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Article

An Innovative Airlift Bioreactor Design with a Baffled Anoxic Zone for Simultaneous Carbon and Nutrient Removal via Various Mechanisms from High-Strength Wastewater

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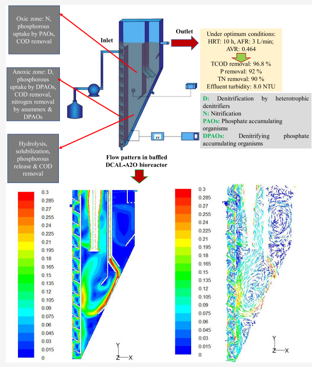
ABSTRACT: This study investigated the concurrent removal of carbon, nitrogen, and phosphorus (CNP) from milk processing wastewater (MPW). The evaluation was carried out using a newly developed single-stage bioreactor known as the baffled dual internal circulation airlift A2O (B-DCAL-A2O) bioreactor. The bioreactor functionality was monitored to determine the impact of three important factors, 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 use of baffles in the anoxic zone identified improved nutrient removal. Specifically, the total chemical oxygen demand (TCOD), total nitrogen (TN), and phosphorus removals of 94.8, 80, and 80%, respectively, as well as a reduction in the effluent turbidity of 8 NTU at optimum circumstances (HRT, AFR, and AVR of 10 h, 3 L/min, and 0.464, respectively) were obtained. From polymerase chain reaction (PCR) analysis, the superior nitrogen removal was attributed to the combined effects of simultaneous nitrification and denitrification (SND), anaerobic ammonium oxidation (anammox), and denitrifying phosphorus-accumulating organisms (DPAOs) and glycogen-accumulating organisms (GAOs). PCR analysis validated the coexistence of diverse functional microbial groups, including ammonia-oxidizing bacteria (AOB: Nitrosospira sp., Nitrosomonas sp., Nitrosococcus), and nitrite-oxidizing bacteria (NOB: Nitrospira [23S rRNA]), denitrifiers (Pseudomonas), anammox bacteria (Candidatus Brocadia and Candidatus Kuenenia), PAOs (Tetrasphaera, Candidatus Microthrix [16S rRNA], and Candidatus Accumulibacter [ppk1]), DPAOs (Pseudomonas), and GAOs (Sphingomonas [16S rRNA]). The phosphorus removal was attributed to the existence of PAOs and DPAOs in a single bioreactor. The results confirmed improved sludge properties, verified with the best results obtained for the turbidity (6 NTU) and SVI (90 mL/g) at an HRT of 15 h, an AFR of 3 L/min, and an AVR of 0.464.



1. INTRODUCTION

Biological nitrogen removal (BNR) processes remove nitrogen and phosphorus as crucial nutrients. Traditional BNR has multiple sequential compartments (generally anaerobic, anoxic, aerobic) and liquid and solid recycles, leading to relatively low loading rates.[1,2] High-rate bioreactors have been developed to address this, which are single-vessel and allow considerable reduction in the energy consumption, operational cost, and required space and volume compared with traditional BNR. The fundamental prerequisite for achieving high removal of nutrients in a single vessel is to facilitate enhancement of the growth of the various functional microbial species that can be provided through employment of efficient approaches.[3−8]

Airlift bioreactors (ALBs) are a robust rival for sequencing batch reactors (SBRs) and incorporate different zones (such as anaerobic, anoxic, and aerobic) into a single vessel. Substantial research has been conducted in optimizing ALBs for performance durability, further exploitation, and feasible practical use. For instance, Guo et al. and Jiang et al. conducted studies via developing and constructing novel variations in ALBs in order to examine the simultaneous elimination of CNP compounds.[6,9] Guo et al. employed a continuous regime known as the airlift intermittent circulation membrane bioreactor, while Jiang et al. utilized an airlift loop reactor. Asadi et al. were the first researchers to develop an ALB that utilized a continuous feed and intermittent discharge (CFID) regime for the treatment of industrial wastewaters.[10,11] Although the proposed ALB revealed a superior performance in the simultaneous removal of COD (98%), TN (>70%), and TP (>60%) from studied wastewaters under varied operating and processing conditions, the need of additional equipment and complexity in operation are substantial limitations. Gholami et al. could continuously operate a new design of the ALBs by the combination of a jet loop bioreactor and ALBs

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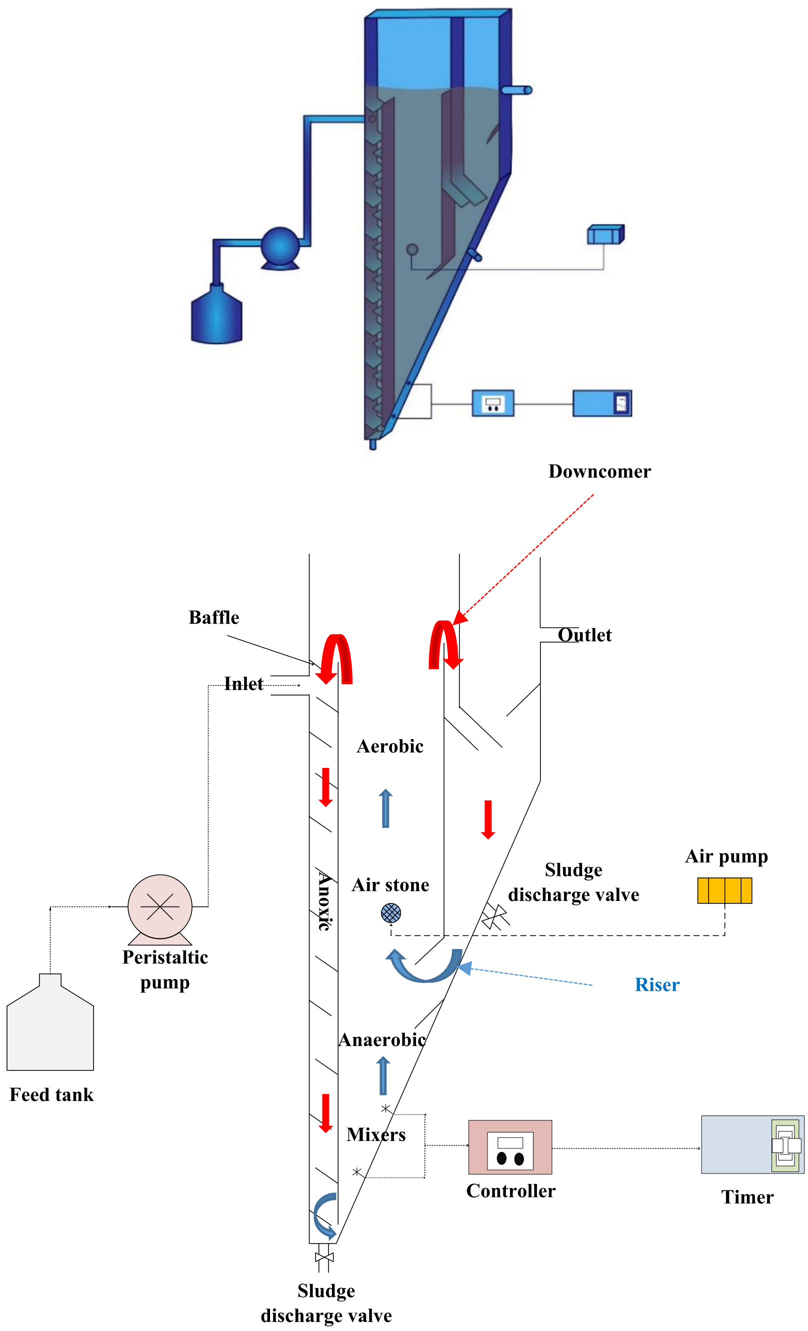

Figure 1. Schematic of the experimental rig.

