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Effect of microbial growth types (suspended and attached) in anoxic zone of innovative one-stage anaerobic/anoxic/oxic bioreactor with airlift regime on nitrogen removal through combined mechanisms and phosphorous removal: A comparative study

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ABSTRACT

The objective of this study was to treat different types of industrial wastewaters (milk processing wastewater, soft drink wastewater, and soybean oil plant wastewater) using modified configurations of a bioreactor called one-stage internal dual circulation airlift A2O (DCAL-A2O) bioreactor. The modification involved the use of physical barriers (baffle and packing media) to enhance the anoxic zone of the bioreactor. The study investigated the performance of bioreactors in simultaneously removing carbon, nitrogen, and phosphorus. The bioreactor was designed in three configurations: ordinary, baffled, and hybrid DCAL-A2O bioreactors, depending on the type of manipulation. Different operating conditions, such as hydraulic retention time (HRT), air flow rate (AFR), and anaerobic volume ratio (AVR), were examined simultaneously. The hybrid DCAL-A2O bioreactor was found to be highly suitable for wastewater treatment due to its superior biological performance, ease of operation, and cost-effectiveness when compared to two other bioreactors. The chosen bioreactor demonstrated high performance in terms of TCOD, TN, phosphorus removal efficiencies, and effluent turbidity. Specifically, it achieved removal efficiencies of 97.0% for TCOD, 92% for TN (with a concentration of 179.4 mg/L), 90% for phosphorus (with a concentration of 50.33 mg/L), and an effluent turbidity of 9 NTU. These results were obtained under optimal conditions, which included a HRT of 10 h, an AFR of 2 L/min, and an AVR of 0.464. The unique setup of the bioreactor demonstrated its undeniable ability to effectively treat wastewater from different feeding points. PCR tests confirmed the co-existence of multiple functional bacterial species, including AOB, NOB, denitrifying bacteria, anammox, PAOs, DPAOs, and GAOs. This provides strong evidence for concurrent removal of organics and nutrients in all three configurations of the integrated unit. In summary, this study emphasizes the need for ongoing research in energy efficiency to safeguard our environmental resources.

1. Introduction

Although the various biological treatment processes have been used steadily to purify wastewater, the excessive release of the nutrients containing wastewater is still a pervasive and ubiquitous environmental challenge due to eutrophication phenomenon and the following destructive implications. Accordingly, the development of biological nutrient removal (BNR)-based technologies has received a considerable deal of attention from the specialists and environment engineers to eliminate the nutrient contents effectively (Abyar et al., 2018a; Amini et al., 2013; Asadi et al., 2012a, 2012c; Cao and Mehrvar, 2011; Chen et al., 2013; Di Bella and Torregrossa, 2013; Mansouri and Zinatizadeh, 2017; Rezaee et al., 2015, 2016; Zinatizadeh and Ghaytooli, 2015). The conventional BNR processes-based bioreactors, in a general view, need three separate compartments in series to provide the required environments to remove nitrogen (in anoxic & aerobic compartments),

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phosphorus (in anaerobic & aerobic compartments) and carbon (in anoxic, anaerobic & aerobic compartment) from the wastewaters. Also, they require the additional apparatus such as the internal recycling and waste sludge discharge pumps. Complexity in the operating as well as high labor and energy cost is regarded as other disadvantages of the conventional BNR systems. To overcome the existing challenges, some researchers designed and established advanced bioreactors with new configurations and high rate features to simultaneously remove the nutrients and carbonaceous contents from various wastewaters in single unit using suspended, attached growth and combination of both. The developed bioreactors need less the input energy and required footprint compared to the conventional BNR processes-based bioreactors. In all, the notable independence of bioreactor performance from high food to microorganism (F/M) ratio and organic loading rate (OLR), as well as short hydraulic retention time (HRT) and solids retention time (SRT), are distinguished attributes of these high-rate bioreactors compared to others (Jimenez et al., 2015; Bonakdari and Zinatizadeh, 2011). For example, Asadi et al. developed and applied a novel structure of the sequence batch reactor (SBR) entitled a high rate single up-flow aerobic/anoxic sludge bed (UAASB) bioreactor with operating regime of continuous feed and intermittent discharge (CFID) to treat a real industrial estate wastewater (Asadi et al., 2012a,b). In the following, benefiting from the granular sludge and the continuous regime, Amini et al. proposed an innovative up-flow aerobic–anoxic flocculated sludge bioreactor (UAASB) to investigate the concurrent CNP removal from dairy wastewater (Amini et al., 2013). Rezaee et al. in their studies tried to augment a single up-flow anaerobic/aerobic/anoxic bioreactor treating milk processing wastewater (MPW) using ultrasound transducer in order to promote the simultaneous CNP removal and improve the sludge settling ability and effluent turbidity via the enhancement in the sludge flocculation compared to once the mechanical mixing was in use (Rezaee et al., 2015, 2016). Abyar et al. also successfully operated a high rate up-flow anaerobic, anoxic and oxic (A2O) bioreactor to concurrently remediate the CNP from meat processing wastewater (Abyar et al., 2018a). It is worth noting that the meat processing wastewater contained average TCOD and TP contents of 1500 mg/L and 45 mg/L, respectively. Inlet TN concentrations were varied at 60, 120, 180, 240, and 300 mg/L to adjust the COD/TN ratio at five levels (4, 8, 12, 16 and 20) as a variable. Although the reported bioreactors were successful in the treatment of various wastewaters, complex operation, the requirement of additional equipment, and the need to accurate supervision have restricted their scale-up studies.

In some reports, the main focus of the researchers has been centered on only the nitrogen removal via the cooperation of various mechanisms such as partial nitrification/anammox (PNA)-partial denitrification/anammox (PDA) in sequence batch bioreactor (SBR), simultaneous nitrification and denitrification (SND) in moving bed biofilm reactor (MBBR) using bacterium of heterotrophic nitrification and aerobic denitrification (HNAD), partial nitrification/anammox (PN/A), and PN/A in a single-stage hybrid airlift BioCAST bioreactor (Guo and Li, 2020; Jia et al., 2020; Li et al., 2020; Mohammadhosseinpour, 2016; Saborimanesh et al., 2019). Providing the convenient conditions for growing the functional bacterial strains requires the exact control of operating conditions, particularly, in the case of MBBR, where, any tension threatens attached biofilm.

Recently, the development of airlift bioreactors (ALBs) configuration towards integration of the various environments (anaerobic/anoxic and oxic zones), required for the functional bacteria growth, in a single unit in order to get the efficient and simultaneous CNP removal has become a special interest due to their salient features. These kinds of the bioreactors have their own distinct merits such as simple design, formation of complete and effective suspension along with low input energy, the appropriate and controllable mixing creating homogeneous media and low shear stress especially for the attached growth and reducing the dead stagnant volumes, and last but not least high mass transfer (Shi et al., 2020). Therefore, these bioreactors have been hailed by the researchers as the most competitive wastewater treatment technology. For instance, Asadi et al. suggested a single airlift bioreactor with CFID regime and investigated the performance of bioreactor in terms of the concurrent CNP removal under varied process and operating conditions (Asadi et al., 2016, 2016a, 2016b). The authors also studied the nitrogenous compounds removal through the SND process and the combination of SND and anammox processes at the applied various process and operating conditions. In another study, the authors assessed the effect of operational modes i.e. batch, continuous and CFID on the function of the single airlift bioreactor (Asadi et al., 2017). Mirghorayshi et al. evaluated the performance of the concerned bioreactor in the nitrogen removal through various mechanisms i.e. the SND, anammox and presumable dissimilatory nitrate reduction to ammonium (DNRA) by changing ratios of $NH_4^+/(NH_4^+ + N\text{-}NO_3^-)$ and HRTs (Mirghorayshi et al., 2018). In other studies, the investigators intended to reduce the surplus sludge as well as the concurrent CN and CNP removal using combination of jet loop bioreactor and airlift bioreactor addressed to jet loop-airlift bioreactor (Gholami et al., 2020; Moradi et al., 2021). In spite of superior performance of the advanced bioreactors, intermittent effluent discharge and the high oxygen consumption are known as limiting factors against practical applications that must be solved in an affordable manner. Currently, Mirghorayshi et al. advised a hybrid structure of airlift bioreactor with an anaerobic section at the base of the bioreactor called hybrid airlift bioreactor (HALBR) to simultaneously eliminate CN contents from refractory wastewater of composting leachate (Mirghorayshi et al., 2021a). Complexity in operation and precise control are considered as main constraints for such high rate bioreactors.

