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Process optimization, sludge characteristics and operating cost evaluation

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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

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HIGHLIGHTS

- Simultaneous CNP removal was investigated in a one-stage DCAL-A2O bioreactor.
- The impact of wastewater biodegradability and feeding location was evaluated on the bioreactor performance.
- The sludge characteristics were explored throughout multiple experimental runs.
- Operational cost estimates were performed under different operating conditions.
- The coexistence and mutualistic development of various functional bacterial were observed in the context of PCR assays.

GRAPHICAL ABSTRACT
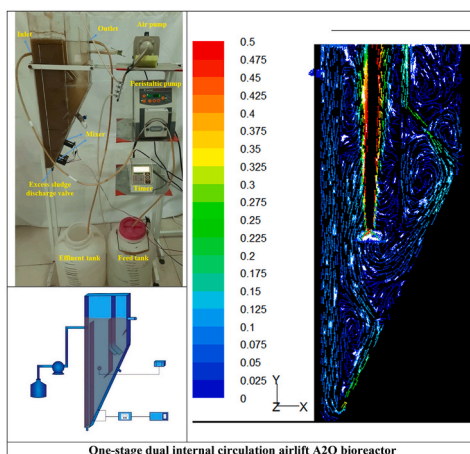
One-stage dual internal circulation airlift A2O bioreactor

ARTICLE INFO

ABSTRACT

In this work, a one-stage dual internal circulation airlift anaerobic/anoxic/aerobic (DCAL-A2O) bioreactor was continuously operated for concurrent removal of nutrients and organics from milk processing wastewater (MPW). Special configuration of the airlift A2O bioreactor created possibility of the formation of desired anaerobic, anoxic and aerobic zones in a single unit. The process functionality of the bioreactor was examined under three influential operating variables i.e. hydraulic retention time (HRT; 7–15 h), air flow rate (AFR; 1–3 L/min) and aerobic volume ratio (AVR; 0.324–0.464). The optimum region was identified at HRT of 13h, AFR of

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Sludge characteristics
Cost evaluation

2L/min and AVR of 0.437, leading to TCOD, TN and TP removal efficiency of 94.5 %, 59.6 %, and 62.2 %, respectively, and effluent turbidity of 8 NTU. The impact of feed biodegradability on the process performance of the bioreactor treating the MPW, soft drink wastewater (SDW) and soybean oil plant wastewater (SOW) was also assessed. From the results, the feed characteristics affected significantly the nutrients removal. Moreover, the feeding location played an effective role in the nutrient removal while treating the MPW at optimum operating conditions. In this study, the change in residual organic matters as soluble microbial products (SMP) was monitored at various operating conditions. In addition, the impact of SMP extracted from sludge, extracellular polymeric substances (EPS) comprising of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS) was analyzed on sludge characteristics as bio-flocculation and settleability properties. According to the obtained data, the increase in operating variables led to the reduction in contents of effluent SMP, sludge SMP, LB-EPS, turbidity, and SVI, thereby, the enhancement in the sludge characteristics. Meanwhile, analysis of microbial communities verified the presence of various functional bacterial species. The cost operating evaluation confirmed the cost effectiveness of the airlift A2O bioreactor in reduction of energy consumption for the MPW treatment.

1. Introduction

More recently, the increasing human activities in industrial and agricultural sectors as well as the issue of the change in the climate status led to the generation of the huge wastewater volume and depletion of water resources. Regarding the polluted water if no proper or improper treatment approach is applied to deal with these pollutant sources, pervasive environmental challenges will arise (Zainal et al., 2018). Various treatment technologies have been utilized to address wastewater with varying characteristics. Biological treatment processes (Zinatizadeh, 2006), photocatalytic oxidation (Zangeneh, et al., 2019), coagulation and flocculation processes (Zinatizadeh, et al., 2017), adsorption (Sethupathi et al., 2015), and membrane separation (Rahimi, et al., 2021) are the most used treatment technologies. Besides, natural treatment systems offer a cost-effective solution for treating municipal and industrial wastewaters. In comparison to mechanical treatment systems, the extended retention time in these systems has led to a reduction in reactor volume yield (Tchobanoglous et al., 2002; Bonak-dari and Zinatizadeh, 2011).

Majority of produced wastewaters contain high organic matter level (C) and crucial nutrients i.e. nitrogen & phosphorous (N & P), giving rise to eutrophication phenomenon and oxygen deterioration in the receiving water bodies which in turn creating a serious threat for the humans' and aqueous organisms' health. Biological nutrient removal (BNR) processes have been adopted successfully in wastewater treatment plants (WWTPs) all over the world to treat CNP enriched wastewaters. The main mechanisms involved in the nitrogen removal in the BNR systems are reliant on simultaneous nitrification and denitrification (SND) process. In the conventional BNR processes, for having these mechanisms, two separate anoxic and aerobic compartments are required tandemly. In the case of phosphorous component, it can be removed over a two-stage mechanism namely phosphorus release by the functional bacteria of phosphorus accumulating organisms (PAOs) under anaerobic conditions, and the subsequent accumulation of the released phosphorous at aerobic environment. Thereby, these bacteria need anaerobic-aerobic conditions in series to do their job. Based on these process descriptions, the CNP elimination from the different wastewaters in the conventional BNR systems takes place in three successive bioreactors of in sequence the anaerobic, anoxic and aerobic giving rise to the increase in footprint and investment. Besides, this design is equipped to additional apparatuses like the internal recycling pump, sludge recycling pump and extra mixers where result in the increase in the energy input. Therefore, the present design of the conventional BNR systems is costly in construction, and complex in the operation management. Totally, the water and wastewater specialists are pursuing the convenient designs by developing the conventional geometries to solve these serious dilemmas. Furthermore, upgrading conventional bioreactors' capacity is another significant perspective followed by researchers.

Considering existing challenges, high rate single stage bioreactors have received a great deal of interest currently. In such bioreactors, the simultaneous removal of CNP is reliant on being provided all the circumstances required for the growth and cooperation of the functional bacterial strains i.e. nitrifiers, denitrifiers, and PAOs in a single unit. Foundation of the single stage bioreactors developed recently is based on employing either intermittent aeration or physical separation as the use of internal tube and baffles. The advanced high rate single bioreactors are operated generally as continuous feed and intermittent discharge (CFID) regime (Asadi et al., 2012a, 2012b; Rezaee et al., 2015, 2016) and continuous regime (Abyar et al., 2018; Amini et al., 2013).

Looking forward current advances, the high rate single stage airlift bioreactors have been developed remarkably over the recent years. The highlighted features of these types of the high rate single bioreactors come back to high mass transfer, simple design, complete and effective mixture with low energy requirement, and low shear stress to attached microbial species (Shi et al., 2020). Therefore, more recently, the single stage airlift-based installations have been used in the wastewater treatment with the benefits of saving in installation space, mitigation in the sludge generation, and the reduction in operating and investment cost apart from the mentioned features. The virtues of such airlift bioreactors are further enlarged via the enhancement in the bioreactor's treatment capacity. Asadi et al. for the first time designed and employed a new design of the single stage airlift bioreactor with CFID regime to treat industrial wastewaters i.e. milk processing wastewater (MPW) and soft drink wastewater (SDW) (A. Asadi et al., 2016; Azar Asadi et al., 2016a, 2016b). In the conducted studies, the simultaneous CNP removal and nitrogen removal via different mechanisms of simultaneous nitrification–denitrification (SND) and anaerobic ammonium anaerobic ammonium oxidation (anammox) were examined under different independent variables. In the following these researches, Mirghorayshi et al. investigated the impact of the wastewater characteristics in terms of the various ratios of $N–NH_4^+/(N–NH_4^+ + N–NO_3^-)$ on the referred bioreactor's performance in nitrogen removal through the different mechanisms (Mirghorayshi et al., 2018). Gholami et al. for the first time proposed a new structure of the single stage airlift bioreactor entitled jet loop-airlift bioreactor to remove CN simultaneously from the SDW (Gholami et al., 2020). In another study, Moradi et al. tried to provide anaerobic zone by changing the location of aerator in order to remove CNP simultaneously from the SDW in the introduced jet loop-airlift bioreactor (Moradi et al., 2021). Mirghorayshi et al. in another study designed and explored an innovative geometry of the single stage airlift bioreactor as anaerobic/anoxic/aerobic hybrid airlift bioreactor (HALBR) employing both attached and suspended microbial growth (Mirghorayshi et al., 2021). The major aim of this research was the investigation of simultaneous biodegradability of carbonaceous substances and nitrogen removal from refractory composting leachate wastewater in the single stage HALBR. Additionally, airlift regime based bioreactors showed the successful performance as the concurrent nitrogen elimination and phosphorous recovery through various mechanisms of partial nitrification-annamox and hydroxyapatite crystallization, respectively, using granular sludge

and attached sludge (bio-carriers) from the synthetic wastewaters under specified operating conditions (H. Chen et al., 2021b, 2021a; Y. Chen et al., 2021; Guo et al., 2022, 2021; Guo and Li, 2020; Liu et al., 2017; Wang et al., 2017).