as a jet loop airlift bioreactor with a settling zone included in a single unit to concurrently eliminate CN (above 80%) from soft drink wastewater (SDW).[12] By providing anaerobic conditions at the bottom of the previous jet loop airlift bioreactor through a movable aerator, simultaneous CNP removal from SDW under various operating conditions was investigated.[13] In spite of the successful functionality of the referred ALB (jet loop airlift bioreactor), a high aeration requirement and an inefficient settling zone are major restrictions against large-scale applications. In order to address these challenges, Mirghorayshi et al. explored the simultaneous elimination of carbon and nitrogen from composting leachate wastewater using a novel configuration of ALBs by benefiting from hybrid growth and anaerobic digestion and incorporating three zones—anaerobic, anoxic, and aerobic—into a single integrated unit.[14] Although the bioreactor achieved high removal efficiencies (90% for carbon 80% for nitrogen), the attachment of plastic packing media to the internal tube's external wall may cause flow channeling during prolonged operation. In another study, the authors demonstrated the enhanced performance of this bioreactor configuration featuring an internal rotating spiral separator for in situ biomass separation during simultaneous nutrient and carbon removal from milk processing wastewater (MPW).[15] Under optimized conditions (HRT: 11.1−16 h; AFR: 2.95−4.4 L/min), the bioreactor achieved remarkable removal efficiencies (>96% sCOD, >75% TN) while maintaining excellent sludge settleability (SVI <90 mL/g) and effluent clarity (<10 NTU), establishing its superiority for high-strength wastewater treatment. Nouri et al. conducted a separate study utilizing

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bioelectrochemical reactions in an airlift bioreactor to enhance nitrogen removal from wastewater with a low C/N ratio of 2.5.[16] Under optimized conditions obtained at a temperature of 16 °C, an HRT of 6 h, a current density of 2 A/m^2, and an NH_4^+−N/TN ratio of 75%, the bioreactor achieved a maximum nitrogen removal efficiency of 73%. This performance was linked to autotrophic denitrification and simultaneous nitrification and denitrification (SND) mechanisms.

Recently, a new configuration of a one-stage airlift internal circulation bioreactor has been developed to employ partial nitritation and anammox using biocarriers and microgranules in order to achieve nitrogen removal and phosphorus recovery from diverse wastewaters across a range of operational parameters.[17−26] Despite successful findings, preserving oxygen-limited conditions, which is a key operating factor for having partial nitritation and anammox mechanisms, makes the operational conditions complex. Also, operating this bioreactor requires more monitoring equipment. In addition, in some of these studies, the authors tried to remove nitrogen and recovery phosphorus at a short HRT and high biomass via culturing concentrated, tiny, highly dispersive, and easily settleable granular sludge by adding external chemicals (calcium and iron ions) into the wastewater, which increases the operating costs and adds TDS (in the case of calcium).[18,24,27] In addition, culturing biofilm and granular sludge is difficult and time-consuming. Recently, we developed an innovative dual internal circulation airlift A2O bioreactor with two distinct configurations differing in their anoxic zone design: an ordinary A2O bioreactor and a hybrid A2O bioreactor.[28,29] The bioreactors were evaluated for simultaneous nutrient and carbon removal from milk processing wastewater (MPW). Results revealed that the hybrid A2O configuration, incorporating biofilm development, significantly enhanced the nutrient removal efficiency through improved microbial growth and boosted the anoxic zone.[29]

According to the provided information, this study is the first documented instance of simultaneously eliminating CNP compounds from milk processing wastewater (MPW) using a one-stage baffled dual internal circulation airlift A2O (B-DCAL-A2O) bioreactor. In this paper, we assess the use of baffles in the anoxic zone, which should lead to an improvement of nutrient removal. This is primarily attributed to the creation of a controlled environment that ensures the presence of anoxic and anaerobic conditions. Consequently, this facilitates the coexistence of anaerobic ammonia-oxidizing (anammox) bacteria, denitrifying bacteria, polyphosphate-accumulating organisms (PAOs), denitrifying PAOs (DPAOs), and glycogen-accumulating organisms (GAOs). The introduction of baffles results in the creation of stagnant regions and an alleviation of the rate of internal circulation, which further supports the proliferation of these microorganisms. This research included an evaluation of sludge features and economic factors, in addition to the assessment of the process performance. The evaluation was conducted by examining the variations in the HRT, AFR, and AVR across different operating circumstances. In this research, the experimental studies were intended using the central composite design (CCD) of the response surface methodology (RSM) with the assistance of Design-Expert software. Through successive generations of high-rate single bioreactors,[10−15] we have systematically enhanced both operating conditions and the overall bioprocess performance.

2. MATERIALS AND METHODS

2.1. Experimental Setup and Process Description. A transparent acrylic sheet was used to construct the one-stage baffled dual internal circulation airlift A2O (B-DCAL-A2O) bioreactor, which consisted of a reaction zone with a working volume of 7 L and an inclined plate settling zone (10 cm × 10 cm × 20 cm as width × length × height) with an effective volume of 2 L, as demonstrated in Figure 1. The reaction zone was segregated into three essential biological zones; anaerobic (10 cm × 10 cm × 22.5 cm; 1.125 L), anoxic (3 cm × 10 cm × 62.5 cm; 1.875 L), and aerobic (10 cm × 10 cm × 40 cm; 4 L). In this configuration, the anoxic zone was tailored using 14 baffles (2 cm × 10 cm) in order to decrease the circulation rate, hence enhancing the nutrient removal. To ensure effective mixing, two mixers were installed in the anaerobic zone. These two mixers were controlled in on/off mode (1 min on/60 min off). An air stone was mounted at the top of the anaerobic zone for oxygenating and providing the airlift mix. The bioreactor was continuously fed with milk processing wastewater (MPW) from the top of the anoxic zone using a peristaltic pump. The sludge was transferred from the settling zone to the anaerobic zone with the assistance of airlift force and the inclined plate. The surplus sludge was discharged regularly over the entire experiment to keep the biomass concentration constant. Wastewater compositions and the inoculum sludge are explained in the Supporting Information (SI).

2.2. Operating Procedure. In summary, the operating procedure was divided into three phases: phase 1 (startup stage), phase 2 (the investigation of the bioreactor's performance at varied experimental runs), and phase 3 (determination of optimum conditions). During the initial phase (phase 1), the bioreactor was operated with an HRT of 7 h, an AFR of 3 L/min, and an AVR of 0.464. These conditions were maintained for approximately 3 weeks to facilitate the startup and achieve steady-state conditions. In the subsequent phase (phase 2), the one-stage airlift bioreactor, enhanced with baffled anoxic and dual internal circulation, was operated based on the experimental design conducted employing Design-Expert software (Stat-Ease Inc., version 10.0). During this phase, the bioreactor's capability to concurrently remove carbon constituents and nutrients was evaluated. The influence of three operating variables, i.e., HRT, AFR, and the aerobic zone volume to working volume ratio (AVR) ranging from 7 to 15 h, 1 to 3 L/min, and 0.324 to 0.464, respectively, was assessed to determine their impact on the bioreactor's performance. The HRT, AFR, and AVR represent critical operational parameters that require careful optimization to achieve an optimal bioprocess performance. The selected ranges for these variables were determined based on experimental evidence from our previously published research findings.[10,11,14,15]

The values of the AVR were adjusted through changing the location of the air stone inside the aerobic zone. In order to get steady responses, each experimental run lasted for 14 or 21 days depending on HRTs and the number of turnovers. Totally, the bioreactor was run for 240 days. The presence of polyphosphate-accumulating organisms (PAOs), denitrifiers, and nitrifiers was confirmed by measuring the dissolved oxygen (DO) concentrations in different zones of the bioreactors. In phase 3, the bioreactor was operated for 14 days under controlled parameters determined as optimal during phase 2.

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Besides, the effect of biodegradability of various wastewaters (as BOD_5/COD) on the bioreactor's functionality was checked using soft drink wastewater (SDW), milk processing wastewater (MPW), and soybean oil plant wastewater (SOW). The influence of the feeding location, specifically at the anoxic and anaerobic zones, was also monitored to assess its influence on the bioreactor's process functionality. Subsequently, the long-term operation of the bioreactor was evaluated over a period of 32 days. Detailed descriptions of chemical analysis, experimental design, mathematical modeling, and statistical analysis can be found in the Supporting Information (SI).