Presently, some researchers are focused on removing the nitrogen using partial nitritation-anammox (PN/A) process from low strength ammonia wastewater in a one-stage airlift internal circulation (AICB) reactor employing bio-carriers, micro-granules and hydroxyapatite (HAP)-cultured syntrophic granules (Chen et al., 2021, 2021a, 2021b; Guo et al., 2021, 2022; Liu et al., 2017; Wang et al., 2017). Guo et al. for the first time examined the phosphorous recovery and the nitrogen removal upon the PN/A process in the referred one-stage bioreactor through granular sludge cultured at the presence of hydroxyapatite (Guo and Li, 2020). In these studies, the PN/A mechanism is sensitive to dissolved oxygen (DO) concentration and that should be kept in low amount. In addition, the start-up of granular sludge-based bioreactors is time consuming. Alimahmoodi et al. effectively eliminated CNP from synthetic wastewater by employing BioCAST technology, utilizing a hybrid growth approach (both suspended and attached growth) over nitrification/denitrification mechanisms and phosphorus removal (Alimahmoodi et al., 2013). In another study, BioCAST technology was used to apply the PN/A mechanism in a single-stage hybrid bioreactor to eliminate nitrogen from synthetic nitrite-limited wastewater with elevated ammonia levels (Saborimanesh et al., 2019).

As far as we are aware, this study is the first report featuring the effect of the anoxic zone manipulated by the physical barriers i.e. packing media and baffles to enhance the simultaneous nitrogen and phosphorous removal in a one-stage dual internal circulation airlift A2O (DCAL-A2O) bioreactor. Therefore, three identical one-stage airlift A2O bioreactors with the difference in the anoxic zone entitled ordinary airlift A2O bioreactor (without manipulation), baffled airlift A2O bioreactor (with installed baffles) and hybrid airlift A2O bioreactor (with packing media) all with dual internal circulation were operated in parallel. It is predicted the effective entrapment of sludge in the anoxic phase by the installed baffles and attached growth formed on the packing media would provide the anaerobic micro-environment and guaranteed anoxic zone for the concomitant removal of nitrogen and phosphorous. In addition, these physical barriers via the reduction in the internal circulation rate, as proved by computational fluid dynamics (CFD), lengthen the retention time of the ample functional and diverse microbes in the anoxic and anaerobic zones of the one-single airlift A2O bioreactor. Thus, the bioreactor performance in terms of CNP removal efficiency would promote. The specific structure of the proposed bioreactor led to the diversity in the microbial community providing various paths for removing nitrogen through the combined mechanisms i.e. SND and anammox. Manipulation of the anoxic zone affected the enhancement degree in the microbial diversity. The sludge characteristics cultured in three one-stage dual internal circulation airlift A2O bioreactors was explored and compared as the relationship between sludge volume index (SVI) and effluent turbidity with SMP and EPS contents measured as total organic carbon (TOC), the protein and carbohydrate contents. Furthermore, the impact of the enhanced anoxic zone on the functionality of the concerned bioreactor was checked when treating diverse wastewaters with varied biodegradability (as BOD_5/COD ratio) at optimum operating conditions, obtained treating milk processing wastewater. Also, the impact of feeding location was studied on the performance of the concerned bioreactors at the determined optimum conditions. The microbial community analysis was conducted for the three dual internal circulation airlift A2O bioreactors. Finally, the operating cost evaluation and energy efficiency were calculated for the three bioreactors.

2. Materials and methods

2.1. Bioreactor description

A one-stage dual internal circulation airlift anaerobic/anoxic/oxic (DCAL-A2O) bioreactor manufactured from transparent Plexiglas was implemented to oxygenate and agitate the mixed liquor suspended solids (MLSS) concurrently due to the special configuration of this type of bioreactor. In a general view, the bioreactor was consisted of two main sinks i.e. reaction phase and settling chamber with the total effective volumes of 7 and 2 L, respectively. The reaction phase itself was comprised of three substantial zones namely anaerobic, anoxic and oxic regions in a single unit with the working volume of 1.125, 1.875 and 4 L, respectively. As represented in Fig. 1, the anoxic zone of the dual internal circulation airlift A2O bioreactor was manipulated using physical barriers (baffles and the packing media) to explore the impact of manipulated anoxic zone on the bioreactor performance in comparison with the airlift A2O bioreactor without any manipulation in the anoxic zone. Accordingly, the airlift A2O bioreactor was manufactured in three various configurations and addressed as ordinary (without manipulation), baffled and hybrid airlift A2O bioreactor in terms of the manipulated anoxic zone. The required air was supplied to the bioreactor employing an air pump through an air stone installed at the bottom of the oxic zone. Air flow rate (AFR) was set using the gas flow gauge. Feeding was introduced continuously to the bioreactor from the top of the anoxic section using a peristaltic pump with adjustable flow. Feeding location was determined to be from the top of the anoxic zone in order to supply the readily biodegradable organic matters for denitrifiers and PAOs, and accelerate the deoxygenation process to provide anoxic and anaerobic conditions. The hydraulic retention time (HRT) was monitored through regulation of the feed flow rate. Two mixers were mounted into the anaerobic zone and turned on once in a while (1 min/h) through a timer to make sure about the adequate mixing. 14 baffles with dimension of 2 cm × 10 cm were installed on both sides of the anoxic zone, 7 baffles on each side spaced about 2 cm apart with angle of 45° to effective entrapment of the biocatalyst in the anoxic zone, as shown in Fig. 1b. In another configuration as shown in Fig. 1c, carriers Kaldnes K2 (with diameter of 15 mm and a specific area of 360 m^2/m^3) were threaded through fishing yarn as 6 strings and hanged out inside the anoxic zone with the aid of support with filling ratio of 30 % to develop the attached growth. The internal recirculation rate of the airlift A2O bioreactor in three configurations was measured through the computational fluid dynamics (CFD) using Gambit and Fluent software. Wastewater compositions and seed sludge details are provided in supplementary information (SI, text S1).

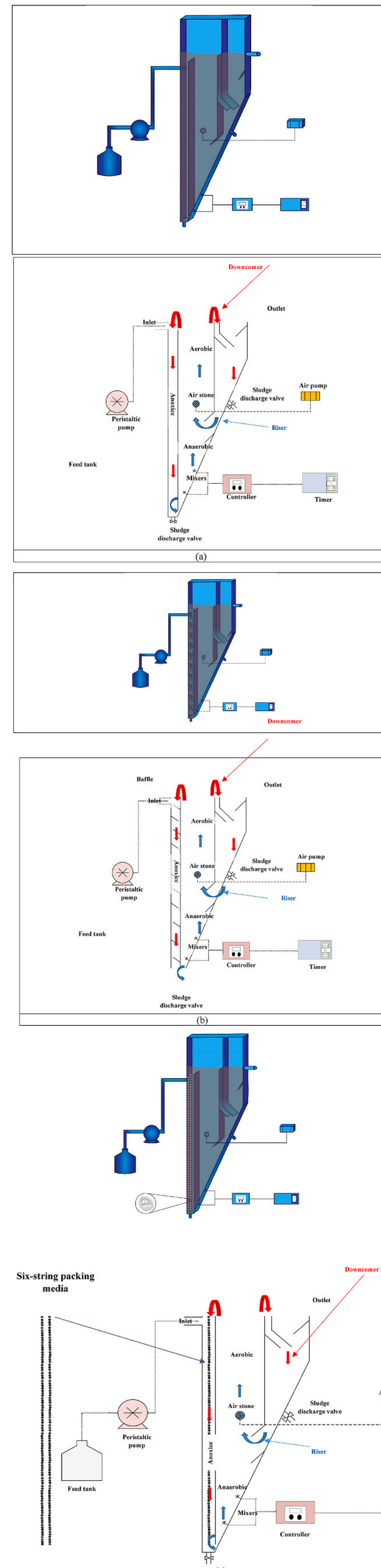


Fig. 1. Bioreactor configuration (a) ordinary DCAL-A2O bioreactor, (b) baffled DCAL-A2O bioreactor, and (c) hybrid DCAL-A2O bioreactor.