Therefore, in this study, we provide a novel one-stage dual internal circulation airlift A2O (DCAL-A2O) bioreactor as a potential solution for the efficient removal of carbon, nitrogen, and phosphorus (CNP) from milk processing industrial wastewater (MPW). This innovative bioreactor offers the advantage of significantly reducing the required footprint and investment cost. The integrated unit of this bioreactor design incorporates multiple redox processes to effectively remove CNP. This study aims to examine the behavior of the DCAL-A2O bioreactor in the treatment of MPW. The study investigates the impact of three significant operating factors on the performance of the bioreactor, the features of the sludge, the evolution of diverse bacterial species, and the operating cost.

2. Materials and methods

2.1. Description of the bioreactor configuration and operating procedure

The wastewater characteristics and inoculum sludge are described in supplementary information (SI). The one-stage dual internal circulation airlift anaerobic/anoxic/oxic (A2O) (DCAL-A2O) bioreactor was designed, made of transparent Plexiglas and employed to treat the milk processing wastewater (MPW). In general, the DCAL-A2O bioreactor was consisted of a reaction zone (including anaerobic ($10 \times 10 \times 22.5$ cm as width $\times$ length $\times$ height)/anoxic ($3 \times 10 \times 62.5$ cm)/aerobic zones ($10 \times 10 \times 40$ cm)) and a clarifier compartment ($10 \times 10 \times 20$ cm) with the working volume of 7 L and 2 L, respectively. The clarifier section was connected to the reaction zone using an inclined plate to facilitate the sludge circulation. In this configuration, the sludge can easily slip into the reaction zone via the junction channel by means of airlift force. The schematic of the lab-scale apparatus was depicted in Fig. 1. An air pump was applied to supply the required oxygen and create the dual internal circulation based on the pre-determined air flow rate (AFR) by regulation of the gas flow gauge in order to balance the biological reactions between bacterial species responsible for CNP removal. Air outlet was injected to the bioreactor through an air diffuser installed at various heights of aerobic zone to set aerobic volume ratios (AVRs). The bioreactor was fed from the top of the anoxic zone at certain feed flow rates adjusted by a manually controllable peristaltic pump according to given hydraulic retention time (HRT). There are two mixers installed in the anaerobic zone to be ensure about the proper mixing managed based on on/off (5s/30min). The pH of the incoming feed was maintained at 6.5–7.0 using NaOH solution. Dissolved oxygen (DO) concentration revealed the significant difference between various zones of the reaction unit i.e. anaerobic, anoxic and aerobic zones under applied operating conditions providing the convenient environment for the co-growth of the functional microbial species in the one stage DCAL-A2O bioreactor. The surplus sludge was discharged daily to stabilize the intended MLSS concentration (around 6000 mg/L) inside the bioreactor. Totally, the DCAL-A2O bioreactor was operated in three main phases. The start-up operation was conducted in phase I for a two-week period. The performance of the bioreactor was evaluated continuously under the influential operating variables (i.e. HRT, AFR and AVR) in phase II for 7 months. The phase II consists of 20 experimental runs according to the experimental design performed by Design Expert software discussed in the following sections with details. In phase III, the process optimization study was accomplished for two weeks. Experimental design, mathematic modeling and statistical analysis, and chemical analysis are included in supplementary data.

2.2. Microbial community analysis

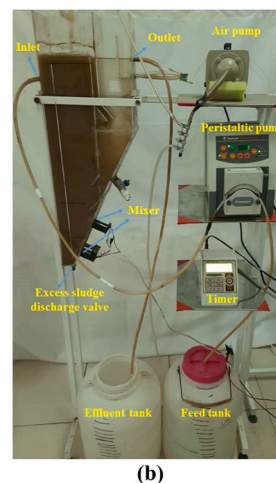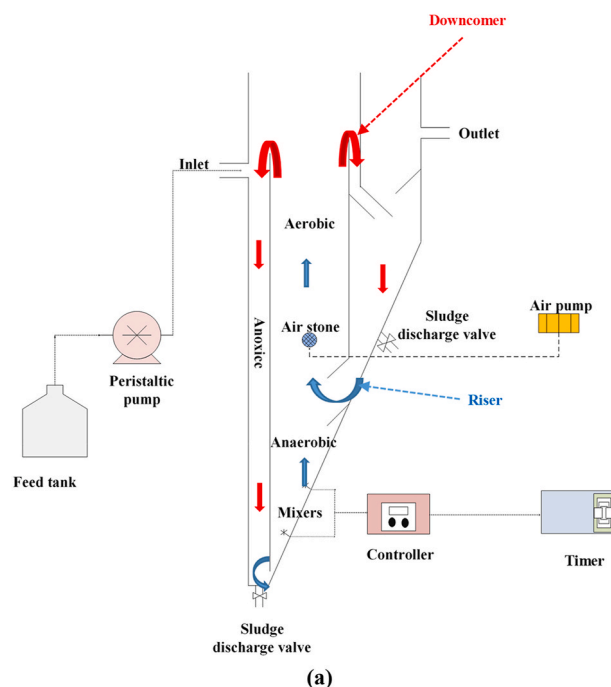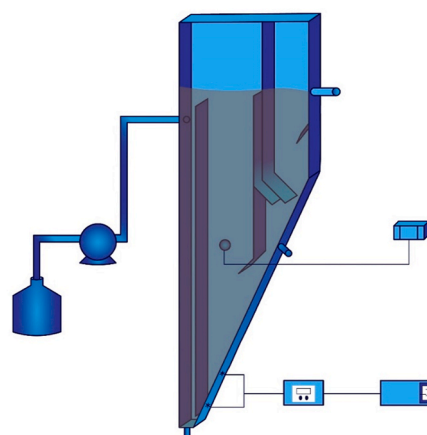This part includes two main sections i.e. DNA extraction and PCR

Fig. 1. (a) Schematic and (b) real picture of the DCAL- A2O bioreactor configuration.

amplification described in the following. For this purpose, biomass samples were taken from DCAL-A2O bioreactor under optimum conditions. To extract DNA, 5 μL of protease-K enzyme and 100 μL of enzyme buffer were added to 100 μL of each biomass sample. The samples were vortexed for 5–10 s, and kept at 72 °C for 5 min. Then, 400 μL of lysis buffer was added to the samples, and vortexed for 15 s. In the next stage, the vortexed samples were maintained at ambient temperature for 5–15 min. Thereafter, 300 μL of precipitation solution was added to the sample, and vortexed for 15 s. The biomass samples were freeze-dried at −20 °C overnight, and then, centrifuged at 13500 rpm for 15 min. Resultant supernatant was removed, and the micro-tube was placed upside down on sterile flame for a short time (until large drops were eliminated). Next, 1 mL of washing buffer was added to the micro-tube. The micro-tube was vortexed for 15 s, and centrifuged at 13500 rpm for 10 min. The supernatant was removed, and the micro-tube was totally dried on the sterile frame. Thereafter, 30 mL of deionized water was added to the micro-tube. The micro-tube was put in dry block at 55–65 °C for 10–15 min. In order to open the secondary structure formed inside the DNA molecules, the heated micro-tube was cooled immediately in an ice bath. Finally, the extracted DNA was preserved at −20 °C for PCR reactions.

The primers of the various bacteria species i.e. anammox, denitrifying bacteria, ammonium oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), phosphate accumulating organisms (PAOs), glycogen-accumulating organisms (GAO), denitrifying PAOs (DPAOs) are

Table 1
The primers of the various bacterial species used.

