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A high-rate A2O bioreactor with airlift-driven circulation and anoxic hybrid growth for enhanced carbon and nutrient removal from a nutrient rich wastewater

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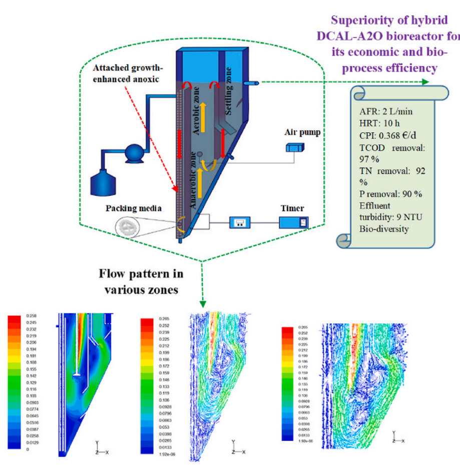
HIGHLIGHTS

- The one-stage hybrid DCAL-A2O bioreactor was superior in simultaneous CNP removal.
- Cooperative consortia of anammox and denitrifying bacteria were harvested in a single unit.
- Growth of PAOs consortium was realized in the one-stage hybrid DCAL-A2O bioreactor.
- The cost-saving and efficient process performance were smoothly feasible in the bioreactor.

GRAPHICAL ABSTRACT



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ABSTRACT

Within this research, a one-stage hybrid dual internal circulation airlift A2O (DCAL-A2O) bioreactor was designed and operated to simultaneously remove carbon, nitrogen and phosphorous (CNP) from milk processing wastewater (MPW) in different operational circumstances. The substantial operating variables monitored in this work were including hydraulic retention time (HRT), airflow rate (AFR) and aeration volume ratio (AVR) ranged from 7 to 15 h, 1–3 L/min and 0.324–0.464, respectively. From the view point of economics and process function, the optimum conditions were obtained at the HRT, AFR and AVR of 10 h, 2 L/min and 0.464, respectively. At the optimum conditions TCOD, TN, TP removal efficiencies and effluent turbidity were reported to be 97 %, 90 %, 92 % and 9 NTU, respectively. The impact of wastewater biodegradability (BOD_5/COD) was

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evaluated on the bioreactor performance using two other wastewaters i.e. soft drink (SDW) and soybean oil plant wastewaters (SOW) in comparison with the MPW. Removal efficiencies for TCOD and TN exceeding 80 % were observed. The feeding location revealed a prominent impact on the TN and phosphorous removal efficiencies (both ≥80 %) related to the availability degree of the readily biodegradable organic substrate to denitrifiers and PAOs. The rise in HRT, AFR and AVR resulted in reducing microbial secretions as SMP present in sludge and bioreactor effluent as well as loosely bounded EPS (LB-EPS), reported to be 26, 28, 32.5 and 194.4 mg/L TOC, respectively. Different bacteria species were present at optimum conditions confirming concurrent CNP removal in a single body. Finally, the operating cost evaluation verified the effectiveness of the hybrid airlift A2O treating the MPW.

1. Introduction

The simultaneous organic and nutrient removal-based processes have attracted considerable attention by water and wastewater's specialists and environmental engineers (Asadi et al., 2016a). Since the presence of excess nutrients in aqueous bodies provides the proper environment for the growth of algae and therefore eutrophication phenomenon (Asadi et al., 2016a).

It is well argued that the biological treatment processes, particularly biological nutrient removal (BNR), are the most widely implemented to purify wastewater effectively (Asadi et al., 2016a). To remove organics and nutrients, the conventional BNR systems require three successive compartments (Yilmaz et al., 2007). These facilities lead to the increase in space and footprint for construction and infrastructure, thus, bioreactor volume and hydraulic retention time (HRT) are increase which has caused lower reactor volume yield in compared with the high rate bioreactors (Chan et al., 2009; Bonakdari and Zinatizadeh, 2011). Moreover, additional equipment such as the use of high number of mixers in anaerobic and anoxic compartments, internal recycling pump and sludge recycling pump are employed (Metcalf, 2003). Therefore, the increase in costs of operating, capital and investment along with high energy consumption due to the mentioned cases have limited the further employment of the BNR systems. With this line, recently, the high rate single bioreactors are being developed successfully in order to concurrently eliminate organic and nutrient contents from the various wastewater and reduce the enumerated challenges (Abyar et al., 2018; Amini et al., 2013; Asadi et al., 2012a, 2012b; Mansouri and Zinatizadeh, 2017; Rezaee et al., 2015, 2016). Despite excellent functionality of the proposed bioreactors, the complexity in the operation owing to control of intermittent aeration and discharge is known as limiting factor in scale-up studies. In addition, the formation of granular sludge is time-consuming (Guo et al., 2021), and the use of ultrasound transducer is not practical in large-scale applications.

More recently, airlift bioreactors (ALBs) as another generation of the high rate bioreactors have gained more interest in the wastewater treatment (Asadi et al., 2016a, 2016b; Mirghorayshi et al., 2021a). These bioreactors possess superior performance in simultaneous removal of carbon and nutrients from the various wastewater (Asadi et al., 2016a, 2016b; Mirghorayshi et al., 2021a). Asadi et al. obtained the sCOD, TN and TP removal efficiencies of 98 %, 85 %, and 78 % under optimum conditions (HRT of 10 h and AFR of 2 L/min) treating milk processing wastewater (MPW) in an airlift bioreactor with continuous feed and intermittent discharge (CFID) regime (Asadi et al., 2016a). The authors, in the same bioreactor, could to get the COD, TN and TP removal efficiencies of 98 %, 72 % and 65 %, respectively, treating soft drink wastewater (SDW) under optimum conditions (HRT of 12h, DO concentration of 3.5 mg/L and internal to external diameters ratio of the column (D_{in}/D_{out}) of 0.5) (Asadi et al., 2016b). The ALBs agitated pneumatically possess distinguished features such as simple design, effective and complete suspension of three phases (solid-liquid-gas) with low energy consumption (Mirghorayshi et al., 2021a; Shi et al., 2020). These bioreactors benefit from controllable mixing, lack of dead stagnant volumes, and high mass transfer (Shi et al., 2020). More importantly, these bioreactors have low shear stress due to well-organized flow pattern that is suitable for sensitive microorganism with attached growth (Shi et al., 2020). In another similar study, the effect of various ratios of NH_4^+-N/(NH_4^+-N + NO_3^--N) (0.25–0.75) and varied HRTs (10–20h) was evaluated on the different mechanisms of the nitrogen removal from the SDW in the same bioreactor (Mirghorayshi et al., 2018). In this study, TN and sCOD removal efficiencies were reported to be 76 and 88 %, respectively, at the optimum conditions (HRT of 20h and NH_4^+-N/(NH_4^+-N + NO_3^--N of 0.25). The nitrogen removal mechanisms were found to be anaerobic ammonium oxidation (Anammox), simultaneous nitrification-denitrification (SND), and presumable dissimilatory nitrate reduction to ammonium (DNRA). While the uniquely configured ALBs demonstrated exceptional performance in simultaneous CNP and CN removal, the CFID regime requires close monitoring to precisely control aeration and discharge times.

Once pursuing a high capacity in high strength wastewater treatment through single bioreactors, substantial perquisite is retaining ample sludge as biocatalyst in the bioreactor. As is broadly known, culturing granular sludge (Chen et al., 2019), formation of biofilm on the packing media (Li et al., 2017) and the use of membrane in the membrane bioreactor (Huang et al., 2016) are fundamental strategies to increase the microbial populations. These strategies reduce HRT and footprint, increase the biomass retention time (SRT), decrease the sludge production and make the monitoring easy (Guo et al., 2021). Despite the relative successes in the usage of the granular sludge and membrane, some serious drawbacks such as being time-consuming and the membrane fouling are mentioned (Cao et al., 2017; Chen et al., 2019; Ding et al., 2018). Although the attached growth system is widely and smoothly adopted in practice, applying proper substrate loading and mass transfer, and low shear stress are vital in order to grow the biofilm and prevent sloughing (Mirghorayshi et al., 2021a). Due to outstanding ALBs' features mentioned in the preceding paragraphs, these bioreactors are able to provide the suitable conditions for the attached growth (Mirghorayshi et al., 2021a). Guo et al. succeeded to culture the granular sludge with exceptional properties (concentrated, highly dispersive, and easily settleable) using hydroxyapatite (HAP) (Guo et al., 2021). The studies were carried out in a one-stage airlift bioreactor to achieve considerable nitrogen removal rate (NRR) and phosphorous recovery at short HRT via partial nitrification-anammox process. The authors reported the nitrogen removal rate (NRR) of 1.28 kg/m^3.d at short HRT of 1h when treating low strength ammonium wastewater with influent NH_4^+-N of 63 mg/L. The researchers in their studies tried to apply bio-carriers (with influent NH_4^+-N of 50 mg/L and nitrogen removal efficiency (NRE) of 70 %) (Chen et al., 2021b), micro-granules (with influent NH_4^+-N of 50 mg/L and NRE of 72.7 %) (Chen et al., 2021a) and hydroxyapatite (HAP)-based syntrophic granules in a one-stage airlift bioreactor (with influent NH_4^+-N of 1140 mg/L and NRE of 88.2 %) (Chen et al., 2021).

Liu et al. for the first time upgraded the symbiotic relationship between Nitrosomanas and anammox bacteria using attached growth in a new single-stage ALB through partial nitritation–anammox process (Liu et al., 2017). This approach achieved a NRE of 81.1% at a DO level below 0.3 mg/L and a nitrogen loading rate (NLR) of 0.5–2.5 kg NH_4^+-N/m^3·d. Wang et al. successfully achieved the high process performance as nitrogen removal efficiency (82 %) at NLR of 0.5–2.5 kg

$N/m^3.d$ in a single-stage ALB by culturing micro-granule (Wang et al., 2017). Mirghorayshi et al. proposed a new hybrid airlift bioreactor with the attached growth developed in anoxic zone, and anaerobic compartment installed at the base of the bioreactor (Mirghorayshi et al., 2021a). The anaerobic compartment was for hydrolysis and acidification process in order to help solubilize young composting leachate containing refractory compounds and promote the nitrogen removal. Under optimal conditions with a HRT of 28.3–30 h and AFR of 1.7–2 L/min, the authors achieved COD and TN removal efficiencies of 90% and 80%, respectively, with an effluent turbidity below 70 NTU. However, in culturing hydroxyapatite-based granular sludge, external chemicals must be added into the inlet wastewater that makes the secondary pollution. In addition, preserving dissolved oxygen concentration at low level for having partial nitritation-anammox mechanism requires more care. Furthermore, in the study of Mirghorayshi et al. (2021a), threading plastic packing media and spinning those around the internal tube would create channeling phenomena over long-term operation (Mirghorayshi et al., 2021a).

Inspired from the literature, in this report, an innovative hybrid dual internal circulation airlift A2O (DCAL-A2O) bioreactor with unique geometry upgraded with a dual-internal circulation and anoxic zone filled with bio-carriers was proposed. The bioreactor in novel configuration was designed to enhance the bioreactor's capacity as simultaneous CNP removal from the milk processing wastewater (MPW). The effect of three independent operating variables namely HRT, AFR and aerobic volume ratio (AVR) was assessed on the function of the concerned bioreactor. The highlighted features of the referenced bioreactor were the use of the bio-carriers in the anoxic zone in order to promote N & P removal via the increase in the hydraulic resistance and contact time of biomass with substrate. The presence of packing media in the anoxic zone provides a safe place for the attached microbial community due to indirect accessibility to bubbling zone, and therefore, reduction in the shear stress.