2.2. Operational description

From a general perspective, the DCAL-A2O bioreactor operation was carried out into 4 phases, explained below. The start-up of the hybrid DCAL-A2O bioreactor in phase 1 was initiated by immersing the packing media in a 6 wt % agar solution for a short time and threading them into six strings using fishing yarn to facilitate biofilm growth. Then, the strings were hanged into the anoxic zone, and continuously fed with the MPW at the richly managed conditions i.e. HRT and AFR of 7 h and 3 L/min at the mean AVR of 0.394 for a month. After the start-up, the biofilm was nicely formed on the packing media with a thickness of ca. 400 μm. In the case of other configurations namely ordinary and baffled DCAL-A2O bioreactors, the start-up period lasted for two weeks in order to ensure about adaptation of the sludge with the MPW, and getting the steady state conditions (negligible variations in CNP removal). Afterwards, in phase 2, all the three configurations of the bioreactor were run at the various experimental conditions defined by Design-Expert® software (Stat-Ease Inc., Version 10.0) based on the substantial independent variables. The studied variables were including hydraulic retention time (HRT), air flow rate (AFR), and aerobic volume ratio (AVR), defined as aeration volume to total reaction volume ratio, ranged from 7 to 15 h, 1–3 L/min and 0.324–0.464, respectively. A movable air stone was employed to set the various AVRs at the length of aeration region. Each experimental run continued for about 2–4 weeks to get the steady state conditions depending on the applied operating conditions. Each bioreactor was operated continuously for 8 months under ambient temperature. In this phase, the optimum operating conditions were obtained for the three bioreactors treating MPW. In the phase 3, the impact of various wastewaters i.e. SDW and SOW with varying biodegradability as the BOD_5/COD ratio on the performance of the bioreactors was evaluated for 2 weeks at optimum conditions obtained for each bioreactor while treating MPW. In Phase 4, the impact of the feeding location was examined on the performance of the bioreactors treating MPW at optimum conditions obtained for each bioreactor while treating MPW in phase 2. The dissolved oxygen (DO) concentrations measured in various zones strongly indicated the coexistence of triple zones within a single unit, essential for residence of functional bacterial species responsible for removing nitrogen and phosphorus.

The sludge characteristics, including sludge settleability (measured as sludge volume index, SVI), bio-flocculation represented by total organic carbon (TOC), protein, and carbohydrate contents, as well as soluble microbial products (SMP) and bound extracellular polymeric substances (EPS) categorized as loosely bound EPS (LB-EPS) and tightly bound EPS (TB-EPS), were measured according to the procedures outlined in a recently published paper (Rahimi et al., 2024). Additionally, detailed information regarding the analysis of microbial community, cost evaluation, and energy efficiency can be found elsewhere (Rahimi et al., 2024).

3. Results and discussion

3.1. TCOD removal

According to the ANOVA output presented in Table S2, the TCOD removal efficiency obtained from all the three airlift A2O bioreactors was matched to a reduced quadratic model. From the model, the linear and positive model terms A, B, and C, representing HRT, AFR, and AVR, respectively, were statistically significant. Furthermore, the negative model terms A^2 and C^2 were mutual for all the three bioreactors. However, the interactive model terms were different for the bioreactors that might be due to the special manipulation created in the anoxic zone that led to appearance of various bacterial species. Noted that the model terms of pertinent quadratic equations were eliminated based on the beneficial simplification and satisfied statistical parameters i.e. R^2, adj. R^2, Pred. R^2 and p-values. Fig. 2 depicts three-dimensional (3-D) response surface plots illustrating the relationship between the operating variables with highest coefficient regardless the sign, while keeping the variable with the lowest impact (lowest coefficient) constant. In this paper, in order to save space, only selected TCOD removal efficiency for each bioreactor was presented and more details can be found elsewhere (Rahimi et al., 2024).

In a general view, from Fig. 2, the bioreactors revealed the superior performance as the TCOD removal efficiency treating the milk processing wastewater (MPW) containing lipid, protein and other organic contents confirming the high capacity of the proposed airlift bioreactor in three various configurations. The increase of the studied variables, i.e., HRT (A), AFR (B), and AVR (C), enriched the heterotrophic bacteria adapted for consuming the relatively persistent organics in the airlift A2O bioreactor with its special geometry. The values of TCOD removal rate were ranged from 1.94 to 4.29 kg/m^3.d for all the three bioreactors. The TCOD removal efficiencies obtained for the three bioreactors are given in Table S3. Generally, the increase in HRT, AFR and AVR led to the enhancement in aerobic volume that is favorable for the increase in oxidation potential, thereby the increase in TCOD removal efficiency. As is well known, ordinary heterotroph organisms responsible for COD consumption can grow easily under aerobic conditions. In a total conclusion, the different behavior of the bioreactors in the COD removal was ascribed to the manipulated anoxic and the change in anaerobic/anoxic/oxic zones. However, the three bioreactors revealed the high potential in the enhancement of the oxygen bubble transfer efficiency even at minimum HRT and AFR owing to the specific configuration of the airlift bioreactors (Asadi et al., 2016c). The major merit of the single bioreactors over the conventional biological treatment technologies is simultaneous activity possibility of heterotrophic bacteria such as denitrifyers, ordinary heterotroph organisms responsible for COD removal and PAOs in a single unit if the right conditions are provided. Abyar et al. attributed the high COD removal efficiency to the anaerobic digestion, and the activity of the denitrifying and heterotrophic bacteria in anoxic and oxic zones using the electron acceptors of NO_3^--N and oxygen, respectively (Abyar et al., 2018a).

3.2. Nitrogenous compounds removal

In this study, the effect of the bioreactor configuration and anoxic zone manipulated with the physical barriers i.e. baffle (baffled airlift A2O bioreactor) and packing media (hybrid airlift A2O) and without manipulation (ordinary airlift A2O bioreactor) was assessed on the nitrogenous compounds removal. Totally, the presence of physical barriers led to the reduction in circulation rate and movement of the sludge between anaerobic, anoxic and oxic zones via the increase in hydraulic resistance, and therefore, the effective entrapment of the sludge in anoxic section. In this situation, the ample functional bacteria i.e. the denitrifiers responsible for converting the produced nitrate into nitrogen gas would be enriched to cooperate in the simultaneous nitrification and denitrification (SND). Furthermore, the appearance of micro-zones in the interior layers of the packing media and the dead regions in the baffled A2O bioreactor due to the installed baffles has created the possibility of the nitrogen removal through anaerobic ammonium oxidation (anammox). The performance of the bioreactors with different anoxic zones was investigated as the nitrogen removal under various operating conditions i.e. varied HRT, AFR and AVR.