Name	Primers	T_A	Product length
Pseudomonas (DPAO) (Denitrifing bacteria)	F-GCAGAGTTTGATCCTGGCTC R- TAAGGAGGTGATCCAGCCG	58	1500
Nitrosospira sp (AOB)	F- AGTAGCTGGTCTGAGAGGAC R- CTGCCACACTCTAGCCTTG	57	381–383
Nitrosamines SP (AOB)	F-TGGGGAATAACGCAACGAAAG R- GTCATCCCCACCTTCCTCC	59	1052
Tetrasphaera (PAOs)	F-GTATTTCATCGGTGACGGGG R- GGGCTCGTGGTGATAGAGG	58	238
Candidatus_Brocadia (Anammox)	F- CTCCTCTACCAACCCACACC R- AATACCCTGACCCGAACACAT	59	415
Candidatus Kuenenia (Anammox)	F- AAGGGCATTTGTAGAAGAACTG R-CCTGTCTCTTCCTTGCTTTG	56	650
Nitrosomonas sp (AOB)	F-GCTTGGAATTTTACGGAGAC R-CTACGAAGTGCTTTGTGAG	54	304
Dechloromonas (DPAOs)	F- ACAAAAGATAAATCGCCAAATC R-TTAGCGTCCCATGGACAA	55	394
Nitrobacter(NOB)	F-GATCTTGGCTCAGAGCGAAC R- ACTCACCTCTCCCGAACTC	58	600
Nitrospira (NOB) (23SrRNA)	F- CTTGTGGGAATCTGGGACGAC R-GGGAGAACCAGCAATCACGAAG	60	435
Candidatus Microthrix (16srRNA) (PAOs)	F- GTTGGGCACTAGGTGTGGG R- CCTATCGTGGACCCCGAAG	60	230
Candidatus Accumulibacter)ppk1((PAOs)	F- GCGACAATCTCAAATTCAAGCCT R- TCGGATTGAAGCTCTGGTAGG	59	129
Sphingomonas (16SrRNA) (PAOs)	F- CGTAACGCGTGGGAATCTG R-GCTTTACAACCCTAAGGCCTTC	59	306
Competibacter (PAOs)	F- GAATTTTACGGAGACATG R- TAGCGTCCCATGGACAACTTC	58	375
Paracoccus (DPAOs)	F- TGATTTTACGGCGGGAATCTG R-GCTTTACAACCCTAAGGCCTTC	57	450
Rhodocyclus (PAOs)	F- CAATTTTACGGAGACGAATCTG R- TGGTGATAGAGGAAGGCCTTC	59	350
Nitrosococcus (AOB)	F- gcAACGCGTGGGAATCTG R- GCTTTAgactctcacGGCCTTC	60	

T_A: Temperature annealing.

presented in Table 1.

For PCR reactions, the PCR solution with a final 25 μL volume was prepared from adding 6 μL of DEPC water, 1 μL of 10 pmol Forward primer solution and 1 μL of 10 pmol Reverse primer solution plus 12 μL of master mix into a micro-tube. Then, 5 μL of the extracted DNA was added to each micro-tube. The micro-tubes were electrophoresed using 1.5 % (W/V) agarose gels, followed by staining (for 1 h) with gel red. Thereafter, the PCR reaction was performed according to the following conditions: Pre-denaturing step for 5 min at 95 °C followed by 30 cycles of denaturing for 1 min at 95 °C, annealing for 1 min, extension for 2 min at 72 °C, and final extension for 2 min at 72 °C.

2.3. Determination of residual organic matters and sludge characteristics

In this research, the contents of residual organic matters from the treated wastewater known as soluble microbial products (SMP) were quantified. The main constituents of SMP are proteins and carbohydrates. In addition, in order to identify the physical sludge characteristics as bio-flocculation and settleability, sludge volume index (SVI), the SMP and extracellular polymeric substances (EPS) extracted from sludge, and some effluent parameters such as TSS and turbidity were measured. The EPS itself is comprised of loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS). The SMP and EPS were extracted from sludge using the protocol followed by the research team of Mei et al. (2014). The quantity of SMP, TB-EPS and LB-EPS was determined as TOC using TOC analyzer (TOC-5000A, Shimadzu). The amounts of proteins and carbohydrates were measured based on Lowery and phenol-sulfuric acid methods using bovine serum albumin (BSA) and dextrose as standard, respectively (Lowry et al., 1951; Dubois et al., 1956).

2.4. Estimation of operating cost

Given that the estimation of the operating cost is an essential factor to check the performance of the bioreactor, in this study, the economic evaluation was conducted. In this regard, the cost performance index (CPI) was calculated considering pumping energy (PE), sludge production (SP), aeration energy (AE), mixing energy (ME) as energy sources and also effluent quality (EQ) (Zhou et al., 2015). The pollutant unit (PU, mg/L) was determined taking into consideration the concentration of four main process parameters i.e. TCOD, TSS, TN and PO_4^{-3}-P and the weighting factor (β) recommended by the Chinese EPA for each parameter (i.e. $\beta_{TSS} = 0.25$, $\beta_{COD} = 1$, $\beta_{TN} = 20$, and $\beta_{TP} = 20$). The parameters of the operating cost evaluation are computed according to the literature (Gernaey and Jørgensen, 2004; Stare et al., 2007; Zhou et al., 2015).

3. Results and discussion

3.1. Carbonaceous compounds removal as TCOD removal efficiency

From the ANOVA analysis (Table S4), a quadratic model was employed to describe the variations trend in TCOD removal efficiency as function of operating variables. Among different model terms, only the model terms (in the order of the highest coefficient) A, B, C with linear and positive effects, A^2 and C^2 with square and negative impacts were regarded as the significant model terms. In general, model terms were screened according to removing the insignificant model terms with the highest p-value based on a beneficial simplification. So that meantime keeping the values of some statistical parameters in a high level (R^2-Adj. R^2, Pre. R^2 and Adequate Precision), the simplest model was selected in order to simplify the interpretation of three dimensional (3-D) response surface plots. The removal stages of the various model terms and their effects on the statistical parameters and the simplification of the proposed 3-D response surface plots were shown in Figure S1. From the Figure, removing three model terms of B^2, AC, AB and BC seemed

reasonable due to the mentioned reasons while omitting model terms BC and C did not appear to influence the statistical parameters significantly.

It should be mentioned the 3-D response surface plots for each response were portrayed as a function of the variables having the most impact on the response based on the obtained regression coefficients (Table S4). Accordingly, the 3-D response surface plots of the TCOD removal efficiency as a function of HRT (A) and AFR (B) at constant AVR (C) were displayed in Fig. 2. As clear from the Figure, the longer HRT, the more TCOD removal efficiency. The impact of the aeration volume ratio (AVR) on the relevant response was somewhat insignificant. In summary, the TCOD content can be consumed in anaerobic, oxic and anoxic phases using electron acceptors of oxygen and N–NO$_3$, respectively, for two latter redox reactions. Such justification was reported by Abyar et al. in the case of the high rate up-flow A2O bioreactor treating meat processing wastewater (Abyar et al., 2018).

The gradient of DO concentration at the various points of the ordinary DCAL-A2O bioreactor as shown in Figure S2 is confirming the co-existence of the anaerobic, anoxic and oxic phases under various operating conditions. The high TCOD removal efficiency is in the respect with the high oxidation potential provided in the new configuration of the bioreactor for heterotrophic bacteria to use the carbonaceous matters for their growth and activities.

In overall, the synergistic effect of HRT, AFR and AVR led to the remarkable enhancement in the TCOD removal efficiency. The maximum amount of response (98.9 %) was observed at the highest value of HRT, AFR and AVR of 15h, 3 L/min and 0.464, respectively. In addition, the distribution of the experimental data close to the straight line of the predicted data was another evidence demonstrating the accuracy of the employed model (Fig. 2d). Asadi et al. could reach more than 90 % of sCOD removal efficiency in an airlift bioreactor with CFID regime treating MPW containing around 1000 mgCOD/L at AFR of 1 L/min and HRT of 10 h (Azar Asadi et al., 2016a). In another study, the authors succeed to get the full sCOD removal efficiency from about 1000 mgCOD/L containing SDW in the introduced bioreactor under optimum conditions obtained in HRT of 12h, internal diameter of 5 cm, and DO of 3.5 mg/L (Azar Asadi et al., 2016b).