2. Materials and methods

2.1. Wastewater compositions and inoculum sludge

The bioreactor was fed with wastewater taken from Bisetoon factory producing milk processing products (Bisetoon, Kermanshah, Iran). At the optimum operating conditions obtained treating the milk processing wastewater (MPW), the performance of the bioreactor was investigated by the two other wastewaters with different biodegradability; soft drink wastewater (SDW) and soybean oil plant wastewater (SOW). Soft drink wastewater (SDW) was collected from Zamzam Company (Kermanshah, Iran) and soybean oil plant wastewater (SOW) was provided from cultivation and industry of vegetable oil (Mahidasht, Kermanshah, Iran). The wastewaters were stored at temperature below 4 °C in a cool room to prevent any changes in the characteristics. The compositions of the various wastewaters are given in Table S1. Due to the low contents of phosphorous (PO_4^{3-}-P) and ammonia nitrogen (NH_4^+-N), KH_2PO_4 and NH_4Cl were added to the feed as supplemental additives to set COD:N:P at 100:13.3:5. The concentrations of NH_4^+-N and PO_4^{3-}-P were in the range of 190–220 mg/L and 63.2–75.7 mg/L, respectively. In the case of the SOW, owing to existence of high level of the phosphorous in the raw wastewater (156.1–192.4 mg/L), did not need to add any external phosphorous source such as KH_2PO_4. The inoculum sludge was provided from aeration tank of a local municipal wastewater treatment plant, Kermanshah, Iran.

2.2. Bioreactor set-up description

Experimental set-up was a single-stage hybrid dual circulation airlift A2O (hybrid DCAL-A2O) bioreactor comprised of a reaction unit with effective volume of 7 L in combination with a settling unit with effective volume of 2 L. The sludge circulated between the settling unit and the reaction unit via the junction channel and internal circulation due to airlift driving force. The schematic of experimental apparatus is displayed in Fig. 1. The reaction unit itself is divided into three zones i.e. anaerobic, anoxic and aerobic zones in an integrated structure. The various zones were in connection with each other through another internal circulation. An air pump with an air stone fixed at various heights of aerobic zone in order to adjust the ratio of aerobic volume to total volume (AVR) was employed to provide the required oxygen and adequate agitation. The air flow rate (AFR) was monitored using an air flow gauge. Two mixers installed in the anaerobic zone were turned on for 2 min once every 2 h in order to ensure the enough mixing. The mixers were monitored through a programmable automated timer. The incoming wastewater was continuously supplied to the bioreactor from the top of anoxic zone using a peristaltic pump according to the feed flow rate regulated by various hydraulic retention time (HRT). The anoxic zone was filled using plastic carriers (Kaldnes K2 with diameter of 15 mm and a specific area of 360 m^2/m^3) as packing media threaded using fishing yarn as six strings. These packing media strings were hanged out inside the anoxic zone with filling ratio of 30 %. To facilitate the fast development of the biofilm, the packing media were firstly immersed inside 2 wt % agar solution to make their surface sticky, and thereby, attach the microorganism.

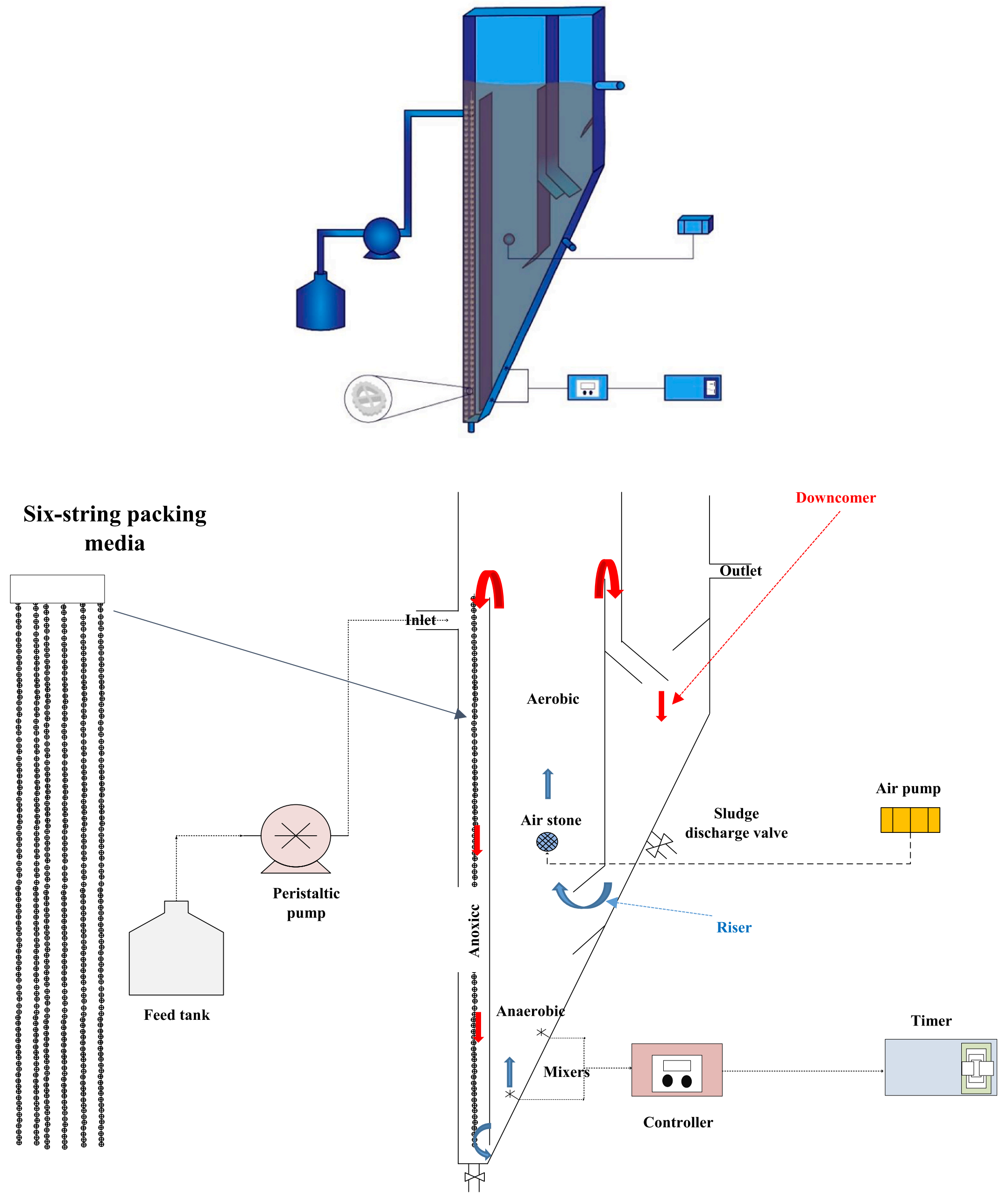
2.3. Experimental design, mathematical modeling and statistical analysis

The design of the experimental conditions, statistical data analysis and determination of bioprocess optimization area were all performed using Design-Expert® software (Stat-Ease Inc., Version 10.0). To get these goals, a central composite design (CCD) of response surface methodology (RSM) was employed. Three numerical effective variables were considered in this study to evaluate the performance of bioreactor in simultaneous CNP removal including hydraulic retention time (A: HRT), air flow rate (B: AFR) and the aeration volume to the total working volume ratio (C: AVR). The HRT reflects indirectly feed flow rate, subsequently, organic/nitrogen/phosphorous loading rate (OLR/NLR/PLR). The AFR influences the superficial gas velocity (J_G) and the dual internal circulation rates between anoxic-anaerobic-aerobic zones and aerobic zone and settling section. The supply of appropriate AFR and the adjustment of volume proportion of various zones counterbalance variant biological reactions i.e. nitrification, denitrification processes and phosphorous removal. The range and level of the controlled independent variables are indicated in Table S2. According to the factorial design and the number of the variables used in this study, the CCD forms a cubic with 2k axial points, 2^k factorial points and 1 central point plus some replications for the central point (here 5 replications) where k is representative of the variables number. The sum of these points determines the number of experimental runs. In overall, 20 experimental runs were defined including 6 axial points, 8 factorial points and 1 central point along with 5 replications as summarized in Table S3.

TCOD removal efficiency, TN removal efficiency, phosphorous removal efficiency, effluent nitrate ($N\text{–}NO_3^-$), effluent turbidity, and sludge properties in terms of bio-flocculation and settleability using sludge volume index (SVI) and the measure of the effluent SMP, sludge SMP, EPS including loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) are considered as the bioprocess responses (or the dependent variables). These responses were fitted with the model to indicate the relationship between independent and dependent variables as well as determination of polynomial coefficients using the following regression equation.

$$Y = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_{ii} X_i^2 + \beta_{jj} X_j^2 + \beta_{ij} X_i X_j \ldots\ldots \tag{S1}$$

where, Y, β_0, and β with different subscripts of i and j stand for the relative response, the intercept, and linear and quadratic regression coefficients depending on the order of X, respectively. For appraisement



(c)

Fig. 1. The schematic of experimental apparatus.

of the effectiveness of the model terms, P-value with confidence level of 95 % was considered. The obtained experimental data were processed using analysis of variance (ANOVA) by Design Expert Software (DOE). Polynomial equations attained on the basis of the coded values (−1, 0, +1) are employed to predict the concerned responses in the design space. These polynomial equations were chosen based on some significant statistical parameters showing degree of fitness of the predicted data and actual data. For example, the high determination coefficient (R^2), adjusted R^2 (Adj. R^2), predicted R^2 (Pred. R^2) (showing lower random noises); high adequate precision (Adeq. Precision) (signal to noise ratio); high coefficient of variation (C.V., showing the standard deviation as a percentage of the mean); high F-value (model's mean square to the mean square error ratio); and low probability values (P-value) reveal the high accuracy and reliability of the proposed models. The regression equations and the results of data analysis obtained from ANOVA for the whole responses after elimination of insignificant model terms were given in Table S4. Three-dimensional (3-D) response surface plots for each response were portrayed to study the simultaneous interaction of the controlled effective variables on the relevant responses.

2.4. Operational procedure

In an overall view, the laboratory studies were conducted during six phases as shown in Table 2.

At the end of start-up phase, the formation of the biofilm with a thickness of ca. 400 μm was observable on the packing media surface. The mixed liquor suspended solids (MLSS) was in the range of

Table 1
Results of verification experiments obtained at optimum operating conditions for the hybrid DCAL-A2O bioreactor.

Optimum conditions		Feasible responses			
		TCOD removal, %	TP removal, %	TN removal, %	Effluent turbidity, NTU
HRT = 10.0 h AFR = 2.0 L/min AVR = 0.464	Experimental value	97.0	90.0	92.0	9.0
	Model values	92.6	80.1	80.1	7.8
HRT = 10.0 h AFR = 2.5 L/min AVR = 0.394	Experimental value	97.3	85.6	84.3	12
	Model values	93.0	86.1	80.1	9.8

Table 2
Various experimental phases.