From the ANOVA results as given in Table S2, the performance of the ordinary, baffled and hybrid DCAL-A2O bioreactors in the TN removal was modeled with a reduced quadratic model for two first bioreactors and a linear model for the latter one. The difference in the significant model terms and the type of the proposed model were related to the manipulated anoxic zone. This modification influenced the TN removal efficiency significantly. The 3-D response surface plots delineated for the various DCAL-A2O bioreactors as a function of the operating factors with the highest impact (the highest statistical coefficients) were represented in Fig. 3. From Table S2 and Fig. 3, the linear reduction in AFR and AVR promoted the anoxic and anaerobic environments required for

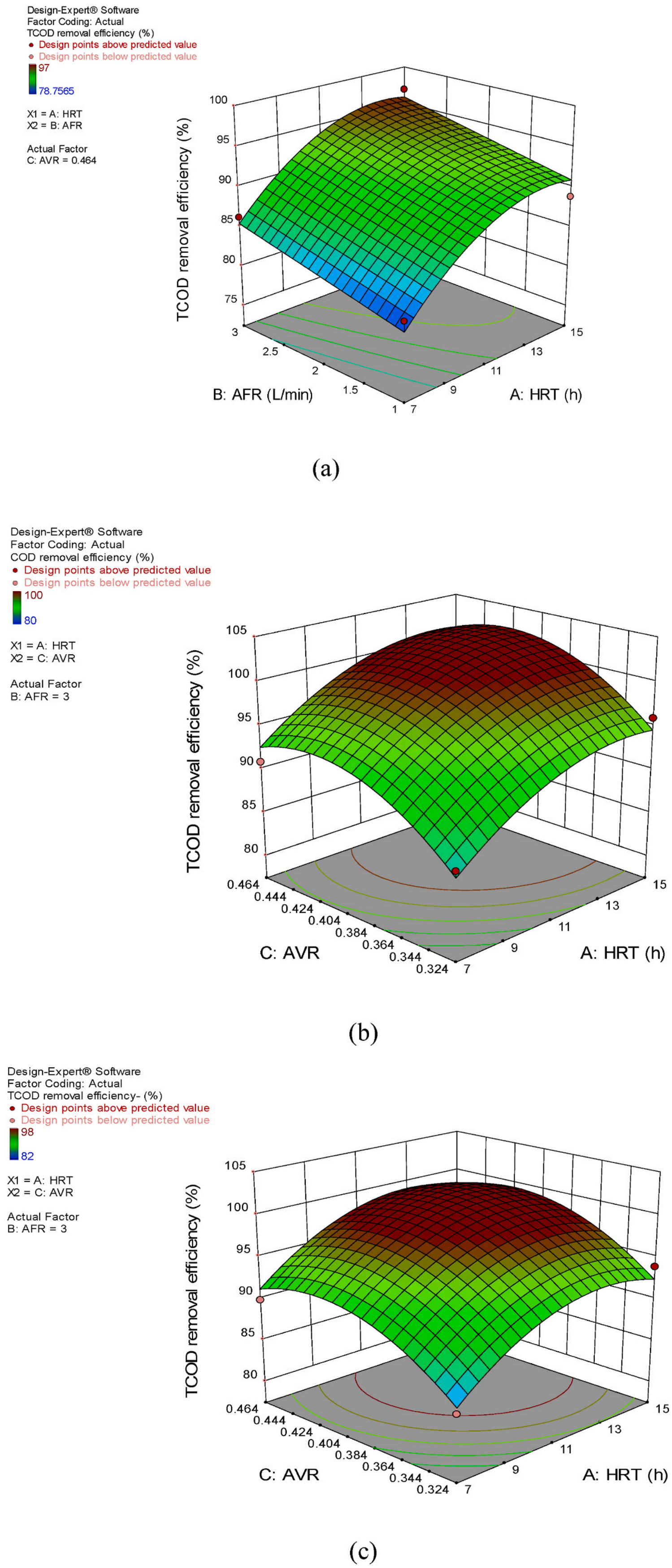
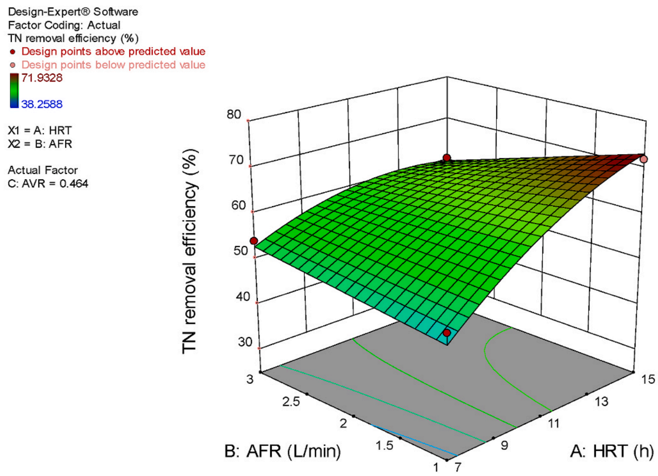
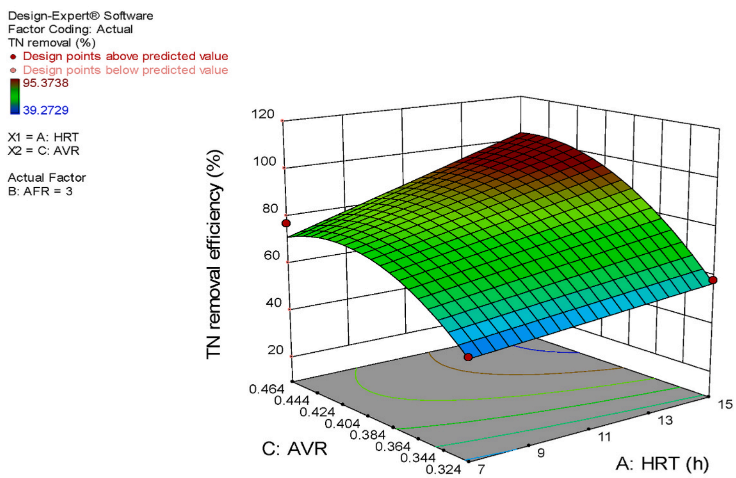


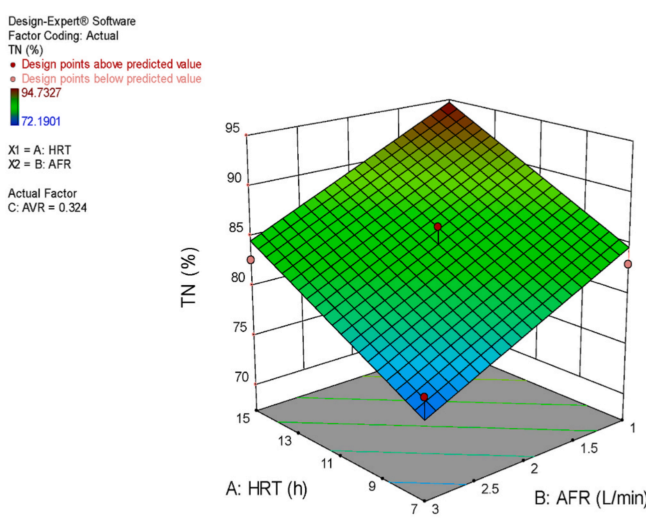
Fig. 2. 3-D response surface plot for TCOD removal efficiency obtained for the airlift A2O bioreactor with manipulated anoxic zone as a function of (a) AFR and HRT at fixed AVR in the ordinary bioreactor, (b) HRT and AVR at constant AFR in the baffled bioreactor, and (c) HRT and AVR at constant AFR in the hybrid bioreactor.