3.2. Nitrogenous compounds removal as TN removal efficiency

From Table S4, the TN removal efficiency was modeled using a reduced quadratic model. The statistical analysis of TN removal efficiency as a function of the operating variables i.e. HRT, AFR and AVR coded as A, B and C, respectively, and beneficial simplification revealed the significance of the linear; interactive; square model terms A, C; AB, AC, BC; and A^2, respectively. The other model terms B^2, B, and C^2 were eliminated in the order of the increase in p-value and their subsequent impact on the variations of the statistical parameters as depicted in Figure S3. The impact of the studied independent variables on the TN removal efficiency was illustrated as the 3-D response surface graphs shown in Fig. 3. These graphs were delineated as a function of the model
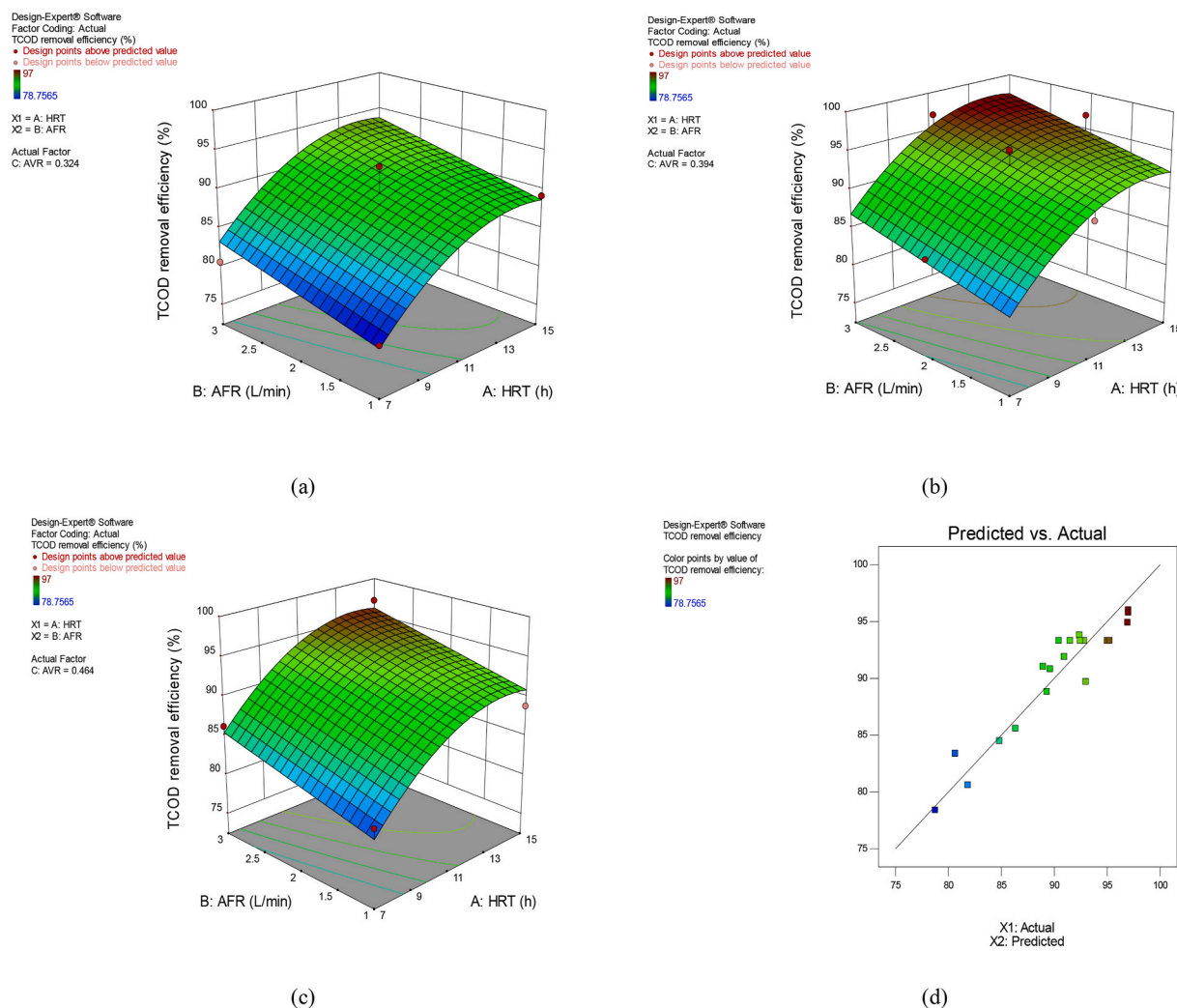

Fig. 2. The 3-D response surface plots of TCOD removal efficiency at various AVRs, (a) 0.324; (b) 0.394; (c) 0.464; and (d) the experimental data versus the predicted ones.
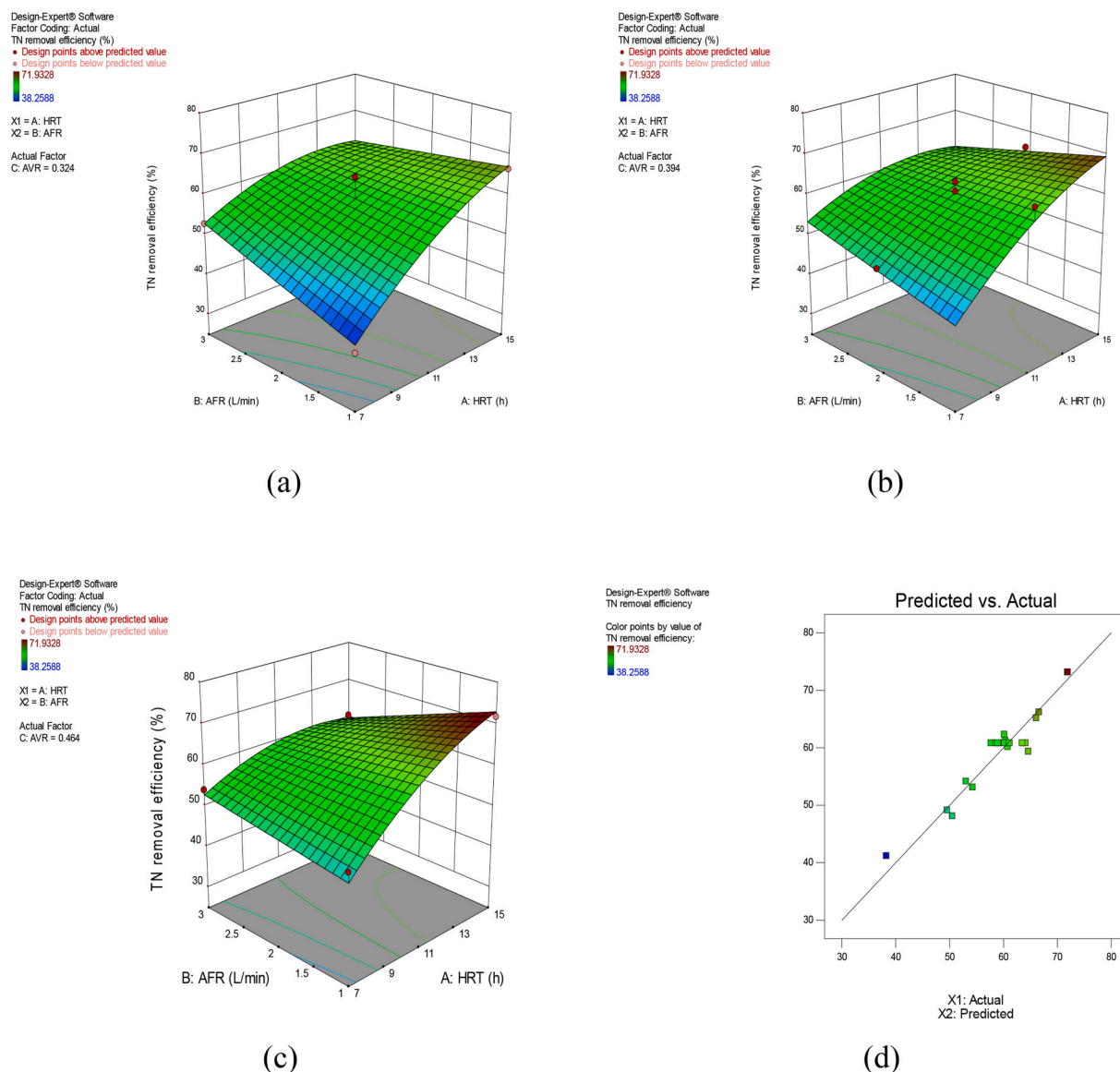
(a)

