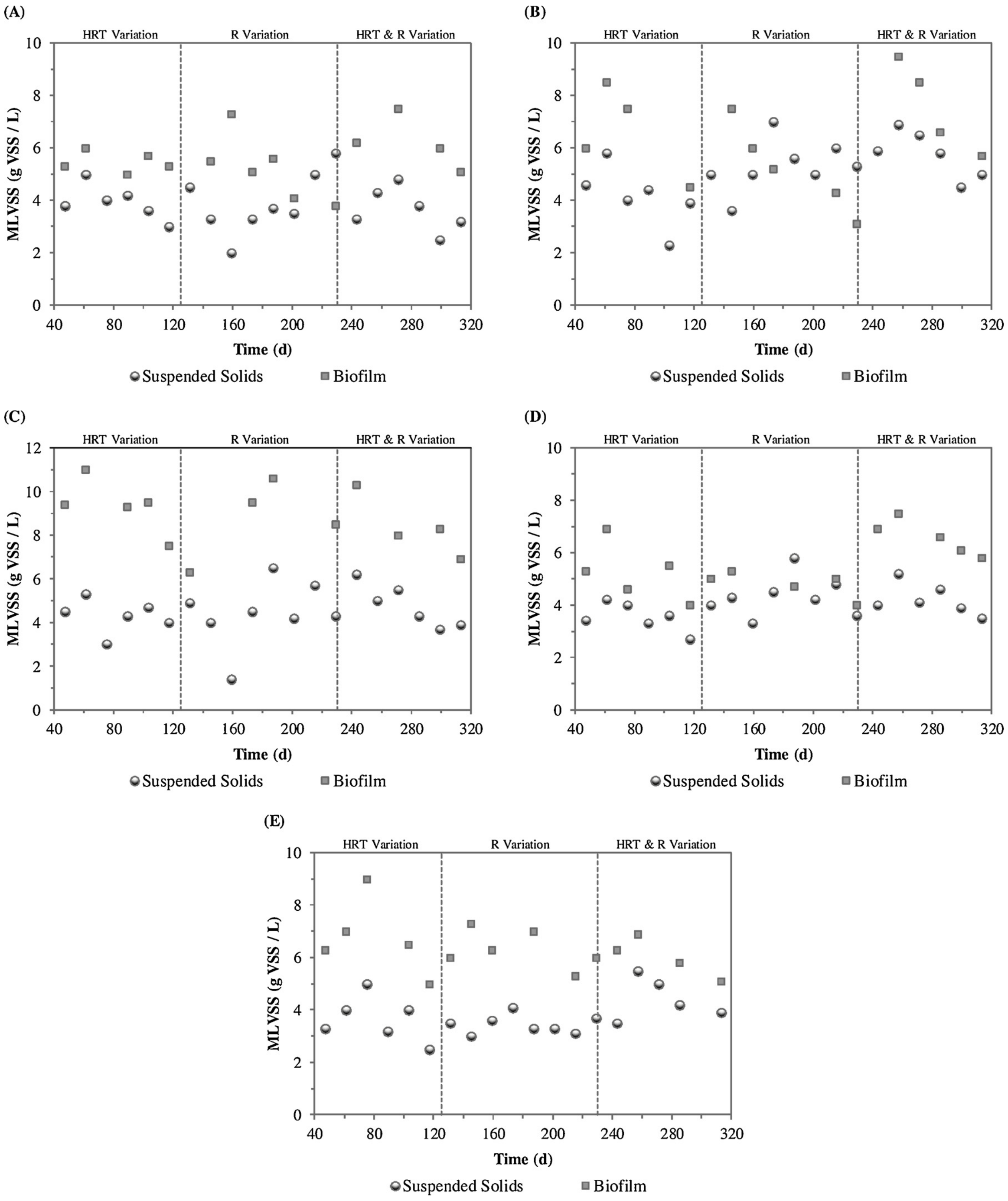

Fig. 7. The Suspended and biofilm biomass concentration at AN (A), Ao-1 (B), Ae-1 (C) Ao-2 (D) and Ae-2 (E) compartments.