Phase no.	Duration, day	Objectives	Operating conditions
I	30	Start-up and development of the biofilm on the packing media	HRT: 7h AFR: 3 L/min AVR: 0.394
Ii	240	Investigation of the bioreactor's capacity in concurrent removal of CNP in a single-stage unit using the milk processing wastewater (MPW)	Designing 30 runs according to Design-Expert® Software (DOE) under HRT: 7–15h AFR: 1–3 L/min AVR: 0.324–0.464
Iii	10	Investigation of the bioreactor's capacity in concurrent CNP removal under the optimum conditions	HRT: 10 h AFR: 2 L/min AVR: 0.394
iV	14	Assessment of the bioreactor's functionality treating various wastewaters with the various biodegradability (as BOD_5/COD ratio) under optimum conditions	HRT: 10 h AFR: 2 L/min AVR: 0.394
V	7	Evaluation of feeding location impact on the process performance of the bioreactor	HRT: 10 h AFR: 2 L/min AVR: 0.394
Vi	7	Investigation of long term performance of the bioreactor	HRT: 10 h AFR: 2 L/min AVR: 0.394

6070–6481.6 mg/L by discharging the surplus sludge daily to keep it approximate 6000 mg/L. The reasonable rationing of the compressed air into the aerobic zone of the hybrid airlift A2O bioreactor provided desired dissolved oxygen (DO) concentration. The suitable DO level provides the anaerobic, anoxic and aerobic zones to cultivate diverse microbial populations (polyphosphate-accumulating organisms (PAOs), nitrifiers, and denitrifiers). The formation of anaerobic and anoxic conditions was guaranteed via the bio-carries occupying the anoxic zone and increasing the hydraulic resistance. The feed pH was maintained around 7.0–7.5 by adding $NaHCO_3$ and NaOH solution. Experimental design, mathematical modeling and statistical analysis are described in SI. In addition, the procedures used to extract soluble microbial products (SMP) and extracellular polymeric substances (EPS) (comprised of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS)) as well as the protein and carbohydrate contents, and TOC values are presented in recently published paper in details (Rahimi et al., 2024). Besides, to identify the diversity of microbial species, protocols of DNA extraction and PCR amplification are explained comprehensively in the recently published paper (Rahimi et al., 2024).

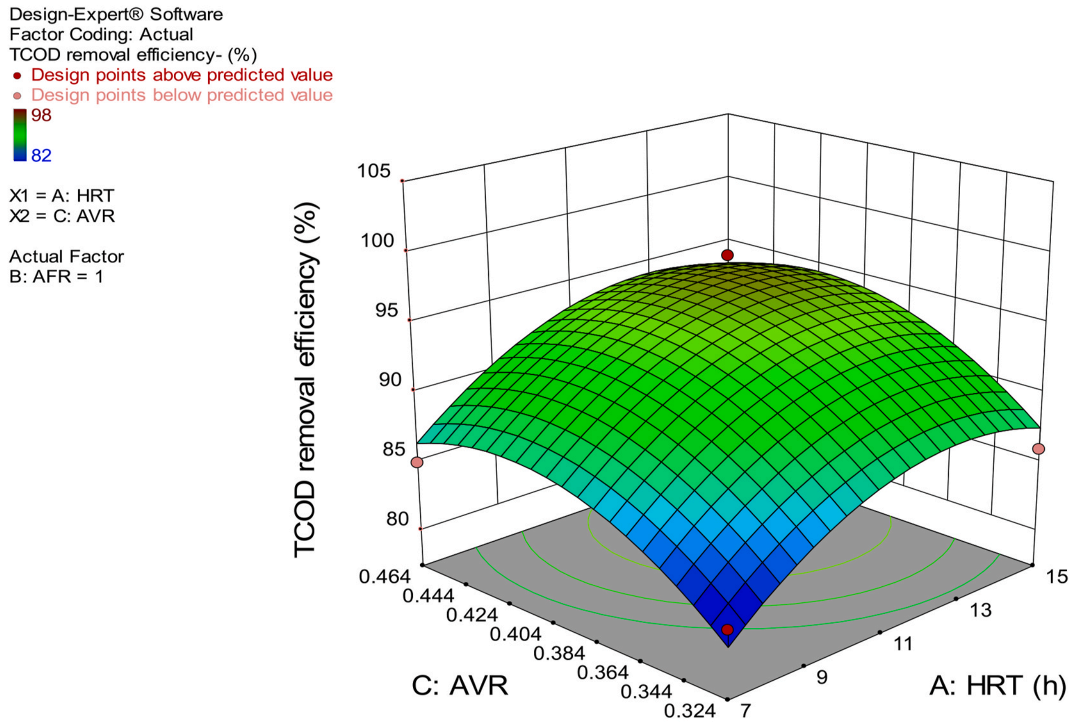
2.5. Analytical approaches

The water samples were taken out from both influent and effluent of the bioreactor to analyze the following parameters. The concentrations of TCOD (using closed reflux, colorimetric method), BOD_5/BOD_{21} (using OxiTOP IS 6 system, WTW, Germany), NO_2^--N, NO_3^--N $^-$, NH_4^+-N, total kjeldhal nitrogen (TKN), organic nitrogen (N-Org), TN, PO_4^{3-}-P and MLSS were detected daily according to the Standard Methods (RBB and Eaton, 2017). The concentrations of TCOD, NO_2^--N, and PO_4^{3-}-P were quantified using spectrophotometer (Jenway 6320D visible range spectrophotometer) at wavelengths of 600, 540, and 690 nm, respectively. The amount of NO_3^--N was determined at the UV region using UV–Vis spectrophotometer at wavelengths of 220 and 275 nm (Agilent 8453 UV–Vis spectrophotometer). The quantity of TKN and NH_4^+-N was measured by TKN meter (Gerhardt Model, Vapodest 10, Germany). N-Org was obtained via deducing NH_4^+-N from the content of TKN. The corresponding meters i.e. portable DO probe (WTW DO Cell OX 330, Germany), air flow meter (101325 Pa), pH meter (Barloworld Scientific Ltd. T/As Jenway 3510, Essex UK), and turbidity meter (2100 P, Hach Co., USA) were employed for the regular control of DO, AFR, pH and turbidity, respectively. The sludge settling properties were studied by measuring sludge volume index (SVI). The amount of oil and grease was calculated on the basis of the solubility in n-hexane described in Standard Methods (RBB and Eaton, 2017).

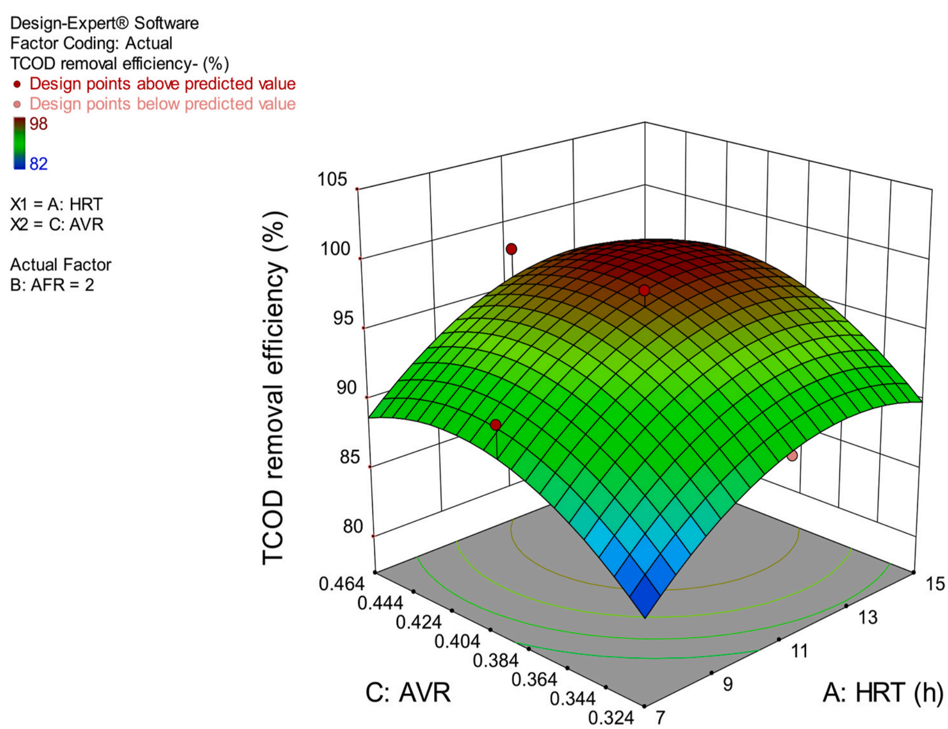
3. Results and discussion

3.1. TCOD removal

From the findings obtained from ANOVA (Table S4), the model terms A (HRT), B (AFR) and C (AVR) revealed the increasing effect on the TCOD removal efficiency. While, the square model terms i.e. A^2 and C^2 left a negative influence on the relevant response. These model terms were chosen based on a beneficial simplification to simplify the interpretation of the proposed equation meanwhile obtaining the desired statistical parameters (Figure S1). According to the results, HRT parameter showed the foremost effect on the response. The impact of HRT and AVR on the TCOD removal efficiency at constant AFR is depicted in Fig. 2a–c as 3-D response surface plots. By increasing the HRT owing to the increase in contact time between substrate and biocatalyst, the aerobic heterotrophic bacteria have the enough opportunity to degrade carbon contents at the presence of adequate DO and appropriate aerobic environment. Another substantial reason was ascribed to unique features of the airlift bioreactors promoting the oxygen bubbles efficiency. Thereby, the hybrid bioreactor revealed the high capacity in TCOD removal efficiency even at the poorly/richly managed situations. The high performance of the hybrid DCAL-A2O bioreactor at the lowest HRT (7 h) (82–85 %) was attributed to the high growth of aerobic heterotrophic bacteria at high F/M (0.82 g-COD/g-MLSS.d) applied to the bioreactor. These observations confirm the high oxidation potential of the proposed bioreactor in the TCOD removal efficiency at all operating conditions. The highest TCOD removal efficiency (98 %) belonged to high AVRs (0.394–0.464), long HRT (11–15 h) and high AFR (2–3 mg/L). Asadi et al. reported the high sCOD removal efficiency at the studied design space (HRTs of 6–14 h and AFR of 1–3 L/min) with the incoming MPW of approximate 1000 mg COD/L in an airlift bioreactor entitled continuous feed and intermittent discharge airlift bioreactor (CFIDALB) as introduced before (Asadi et al., 2016a). Mirghorayshi et al. achieved 90 % COD removal efficiency (equals to 3600 mg/L COD removed) under optimum circumstances (HRT and AFR corresponding to 28.3–30 h and 1.7–2 L/min) in a hybrid

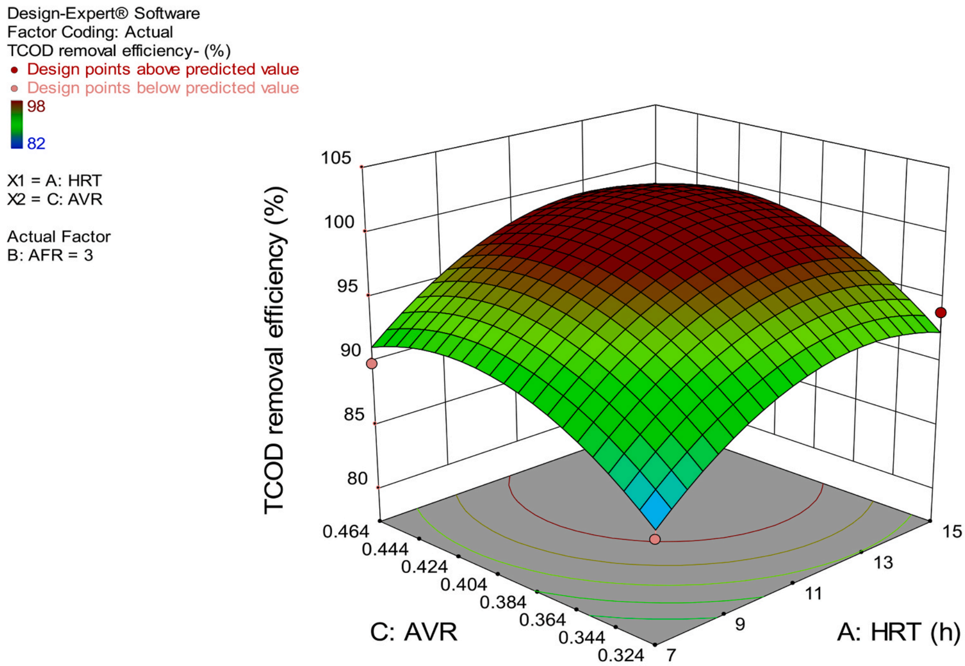


(a)



(b)

Fig. 2. The changes in TCOD removal efficiency under varied AFRs (a–c) and (d) comparison between actual and predicted data.