2.3. Specific Activity Experiments and Microbial Community Analysis. Specific activity experiments of various bacterial species such as specific ammonia oxidation activity (SAOA, g-N/g-MLSS.d), specific nitrite oxidation activity (SNOA, g-N/g-MLSS.d), specific nitrate oxidation activity (SNAOA), and specific anammox activity (SAA as g-N/g-MLSS.d) were conducted under optimum operating conditions in Erlenmeyer flasks of 200 mL at a temperature of 30 ± 1 °C.[30] This study placed particular emphasis on investigating the microbial community by means of DNA extraction and PCR amplification. The methodology and specific primers employed for different bacterial species can be found in the newly published study.[28,29,31] Furthermore, the recently published paper provides comprehensive information on protein and polysaccharide extraction, as well as operational cost estimation.[28,29]

3. RESULTS AND DISCUSSION

3.1. Investigation of the Biological Functionality. *3.1.1. TCOD Removal.* The results of ANOVA for the modified models are summarized in Table 1. As indicated in the table, the model terms, A, B, and C, as representatives of the HRT, AFR, and AVR, respectively, exhibited a positive influence on the elimination of the total chemical oxygen demand (TCOD) as the response. The selection of significant model terms was based on a substantial reduction in model R^2 (Figure 2). Figure 3 presents a three-dimensional response surface plot depicting the relationship between the TCOD removal efficiency and the most impactful model terms with the highest regression coefficient. Totally, the new baffled airlift A2O bioreactor showcased high capacity in TCOD removal ranging from 80 to 100% with a high organic loading rate (OLR) and a superficial gas velocity of 1.98−4.3 kg of COD/m^3.d and 0.1−0.3 m/min, respectively. Such high TCOD removal was enhanced due to the high oxidation potential and efficient retention of MLSS within the anoxic and anaerobic zones, provided by manipulating the anoxic zone by installing some plates as baffles.

Since milk processing wastewater (MPW) contains protein, oil, and fatty acid, trapping sludge in the anoxic zone by means of installed baffles can create a local anaerobic environment for hydrolysis and acidification. This solubilization can lead to an improvement in TCOD removal. Furthermore, the presence of these plates increased the hydraulic resistance (decrease in the circulation rate), therefore lengthening the HRT of the anoxic zone. Moreover, sludge accumulation occurred in the anaerobic zone especially at minimum AFR (1 L/min). For instance, at an HRT of 8 h and an AFR of 1 L/min, the MLSS concentrations were approximately 7400 mg/L (aerobic zone), 5120 mg/L (anaerobic zone), and 6230 mg/L (entire bioreactor). While the anoxic zone's MLSS was not directly measured, it could be derived from the measured values of the

Table 1. ANOVA Results for the Modified Model Equation as Coded Factors Acquired for the Studied Responses in the Baffled DCAL-A2O Bioreactor

response	modified model equation as coded factors	*F*-value	probability	probability for lack of fit	R^2	adj. R^2	pred. R^2	adeq. precision	C.V.%	press
TCOD removal	$97.7 + 4.18A + 2.64B + 3.20C-2.83A^2-3.83C^2$	22.66	<0.0001	0.8952	0.89	0.85	0.79	16.992	2.28	123.38
TN removal	$76.88 + 10.15A + 7.02B + 17.40C + 5.75AC-13.67C^2$	26.04	<0.0001	0.1869	0.90	0.87	0.78	18.996	9.49	1391.72
phosphorus removal	$75.5 + 6.92A + 16.71B + 17.29C-21.82C^2$	47.85	<0.0001	0.8008	0.93	0.91	0.87	24.360	10.40	1226.18
effluent turbidity	$14.56 - 10.70A-8.0B-8.4C + 4.38AC + 6.19 A^2 + 3.69C^2$	21.59	<0.0001	0.7272	0.91	0.87	0.75	18.912	24.84	824.26

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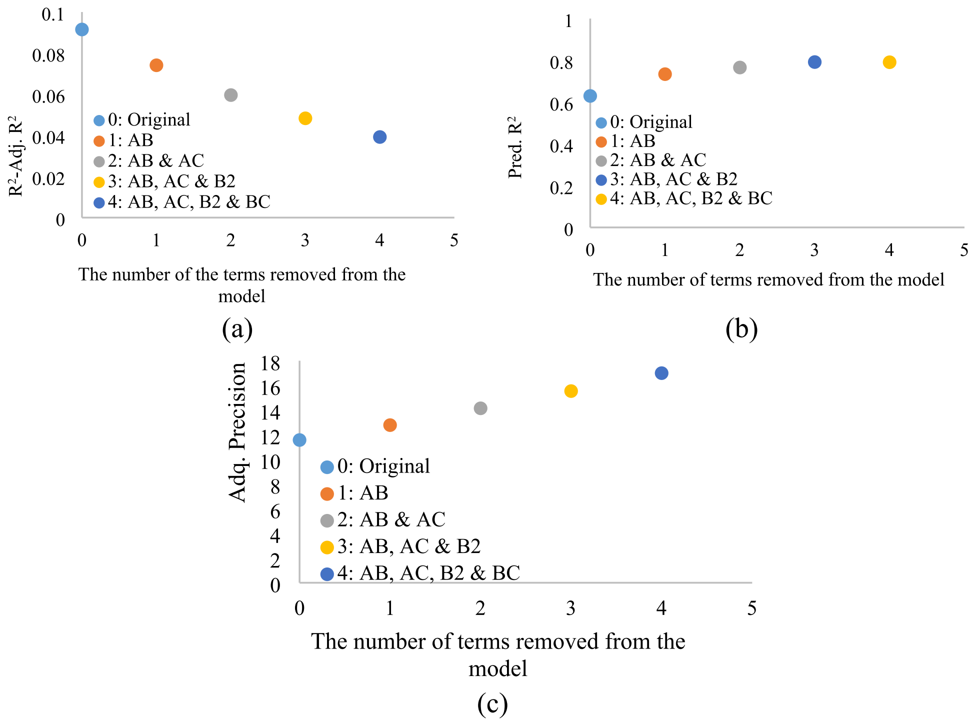

Figure 2. Removal of the insignificant model terms from the TCOD removal efficiency equation as a function of the statistical parameters. (a) R^2-Adj. R^2, (b) Pred. R^2, and (c) Adeq. Precision.

anaerobic, aerobic, and total bioreactor MLSS. For the total MLSS measurement, the entire bioreactor sludge was thoroughly drained and homogenized. The increase in the MLSS concentration measured at the anaerobic zone and unpleasant and foul odors during sludge sampling all confirmed the accumulation of sludge at anaerobic zones. In this study, although pH and ORP were not directly measured, the sour and unpleasant odor of the sludge discharged from the anaerobic zone to measure MLSS suggests acidogenic activity and a potentially acidic pH, while the increased alkalinity confirms active denitrifiers and an anoxic pH of 7−8. The DO levels in the anaerobic (0 mg/L), anoxic (>0.1 mg/L), and aerobic (3−4 mg/L) zones indirectly indicate ORP ranges of <−100, −100 to +100, and > + 100 mV, respectively. The results confirmed the establishment of distinct zones, facilitating the growth of specialized bacterial communities essential for TCOD removal. Another substantial reason behind such a behavior was related to the distinctive feature of the baffled airlift A2O bioreactor in the enhancement of oxygen bubble efficacy (Asadi et al., 2016c). The findings of this study showed similar results to those reported by Asadi et al. (80−100%) in terms of TCOD removal (Asadi et al., 2016c). They achieved these results using a continuous feed and intermittent discharge airlift bioreactor (CFIDALB) with the HRT and AFR ranging from 6 to 14 h and 1 to 3 L/min, respectively. From Figure 3d, it is observed that the actual data showed good consistence with the predicted data.