(b)

(c)

(d)

Fig. 3. The 3-D response surface plots of TN removal efficiency at various AVRs, (a) 0.324; (b) 0.394; (c) 0.464; and (d) the experimental data versus the predicted ones.

terms A (HRT) with the highest positive coefficient of 8.01 and the model term B (AFR) with interactive effects appeared as the negative model terms AB and BC. As evident from the Figure, the TN removal efficiency in the ordinary airlift A2O bioreactor treating the milk processing wastewater (MPW) exhibited the similar variations trend at different AVRs ranged from 38.2 to 71.9 %. Generally, the specific design of the airlift A2O bioreactor equipped with a dual internal circulation provided the convenient environment required for the growth of nitrifying and denitrifying bacterial species, and anaerobic bacteria responsible for the nitrogen removal over the simultaneous nitrification and denitrification process (SND), and anaerobic ammonium oxidation (anammox), respectively. Obviously, the increase in HRT (A) and AVR (C) resulted in the considerable increase in the TN removal efficiency. While, the increase in the AFR (B) indicated reverse impact on the response. The reduced AFR led to the mitigation of the circulation turnover between the anaerobic, anoxic and oxic zones, thereby, the enhancement of denitrification and anammox processes, and finally the nitrogen removal efficiency. Considering the bioreactor with the special geometry had the superior capacity in the TCOD removal, therefore, the fast depletion of the organic compounds makes an opportunity for the

nitrifying bacteria to do nitrification process using ammonia nitrogen. The changes trend in the nitrification process was in a direct proportion with TCOD removal, so that the lowest nitrification rate (0.15 g-N/L.d) was related to the lowest TCOD removal efficiency (78.8 %) occurred at HRT, AFR and AVR of 7h, 1L/min and 0.324, respectively. In addition, the nitrification process is influenced directly by AFR and AVR. So that in some runs, in spite of the short HRT (7 h) and high OLR (4.6–4.7 g-COD/L.d), the nitrification process was promoted due to the extended aerobic conditions (AVR of 0.464 & 0.392) and adequate oxygen (AFR of 3 & 2 L/min) created the suitable circumstances for high oxidation potential and fate of the organics, therefore, the growth of nitrifying autotrophic bacteria. In a study conducted by Abyar et al., the HRT showed a neutral role in the TKN removal (Abyar et al., 2018). However, the authors pointed out the TN concentration was significantly decreased from 178.76 to 16 mg/L only in the HRT of 5 h. In the present study, the highest TN removal efficiency of 71.9 % was appeared at the greatest AVR (0.464) and HRT (15 h) and the lowest AFR (1 L/min). In this study, the TN removal is mainly governed by HRT. Asadi et al. and Mirghorashyi et al. also in their studies reported the effective role of HRT in the improvement of the TN removal efficiency nicely (Azar Asadi

et al., 2016c; Mirghorayshi et al., 2021). Asadi et al. reported the TN removal efficiency of 85 % in an airlift A2O with CFID regime treating the MPW with inlet TN ranged from 190 to 204 mg/L under optimum conditions (HRT of 10 h and AFR of 2 L/min) (Azar Asadi et al., 2016a). Also, the authors obtained the TN removal efficiency of 72 % from the SDW with inlet TN of 159–171 mg/L under optimum conditions found at HRT of 12 h, D_{in}/D_{out} of 0.5 and DO of 3.5 mg/L (Asadi et al., 2016b).

The nitrogen contents and contribution of various reactions i.e. the nitrification and denitrification processes, and cell synthesis at various operating conditions were represented in Figure S4. The nitrification (mg-N/L), denitrification (mg-N/L) and cell synthesis (N_{cell}, as mg-N/L) are calculated using below expressions.

$$N_{denitrification} \text{ (mg-N/L)} = TN_{in} - TN_{out} - N_{cell}$$

$$N_{acidification} \text{ (mg-N/L)} = N_{denitrification} + NO_3\text{-}N_{out} + NO_3\text{-}N_{in}$$

$$N_{cell} \text{ (mg-N/L)} = 0.12 \, ((MLVSS.V)/(SRT.Q_{in}))$$

where, V represents the volume of the bioreactor, while Q_{in} denotes the influent feed rate.

The cell synthesis showed the most impact at the shortest HRT while the contribution of the denitrification process was clearer at the longest HRT. The best balance between nitrification (152.3 mg/L) and denitrification (146.4 mg/L) processes was observed at AVR of 0.464, AFR of 1 L/min and the HRT of 15 h. In general, the high AVR, long HRT and low AFR guaranteed the high TN removal efficiency as a result of balance between nitrification and denitrification processes, and growth of anammox bacteria. Although, anammox bacteria are inhibited by the organic contents, they can coexist with denitrifying bacteria in one reactor under the properly monitored operating conditions where help realize the concurrent nitrogen and COD removal (Y. Chen et al., 2021). Throughout the experimental runs, nitrite oxidizing bacteria (NOB) were active at various DO concentrations showing the appropriate DO level for the nitrification. The obtained results were in accordance with the findings documented in the existing scientific literature (Chen et al., 2021b; Lu et al., 2023).

Therefore, in a final conclusion, it can be said to get high process performance of the ordinary airlift A2O bioreactor in terms of the high TN removal efficiency, two solutions can be adopted. In this regard, reduction in influent TN concentration or the enhancement in the anoxic zone can be considered as effective solutions to reach the high TN removal efficiency. Chen et al. in their study investigated the performance of a one-stage airlift internal circulation reactor treating low strength ammonia wastewater (50 mg/L N–NH_4^+) by providing partial nitritation and anammox process using attached growth and micro-granular sludge (H. Chen et al., 2021b, 2021a). The authors obtained nitrogen removal efficiency of 70 % and 72.7 % at low DO concentrations of 0.05–0.15 mg/L and 0.1–0.2 mg/L. Guo et al. for the first time could reach high nitrogen removal rate (1.28 kg/m^3.d) at low HRT of 1 h treating synthetic low strength ammonia wastewater (63 mg/L) in an airlift bioreactor via partial nitritation and anammox process provided by tiny and concentrated granular sludge with excellent settleability cultured by hydroxyapatite (HAP) (Guo et al., 2021). Also, Chen et al. using the one-stage partial nitritation and anammox process provided by the hydroxyapatite (HAP)-cultured syntrophic granules and denitrification were successful to treat the fish processing wastewater pre-treated by a self-agitated anaerobic baffled reactor (Y. Chen et al., 2021). The authors got high nitrogen removal rate and nitrogen removal efficiency of 1.51 ± 0.10 kg N/m^3d and 88.2 %, respectively. Guo et al. also in their studies reported the nitrogen removal via aforementioned mechanisms in the referred bioreactor feeding actual municipal wastewater as low strength ammonia wastewater pre-treated in anaerobic membrane bioreactor (AnMBR) (Guo et al., 2022). In another study conducted by Rong et al., a combination of submerged AnMBR and biofilm partial nitritation-anammox (PNA) systems at a pilot-scale could be able to treat the real low strength municipal wastewater during