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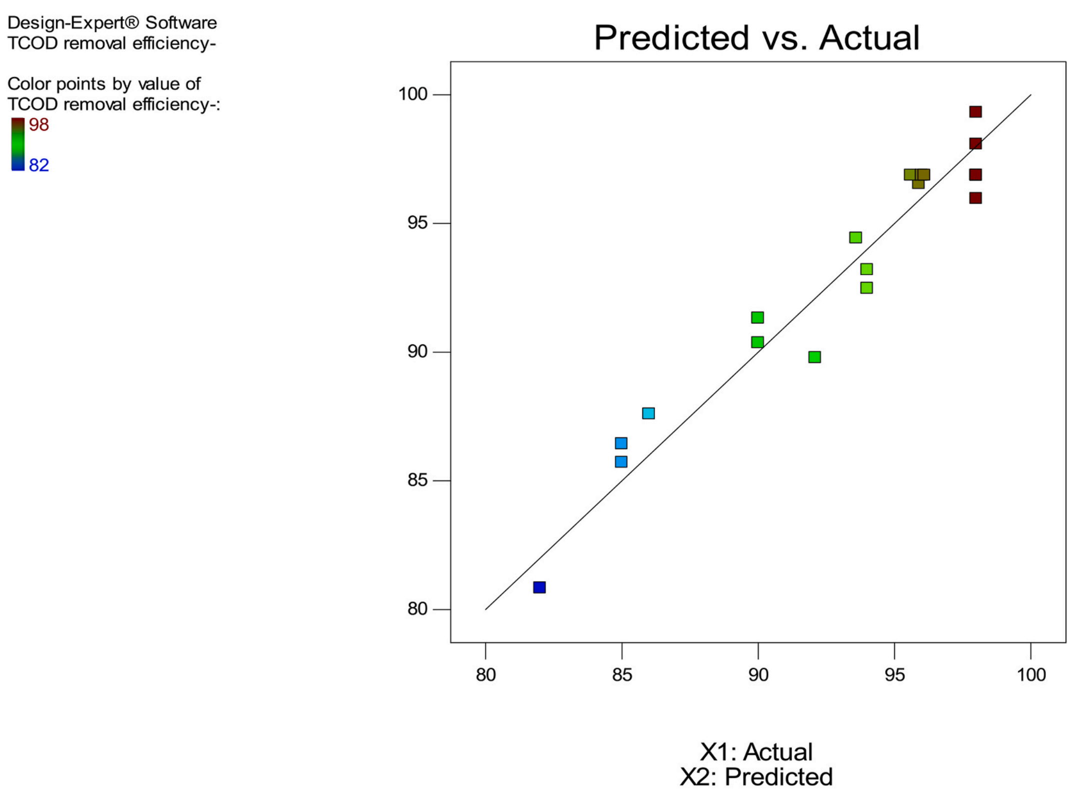
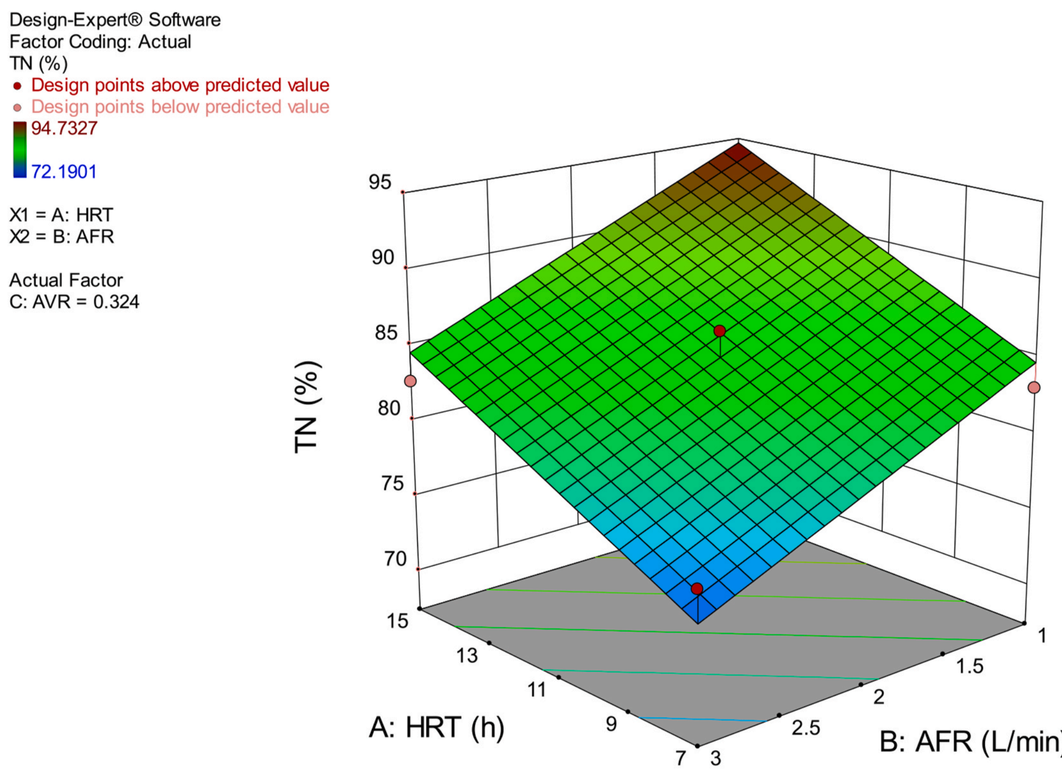


(d)

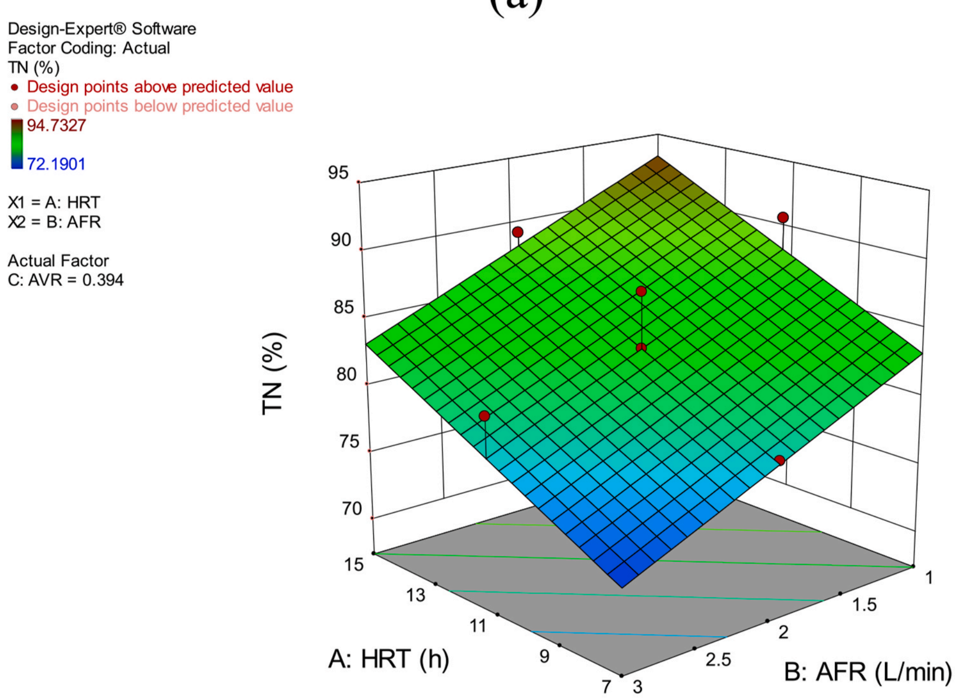
Fig. 2. (*continued*).

airlift bioreactor equipped by an anaerobic chamber to do the solubilization process from young compositing leachate (Mirghorayshi et al., 2021b). A high removal efficiency of COD (97% & 98 %) was reported when using a jet-loop airlift bioreactor to treat SDW with inlet COD of 1000 mg/L (Gholami et al., 2020; Moradi et al., 2021).

In general, the protein and organics containing wastewater creates the suitable media for the microbial growth and therefore the TCOD elimination. Fig. 2d shows that the experimental data are closely aligned

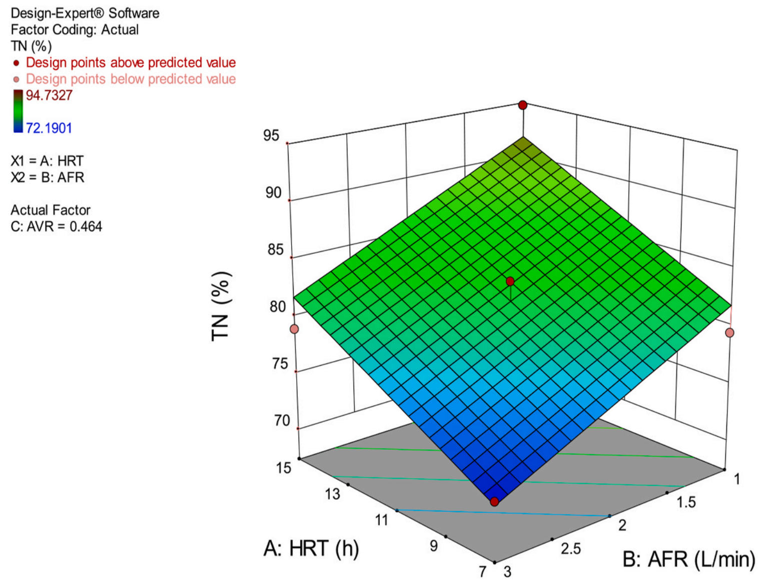


(a)



(b)

Fig. 3. The changes in TN removal efficiency under varied AVRs (a–c) and (d) comparison between actual and predicted data.



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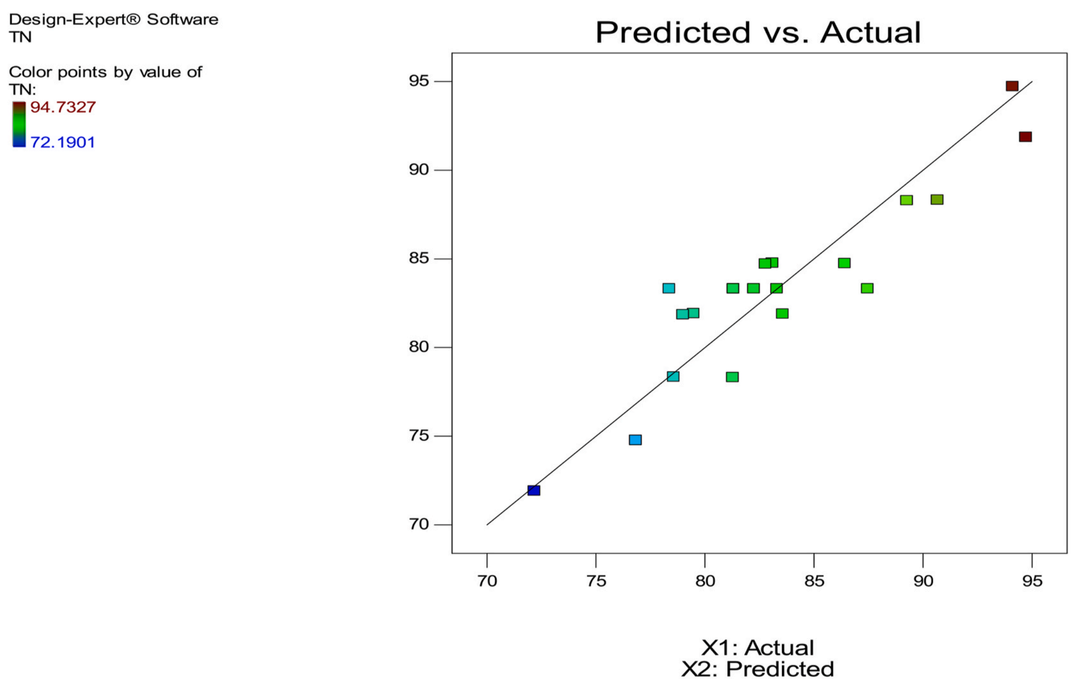


(d)

Fig. 3. (*continued*).

with the straight line representing the predicted data, confirming the model's goodness of fit.