3.1.2. Nitrogen Removal. The bioreactor performance was evaluated in nitrogen removal over the entire experiment under various operating conditions. From Table 1 obtained from beneficial simplification (Figure S1), the model terms A, B, C, AC, and C^2 are chosen as the significant model terms with the positive regression coefficients for the first four model terms and negative regression coefficients for the latter one. The distinctive geometry of the airlift A2O bioreactor, incorporating baffled anoxic and dual internal circulation, facilitated favorable conditions for simultaneous nitrification and denitrification (SND). This was achieved through the introduction of physical barriers (the installed baffles), which increased the hydraulic resistance and, consequently, extended the HRT within the anoxic zone. More importantly, the population of the denitrifying heterotrophic bacteria in the anoxic zone was increased through the entrapment of sludge at angles of mounted plates providing dead regions. Retaining sludge in these dead sections caused anaerobic microzones that facilitated solubilization of the feed during the acidification and hydrolysis process. Such readily biodegradable organic compounds resulted in the enhancement in the denitrification process. Furthermore, autotrophic nitrifiers were nicely cultured in an aerobic medium due to adequate oxygen and high rate of the organic matter. Figure 4 presents the three-dimensional (3D) response surface plots illustrating the relationship between the TN removal efficiency and variables A and C while keeping variable B fixed with its lowest regression coefficient value of 7.02. Generally, increasing the HRT, AFR, and AVR exhibited a positive impact on the TN removal efficacy by maintaining a balanced anoxic and aerobic zone. The maximum TN removal efficiency value (95.4%) was observed when the HRT, AFR, and AVR were set at their maximum values of 15 h, 3 L/min, and 0.464, respectively, according to the obtained results.

At a low OLR and high AFR and AVR, there is more chance for a complete nitrification process owing to high depletion of the substrate. Also, at a high HRT, the denitrifying bacteria have more opportunity to use readily biodegradable organics. In addition, at the maximum level of the AFR (3 L/min), the rise in the HRT promoted the TN removal efficiency because of the decline in the level of the OLR, the increase in the nitrification process, and the enhancement in the denitrification process. Interestingly, for all plots presented here, at the

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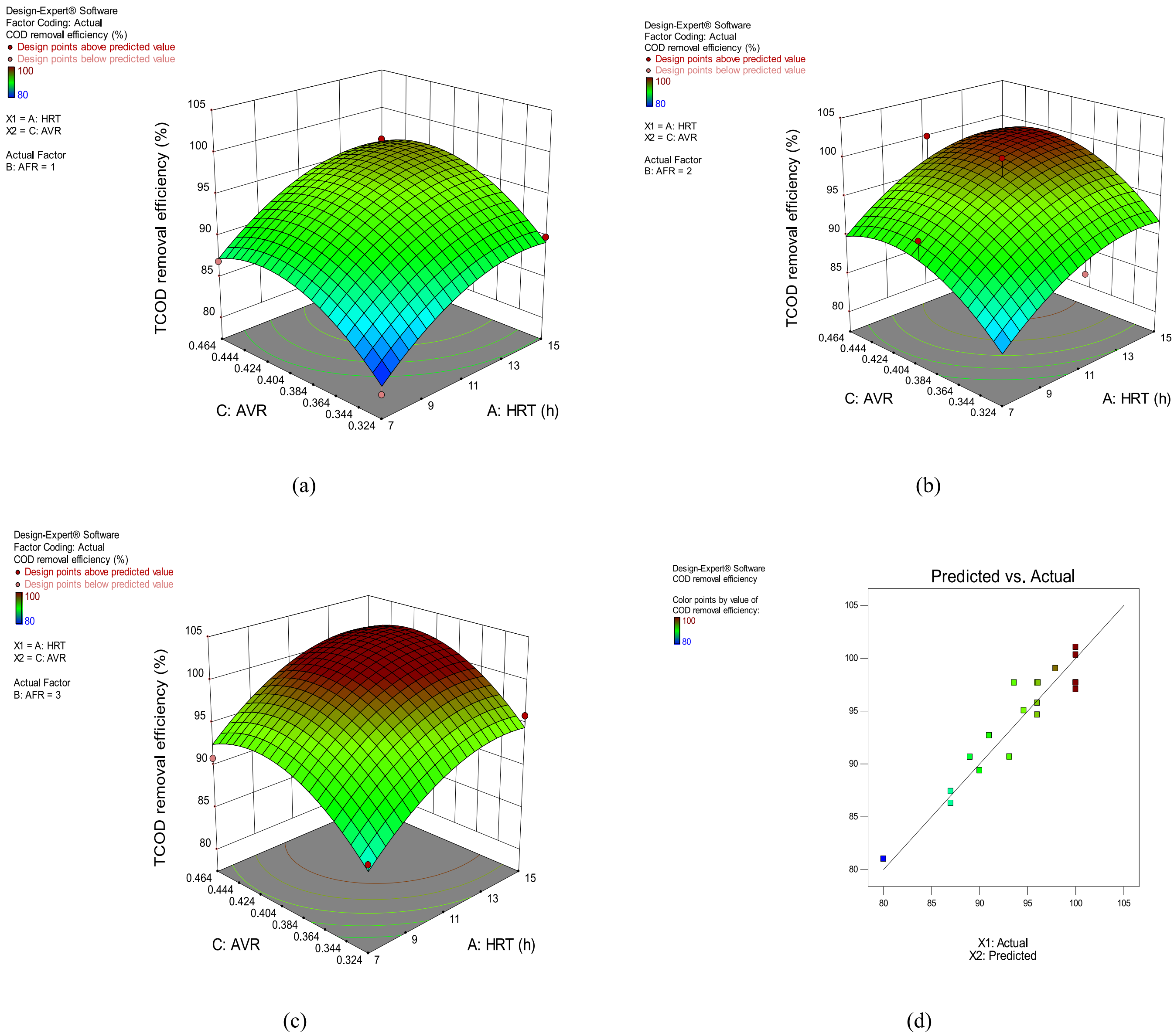

Figure 3. 3D response surface plots revealing the variations in the TCOD removal efficiency at varied AFRs of (a) 1, (b) 2, and (c) 3 L/min and (d) the actual data versus the predicted data.

lowest of the HRT (7 h), the increase in the AVR until 0.444 showed an upward trend since at an AVR above 0.444, the suitable balance of anoxic and aerobic zones was disturbed as a result of more enhancement in the aerobic zone and weakness in the anoxic zone. The positive impact of the AFR on the response correlates that enough oxidation potential intensified the conversion of $N-NH_4^+$ to $N-NO_3^-$. Additionally, adequate accessibility to the substrate at a high HRT created a favorable environment for the heterotrophic denitrifying species growth.

Furthermore, retaining the ample and effective biocatalyst in anoxic and anaerobic zones led to culturing anaerobic ammonium oxidizing (anammox) bacteria, denitrifying phosphorus-accumulating organisms (DPAOs), and glycogen-accumulating organisms (GAOs) as confirmed by PCR analysis that will be discussed in the following paragraphs. From the literature, DPAOs and GAOs contribute in the endogenous denitrification.[32,33] The variation in the NO_2^--N concentration could be additional evidence for the presence of anammox bacteria. From Figure 4d, it is seen that there is a strong correlation between the actual and forecasted data, verifying the accuracy of the experimental results.

The contributions of various mechanisms (cell synthesis and denitrification process) and nitrogen contents as effluent $NO_2^{3-}-N$, effluent $NO_2^-{-}N$, and influent TN concentrations in nitrogen removal are illustrated in Figure S2. As mentioned above, aerobic heterotrophic bacterial species consume a portion of influent TN, especially at low HRTs, for their growth.