biological reactions of anaerobic digestion (AD), denitrification, partial nitritation and anammox processes under ambient temperature (Rong et al., 2021). The authors obtained the maximum COD and TN removal efficiencies of 95.1 % and 81.7 %, respectively. The research teams of Liu et al. and Wang et al., tried to treat synthetic nitrogen rich wastewater with no organic carbon at various nitrogen loading rate (NLR) and HRTs at 25 °C via symbiosis of ammonia oxidizing bacteria (AOB) and anammox bacteria in a one-stage airlift bioreactor using biofilm growth and granular sludge, respectively (Liu et al., 2017; Wang et al., 2017). The maximum nitrogen removal efficiency was obtained to be 81.1 % and 82 % at NLRs of 0.5–2.5 kg-N-NH_4^+/m^3.d. Guo et al. for the first time could reach simultaneous nitrogen removal and phosphorous recovery from synthetic digestion effluent using combination of partial nitritation/anammox process and hydroxyapatite crystallization in a single airlift bioreactor (Guo and Li, 2020). The authors investigated the performance of the single bioreactor at various ammonia and calcium concentrations at constant HRT of 6 h. The authors in another study investigated the performance of the relevant bioreactor treating synthetic low strength ammonia wastewater under 5 phases at various HRTs with varied N–NH_4^+, phosphorus and $CaCl_2$ concentration (Guo et al., 2021). The hydroxyapatite augmented granular sludge with high settleability, high concentration and effective substance transfer realized high nitrogen removal rate. Guo et al. recently released a report regarding simultaneous removal of nitrogen and phosphorous content from permeate of anaerobic membrane bioreactor (AnMBR) (COD-reduced municipal wastewater) in an airlift bioreactor based on partial nitritation anammox and iron phosphate crystallization (Guo et al., 2023). The authors reached to nitrogen and phosphorous removal efficiencies of 37.6 and 62.7 %, respectively, treating the wastewater containing 50 mg/LN-NH_4^+ and 5.4–7.1 mg/L P.

However, the novelty of our work is related to the simultaneous CNP removal from a real high strength milk processing wastewater without adding any external inorganic matters in a one-stage dual internal circulation airlift A2O (DCAL-A2O) bioreactor.

3.3. Phosphorus removal

Totally, in the one-stage bioreactors, a variety of functional bacteria responsible for removing carbon and nutrients (N & P) start their activity simultaneously if the required conditions are provided. While, in the conventional biological nutrient removal (BNR) systems, pollutants of wastewater (as C, N & P) can be removed independently in the separate compartments. To remove phosphorus (P) in single integrated bioreactors, co-existence of two anaerobic and aerobic media in an appropriate proportion is necessary. The imbalance between the mentioned environments gives rise to the reduction in the phosphorus removal efficiency.

From Table S4, the interactive model terms AB, AC, BC and the square model term B^2 both sets with the negative impact are known as the significant model terms. These model terms are selected based on the statistical parameters and beneficial simplification as shown in Figure S5.

Interestingly, the changes trend in phosphorus removal efficiency was different at various AFRs visualized in Fig. 4 as 3-D response surface plots. The highest phosphorus removal efficiency was attained at the mean AFR ascribed to the sufficient mixing leading to balance the anaerobic and aerobic conditions. At mean AFR (2 L/min), the HRT exhibited the reverse effect on the phosphorus removal efficiency at the highest AVR of 0.464 due to the imbalance of the anaerobic and aerobic conditions as a result of the enhanced aerobic zone. While at the lowest AVR of 0.324, corresponding with the increase in anaerobic and anoxic zones, and the mean AFR of 2 L/min, the HRT revealed the direct influence on the response due to the increase in duration of mentioned zones. According to Abyar et al., nitrate acts as an electron acceptor for the absorbance of the phosphorus under anoxic conditions (Abyar et al., 2018).
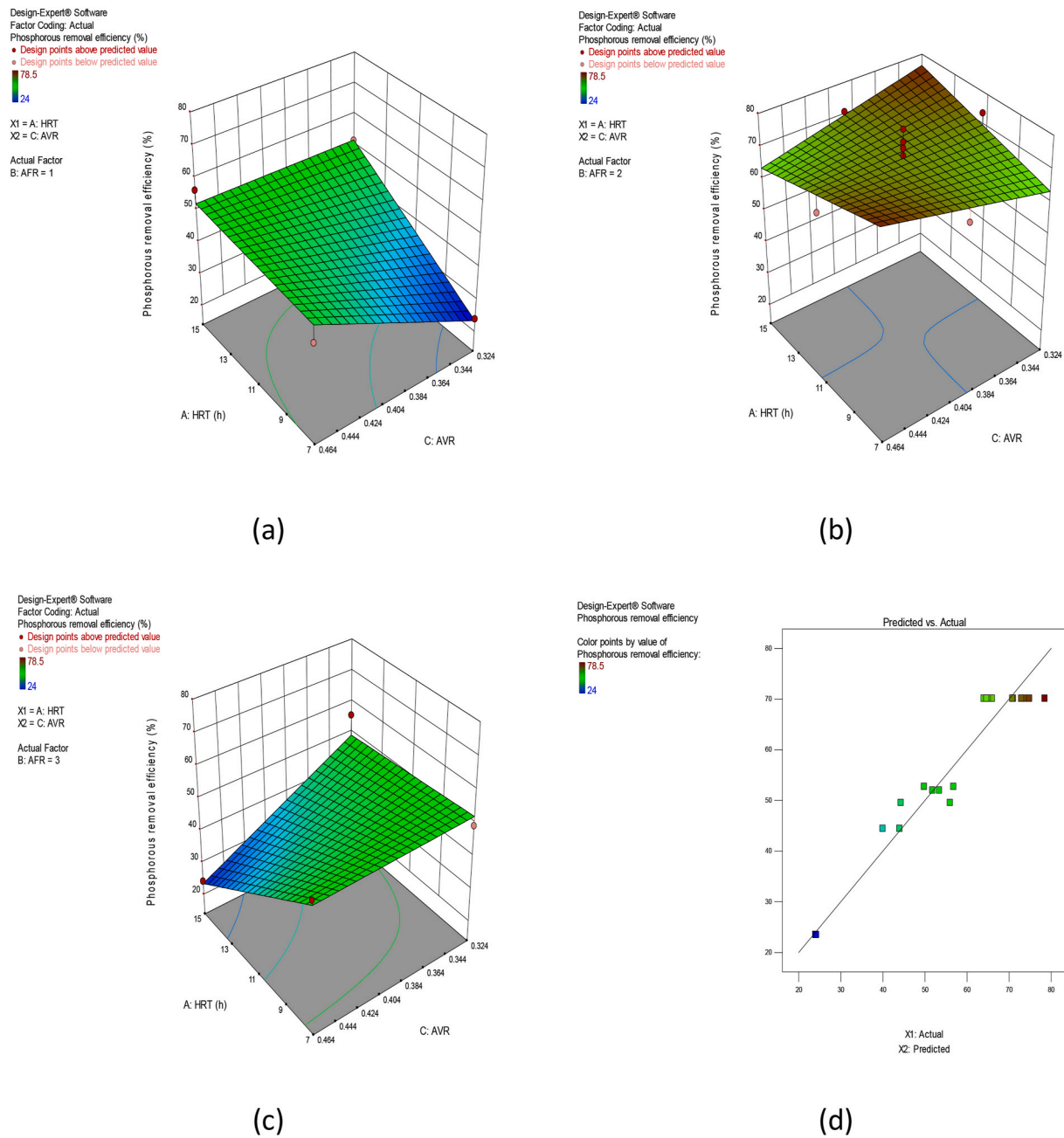
(a)

(b)

(c)

(d)

Fig. 4. The 3-D response surface plots of phosphorus removal efficiency at various AFRs, (a) 1 L/min; (b) 2 L/min; (c) 3 L/min; and (d) the experimental data versus the predicted ones.