3.2. Nitrogen removal

The nitrogen removal takes place through various mechanisms. In this study, the SND and anammox processes happen in an integrated unit by providing guaranteed anoxic conditions via physical separation created by designing a new geometry of airlift bioreactor and the use of packing media in the anoxic zone. In the hybrid dual internal circulation airlift A2O (DCAL-A2O) bioreactor, the existence of the plastic media in the anoxic part plays two significant roles (i) creation of proper environment for the biofilm formation; (ii) the increase in the hydraulic resistance (reduction in circulation rate) leading to the enhancement in anoxic and anaerobic conditions. In this study, the attached biofilm was developed well at the operating conditions applied due to unavailability of the attached biofilm to the shear stress. In fact, the developed biofilm creates the right conditions for the slow growing anammox bacteria that are responsible for the nitrogen removal under anaerobic conditions.

Based on the Table S4, the linear model proposed was a function of A (with intensive positive effect), B and C with the negative coefficients. In this case, no need to do the beneficial simplification since the proposed model was linear. Fig. 3 illustrates the interactive effects of HRT (A) and AFR (B) at different AVRs (C) as 3-D response surface plots. As indicated in the Figure, TN removal efficiency exhibited similar variations trend under the varied AVRs, ranging from 72% to 95%. As the AFR (B) increased, the circulation rate between the anoxic-anaerobic-oxic zones also increased. This led to an imbalance in the existing zones due to a reduction in hydraulic resistance compared to when lower AFRs were applied. Interestingly, the hybrid dual internal circulation airlift A2O (DCAL-A2O) bioreactor had sufficient oxidation capability for nitrification process even at the lowest AVR due to the supply of the required DO and high depletion of the TCOD. However, the increase in the AVR as the model term C restricts the anoxic conditions for the denitrification process, while, that encourages the aerobic conditions for the nitrification process. In overall, the nitrification process is influenced by various factors such as HRT, AFR, OLR and AVR. In some cases, only one of these factors is more dominant depending on the operating conditions and the bioreactor configuration. In the present study, the nitrogen elimination was further governed by the HRT. The more HRT, the more the TN removal efficiency is obtained. Superiorly, the innovative hybrid airlift A2O bioreactor indicated the distinguished functionality in the TN removal contributed to the unique geometry and the presence of bio-carriers in the anoxic zone. Considering autotroph bacteria.

(nitrifiers and anammox) have slow growth compared to the heterotroph bacteria (denitrifiers and ordinary heterotroph bacteria responsible for BOD removal), long HRT is suitable for enriching these bacteria inside the bioreactor. In addition, the presence of packing media and the biofilm growth led to the increase in the microbial population and creation of anoxic zone. Furthermore, biodiversity in microbial community as coexistence of DPAOs, GAOs, denitrifers, anammox due to unique geometry of bioreactor, attached growth and long HRT resulted in the enhancement in the TN removal efficiency. So that, the TN removal efficiency was above 70 % throughout experimental runs. The reason behind such improved TN removal at low HRT was related to the more biofilm thickness at higher OLR, subsequently, well-developed anoxic environment. In addition, as clear from Fig. 3d, the experimental data were in a close agreement with the predicted data (straight line).

Figure S2 illustrates the nitrogen amounts and the participation of various reactions in TN removal. Generally, the heterotrophic cell growth responsible for the BOD removal represents large share in the nitrogen removal at the richly managed conditions i.e. the lowest HRT (7h), the highest AFR (3 L/min) and AVR (0.464). The cause of the nitrite and nitrate production in the bioreactor's effluent in some experimental runs was related to the increased AFR and AVR disrupting the normal cycle of the denitrification process. Furthermore, the reason of incomplete nitrification and remaining ammonia nitrogen in the effluent were explained by deficiency of aeration and lack of long HRT required for the slow growing autotrophic bacteria. The largest nitrification rate (0.64 g-N/L.d) and denitrification rate (0.62 g-N/L.d) were obtained corresponding to HRT, AFR and AVR of 7 h, 3 L/min and 0.464 (Run no. 14), and 7 h, 1 L/min and 0.324 (Run no. 15), respectively, at the high organic loading rate (OLR: 4.5–4.7 g-COD/L.d), favorable for biofilm growth and development of the anoxic environment.

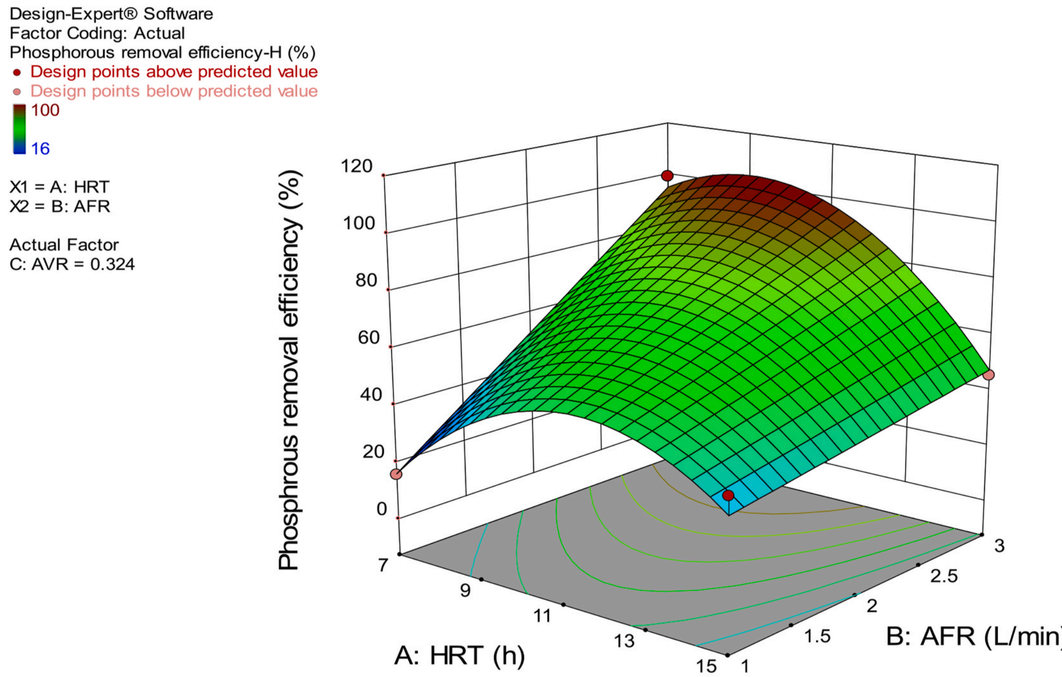
The highest level of denitrification (207.6 mg-N/L) was obtained at HRT of 15 h, AFR of 1 L/min and AVR of 0.324. This high value was owing to the enhancement in anoxic zone as a result of the increase in anoxic space, the reduction in circulation rate due to the presence of bio-carriers, and therefore, the high contact time between feed and formed biofilm. The TN removal efficiency, in overall, was as high as ranging from 72.2 to 94.7 % that reveals the high capacity of the hybrid dual internal circulation airlift A2O bioreactor in the nitrogen removal. Interestingly, the variations trend in the nitrification and denitrification amounts was not similar to the TN removal efficiency, showing another mechanism is involved in nitrogen removal other than SND. This mechanism is attributed to anaerobic ammonium oxidation (anammox) bacteria activity in some experimental runs guaranteed by the presence of the attached growth. Asadi et al. achieved a TN removal efficiency of 85% using an airlift A2O bioreactor treating MPW, with an HRT of 10 h and an AFR of 2 L/min (Asadi et al., 2016c). The authors in another work succeed to get TN removal efficiency of 72 % at HRT, Din/Dout ratio and DO concentration of 12 h, 0.5 and 3.5 mg/L, respectively (Asadi et al., 2016b). This study was conducted in the same airlift bioreactor treating the SDW under various operating conditions. The high TN removal efficiency of 80 % was reported in hybrid airlift bioreactor treating leachate wastewater (Mirghorayshi et al., 2021a). Several researchers investigated nitrogen removal from synthetic or real low-strength ammonia wastewaters (36–63 mg NH_4^+-N/L) by employing partial nitritation and anammox mechanisms (Chen et al., 2021a, 2021b; Guo et al., 2021, 2022, 2023; Rong et al., 2021). T hese mechanisms were facilitated within bio-carriers (NRE of 70 % with inlet ammonium of 50 mgNH_4^+-N/L) (Chen et al., 2021b), micro-granules, and granules cultivated with hydroxyapatite (HAP) (NRR of 1.28 kg/m^3.d with inlet ammonium of 63 mgNH_4^+-N/L and HRT of 1h) (Guo et al., 2021). These experiments were conducted in a one-stage airlift internal circulation bioreactor, with precise control of dissolved oxygen (DO) concentration (ranging from 0.05 to 0.21 mg/L). The proposed bioreactor displayed the best performance in removing the nitrogen (63.38–81.7 % and 1.28 kg/m^3.d) at short HRTs (1, 2, 4h). Furthermore, the bioreactor was able to treat high strength ammonia wastewaters (250–1470 mg/L) with high nitrogen removal (>80 %) at HRTs of 6, 12, 15h, using granular sludge and bio-carriers under low DO concentration (<0.3 mg/L) (Y. Chen et al., 2021; Guo and Li, 2020; Liu et al., 2017; Wang et al., 2017). Although, the introduced bioreactor acted successfully in the treatment of ammonia containing wastewaters, the culture of granular sludge with distinguished feature of tiny size, excellent settleability and concentrated biomass faces some challenges. For example, that is time-consuming, and needs addition of external chemicals (hydroxyapatite (HAP) producing secondary pollution. In addition, the bioreactor performance is very sensitive to the DO concentration and requires more supervision. While, our bioreactor had superior capacity to remove simultaneously CNT from high strength wastewater using hybrid growth at ambient temperature without adding any external enhancing chemicals.

3.3. Phosphorous removal

Benefiting from developing the bioreactor configuration and going forward the single bioreactors, conventional two mechanisms are doable in an integrated unit through simultaneously creating anaerobic and aerobic zones. These advanced bioreactors are of interest from the economic viewpoint owing to the decline in the required space and operating cost.

Table S4 identifies model terms A, B, C, AB, BC, and A^2 as significant factors for assessing the performance of the hybrid dual internal circulation airlift A2O (DCAL-A2O) bioreactor in relation to phosphorus removal efficiency. These model terms were chosen according to their ability to simplify the model while still providing the desired statistical parameters and facilitating ease of interpretation of the proposed model. The results of simplification are shown in Figure S3. Among these significant model terms, only model term B (AFR) exhibited the intensive positive impact on the relevant response.