(a)



(b)



(c)

Fig. 3. The changes in TN removal efficiency as 3-D response surface plots obtained for the airlift A2O bioreactor with manipulated anoxic zone in terms of (a) HRT and AFR at fixed AVR in ordinary bioreactor, (b) HRT and AVR at fixed AFR in baffled bioreactor, and (c) HRT and AFR at fixed AVR in hybrid bioreactor.

the implementation of the various mechanisms of the nitrogen removal i.e. SND and anammox in the hybrid DCAL-A2O bioreactor. To be noted in order to save space and make a useful comparison; only graphs showing the best performance of each bioreactor in TN removal were presented here. From a comparative view, the hybrid DCAL-A2O bioreactor revealed the superior functionality in the TN removal throughout the experimental runs ranged from 72.2 to 94.7 %. The concerned hybrid DCAL-A2O bioreactor showed a distinguished behavior and led to the enhanced TN removal efficiency with increase in HRT and reduction in AFR and AVR. However, the change in VAR with lowest coefficient (−1.43) showed the little effect on the response. The reduction in AFR and the presence of packing media created a hydraulic resistance and the decrease in air velocity resulting in the decline of the circulation turnover and the movement of sludge. Thereby, the increase in contact time between the functional bacteria and substrate, and development of the attached microflora growth. The presence of the baffle in the anoxic section indicated the effective role in the TN removal with linear increase in the significant model terms A (HRT), B (AFR) and C (AVR). The installed baffles made the high hydraulic resistance especially at the low AFR, giving rise an imbalance in the conditions necessary for effective nitrogen removal. The TN removal efficiency in such a bioreactor was in the range of 39.3–95.4 %. On the other hand, the ordinary airlift A2O bioreactor without having any physical barrier exhibited the lowest TN removal efficiency (33–72 %) than that of the others.

In a glance view, the pronounced changes trend in the response for the ordinary airlift A2O bioreactor was obtained at AFR of 1 L/min at constant AVR by increasing HRT, as shown in Fig. 3a. Indeed, a rise in AFR and reduction in HRT showed a reducing effect on the response due to the high circulation rate (superficial gas velocity) leading to restricting the anoxic zone and deteriorating the denitrification process. In the baffled DCAL-A2O bioreactor, a sharp upward trend was observed at an HRT of 15 h and an AFR of 3 L/min. The increase in AVR indicates a balance between the nitrification and denitrification processes, leading to the improvement in TN removal. Both SND and anammox mechanisms were incorporated in the TN removal for the three bioreactors. However, it can be said that the presence of micro-anaerobic zones created in the dead regions of installed baffles and interior layer of the biofilm grown on the packing media guaranteed the contribution of SND and anammox mechanisms more effectively compared to the ordinary bioreactor, evident by the improvement in nitrogen removal. Generally, depending on the operating conditions, one of the mentioned mechanisms could be dominant in the nitrogen removal. The use of the physical barrier, specially packing media, enhanced the anoxic zone remarkably as verified by the increase in the nitrification and denitrification amounts and reduction in the effluent nitrite and nitrate. The maximum nitrification amount was reported to be 152.3, 194.7 and 205.5 mg-N/L, for the ordinary, baffled and hybrid DCAL-A2O bioreactors, respectively. Additionally, the denitrification capacity was obtained to be 146.4, 198.4 and 207.6 mg-N/L for the ordinary, baffled and hybrid DCAL-A2O bioreactors, respectively. Some researchers reported that SND is the main mechanism in TN removal, based on the contribution degree of cell synthesis, nitrification, and denitrification processes (Asadi et al., 2016c; Mirghorayshi et al., 2021a). Abyar et al. achieved a TN removal efficiency of 86.95% in their studies, using a high aerated volume fraction and a COD/TN ratio of 100:16 (Abyar et al., 2018a).

3.3. Phosphorous removal

Generally, to remove phosphorus from wastewater, it is necessary to provide anaerobic and aerobic conditions in sequence. In the anaerobic zone, the functional bacteria of the phosphorous removal i.e. phosphorous accumulating organisms (PAOs) will start their job firstly with the simultaneous release of phosphorous into the surrounding media and accumulation of the readily biodegradable sCOD (rb-sCOD) as polyhydroxyalkanoates (PHAs), used as intracellular carbon and energy storage in the next stage. So, in this condition, the presence of the proper rb-sCOD is urgently required. In the following, the PAOs to complete their activity, they must be moved towards the aerobic circumstances to absorb the released phosphorous using consumption of the PHAs previously accumulated.

From Table S2, reduced quadratic models with the various model terms described the phosphorous removal efficiencies obtained from the different bioreactors. The significant model terms were chosen based on the simplification of the proposed models and the rational statistical parameters. Fig. 4a–c displayed the variations in the phosphorous removal efficiency as 3-D response surface plots under the varied operating conditions for the three DCAL-A2O bioreactors. It is noted that in these plots, the model term with the lowest impact on the response (the lowest coefficient) was considered constant. From Fig. 4a, the more phosphorous was removed at the middle conditions for the ordinary DCAL-A2O bioreactor. These observations were in the relation with the circulation speed between anaerobic and aerobic phases. At the average AFR (2 L/min) with a superficial gas velocity of 0.2 m/min, a balance was achieved to facilitate the removal of the targeted response by ensuring suitable circulation and an adequate oxidation environment. However, deviations from this balance, such as the low and high mixing rates corresponding to AFRs of 1 L/min and 3 L/min (superficial gas velocity of 0.1 m/min and 0.3 m/min, respectively), resulted in diminished phosphorus removal efficiency. The increase in AFR induces the turbulence, and thus, restricts the anaerobic zone, causing disruption in the phosphorus elimination. Conversely, aerobic conditions have been limited at low AFRs due to laminar flow, thereby affecting the oxidation potential necessary for achieving desired phosphorus removal. Overall, phosphorus removal efficiencies were ranged from 24% to 78.5%.

In the baffled DCAL-A2O bioreactor, at the low AFRs, the presence of the installed baffles resulted in the reduction in circulation rate and mixing of the aerobic and anaerobic zones, and therefore, expansion of the anaerobic zone. The increase in the AVR and HRT provided the long aerobic conditions for the uptake of the phosphorus released in the anaerobic conditions. Additionally, the long HRT gave rise to mitigating the F/M ratio that was a good opportunity for the PAOs to do their job without competition with the aerobic heterotrophs. As a matter of fact, the existence of the baffles in the anoxic section created reliable anaerobic circumstances via the increase in the hydraulic resistance and the reasonable decrease in circulation turnover at the highest AFR (3 L/min). The range of the phosphorus removal was reported to be 14.3–100 %.

As indicated in Fig. 4c, for the hybrid DCAL-A2O bioreactor, in an overall view, by increasing the AFR, the reasonable balance was created between anaerobic and aerobic zones. While, at high HRT, sufficient feed was not available for slow growing heterotrophic bacteria i.e. PAOs. Furthermore, the increment in aerobic zone by the increase in AVR restricted the anaerobic zone. The further increase in the HRT to 15 h was contributed to the decrease in the F/M ratio and the increase in competition between the slow growing PAOs and other fast growing heterotrophs to reach the substrate at the anaerobic conditions, resulting in the reduction in the phosphorus removal. Furthermore, the high AVR of 0.464 led to a disturbance in the balance of the anaerobic and aerobic phases, thereby, interfering with the release and uptake of the phosphorous. In this configuration, the phosphorous removal efficiency was in the range of 16–100 %.