From Fig. 4 a, the increase in the AVRs and HRTs at the AFR of 1 L/min resulted in being enhanced the response as a reason of the promoted aerobic conditions and the increase in the duration of the phosphorus absorbance process under the reduced F/M ratio. In general, the low mixing rate (at AFR of 1 L/min corresponding to superficial gas velocity of 0.1 m/min) and high mixing rate (at AFR of 3 L/min corresponding to the superficial gas velocity of 0.3 m/min) of the mixed liquor suspended solids (MLSS) created through the airlift force between anaerobic and aerobic zones led to the reduction in the phosphorus removal. Since the turbulent flow happened in the high AFR restricts the anaerobic zones at the beginning of the relevant run, thereby, makes a disturbance in the phosphorus release process. In addition, laminar flow appeared at the low AFR limits the aerobic zone and hence required oxidation potential to obtain satisfactory phosphorus absorption. While, at the mean AFR (2 L/min corresponding with superficial gas velocity of 0.2 m/min), an

appropriate balance was created between the required environments for the removal of the relevant response due to suitable circulation and proper oxidation potential. From Fig. 4d, the experimental data indicated the good agreement with the actual data. Asadi et al. successfully obtained the maximum phosphorus removal efficiency of 78 % at the mean HRT of 10 h and the highest AFR of 3 L/min ascribed to the extended aerobic conditions in an airlift bioreactor with CFID regime (Asadi et al., 2016c). The authors stated the further increase in HRT (14 h) at the mean and high AFRs of 2 and 3 L/min, respectively, give rise to reducing the response. In another study, the researchers succeed to remove the phosphorus in a high level of 85 % under special operating conditions (HRT of 14 h; D_{in}/D_{out} of 0.5 and DO concentration of 2 mg/L) (Asadi et al., 2016b). In a study conducted by Abyar et al., the maximal phosphorus removal of 94.2 % was reported at the lowest HRT (7.5 h) and aerated volume fraction (50 %), and the highest COD/TN

ratio (100:16) treating the meat processing wastewater in a high rate up-flow A2O bioreactor (Abyar et al., 2018).

3.4. Effluent turbidity and sludge bio-flocculation

Generally, the effluent turbidity known as index of effluent TSS examination is regarded as a quantitative indicator to detect the level of suspended solids in the clarified effluent, and shows the sludge bio-flocculation and functionality of the settling tank. According to Table S4, the negative linear model terms A, B and C, the positive interactive model term AB and the square model term C^2 were recognized as the significant and effective model terms. The reason of the selection of these model terms was simplifying the 3-D surface response plots at the high level of the statistical parameters as delineated in Figure S6.

The 3-D surface response plots of the effluent turbidity as a function of the operating variables (A & B at constant C) are displayed in Figure S6. Among the various model terms, A and B revealed the highest direct negative impact on the response with the coefficients of −15.0 and −20.20, respectively, whereas, the model term C had the lowest direct effect with the coefficient of −6.50. From the Figure, the decrease in the HRT was brought about deterioration of the effluent quality based on the turbidity indicator, specially, in the lowest AVR (0.324). The decrease in the HRT corresponds with the increase in the F/M ratio and the OLR that have a negative influence on the effluent turbidity via the imposition of the sudden shock to the airlift A2O bioreactor. The worst effluent turbidity (96 NTU) was reported at HRT, AFR and AVR of 7 h, 1 L/min and 0.324, respectively, corresponded to the lowest solids retention time (SRT, 6.4 d), the highest F/M ratio (0.78 1/d) and OLR (5.2 g-COD/L.d). While, the lowest effluent turbidity (4 NTU) was related to the highest HRT (15 h), AFR (3 L/min) and AVR (0.464) with the high SRT of 33 d and low OLR of 2.2 g-COD/L.d. According to Asadi et al., the increase in the DO concentration results in the formation of stable and dense flocs, thereby, the reduction in the effluent turbidity, while, the low DO and HRT courage the expansion of the sludge and the increase in the turbidity (Asadi et al., 2016b, 2016c). The obtained results were comparable with those reported by research teams of Asadi et al. and Mirghorayshi et al. (Azar Asadi et al., 2016b; Mirghorayshi et al., 2021).

3.5. Sludge volume index (SVI)

Given that the issue of bulking and floating sludge is known as a big challenge in the wastewater treatment plant, in this study, the settling ability of the sludge was monitored via the measure of the sludge volume index (SVI). The results obtained for ordinary DCAL-A2O bioreactor are presented in Figure S7. The reduction in the SVI was in a good agreement with the decreased effluent turbidity values. The decline in the OLR and F/M due to the increase in HRT gave rise to the enhancement in the sludge settling properties corresponded to the reduced SVI. Furthermore, the mitigation in AFR and DO concentration along with the reduction in AVR (reduced aerobic space) resulted in the decrease in SVI values. From the results, the lowest SVI value (80 mL/g) was gained under favored operational conditions dictated to the airlift bioreactor i. e. HRT of 15 h, AFR of 3 L/min and AVR of 0.464. Abyar et al. in their studies got to a desirable range of 75.9–166.33 mL/g, where, the lowest SVI was contributed to the lowest effluent turbidity (1.5 NTU) confirming the effective accumulation of the sludge inside the A2O bioreactor and inhibited wash-out treating the meat processing wastewater (Abyar et al., 2018).

Surprisingly, in spite of the high SVI values in some runs, the effluent turbidity was satisfactory. The reason of such observations was described by the presence of the internal circulation between the settling tank and the reaction zone as well as on time discharging the surplus sludge that led to retaining the sludge level below the bioreactor' outlet. Thereby, the sludge wash-out was monitored considerably.

3.6. Sludge bio-flocculation and settleability properties

To further study on the sludge bio-flocculation and settleability properties, the characteristics of the treated wastewater and the sludge were investigated using more analysis. Although the biological treatment-based processes are extensively employed to treat various wastewaters, the treated wastewaters still contain a variety of soluble organic matters including proteins, carbohydrates and humic acids etc., deriving from various mechanisms over the feed metabolism, extracellular polymeric products (EPS) hydrolysis, microbial growth and decay (Shon et al., 2006). The physical and chemical properties of such residual organic compounds leave a significant impact on the following treatment approaches, the effluent discharge level and final environmental complications, and water reuse applications. Accordingly, the determination of the concentration and characteristics of the bioreactor effluent contents must be explored deeply. More importantly, the concentration level of these components has a direct relationship with the effluent TSS, consequently, effluent turbidity and sludge bio-flocculation. In overall, the concentration of organic residues from the bioreactor effluent is measured as COD and TOC (Aquino and Stuckey, 2002). On the other hand, a significant portion of the treated wastewater is weakly identified chemically that might be contributed to the low concentration of these compounds. In addition, the sludge characteristics as SMP, LB-EPS and TB-EPS influence the sludge bio-flocculation and settleability properties. In the present study, the relationship between the protein and carbohydrate contents of the effluent SMP, sludge SMP, LB-EPS and TB-EPS with the effluent TSS (index of sludge bio-flocculation examination) and SVI (index of sludge settleability examination) were studied under the various operating conditions. From the literature report, the proteins and carbohydrates are known as the main constituents of residual organic matters (Zhang et al., 2008). That's why, in this study, the changes in amounts of only these two components were checked. Despite the importance of characteristics identification of the sludge and the treated wastewater effluent based on microbial secretions (SMP & EPS), only few work has been published in this field (Li and Yang, 2007; Zhang et al., 2008). In a study conducted by Li et al., there was a reverse correlation between the protein and carbohydrate concentrations and pre-determined SRTs (5, 10, and 20 d) in glucose-fed and acetate-fed bioreactors (Li and Yang, 2007). The bioreactors were operated as batch wise with MLSS of 2000 mg/L and COD:N:P of 100:5:1. Nonetheless, in the present study, other than the SRT, the impact of other variables i.e. HRT, AVR and AFR was investigated on the sludge characteristics. The results obtained from the protein and carbohydrate concentrations of effluent SMP, sludge SMP, LB-EPS and TB-EPS are given in Table S5.

From the Table, there is a direct relationship between effluent TSS (effluent turbidity) and the protein and carbohydrate concentrations derived from effluent SMP, sludge SMP, and LB-EPS. As clear from the Tables, the increase in AFR, AVR and HRT had descending effect on the protein and carbohydrate concentrations, and SMP and LB-EPS concentrations.

In this study, the high amounts of protein and the carbohydrate are attributed to the difference in the operating conditions and the type of influent feed. According to Li et al., the glucose-based feeding resulted in production of more EPS compared to the acetate-based feeding confirming the role of feed carbon source in the EPS production (Li and Yang, 2007). The authors stated the sodium acetate follows simple metabolic pathway, and directly enters the citric acid cycle, while, glucose-based feed prior to entering the citric acid cycle, firstly needs to be degraded to pyruvate, and then oxidized into acetyl CoA. Thereby, due to the complexity in metabolism of glucose, the more extracellular enzymes are expected to be produced that is a sign of the high EPS production compared to another feed.