Fig. 4 showcased the variations trend in phosphorus removal efficiency with the controlled variables as a 3-D response surface plot. By increasing the AFR, the reasonable balance was created between anaerobic and aerobic zones. While, at high HRT, sufficient feed was not available for slow growing heterotrophic bacteria i.e. PAOs. Furthermore, the increment in aerobic zone by the increase in AVR restricted the anaerobic zone. Generally, the presence of the packing media in the anoxic zone triggered the rise in the hydraulic resistance, therefore, the increase in the contact time between the substrate and the functional microbial community (PAOs). In addition, the local anaerobic zones created by the attached biofilm play a crucial role in the phosphorus



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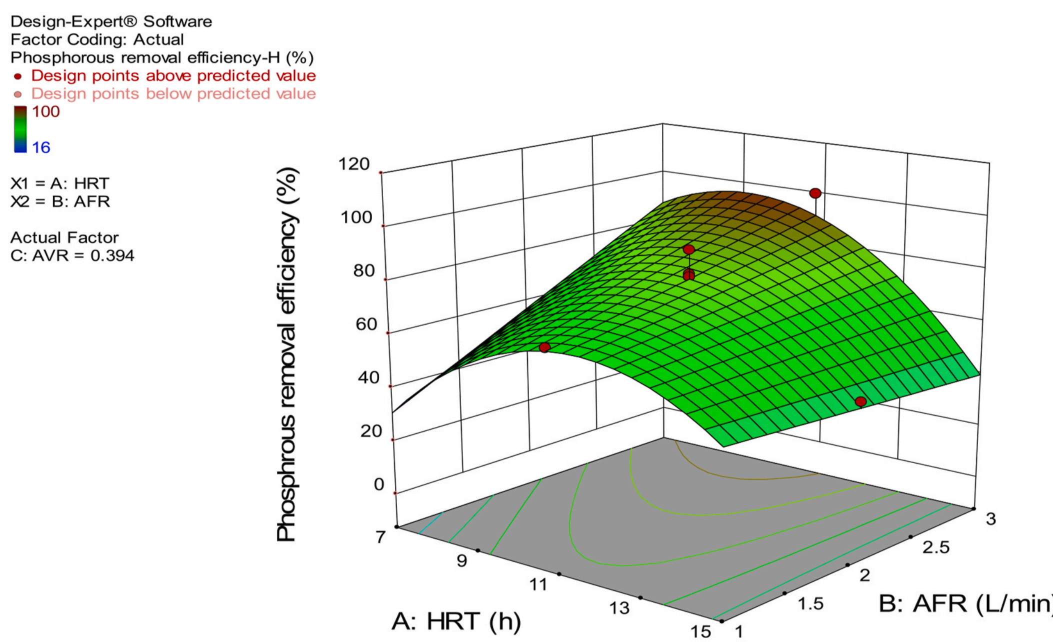
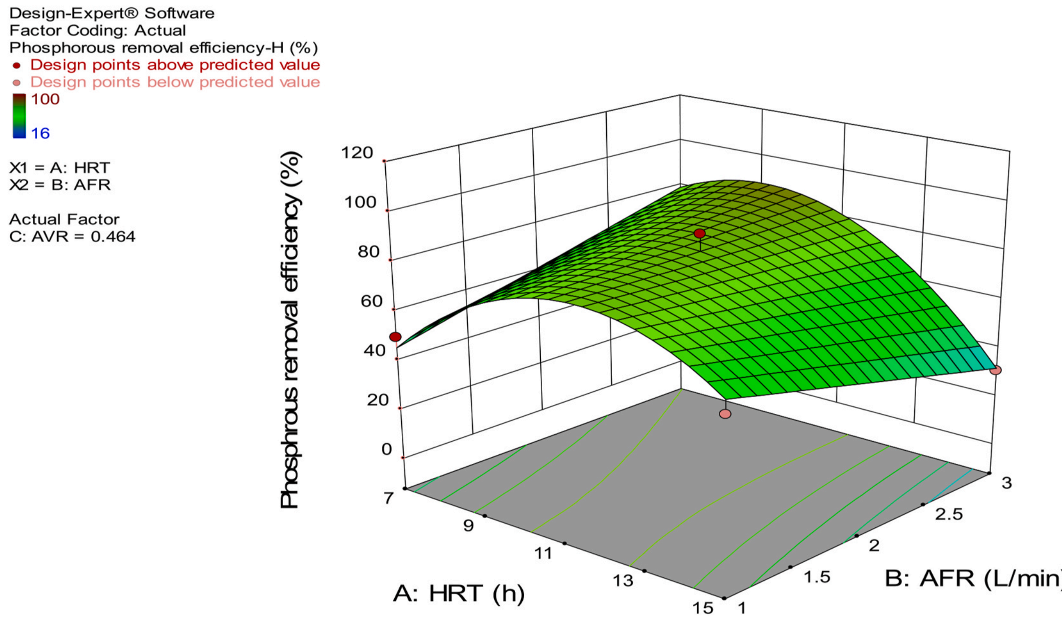


(b)

Fig. 4. The changes in the phosphorus removal efficiency under varied AVRs (a–c) and (d) comparison between actual and predicted data.

removal. On this basis, at the shortest HRT by increasing the AFR from 1 to 3 L/min, the biofilm thickness was increased due to accessibility to the abundant feed (high F/M of 0.98 g-COD/g-VSS.d) that provided proper circumstances for the phosphorus removal.

Another effective impact of the increased AFR was related to the increase in the circulation rate and the transfer of the anaerobic sludge into the aerobic conditions. Since sufficient mixing has a significant effect on the bioreactor's efficacy via the improvement in the mass transfer between feed and biomass (Amini et al., 2013). Totally, the phosphorus removal efficiency was ranged from 16 to 100 %. In a study



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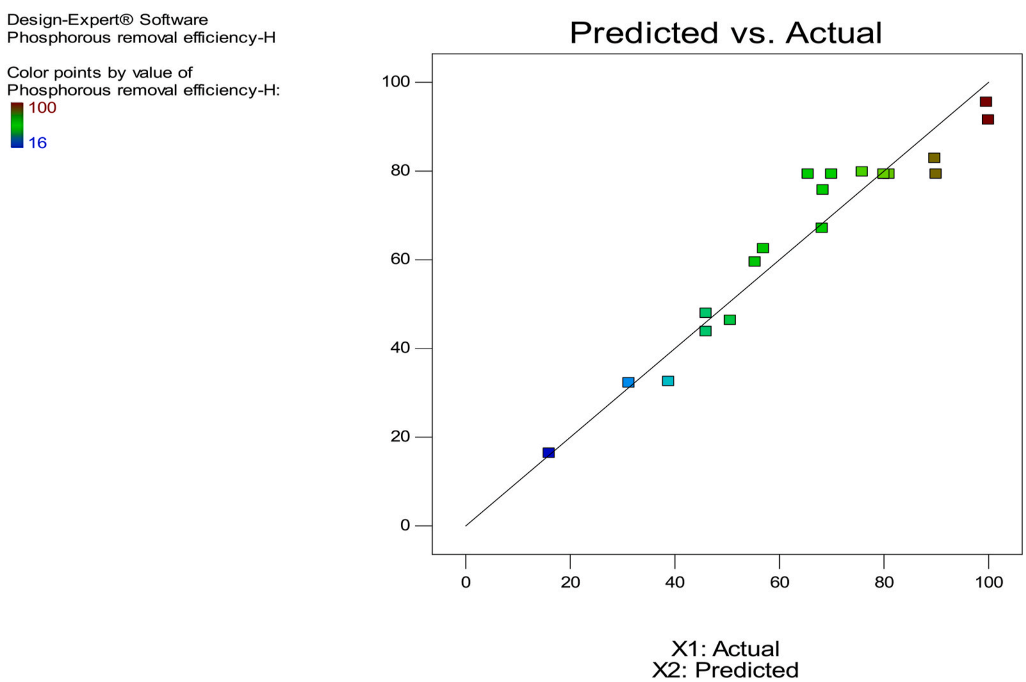


(d)

Fig. 4. (*continued*).

of Asadi et al., the removal efficiency of TP varied between 26% and 80%, with influent TP concentrations ranging from 48 to 50 mg/L in the CFIDALB system (Asadi et al., 2016c). In another study, Abyar et al. achieved a high TP removal efficiency ranging from 52.6% to 94.2% in an up-flow A2O bioreactor (Abyar et al., 2018). The maximum TP removal was achieved to be 94.2 % at short HRT of 8 h. Guo et al. conducted studies by focusing on the synchronous removal of nutrients (N & P) from digestion effluent and mainstream wastewater (Guo et al., 2023; Guo and Li, 2020). They employed a one-stage internal circulation airlift bioreactor, utilizing granules cultivated through hydroxyapatite and iron phosphate crystallization. The authors gained phosphorous removal efficiency of 83 and 63 % with low inlet phosphorus of 20 mg/L and 7 mg/L at HRT of 4 and 6h, respectively.

In overall, the hybrid dual internal circulation airlift A2O bioreactor showed the superiority in the phosphorus removal contributed to the employment of the packing media as well as the special design of the hybrid dual internal circulation airlift A2O bioreactor. The presence of packing media enhanced the anoxic and anaerobic zones via the formation of attached biofilm and the decrease in the circulation rate. The sludge characteristics, including turbidity, SVI, and SMP and EPS contents, are detailed in the supplementary information (SI).

3.4. Process optimization and verification

In this section, in order to ascertain the optimum values for the controlled independent variables i.e. HRT, AFR and AVR with the aim of getting the high effluent, process optimization was performed using

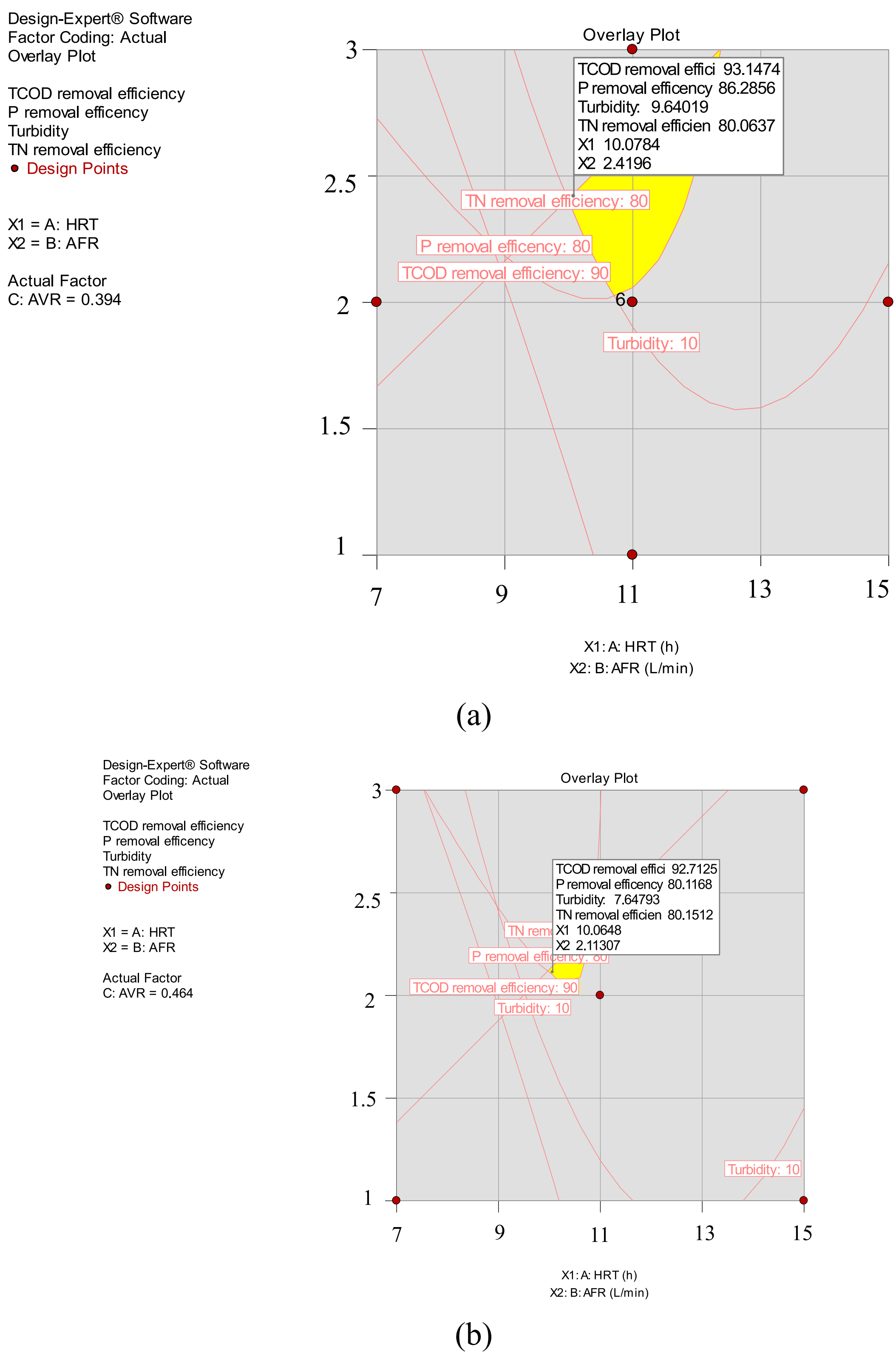


Fig. 5. Overlay plots visualized for hybrid DCAL-A2O bioreactor at the optimum region with different upper and lower limits for the various responses at (a) normal conditions, (b) more strict conditions.