liquid with higher shear force [60]. Correspondingly, there was an increase in suspended biomass concentration originated from the biofilm detachment. Biofilm and suspended biomass concentrations displayed a minor fluctuation during runs 5 to 9 (R variation) in other compartments. Fig. 8 illustrates the mean values of the suspended and biofilm concentrations inside all compartments. Biofilm concentration at aerobic compartments (Ae-1 and Ae-2) is higher than in other compartments. The secretion and release of extracellular polymeric substance (EPS) by microorganisms can have an effective role in the stability of biofilms against hydraulic stresses [61]. EPS had great influences on biofilm formation and adhesion [19]. Previous studies reported that some microorganisms had a stronger EPS secretion capacity under aerobic environment compared with anoxic and anaerobic environments [62,63]

Recently, in order to provide an insight on the cost effectiveness of nutrient removal, Bashar et al. [64] considered six full-scale treatment scenarios focused on TP. The processes considered were: (S1) Modified University of Cape Town (MUCT) process, (S2) 5-stage Bardenpho Process, (S3) membrane bioreactors (MBRs), (S4) Integrated Fixed-Film Activated Sludge Systems with biofilm Enhanced Biological Phosphorus Removal (IFAS-EBPR), (S5) struvite recovery by chemical precipitation, and (S6) tertiary media filtration. Although 5-stage Bardenpho process appeared to be a very attractive option for enhanced N removal, the cost for P removal ($46.01/lb-P removed) was higher than most of the other scenarios (S1 $42.25/lb-P, S4 $42.22/lb-P, S5 $44.60/lb-P, S6 $44.04/lb-P). MBRs resulted in the highest cost of all scenarios evaluated. IFAS-EBPR was one of the most cost-effective configuration due to low chemical requirement and flexibility to adjust SRT with the

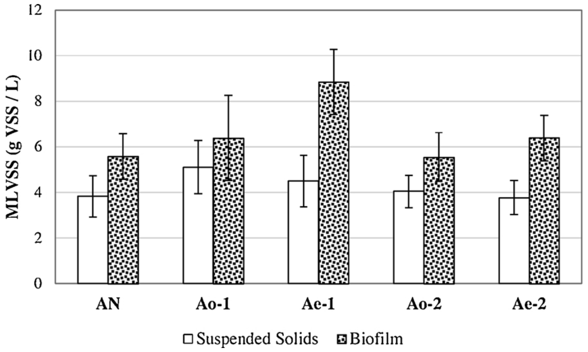

Fig. 8. Suspended and biofilm biomass concentrations inside all process compartments.AN: anaerobic, Ao-1:anoxic 1, Ae-1: aerobic 1, Ao-2: anoxic 2, Ae-2: aerobic 2.

contribution of the biofilm without impacting the nitrogen removal capacity of the system. Therefore, it can be inferred that hybrid 5-stage Bardenpho - MBBR might be even more cost effective for both TN and TP removal than the aforementioned technologies.

4. Conclusions

The hybrid 5-stage Bardenpho - MBBR system exhibited maximum performance for both organics and nutrients removal under a total HRT of 15.74 h corresponding to an HRT in the anaerobic compartment of 2 h and a nitrate recycle ratio of 2. Satisfactory COD, NH_4^+-N, TN and TP removal efficiencies were obtained of about 98.20%, 96.50%, 92.54% and 94.70%, respectively. Two quadratic response surface models for TN and TP removal displayed a satisfactory and statically significant fitting between the experimental and the predicted values. At a constant HRT, TN removal was promoted when R increased from 0 to 2. TP removal presented a positive correlation with higher values of R. Moreover; the attached biofilm enhanced the TN and TP removal by simultaneous nitrification-denitrification (SND) and denitrifying phosphorus removal. Nitrification was 80% accomplished by the attached biofilm and 20% by the suspended biomass.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jece.2018.102861.

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