In contrast to the ordinary DCAL-A2O bioreactor without any physical barrier, the free circulation and rapid mixing of the sludge between various reaction zones was limited somewhat depending on the operating conditions for the baffled and hybrid DCAL-A2O bioreactors. Thereby, different levels of phosphorus removal were observed in the three bioreactors. Abyar et al. got the highest phosphorus removal efficiency at the optimum conditions as HRT, COD/TN ratio and aerated volume fraction of 8 h, 100:14 and 65 %, respectively, in a one-stage A2O bioreactor (Abyar et al., 2018a). Asadi et al. successfully

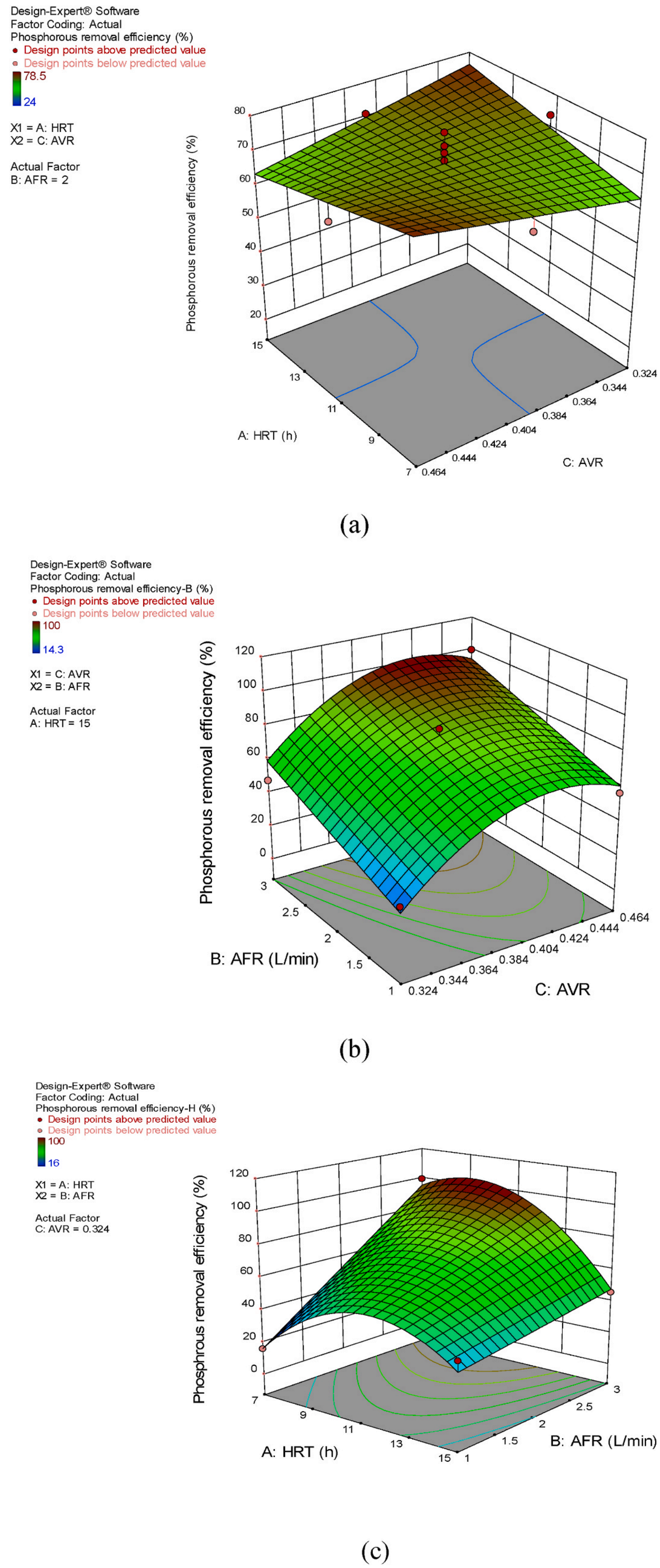


Fig. 4. 3-D response surface plots for phosphrous removal efficiency obtained for the DCAL-A2O bioreactor with manipulated anoxic zone as a function of (a) HRT and AVR at fixed AFR in the ordinary DCAL-A2O bioreactor, (b) AFR and AVR at fixed HRT in the baffled DCAL-A2O bioreactor, and (c) HRT and AFR at fixed AVR in the hybrid DCAL-A2O bioreactor.

removed phosphorus (26–80%) in an integrated airlift bioreactor using the CFID-based regime (Asadi et al., 2016c). Rezaee et al. reported the highest phosphorus removal efficiency of around 70 % at MLSS, HRT and aeration mode of 7000 mg/L, 6 h, and 1, respectively, in a single bioreactor augmented with ultrasound (Rezaee et al., 2015). In a brief conclusion, all the reasons and causes presented for the alteration in the phosphorus removal efficiency were in a consistent agreement with the referred literature.

3.4. The sludge characteristics

3.4.1. Effluent turbidity

Typically, the amount of suspended solids (SS) in the treated wastewater is examined as effluent turbidity. Low effluent turbidity confirms the effectiveness of sludge bio-flocculation and settling.

The ANOVA findings and 3-D surface response plots illustrating the relationship between effluent turbidity and the operational parameters for the three bioreactors are presented in Table S2 and Fig. 5, respectively. To provide a concise and focused comparative study, only the graphs demonstrating the best performance of the bioreactors have been presented here. Based on the Figure, it's obvious that decreasing the HRT led to a decline in effluent quality, as indicated by turbidity, especially pronounced at the lowest AVR (0.324). This reduction in HRT correlates with an increment in both F/M ratio and OLR, which negatively impacted effluent turbidity by imposing sudden shocks on the airlift A2O bioreactors.

For the three bioreactors, the highest amount of the effluent turbidity was observed to be 96, 60 and 34 NTU at the lowest level of the variables, corresponding to the low solids retention time (SRT) (6.4, 8.0 and 8.9 d), the highest F/M ratio (0.90, 0.87 and 0.83 gCOD/gVSS.d) and OLR (4.2, 4.1 and 4.7 g-COD/L.d) for the ordinary, baffled and hybrid DCAL-A2O bioreactors, respectively. It is important to note that this study exclusively considered suspended growth biomass for calculating SRT. Meanwhile, the lowest effluent turbidity (4, 6, and 4 NTU) was associated with the highest level of variables along with high SRTs (33, 30.6, 33.1 days) and low OLRs (2.2, 2.4, and 2.1 g-COD/L.d) for the ordinary, baffled, and hybrid DCAL-A2O bioreactors, respectively. From results obtained by Asadi et al., higher DO concentrations promoted the formation of stable and dense flocs, leading to decreased effluent turbidity. Conversely, low DO levels and HRT encouraged sludge expansion, consequently increasing turbidity levels (Asadi et al., 2016b, 2016c). The outcomes obtained in this study were consistent with findings reported by literature (Asadi et al., 2016b; Mirghorayshi et al., 2021b).

Totally, the less effluent turbidity, the more bio-flocculation happens confirmed by the reduction in soluble microbial product (SMP), and extracellular polymeric substances (EPS) including LB-EPS and TB-EPS, and effluent TSS as discussed in the following section.

3.4.2. Sludge volume index (SVI)

In this study, the sludge settling ability was monitored in order to address bulking and floating sludge phenomenon, known as a serious challenge in wastewater treatment plants. This was achieved by measuring the sludge volume index (SVI) over a settling time of 30 min. Fig. 6 reveals the SVI values obtained from the three DCAL-A2O bioreactors. The changes in the SVI were kept in pace with variations in the effluent turbidity. An improvement in the sludge settling ability was observed by decreasing OLR and F/M as a result of an increase in HRT as indicated by the mitigated SVI. Additionally, the lower SVI was associated with the reduction in the AFR and AVR values. Based on the findings, the three bioreactors showed the lowest SVI values as 80, 90 and 72.5 mL/g for ordinary, baffled and hybrid DCAL-A2O bioreactors, respectively, at the highest amounts of operating variables. The SVI values reported by Abyar et al. were in the range of 75.9–166.33 mL/g (Abyar et al., 2018a). The authors concluded that the lowest value of SVI was associated with the lowest effluent turbidity (1.5 NTU). These results indicate effective sludge bio-flocculation and inhibited wash-out phenomena during the treatment of meat processing wastewater. In the present study, apart from the negative impact of high OLR and F/M on the response, the excellent capacity of the baffled and hybrid bioreactor in the nitrogen removal via the generation of N_2 as a result of the excess nitrite or nitrate concentrations was brought about the increase in the SVI value in some runs (Abyar et al., 2018a).