Design-Expert® software (Version 10). In this context, the airlift A2O bioreactor was optimized considering the critical dependent variables (responses) of TCOD, TN, TP removal efficiencies and effluent turbidity. These responses play a vital role in the enhancement of the effluent quality. Accordingly, the graphical optimization is substantiated as the overplay plot as depicted in Fig. 5. In this plot, the areas meeting the optimization criteria as the maximum removal efficiencies of TCOD, TN and TP, and the minimum amount of the effluent turbidity are shaded in yellow as demonstrated in the Figure. On the basis of the optimization criteria, the lower acceptable limit for three first responses (TCOD > 90 %, TN > 80 %, TP > 80 %) and the upper acceptable limit for the later one (effluent turbidity < 10 NTU) were considered. Accordingly, two experimental runs were proposed in the factors space and verified. The suggested conditions for the controlled independent variables plus the experimental and predicted data are represented in Table 1. As clear from the Table, the actual data are in a good consistent with those modeled. Given that the main aim of the bioreactor development is the reduction in both energy consumption (via decline in the AFR) and the investment costs (via diminish in the HRT and therefore bioreactor volume), the optimum conditions that met these criterial were chosen to further study the bioreactor. Accordingly, the optimum conditions were chosen to be HRT of 10h and AFR of 2.0 L/min. In this section, the impact of the AVR was not as important as two other operating variables as this parameter was insignificant economically.

The impact of the wastewater composition and of feeding location on the process performance of the bioreactor at optimum conditions are discussed in SI.

3.5. Microbial community at optimum conditions

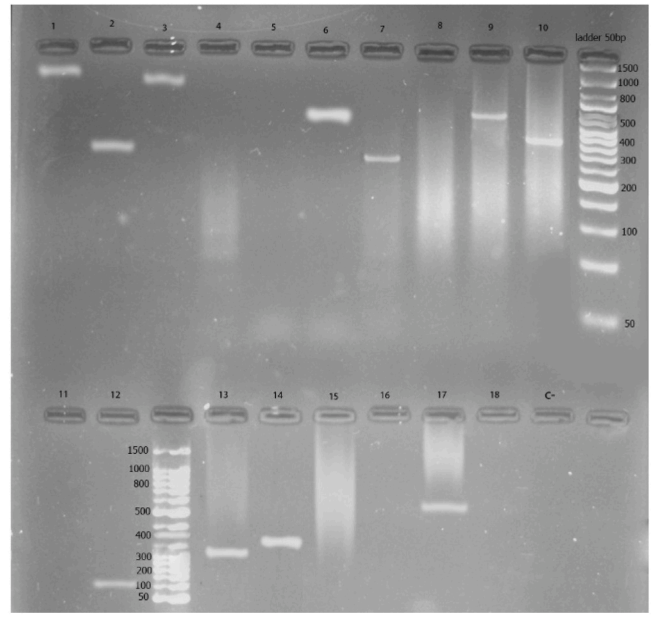
To assess the microbial community composition in both the bacterial suspended and attached sludge, PCR testing was performed. The PCR outcomes from analyzing 19 primers are illustrated in Fig. 6 and detailed in Table 3. As shown in the Table, the presence of variant bacterial community, identifiable via the bands appearing in the Figure, was confirmed as a positive indication. The findings demonstrated the manupulation in anoxic zone influnced the bacterial community.

In the hybid DCAL-A2O bioreactor, for suspended growth, functional species responsible for phosphorous removal, nitrification, denitrification and anammox were identified to be PAOs (Candidatus Accumulibacter (ppk1)(; AOB and NOB (Nitrosospira sp, Nitrosomonas SP, and Nitrosococcus; NOB: Nitrobacter and Nitrospira (23SrRNA)); denitrifying bacteria (Pseudomonas), and Anammox bacteria (Candidatus Kuenenia), respectively. In addition, the characterized genus for GAOs (Competibacter and Defluviicoccus) confirmed nitrogen removal via simultaneous partial nitrification denitrification (Yuan et al., 2020). The functional genus detected in the attached growth were to be DPAOs (Dechloromonas, Paracoccus, and Pseudomonas); PAOs (Rhodocyclus); NOB (Nitrobacter and Nitrospira(NOB) (23SrRNA)); denitryfing bacteria (Pseudomonas); anammox (Candidatus_Brocadia and Candidatus Kuenenia) and GAOs (Competibacter). The obtained findings verified the co-existence of functional bacteria in a one-stage bioreactor for synchronous CNP removal.

The long-term performance of the hybrid DCAL-A2O bioreactor at optimum conditions and assessment of the operating cost are explained in SI.

4. Conclusions

This research demonstrates the effectiveness of a one-stage hybrid dual internal circulation airlift A2O (DCAL-A2O) bioreactor in simultaneously removing carbon, nitrogen, and phosphorus (CNP) from MPW. Optimal conditions were achieved with the HRT of 10 h, AFR of 2 L/min, and AVR of 0.464, resulting in removal efficiencies of 97%, 92%, and 90% for CNP, with effluent turbidity of 9 NTU. The bioreactor's unique design, along with the attached growth and oxygen gradients in the biofilm, enhanced nitrogen removal through biological processes like SND and anammox. Using packing media in the anoxic zone improved the bioreactor's performance by reducing shear stress and optimizing the anoxic and anaerobic zones. The biodegradability of the wastewater influenced phosphorus and nitrogen removal, with SDW providing better phosphorus removal due to its readily biodegradable carbon, while MPW and SOW showed higher TN removal. Feeding from the anoxic zone improved TN removal, while feeding from the anaerobic zone improved phosphorus removal. At optimal conditions, significant reductions in effluent soluble microbial products (SMP), sludge SMP, and LB-EPS were observed, indicating improved sludge flocculation and settleability. The study also identified various bacterial species supporting synchronous CNP removal, confirming the cost-effectiveness and potential scalability of the hybrid DCAL-A2O bioreactor.



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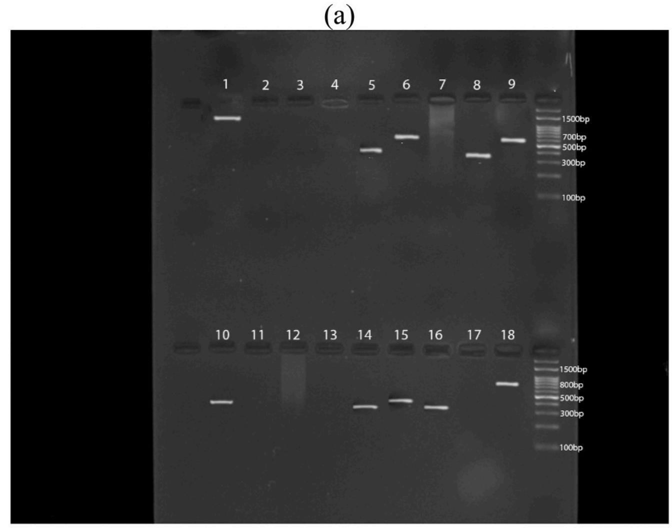

(b)

Fig. 6. The PCR results obtained from (a) suspended growth; and (b) attached growth.

CRediT authorship contribution statement

Zahra Rahimi: Writing – original draft, Validation, Software, Methodology, Investigation, Data curation, Conceptualization. **Ali**

Table 3
The identified species in suspended growth and attached growth under optimum conditions.

No.	Name	Product length	Suspended growth	Attached growth
1	Pseudomonas (denitrifying bacteria)	1500	+	+
2	Nitrosospira sp (AOB)	381	+	−
3	Nitrosomonas SP (AOB)	1052	+	−
4	Tetrasphaera (PAOs)	238	−	−
5	Candidatus_Brocadia (Anammox)	415	−	+
6	Candidatus Kuenenia (Anammox)	650	+	+
7	Nitrosomonas sp (AOB)-Family	304	+	−
8	Dechloromonas (DPAOs)	394	−	+
9	Nitrobacter(NOB)	600	+	+
10	Nitrospira(NOB) (23SrRNA)	435	+	+
11	Candidatus Microthrix (16srRNA) (PAOs)	230	−	−
12	Candidatus Accumulibacter (ppk1((PAOs)	129	+	−
13	Sphingomonas (16SrRNA) (GAOs)	306	+	−
14	Competibacter (GAO)	375	+	+
15	Paracoccus (DPAOs)	450	−	+
16	Rhodocyclus (PAOs)	350	−	+
17	Nitrosococcus (AOB)	521	+	−
18	Pseudomonas (DPAOs)	850	−	+
19	Defluviicoccus (GAO)	447	+	−

Akbar Zinatizadeh: Writing – review & editing, Validation, Supervision, Software, Methodology, Investigation, Conceptualization. **Sirus Zinadini:** Writing – review & editing. **Mark van Loosdrecht:** Writing – review & editing. **Damien J. Batstone:** Writing – review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.143811.

Data availability

Data will be made available on request.

References

Abyar, H., Younesi, H., Bahramifar, N., Zinatizadeh, A.A., 2018. Biological CNP removal from meat-processing wastewater in an innovative high rate up-flow A2O bioreactor. Chemosphere. https://doi.org/10.1016/j.chemosphere.2018.09.047.

Amini, M., Younesi, H., Zinatizadeh, A.A., Najafpour, G., 2013. Determination of optimum conditions for dairy wastewater treatment in UAASB reactor for removal of nutrients. Bioresour. Technol. 145, 71–79. https://doi.org/10.1016/j.biortech.2013.01.111.

Asadi, A., Zinatizadeh, A.A., Van Loosdrecht, M., 2016a. High rate simultaneous nutrients removal in a single air lift bioreactor with continuous feed and intermittent discharge regime: process optimization and effect of feed characteristics. Chem. Eng. J. 301, 200–209. https://doi.org/10.1016/j.cej.2016.04.144.

Asadi, A., Zinatizadeh, A.A., Van Loosdrecht, M., 2016b. A novel continuous feed and intermittent discharge airlift bioreactor (CFIDAB) for enhanced simultaneous removal of carbon and nutrients from soft drink industrial wastewater. Chem. Eng. J. 292, 13–27. https://doi.org/10.1016/j.cej.2016.01.110.

Asadi, A., Zinatizadeh, A.A., Van Loosdrecht, M., 2016c. High rate simultaneous nutrients removal in a single air lift bioreactor with continuous feed and intermittent discharge regime: process optimization and effect of feed characteristics. Chem. Eng. J. 301, 200–209. https://doi.org/10.1016/j.cej.2016.04.144.