Based on the figure, the highest values of nitrification (194.7 mg of N/L) and denitrification (198.4 mg of N/L) were achieved after the bioreactor was run at the maximum values of the HRT, AFR, and AVR. These results provide confirmation of the effectiveness of the simultaneous nitrification and denitrification (SND) mechanism. Apart from the SND, the anammox, DPAOs, and GAOs contributed in the nitrogen removal as well due to the entrapment of sludge into the anaerobic dead zones created by means of baffles. The increase

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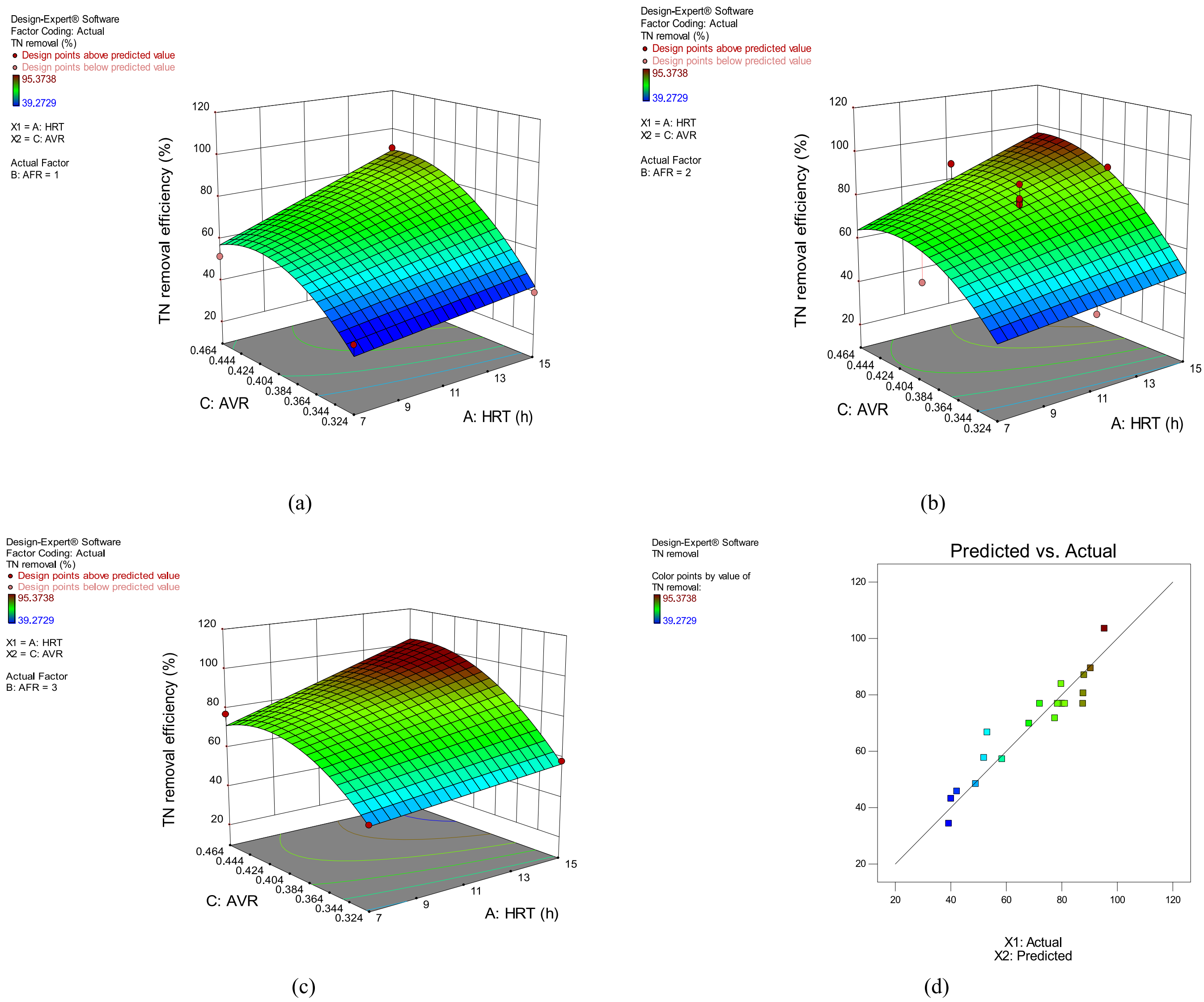

Figure 4. 3D response surface plot for the TN removal efficiency at varied AFRs of (a) 1, (b) 2, and (c) 3 L/min and (d) actual data versus predicted data.

in the effluent NO_2^-−N and NO_3^-−N concentrations in some runs was related to the imbalance of nitrification and denitrification reactions and the disturbance in the activity of anammox bacteria under various operating conditions.

Recently, some researchers disclosed reports regarding nitrogen removal from low/high-strength ammonia-containing wastewaters via the partial nitritation and anammox mechanism in a one-stage airlift internal circulation bioreactor.[17−24,27,34] The authors were successful in providing the concerned mechanism using biofilm growth and hydroxyapatite-augmented granules under limited DO. The main goal pursued in these studies was associated with the rise in the nitrogen removal rate by the decrease in HRT values. However, the disadvantages of these studies come back to the complexity in operation control and production of effluents with high calcium concentrations over the hydroxyapatite production process.

3.1.3. Phosphorus Removal. The A2O bioreactor with baffled anoxic and dual internal circulations enables the presence of both anaerobic and aerobic zones within a single unit. By analyzing Table 1, which was derived from Figure S3 through a useful simplification, it was observed that the model terms A, B, and C have a positive influence on phosphorus removal. Among these terms, terms B (AFR) and C (AVR) have a greater impact on the response, which is reasonable, considering that these parameters directly relate to changes in aerobic and anaerobic conditions.

Figure 5 illustrates 3D plots representing the relationship between the phosphorus removal efficiency and two variables of the AFR and AVR while maintaining the HRT constant. Figure 1 clearly shows that the highest level of phosphorus removal occurs when the AFR and AVR are set to high values at any HRT within the studied range. This can be attributed to achieving a balance between anaerobic and aerobic conditions and ensuring the availability of the substrate. The phosphorus removal efficiency continues to increase as AFR and AVR values rise until they reach approximately 2.9 L/min and 0.418, respectively. Beyond these values, there may be minimal further improvement or even a decline in the phosphorus removal efficiency. The reason for such a behavior was in association with the greater enhancement in the aerobic conditions and fast circulation rate, which restrict the

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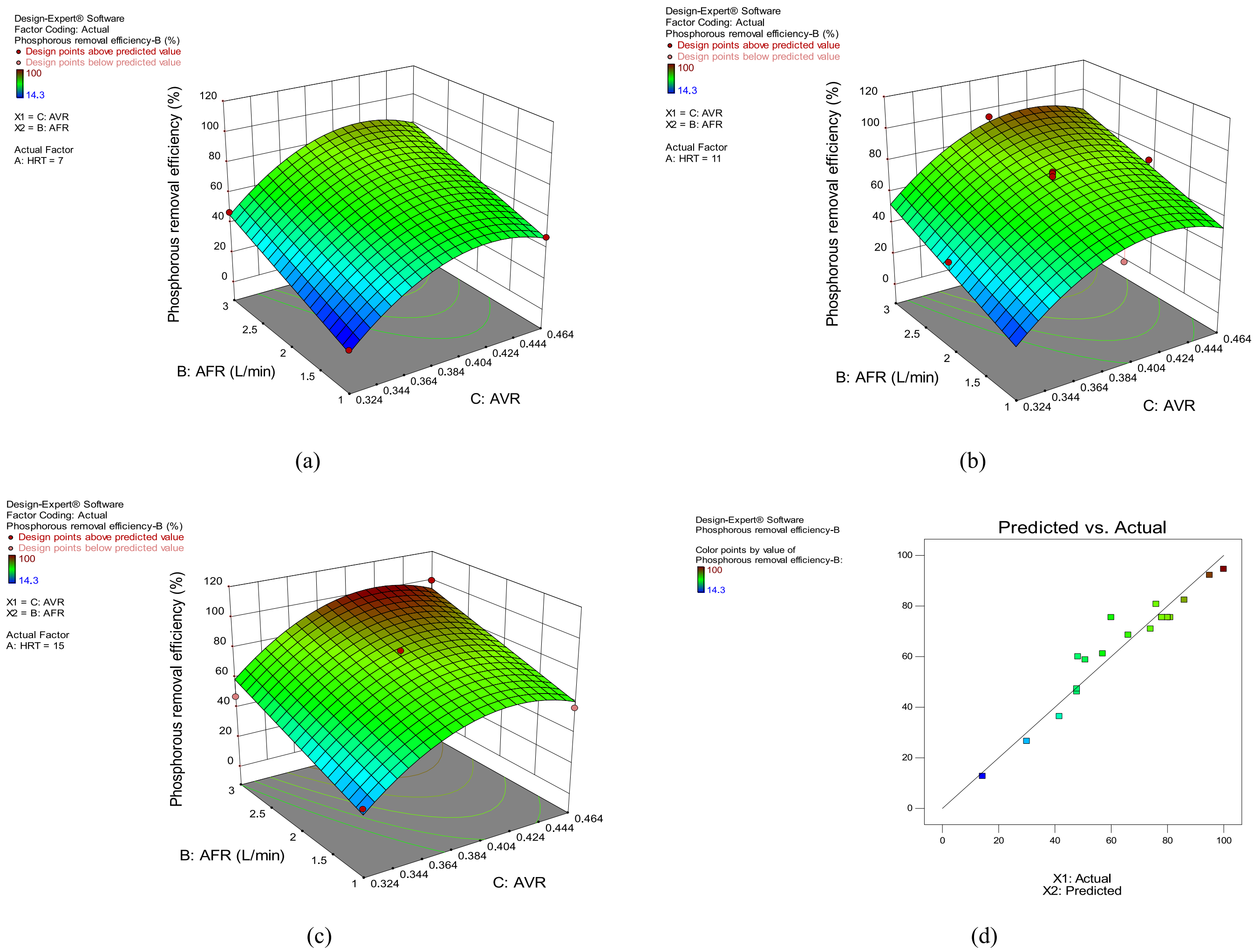