Interestingly, even though some runs exhibited high SVI values, the effluent turbidity stayed within acceptable limits due to internal circulation between the settling and reaction zones, as well as the timely removal of excess sludge. These measures helped maintain the sludge level below the bioreactor's outlet, effectively monitoring sludge washout. In general, the effective entrapment of the sludge in the baffled and hybrid airlift A2O bioreactors owing to the physical barriers employed in the anoxic zone led to the reduction in SVI values compared to the unmanipulated bioreactor (ordinary airlift A2O bioreactor).

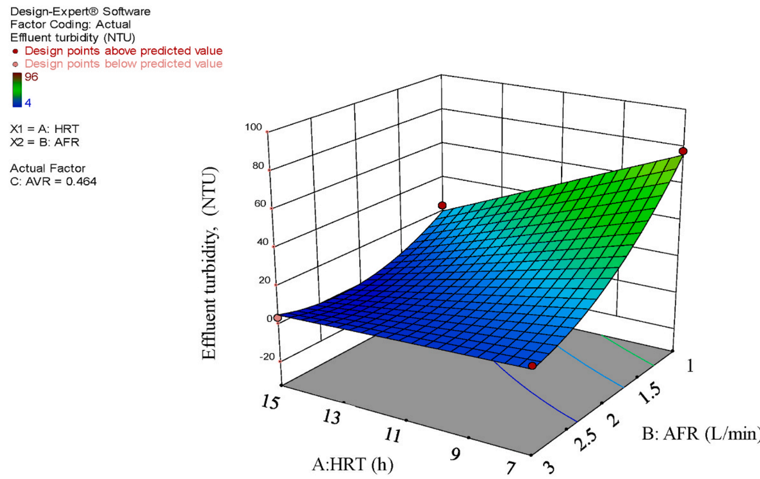
3.4.3. Sludge bio-flocculation and settleability properties

The bio-flocculation properties and settleability of the sludge were further studied by measuring the amounts of protein and carbohydrates present in the SMP present in bioreactors effluent and sludge, LB-EPS and TB-EPS under the varying operating circumstances. Moreover, the correlation of these parameters with the effluent TSS and SVI values known as indicators of sludge bio-flocculation and sludge settleability was determined. Although identifying sludge characteristics and treated wastewater effluent quality as the production of the microbial secretions (SMP & EPS) is significant from the view point of water reuse, there are only a few reports in this area (Li and Yang, 2007; Zhang et al., 2008). Li et al. investigated the impact of SRT values (5, 10, and 20 days) on the amounts of protein and carbohydrate in bioreactors fed batch wise with glucose and acetate at fixed MLSS (2000 mg/L) and COD:N:P ratio (100:5:1) (Li and Yang, 2007). The authors claimed that there is an inverse relationship between the contents of protein and carbohydrate with various SRTs.

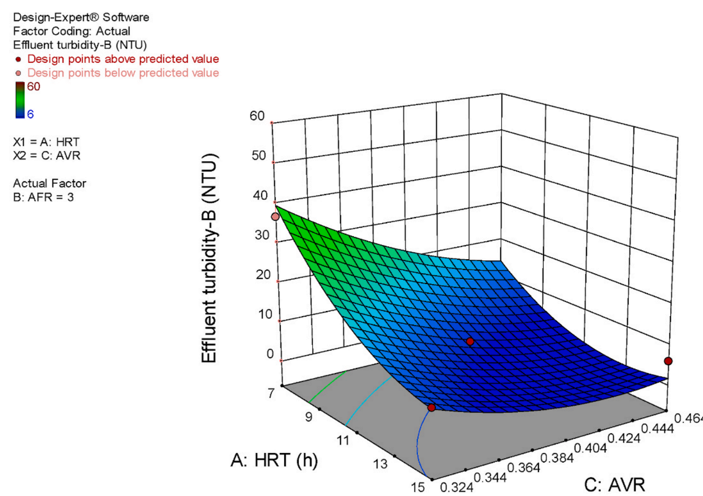
In this work, the influence of three substantial factors i.e. HRT, AVR and AFR on the sludge attributes was assessed. Based on the outcomes, a direct relationship between effluent TSS and contents of the protein and carbohydrate present in SMP of bioreactor effluent and sludge, and LB-EPS was identified. An increment in variables resulted in a decrease in the concentrations of the protein and carbohydrate, SMP and LB-EPS. In summary, increasing AFR and AVR at an appropriate HRT (11–14 h) creates favorable circumstances for growing aerobic bacteria that contribute in removal of a significant fraction of the COD. This outcome is attributed to greater substrate utilization rate plus higher growth rates of aerobic bacteria than other types. Therefore, the optimal sludge bio-flocculation and settleability were achieved at the highest level of the studied variables. In these conditions, the lowest contents of SMP present in bioreactor effluent and sludge, LB-EPS and TB-EPS were reported as follows: 33.2, 37.8, 42.0 and 229.4 mg-TOC/L; 30.0, 32.0, 35.7, and 210 mg-TOC/L; and 26, 28, 32.5 and 194.4 mg-TOC/L for the ordinary, baffled and hybrid DCAL-A2O bioreactors, respectively, as shown in Table 1. The hybrid DCAL-A2O bioreactor showed the lowest microbial secretions compared two other bioreactors ascribed to the use of the hybrid growth.

3.5. Process optimization and verification

Typically, the aim of bioreactor design is to meet effluent discharge standards, which is the primary concern for industrial owners. Accordingly, the optimum process conditions are attained via constraining independent operating variables, and portraying predicted data using overlay plots. The process optimization was performed using Design-Expert® software (Version 10.0). The optimum conditions are determined by selection of influential responses namely TCOD removal, phosphorus removal, TN removal and effluent turbidity. In order to meet the targeted conditions, the maximum values in terms of TCOD,



(a)



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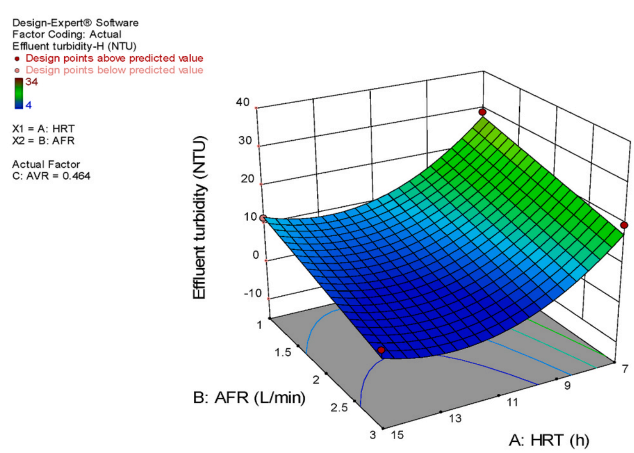

(c)

Fig. 5. 3-D response surface plots for the effluent turbidity obtained for the DCAL-A2O bioreactor with manipulated anoxic zone under various operating conditions in terms of (a) HRT and AFR at fixed AVR in the ordinary DCAL-A2O bioreactor, (b) HRT and AVR at fixed HRT in the baffled DCAL-A2O bioreactor, and (c) HRT and AFR at fixed AVR in the hybrid DCAL-A2O bioreactor.

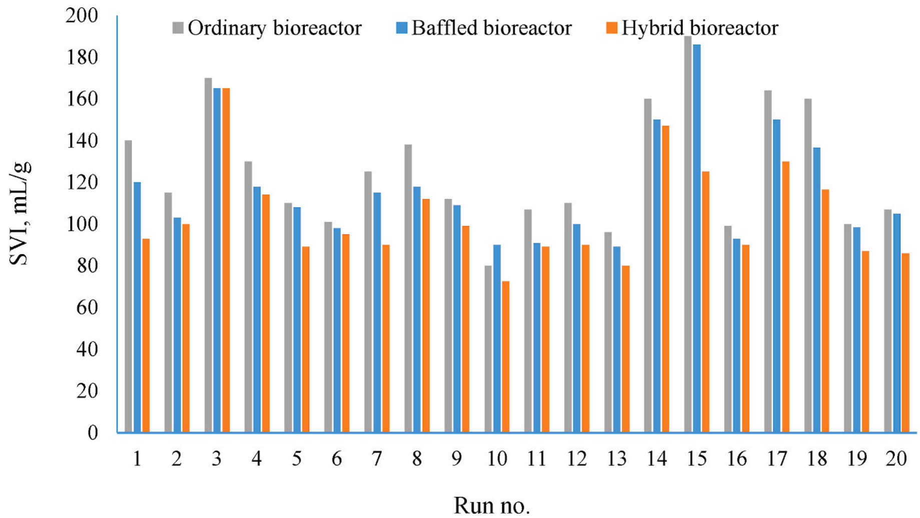

Fig. 6. The trend of variations in SVI under various operating conditions obtained for the DCAL-A2O bioreactor in three different configurations.