Asadi, A., Zinatizadeh, A.A., Hasnain Isa, M., 2012a. Performance of intermittently aerated up-flow sludge bed reactor and sequencing batch reactor treating industrial estate wastewater: a comparative study. Bioresour. Technol. 123, 495–506. https://doi.org/10.1016/j.biortech.2012.07.013.

Asadi, A., Zinatizadeh, A.A., Sumathi, S., 2012b. Simultaneous removal of carbon and nutrients from an industrial estate wastewater in a single up-flow aerobic/anoxic sludge bed (UAASB) bioreactor. Water Res. 46, 4587–4598. https://doi.org/10.1016/j.watres.2012.06.029.

Bonakdari, H., Zinatizadeh, A.A., 2011. Influence of position and type of Doppler flow meters on flow-rate measurement in sewers using computational fluid dynamic. Flow Meas. Instrum. 22, 225–234. https://doi.org/10.1016/j.flowmeasinst.2011.03.001.

Cao, Y., van Loosdrecht, M.C.M., Daigger, G.T., 2017. Mainstream partial nitritation–anammox in municipal wastewater treatment: status, bottlenecks, and further studies. Appl. Microbiol. Biotechnol. 101, 1365–1383. https://doi.org/10.1007/s00253-016-8058-7.

Chan, Y.J., Chong, M.F., Law, C.L., Hassell, D.G., 2009. A review on anaerobic-aerobic treatment of industrial and municipal wastewater. Chem. Eng. J. 155, 1–18. https://doi.org/10.1016/j.cej.2009.06.041.

Chen, H., Wang, H., Chen, R., Chang, S., Yao, Y., Jiang, C., Wu, S., Wei, Y., Yu, G., Yang, M., Li, Y.Y., 2021a. Unveiling performance stability and its recovery mechanisms of one-stage partial nitritation-anammox process with airlift enhanced micro-granules. Bioresour. Technol. 330, 124961. https://doi.org/10.1016/j.biortech.2021.124961.

Chen, H., Wang, H., Yu, G., Xiong, Y., Wu, H., Yang, M., Chen, R., Yang, E., Jiang, C., Li, Y.Y., 2021b. Key factors governing the performance and microbial community of one-stage partial nitritation and anammox system with bio-carriers and airlift circulation. Bioresour. Technol. 324. https://doi.org/10.1016/j.biortech.2021.124668.

Chen, R., Ji, J., Chen, Y., Takemura, Y., Liu, Y., Kubota, K., Ma, H., Li, Y.Y., 2019. Successful operation performance and syntrophic micro-granule in partial nitritation and anammox reactor treating low-strength ammonia wastewater. Water Res. 155, 288–299. https://doi.org/10.1016/j.watres.2019.02.041.

Chen, Y., Sanjaya, E.H., Guo, G., Li, Y.Y., 2021. High nitrogen removal performance of anaerobically treated fish processing wastewater by one-stage partial nitritation and anammox process with hydroxyapatite (HAP)-based syntrophic granules and granule structure. Bioresour. Technol. 338, 125526. https://doi.org/10.1016/j.biortech.2021.125526.

Ding, S., Bao, P., Wang, B., Zhang, Q., Peng, Y., 2018. Long-term stable simultaneous partial nitrification, anammox and denitrification (SNAD) process treating real domestic sewage using suspended activated sludge. Chem. Eng. J. 339, 180–188. https://doi.org/10.1016/j.cej.2018.01.128.

Gholami, F., Zinatizadeh, A.A., Zinadini, S., McKay, T., Sibali, L., 2020. An innovative jet loop-airlift bioreactor for simultaneous removal of carbon and nitrogen from soft drink industrial wastewater: process performance and kinetic evaluation. Environ. Technol. Innov. 19, 100772. https://doi.org/10.1016/j.eti.2020.100772.

Guo, Y., Li, Y.Y., 2020. Hydroxyapatite crystallization-based phosphorus recovery coupling with the nitrogen removal through partial nitritation/anammox in a single reactor. Water Res. 187, 116444. https://doi.org/10.1016/j.watres.2020.116444.

Guo, Y., Luo, Z., Rong, C., Wang, T., Qin, Y., Hanaoka, T., Sakemi, S., Ito, M., Kobayashi, S., Kobayashi, M., Li, Y.Y., 2022. The first pilot-scale demonstration of the partial nitritation/anammox-hydroxyapatite process to treat the effluent of the anaerobic membrane bioreactor fed with the actual municipal wastewater. Sci. Total Environ. 807, 151063. https://doi.org/10.1016/j.scitotenv.2021.151063.

Guo, Y., Sanjaya, E.H., Wang, T., Rong, C., Luo, Z., Xue, Y., Chen, H., Li, Y.Y., 2023. The phosphorus harvest from low-temperature mainstream wastewater through iron phosphate crystallization in a pilot-scale partial nitritation/anammox reactor. Sci. Total Environ. 862, 160750. https://doi.org/10.1016/j.scitotenv.2022.160750.

Guo, Y., Xie, C., Chen, Y., Urasaki, K., Qin, Y., Kubota, K., Li, Y.Y., 2021. Achieving superior nitrogen removal performance in low-strength ammonium wastewater treatment by cultivating concentrated, highly dispersive, and easily settleable granule sludge in a one-stage partial nitritation/anammox-HAP reactor. Water Res. 200, 117217. https://doi.org/10.1016/j.watres.2021.117217.

Huang, X., Sun, K., Wei, Q., Urata, K., Yamashita, Y., Hong, N., Hama, T., Kawagoshi, Y., 2016. One-stage partial nitritation and anammox in membrane bioreactor. Environ. Sci. Pollut. Res. 23, 11149–11162. https://doi.org/10.1007/s11356-016-6309-x.

Li, X., Sun, S., Yuan, H., Badgley, B.D., He, Z., 2017. Mainstream upflow nitritation-anammox system with hybrid anaerobic pretreatment: long-term performance and microbial community dynamics. Water Res. 125, 298–308. https://doi.org/10.1016/j.watres.2017.08.048.

Liu, Y., Niu, Q., Wang, S., Ji, J., Zhang, Y., Yang, M., Hojo, T., Li, Y.Y., 2017. Upgrading of the symbiosis of Nitrosomanas and anammox bacteria in a novel single-stage partial nitritation–anammox system: nitrogen removal potential and Microbial characterization. Bioresour. Technol. 244, 463–472. https://doi.org/10.1016/j.biortech.2017.07.156.

Mansouri, A.M., Zinatizadeh, A.A., 2017. A comparative study of an up-flow aerobic/anoxic sludge fixed film bioreactor and sequencing batch reactor with intermittent aeration in simultaneous nutrients (N, P) removal from synthetic wastewater. Water Sci. Technol. 76, 1044–1058. https://doi.org/10.2166/wst.2017.261.

Metcalf, E., 2003. Wastewater Engineering: Treatment, Disposal, Reuse, fourth ed. McGraw- Hill, Singapore.

Mirghorayshi, M., Zinatizadeh, A.A., van Loosdrecht, M., 2021a. Simultaneous biodegradability enhancement and high-efficient nitrogen removal in an innovative single stage anaerobic/anoxic/aerobic hybrid airlift bioreactor (HALBR) for composting leachate treatment: process modeling and optimization. Chem. Eng. J. 407, 127019. https://doi.org/10.1016/j.cej.2020.127019.

Mirghorayshi, M., Zinatizadeh, A.A., van Loosdrecht, M., 2021b. Simultaneous biodegradability enhancement and high-efficient nitrogen removal in an innovative single stage anaerobic/anoxic/aerobic hybrid airlift bioreactor (HALBR) for composting leachate treatment: process modeling and optimization. Chem. Eng. J. 407, 127019. https://doi.org/10.1016/j.cej.2020.127019.

Mirghorayshi, M., Zinatizadeh, A.A., Van Loosdrecht, M., 2018. Evaluating the process performance and potential of a high-rate single airlift bioreactor for simultaneous carbon and nitrogen removal through coupling different pathways from a nitrogen-rich wastewater. Bioresour. Technol. 260, 44–52. https://doi.org/10.1016/j.biortech.2018.03.048.

Moradi, S., Zinatizadeh, A.A., Zinadini, S., Gholami, F., 2021. High-rate CNP removal from wastewater in a single jet loop air lift bioreactor: process modeling and optimization with four process and operating factors. J. Water Process Eng. 40, 101980. https://doi.org/10.1016/j.jwpe.2021.101980.

Rahimi, Z., Zinatizadeh, A.A., Zinadini, S., van Loosdrecht, M., Jbatstone, D., 2024. Concurrent removal of carbon and nutrients in a one-stage dual internal circulation airlift A2O bioreactor from milk processing industrial wastewater: process optimization, sludge characteristics and operating cost evaluation. Chemosphere 355, 141804. https://doi.org/10.1016/j.chemosphere.2024.141804.

Rbb, E.W.R., Eaton, A.D., 2017. Standard methods for the examination of water and wastewater. Am. Public Heal. Assoc. Am. Water Work. Assoc. Water Environ. Fed. Washington, DC.

Rezaee, S., Zinatizadeh, A.A., Asadi, A., 2016. Comparative study on effect of mechanical mixing and ultrasound on the performance of a single up-flow anaerobic/aerobic/anoxic bioreactor removing CNP from milk processing wastewater. J. Taiwan Inst. Chem. Eng. https://doi.org/10.1016/j.jtice.2015.06.012.

Rezaee, S., Zinatizadeh, A.A., Asadi, A., 2015. High rate CNP removal from a milk processing wastewater in a single ultrasound augmented up-flow anaerobic/aerobic/anoxic bioreactor. Ultrason. Sonochem. 23, 289–301. https://doi.org/10.1016/j.ultsonch.2014.10.018.

Rong, C., Luo, Z., Wang, T., Guo, Y., Kong, Z., Wu, J., Ji, J., Qin, Y., Hanaoka, T., Sakemi, S., Ito, M., Kobayashi, S., Kobayashi, M., Li, Y.Y., 2021. Chemical oxygen demand and nitrogen transformation in a large pilot-scale plant with a combined submerged anaerobic membrane bioreactor and one-stage partial nitritation-anammox for treating mainstream wastewater at 25 °C. Bioresour. Technol. 341. https://doi.org/10.1016/j.biortech.2021.125840.

Shi, Y., Wu, S., Ren, H., Jin, M., Wang, L., Qiao, N., Yu, D., 2020. Computational fluid dynamics and factor analysis of a novel swirling demulsified airlift loop reactor for the treatment of refined soybean oil wastewater. Bioresour. Technol. 296, 122316. https://doi.org/10.1016/j.biortech.2019.122316.

Wang, S., Liu, Y., Niu, Q., Ji, J., Hojo, T., Li, Y.Y., 2017. Nitrogen removal performance and loading capacity of a novel single-stage nitritation-anammox system with syntrophic micro-granules. Bioresour. Technol. 236, 119–128. https://doi.org/10.1016/j.biortech.2017.03.164.

Yilmaz, G., Lemaire, R., Keller, J., Yuan, Z., 2007. Effectiveness of an alternating aerobic, anoxic/anaerobic strategy for maintaining biomass activity of BNR sludge during long-term starvation. Water Res. 41, 2590–2598. https://doi.org/10.1016/j.watres.2007.02.011.

Yuan, C., Wang, B., Peng, Y., Hu, T., Zhang, Q., Li, X., 2020. Nutrient removal and microbial community in a two-stage process: simultaneous enhanced biological phosphorus removal and semi-nitritation (EBPR-SN) followed by anammox. Bioresour. Technol. 310, 123471. https://doi.org/10.1016/j.biortech.2020.123471.