In general, the increase in AFR and AVR at the presence of suitable HRT (11–14 h) provides the favorable conditions for the further growth of the aerobic microorganisms which are responsible for removing the

main portion of the influent COD owing to having higher substrate utilization and microbial growth rates compared to other microorganisms. On the other hand, short HRT imposes the high organic loading rate to the bioreactor giving rise to excessive metabolic activity and therefore the more EPS production which in turn more excretion of SMPs into the surrounding environment. These outcomes are reliable evidences for the deterioration in the sludge bio-flocculation and settleability observed in some experimental runs. Accordingly, the best sludge bio-flocculation and settleability appeared at the longest HRT, highest AFR and AVR. For ordinary DCAL-A2O bioreactor, the lowest effluent SMP, sludge SMP, LB-EPS and TB-EPS were reported to be 33.2, 37.8, 42.0 and 229.4 mg-TOC/L, respectively, at the mentioned favored operating conditions.

Li et al. pointed out the high production of LB-EPS at the shortest SRT (5 d) deteriorated the attachment between cells and weakened the aggregate structure resulting in cell erosion from the sludge floc under turbulent situations. The authors declared the LB-EPS amounts were decreased with the increase in the SRT regardless the type of substrate used, while, the TB-EPS amounts remained constant. Furthermore, they stated although TB-EPS amounts were much higher than LB-EPS that is only LB-EPS contents having an impact on the sludge properties since that might probably act as the primary surface for the attachment of the bacterial cells. Zhang et al. in their study examined the protein and carbohydrate contents from the effluent of various bioreactors using resonance light-scattering method (Zhang et al., 2008). The concentration of protein and carbohydrate was reported to be less than 10 mg/L. Such low amounts might be in the respect with the type of wastewater used.

The high contents of microbial secretions (as SMP and EPS) are in respect with deterioration of cells attachment and integrity leading to the weakness in the sludge settlement. The obtained results confirmed the higher values of protein over other components ascribed to the presence of the extracellular enzymes due to the complex metabolism of the MPW.

3.7. Process optimization and verification

As far as getting the high effluent quality is the final target of the wastewater treatment specialists, therefore, the suitable values of the independent and influential variables must be determined using the process optimization. In this study, four significant dependent variables i.e. TCOD, TN and phosphorus removal efficiencies and the effluent turbidity were considered to obtain the optimum conditions employing Design-Expert® software (Version 10). The overlay plot visualizing the region of viable responses in the design space via delineation of graphical optimization is shown in Figure S8. The process optimization was identified considering the maximum TCOD, TN and phosphorus removal efficiencies and the minimum effluent turbidity. Accordingly, the high acceptable lower limit for the first three responses i.e. TCOD, TN and phosphorous removal efficiencies and the low acceptable upper limit for the latter were quantified. From the graphical optimization of the overlay plot, the regions shaded in yellow and gray are the representative of the areas that do meet the optimization criteria and do not fit the desired constraints, respectively. Based on satisfied criteria i.e. TCOD>90 %, TN>60 %, phosphorous > 60 % and effluent turbidity <10NTU, the optimum zone lies between HRT of 13–15h and AFR of 2 L/min. Since the main target followed in the bioreactor configuration development is the reduction in the bioreactor volume and energy consumption through the decrease in the HRT and AFR, respectively, meanwhile maintaining the high biological performance, therefore, the highest lower constraint for the TCOD, TN and TP removal efficiencies while the lowest upper limit for the effluent turbidity were taken account for achieving the intended target as well as enforcing the relevant limitations more strictly. In this study, by considering the design criteria, HRT, AFR and AVR of 13 h, 2 L/min and 0.437, respectively, were chosen as the optimum conditions for the further investigation. To

confirm the accuracy of the proposed optimization region and validation of predicted results, a verification experiment was conducted at the optimum conditions. The proposed optimum conditions in terms of HRT, AFR and AVR, plus predicted and actual results are presented in Table S6. As clear from the Table, the results attained from the experimental data were strongly consistent with those predicted by the model. The effect of wastewater biodegradability and feeding location on the bioreactor performance along with cost evaluation are discussed in supplementary information. The obtained results are comparable with the literature (Table S7).

3.8. Microbial community

To evaluate the various microbial community (as given in Table 1) in the bacterial biomass of the ordinary DCAL-A2O bioreactor, PCR test was conducted. The PCR results obtained with 18 primers are presented in Figure S9 and Table 2. From the Table, the existence of various bacterial species detectable through the band appeared in the Figure was determined as positive sign. The symbiotic of the anammox bacteria (Candidatus_Brocadia) and denitrifing bacteria (Pseudomonas) in a single unit under optimum operating conditions was a strong evidence for nitrogen removal via SND and anammox mechanisms. Furthermore, phosphorus removal was confirmed by the presence of two PAOs species (Candidatus Accumulibacter and Rhodocyclus). While appearance of glycogen accumulating organisms (GAO) (Sphingomonas) showed simultaneous partial nitritaion-denitrification (SPND). In all, it can be deduced that optimum operating conditions provided suitable circumstances for co-growth of anammox, nitrifying and denitrifying bacteria to remove CNP concomitantly from an industrial wastewater.

4. Conclusions

In this study, the impact of influential key variables governing the behavior of a one-stage dual internal circulation airlift A2O (DCAL-A2O) bioreactor was evaluated on the process performance, sludge characteristics, microbial community and operating cost. From a holistic perspective, the insights gained from this study were particularly notable in relation to the unique design of the A2O bioreactor, characterized by a dual internal circulation system that encompasses three distinct zones: anaerobic, anoxic, and oxic. This specific configuration of the bioreactor has effectively facilitated the simultaneous removal of carbon, nitrogen, and phosphorus (CNP) from high-strength wastewaters within a single unit using culturing various functional microbial community. The process functionality of the bioreactor throughout the

Table 2
The bacterial species identified (+) and those not found (−) in ordinary DCAL-A2O bioreactor.

No.	Name	Product length	Ordinary
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	+

experimental runs were ranged from 78.8 to 97.0 %, 38.2–71.9 %, 24.0–78.5 %, 4–96 NTU for TCOD, TN, phosphorus removal efficiencies and effluent turbidity, respectively. Specific geometry of the DCAL-A2O bioreactor provided this possibility for co-existence of anaerobic, anoxic and aerobic zones inside a single unit via the DO concentration gradient. The increase in HRT while the reduction in AFR played an essential role in the enhancement of the process performance compared to the AVR. The optimum region was explored at HRT of 13h, AFR of 2 L/min and AVR of 0.437 to reach the best functionality as TCOD (94.5 %), TN (59.6 %), phosphorus (62.2 %) removal efficiencies and effluent turbidity (8 NTU). Moreover, the performance of the bioreactor was evaluated using three various wastewaters (MPW, SDW and SOW) with different biodegradability (BOD_5/COD ratio). From the results, the MPW- and SOW-fed bioreactor showed the high capacity in the TN removal, whereas, the SDW-fed bioreactor led to further removal of the phosphorous. These observations were attributed to the difference in characteristics and readily biodegradable contents of the wastewater. More importantly, the feeding location (from the top of the anoxic zone or the anaerobic zone) elaborated a significant effect on the TN and phosphorous removal efficiencies contributed to the accessibility level of feed to functional and responsible bacterial species to do their job. Based on the economic assessment, the low energy was required for aerating, pumping and mixing due to the special configuration of the single unit DCAL-A2O bioreactor giving rise to elimination of additional apparatuses. The presence of various bacteria species (nitrifying, denitrifying, anammox and PAOs) in a single unit was confirmed via PCR test. In summary, the acquired findings in terms of the effluent quality encouraged us to modify DCAL-A2O bioreactor for the realization of the further nitrogen and phosphorous removal via the enhancement in the anoxic and anaerobic zones in future work.

CRediT authorship contribution statement

Zahra Rahimi: Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. **Ali Akbar Zinatizadeh:** Writing – review & editing, Supervision, Methodology, Investigation, Conceptualization. **Sirus Zinadini:** Writing – review & editing, Methodology. **Mark van Loosdrecht:** Writing – review & editing. **Damien JBatstone:** 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.

Data availability

Data will be made available on request.

Acknowledgment

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.chemosphere.2024.141804.

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