Figure 5. 3D response surface plots for the phosphorus removal efficiency as a function of the AFR and AVR at different HRTs: (a) 7, (b) 11, and (c) 15 h and (d) the actual data versus the predicted data.

anaerobic zone that is known as the “selector” for the proliferation of PAOs. However, the HRT demonstrated a direct positive effect on the response, so that the more the HRT, the more phosphorus content can be removed because of the longer aerobic conditions needed to enhance the absorption of the released phosphorus. The high phosphorus removal was in relation with the proliferation of PAOs and DPAOs identified using the PCR test discussed in the following section.[32,33]

Moradi et al. reported an impressive removal of total phosphorus (TP) (81%) in a single jet loop airlift bioreactor while treating soft drink wastewater. This achievement was observed under ideal circumstances, including an HRT of 14 h, an anaerobic volume to total volume ratio (V_{An}/V_T) of 0.06, an AFR of 5.5 L/min, and an influent nitrogen concentration of 225 mg/L (Moradi et al., 2021).

The process of extracting and reclaiming phosphorus from wastewater was examined in a single-stage airlift dual circulation reactor using granular sludge that was cultured with hydroxyapatite and iron ions.[20,24,27] However, the disadvantages of the previous work were related to the use of calcium and iron ions and the production of secondary pollution to remove low phosphorus concentrations (20 mg/L and <10 mg/L, respectively). In addition, retaining the stability of the granular sludge needs precise control compared to the suspended sludge. Therefore, the enlightenment of this work comes back to the special configuration of the airlift bioreactor as a result of the baffles installed in the anoxic part, resulting in the improvement of anaerobic and anoxic environments. Therefore, the bioreactor showed a high phosphorus removal efficiency treating high phosphorus-containing wastewater without incorporating any external chemicals.

3.2. Sludge Characteristics. *3.2.1. Effluent Turbidity and Sludge Bioflocculation Properties.* The functionality of the proposed bioreactor in the improvement of effluent quality and its ability in settling suspended solids are evaluated via the measure of effluent turbidity and ability of sludge sedimentation as the sludge volume index (SVI). Based on the simplified representation depicted in Figure S4, the data listed in Table 1 indicate that the factors A, B, and C had a detrimental influence on the response. Conversely, the interactive model terms AC, A^2, and C^2 had a positive influence on the clarity of the effluent.

The changes in the effluent turbidity come from the unique geometry of the proposed baffled airlift A2O bioreactor. Figure 6 illustrates the changes in effluent turbidity based on numerical variables with the highest regression coefficients, regardless of their positive or negative impact. The concurrent rises in the HRT, AFR, and AVR caused a reduction in effluent turbidity. This decrease can be attributed to improvements in

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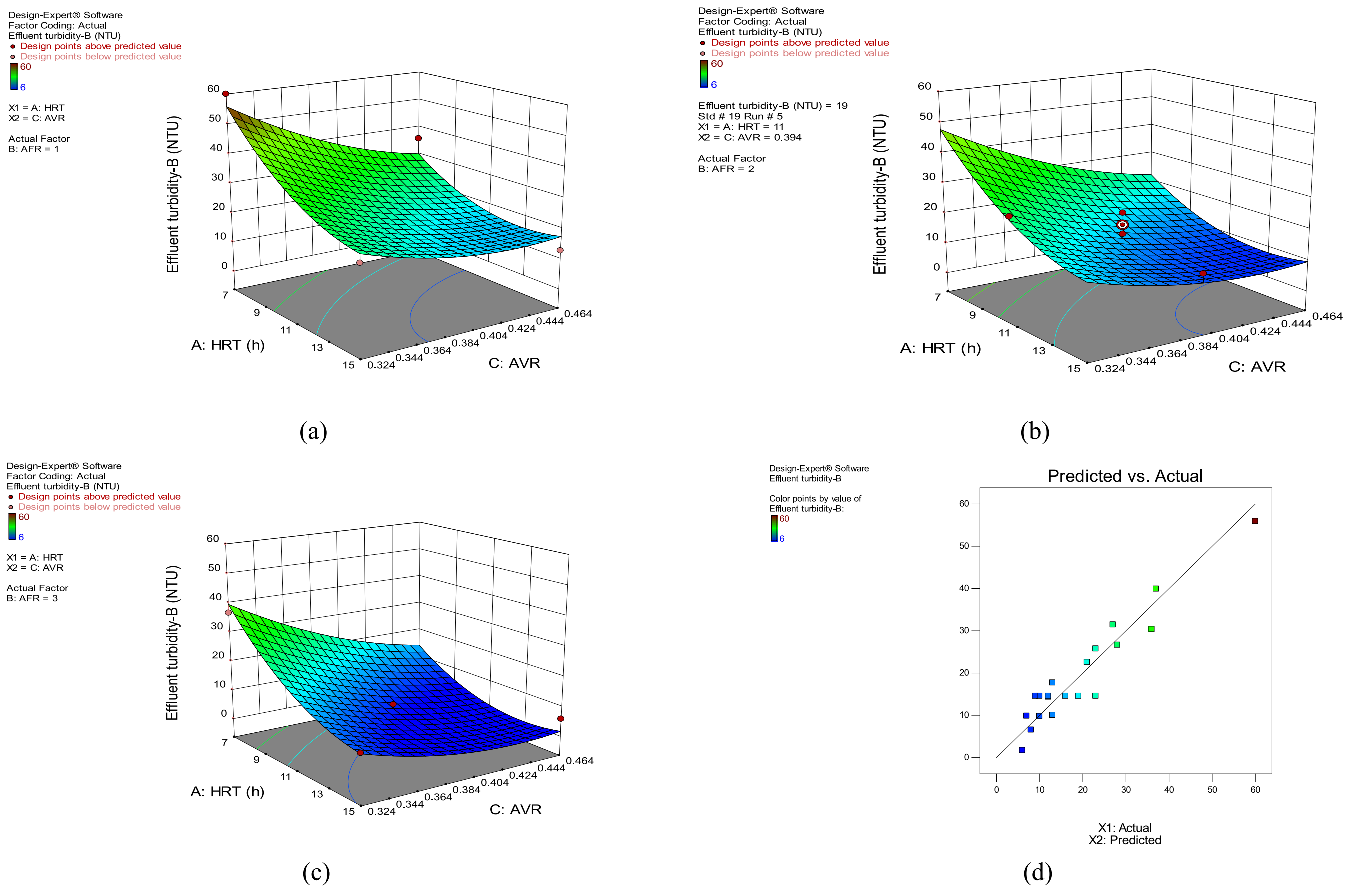

Figure 6. 3D response surface plots for effluent turbidity versus the HRT and AVR at varied AFRs of (a) 1, (b) 2, and (c) 3 L/min and (d) actual data versus predicted data.

sludge bioflocculating and settleability capacity. Nonetheless, the low AFR and HRT values due to reduction in the mixing rate of the sludge suspension, insufficient contact time between the feed and the biocatalyst, and the high OLR, respectively, deteriorate the effluent turbidity.

The effective entrapment of sludge and the high sludge retention time (SRT) are influential factors for effluent turbidity. The lowest level of effluent turbidity (6 NTU) was observed when the HRT, AFR, and AVR were set to 15 h, 3 L/min, and 0.464, respectively. The improvement in the effluent turbidity was in relation with the longest SRT (30.6 d) and the lowest OLR (2.4 kg COD/m^3.d). These observations showed good consistency with the phosphorus removal results. The removed maximum amounts of phosphorus observed at the largest values of the studied variables provide strong evidence for the enhancement of sludge bioflocculating and settleability capacity. This is supported by the formation of dense flocs by polyphosphate-accumulating organisms (PAOs), which contribute to improved settling properties and efficient phosphorus removal.[35] The effluent turbidity ranged from 6 to 60 NTU in the studied space. Figure 6d demonstrates a high level of agreement between the actual data and the predicted data.

3.2.2. Sludge Volume Index (SVI). In the present investigation, the sedimentation properties of the sludge were evaluated through assessing the sludge volume index (SVI) due to the common challenges of bulking and floating sludge in wastewater treatment plants. The results, as shown in Figure S5, indicate a consistent decrease in the SVI corresponding to the reduction in effluent turbidity. This improvement in settling properties can be attributed to the reduction in the organic loading rate (OLR) and food-to-microorganism ratio (F/M) resulting from an increase in the HRT. Additionally, the decrease in the dissolved oxygen (DO) concentration and aerobic space, achieved by reducing the AFR and AVR, respectively, contributed to the decline in SVI values. The SVI value was reduced to 90 mL/g at the maximum levels of the independent operating variables (HRT of 15 h, AFR of 3 L/min, and AVR of 0.464). In an investigation carried out by Abyar et al., the SVI values ranged from 75.9 to 166.33 mL/g.[36] The successful sludge accumulation within the A2O bioreactor played a crucial role in preventing sludge loss while it treated meat processing wastewater. This accumulation was associated with the lowest sludge volume index (SVI) and resulted in minimal effluent turbidity (1.5 NTU). Conversely, higher SVI values were observed when there was an excess of nitrate or nitrite, which facilitated the denitrification and anammox processes. This led to the generation of nitrogen gas, demonstrating the superior performance of the bioreactor in removing TN (Abyar et al., 2018). Bioflocculation and settleability are discussed in the SI.

3.3. Optimization and Verification Studies. In general, the primary goal in the development of bioreactor designs is to determine the optimal capacity that enables the attainment of the highest possible quality effluent. This represents a significant priority for wastewater treatment specialists, who place great emphasis on ensuring the highest standard of effluent quality. Therefore, the optimal process conditions are achieved by controlling independent operating variables,

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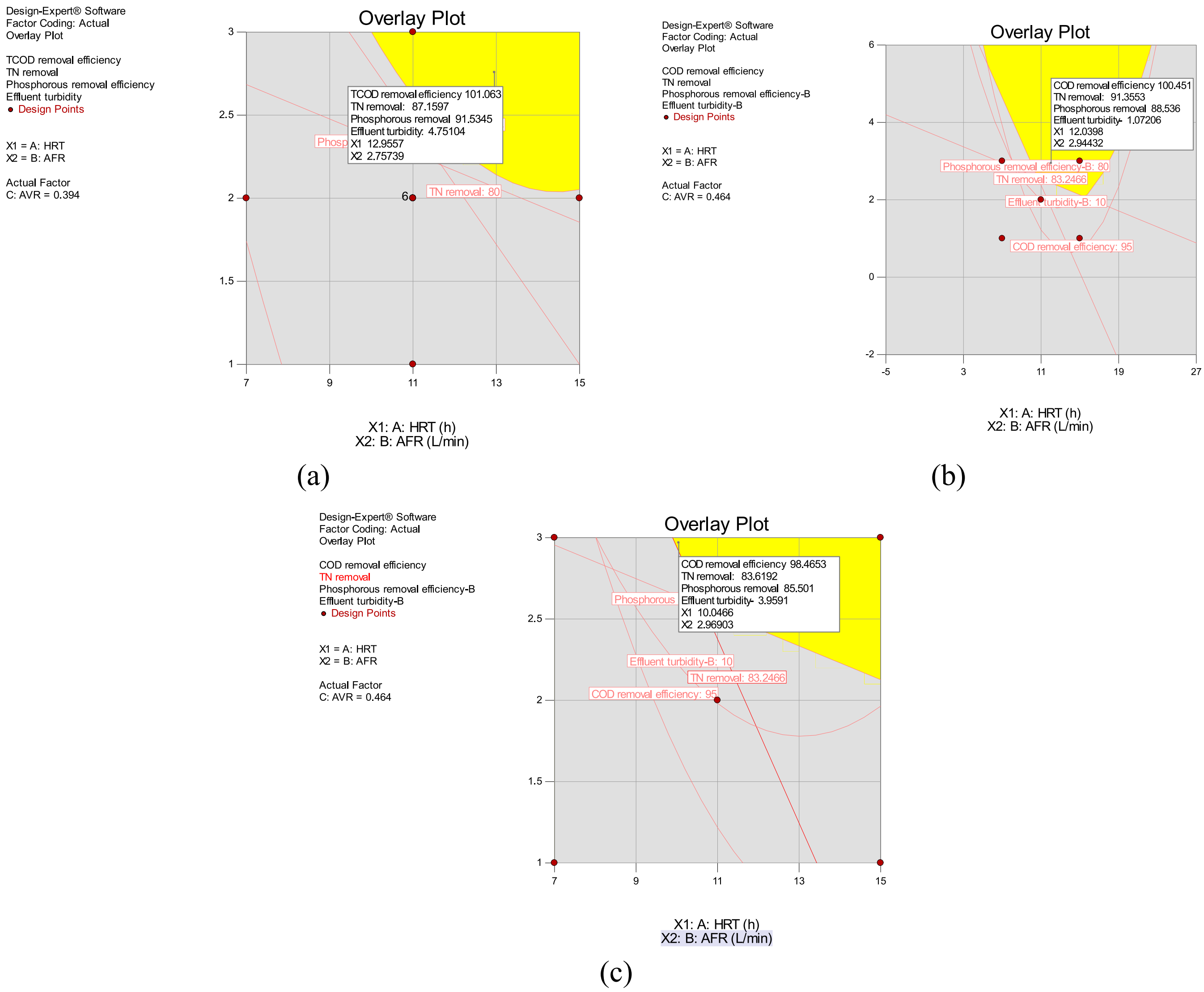

Figure 7. Overlay plots for the baffled DCAL-A2O bioreactor, visualized at the optimum region under varying upper and lower limits for different responses: (a, b) Under normal conditions and (c) at stricter circumstances.

Table 2. Verification Experiment Results under Optimum Operating Conditions for the Baffled DCAL-A2O Bioreactor

optimum conditions		feasible responses			
		TCOD removal, %	phosphorus removal, %	TN removal, %	effluent turbidity, NTU
HRT = 13 h	experimental value	95.2	82.5	83.5	5.0
AFR = 2.75 L/min; AVR = 0.394	model values	101.0	91.5	87.2	4.8
HRT = 12 h	experimental value	97.7	87.0	84.0	7.0
AFR = 3.0 L/min; AVR = 0.464	model values	100	88.5	91.4	1.1
HRT = 10 h	experimental value	96.8	92.0	90.0	8.0
AFR = 3.0 L/min; AVR = 0.464	model values	98.5	85.5	83.6	4.0

modeling both experimental and predicted data, and presenting overlay plots. The selection of key responses, such as TCOD, phosphorus, TN removal efficiency, and effluent turbidity, is used to identify the optimal circumstances. The compromised optimization areas are illustrated in the overlay plot, as depicted in Figure 7a−c. The figure shows regions highlighted in yellow that align with the optimization criteria, while the shaded gray areas denote regions that do not meet the specified limits. To validate the forecasted data from the defined optimum circumstances and assess the precision of the models, three verification experiments are considered and conducted. The operating variables controlled in this study, including the HRT, AFR, and AVR, coupled with the experimentally attained data and their corresponding forecasted values, are provided in Table 2. Collectively, the experimental findings closely aligned with the predicted data, except for the effluent turbidity. The observed percentage differences between predicted and experimental turbidity values (4, 84.3, and 50% for 5, 7.0, and 8 NTU, respectively) likely stem from the dynamic nature of particle aggregation processes, which are challenging to model mathematically. The suggested models therefore are reliable enough for modeling

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the actual data in the determined design space. The primary aim of high-rate bioreactor development is to lower operating expenses and energy consumption by mitigating the bioreactor footprint (resulting in a shorter HRT) and minimizing the aeration energy (leading to a lower AFR). At the same time, the goal is to improve the overall performance of the bioreactor. In this scholarly work, taking into account all of the design criteria, HRT and AFR values of 10 h and AFR of 3 L/min, respectively, were selected as the optimal circumstances for further investigation.

The specific activity of bacterial species, the effect of the feeding location, and the wastewater biodegradability on the bioreactor performance are explained in the SI.

3.4. Identification of Bacterial Communities at Optimum Conditions. The presence of different microbial species, as listed in Table 3, was confirmed in the one-stage

Table 3. Bacterial Species Detected (+) and Those Absent (−) within the Baffled DCAL-A2O Bioreactor

no	name	product length	status of species
1	pseudomonas	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	304	+
8	dechloromonas (denitrifying PAOs)	394	-
9	nitrobacter (NOB)	600	-
10	nitrospira (23S rRNA) (NOB)	435	+
11	candidatus Microthrix (16S rRNA) (PAOs)	230	+
12	candidatus Accumulibacter ppk1 (PAOs)	129	+
13	sphingomonas (16S rRNA) (GAO)	306	+
14	competibacter (GAO)	375	-
15	paracoccus (denitrifying PAOs)	450	-
16	rhodocyclus (PAOs)	350	-
17	nitrosococcus (AOB)	521	+
18	pseudomonas (DPAO)	850	+

baffled DCAL-A2O bioreactor under optimal conditions by using PCR testing. The results of electrophoresis and PCR analysis are shown in Figure 8 and Table 3, respectively. The presence of microbial species was determined by observing bands in the electrophoresis experiments (as shown in Figure 8) and positive indications listed in Table 3. The findings revealed the existence of various bacterial species, including anammox bacteria (Candidatus_Brocadia, Candidatus Kuenenia), PAOs (Tetrasphaera, Candidatus Microthrix (16S rRNA), Candidatus Accumulibacter (ppk1)), denitrifying PAOs (Pseudomonas), AOB (Nitrosospira sp, Nitrosomonas SP, and Nitrosococcus), as well as NOB (Nitrospira). This indicates that the one-stage baffled DCAL-A2O bioreactor successfully established triple zones, allowing the growth of diverse functional bacteria for synchronious CNP removal.

The results demonstrated that the manupulation of the anoxic zone by installing a baffle provided suitable conditions for the symbiosis of various anammox and PAO species as a result of the decreased circulation rate and creation of useful dead zones leading to the guaranteed anaerobic regions. The

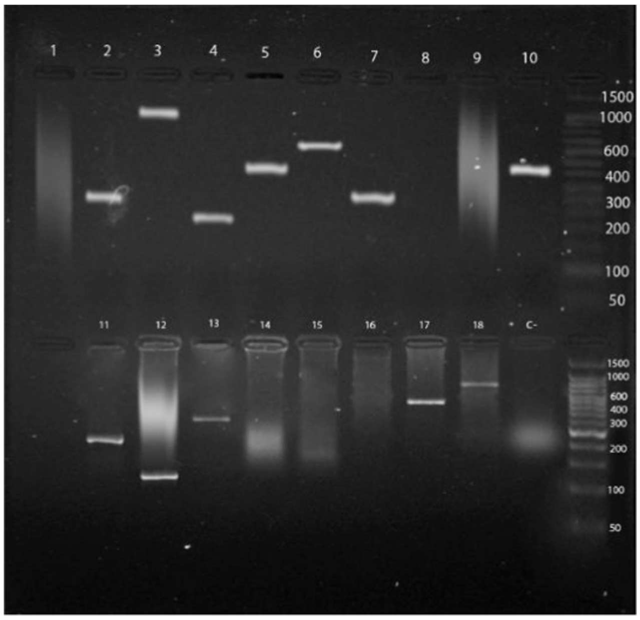

Figure 8. PCR analysis of the baffled DCAL-A2O bioreactor obtained under optimal circumstances.

long-term performance and operating cost assessment are discussed in the Supporting Information (SI).

4. CONCLUSIONS

This study presents the successful design and operation of a new single baffled dual internal circulation airlift A2O (DCAL-A2O) bioreactor for synchronously eliminating CNP from MPW. The inclusion of physical obstacles, such as baffles, in the design of the bioreactor facilitated an effective manipulation of the anoxic zone. This resulted in improved removal efficiencies of TN and phosphorus by reducing the internal circulation rate and enhancing sludge retention in the relevant regions (anoxic and anaerobic). The bioreactor functionality was appraised based on three key parameters that are HRT (7−15 h), AFR (1−3 L/min), and AVR (0.324−0.464). The involved mechanisms in the TN removal were realized to be due to SND, DPAOs, GAOs, and anammox because of the unique geometry of the bioreactor, and the presence of the baffles provided local anaerobic and anoxic environments at the stagnant areas formed in the angles of the installed baffles for growing denitrifiers and bacteria responsible for the anammox process. The PAOs were responsible for phosphorus removal. Under the optimal operating circumstances (gained at the HRT, AFR, and AVR of 10 h, 3 L/min, and 0.464, respectively), the bioreactor demonstrated an impressive performance. The removal efficiencies reached 94.8, 80, and 80% for TCOD, TN, and phosphorus, respectively. The effluent turbidity was measured at 8 NTU. As the independent variables (HRT, AFR, and AVR) increased, there was a reduction in bacterial secretions, including effluent SMP, sludge SMP, and LB-EPS, together with protein and carbohydrate contents. This reduction indicates an improvement in the bioflocculating and settleability capacity of the sludge. Furthermore, feeding the bioreactor from the anoxic part brought about an improvement in the TN removal efficiency. However, once it was fed from the anaerobic zone, the phosphorus removal efficiency was enhanced. These results were attributed to the readily biodegradable substrate being

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accessible to functional bacterial species (denitrifiers and PAOs). In addition, the sludge fed with soft drink wastewater (SDW) demonstrated higher phosphorus removal but a lower TN removal efficiency compared to those of the bioreactor-treated milk processing wastewater (MPW) and soybean oil plant wastewater (SOW). This was because of the higher BOD content of SDW (BOD5/COD ratio of 0.8), which created favorable conditions for PAOs. Nonetheless, the TN removal efficiency was reduced when treating SDW, owing to the rapid depletion of simple molecules (sugars) by fast-growing aerobic heterotrophic bacteria. A notable distinction was observed in nutrient removal between the sludge fed with MPW and SOW compared with SDW. PCR tests validated the successful development of triple anaerobic, anoxic, and aerobic regions within the single unit by identifying various microbial communities, such as anammox bacteria, AOB, NOB, denitrifiers, and PAOs. Furthermore, the operating cost estimation identified the innovative baffled DCAL-A2O bioreactor as an economical solution, demonstrating its potential for further studies.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.iecr.5c00570.

Removal of the insignificant model terms from the TN removal efficiency equation as a function of the statistical parameters (Figure S1); nitrogen contents and contribution of the various reactions in the nitrogen removal at the different experimental runs (Figure S2); removal of the insignificant model terms from the phosphorus removal efficiency equation as a function of the statistical parameters (Figure S3); removal of the insignificant model terms from the effluent turbidity equation as a function of the statistical parameters (Figure S4); changes of the SVI at various experimental runs (Figure S5); specific activity of various active bacterial species in the one-stage baffled DCAL-18 A2O bioreactor (Figure S6); long-term bioprocess performance of the baffled DCAL-A2O bioreactor under optimal conditions over 32 days (Figure S7); compositions of various industrial wastewaters used in this study (Table S1); range and level of the controlled variables studied in this research (Table S2); experimental conditions and the achieved results in the baffled DCAL-A2O bioreactor (Table S3); analysis of the sludge properties over various experimental runs (Table S4); impact of wastewater biodegradability on the bioprocess performance of the baffled DCAL-A2O bioreactor under optimal conditions (Table S5); and evaluation of the operating costs of the baffled DCAL-A2O bioreactor under various operating conditions (Table S6) (PDF)

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Notes

The authors declare no competing financial interest.

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