Table 1
The quantities of protein and carbohydrate of effluent SMP; SMP, LB-EPS and TB-EPS extracted from biomass; and the effluent TSS obtained from ordinary, baffled and hybrid DCAL-A2O bioreactors at HRT of 15 h, AFR of 3 L/min and AVR of 0.464 (P and C refer to protein and carbohydrate).

Type of bioreactor	Effluent SMP, mg/L		Sludge SMP, mg/L		LB-EPS, mg/L		TB-EPS, mg/L		Effluent TSS, mg/L
	P	C	P	C	P	C	P	C	
Ordinary	10.7	9.2	12	7.89	13.85	7.8	136	26	10.7
Baffled	12.4	10.6	15.36	11.25	18.65	13.6	140	30	28.9
Hybrid	15	13.2	19	14.89	23.85	18.8	158	48	6.6

phosphorus and TN removal efficiencies, and the minimum value of the effluent turbidity are considered based on the experimental results. The optimization regions are graphically visualized as the overlay plots for the three DCAL-A2O bioreactors shown in Fig. 7a–c. As can be seen from the Figure, the yellow-dyed and the shaded gray-dyed areas are representative of regions fitting the optimization criteria and not fitting the constraint, respectively. In order to verify the predicted data obtained from defined optimum conditions and the accuracy of models, one, two and three verification experiments are designed and carried out for the ordinary, baffled and hybrid DCAL-A2O bioreactors, respectively. In general, the primary objective of the new bioreactor design is to lessen investment costs and energy consumption by reducing bioreactor volume (thus reducing HRT) and aeration energy (resulting in reduced AFR) as well as enhancing bioreactor performance. In the present work, the selected values of the controlled operating variables i.e. HRT, AFR, and AVR as well as the actual and predicted data are given in Table 1. According to the Table, the attained experimental data agreed well with the predicted data for each response, verifying the suggested models accuracy.

As a conclusion, among the three DCAL-A2O bioreactors, the hybrid DCAL-A2O bioreactor showed the best process performance at desired optimum conditions with HRT and AFR as low as 10 h and 2 L/min, respectively, and AVR of 0.464.

3.6. The impact of the wastewater characteristics on the bioreactors performance at the optimum conditions

In summary, the wastewater characteristics, as biodegradability degree of carbon source (BOD_5/COD ratio), are of the most important factors influencing the process performance of bioreactor, in particular biological nutrient removal (BNR) processes. Accordingly, this matter must be urgently considered while developing the bioreactor design. In this study, the focus was on examining wastewater characteristics using various types of wastewater. To achieve this, the performance of the bioreactors was evaluated and compared not only with MPW but also with two other types of wastewater—soft drink wastewater (SDW) and soybean oil plant wastewater (SOW)—under the optimum conditions determined from the treatment of MPW. These wastewaters were applied to DCAL-A2O bioreactors with different configurations: ordinary DCAL-A2O, baffled DCAL-A2O, and hybrid DCAL-A2O, at the optimum conditions obtained once the bioreactors were fed with MPW. The characteristics of three wastewaters are given in Table S1. The BOD_5/COD ratios for MPW, SDW and SOW were obtained to be 0.7, 0.8 and 0.4, respectively. The functionality of each bioreactor was investigated in terms of TCOD, TN, phosphorus removal efficiencies and effluent turbidity. The acquired outcomes are displayed in Table 3. Obviously, all the three airlift A2O bioreactors showed superior capability in the TCOD removal ($>$ 90 %) for all the three wastewaters with varied biodegradability indicating the high oxidation potential of the relevant bioreactor due to its special design, leading to occurrence of the heterotrophic bacterial species for biodegradation of the slowly biodegradable organic matters. However, the increase in the TCOD removal efficiency was in the order of SDW, MPW and SOW due to the easier biodegradation of the SDW and its higher BOD_5 decomposition rate constant compared to two other wastewaters. The BOD_5 decomposition rate constant for the SDW, MPW and SOW were obtained to be 0.38, 0.18 and 0.03 1/d, respectively.

The bioreactors exhibited the distinguished behavior in the TN removal efficiency, so that, the MPW-fed bioreactor showed the highest TN removal efficiency, then, in sequence the SOW-fed bioreactor and the SDW-fed bioreactor. According to literature report, these results were related to the slow decomposition rate of protein containing MPW compared to carbohydrate containing SDW, resulting in the preservation of carbon source for the denitrifiers to do their job (Adav et al., 2010; Asadi et al., 2016c). Another reason for the extremely reduced TN removal efficiency in the case of the SDW-fed ordinary A2O bioreactor was attributed to the long HRT (13 h) verifying the fast depletion of the required carbon for the denitrification process (Asadi et al., 2016c). On

Table 2
Results of verification experiments obtained at optimum operating conditions for the three DCAL-A2O bioreactors.

Optimum conditions		Feasible responses			
		TCOD removal, %	Phosphorus removal, %	TN removal, %	Effluent turbidity, NTU
Ordinary DCAL-A2O bioreactor					
HRT = 13 h	Experimental value	94.5	59.6	62.2	8.0
AFR = 2 L/min AVR = 0.437	Model values	95.0	65.0	68.2	4.4
Baffled DCAL-A2O bioreactor					
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
Hybrid DCAL-A2O bioreactor					
HRT = 10.0 h	Experimental value	97.0	90.0	92.0	9.0
AFR = 2.0 L/min AVR = 0.464	Model values	92.6	80.1	80.1	7.8

the other hand, the SOW contains slowly biodegradable organic matters (oil and grease), not favorable for the denitrifiers. However, SOW-fed baffled and hybrid DCAL-A2O bioreactors showed the considerable TN removal efficiency due to decomposition of the slowly biodegradable organic contents in the enhanced anaerobic zone by the installed baffles and packing media at short HRT (10 h).

The opposite observations were reported for the phosphorus removal while feeding the bioreactor with various wastewaters. Given that the SDW contains high carbohydrates amounts that are readily biodegradable organic matters, therefore, the SDW-fed bioreactor displayed the highest the phosphorus removal (Asadi et al., 2016c). Since the presence of readily biodegradable organic matters in the anaerobic phase is vital for the PAOs to start their activity and store PHB through easy fermentation of sugar to volatile fatty acid (VFA), known as the main substrate for the PAOs. However, the lowest phosphorus removal efficiency was realized for the SOW-fed bioreactor. There is a reason behind such results, related to high phosphorus contents in the raw wastewater (116.4–185.03 mg/L). Nontheless, the effluent turbidity of the baffled DCAL-A2O bioreactor was increased once it was fed with the SOW that might be related to the high activity of anaerobic bacteria in more secretion of exteracellular enzymes for hydrolyzing the slowly biodegradable organics. The bioreactors showed approximate the similar effluent turbidity when fed with MPW and SDW that was in the relation with the adequate contact time (HRT), proper F/M ratio and high BOD_5/COD ratio.

Indeed, all these results are verifying the satisfactory performance of the dual internal circulation airlift A2O bioreactor after manipulation of anoxic zone using physical barriers (baffle and packing media) treating the various wastewaters with difference in biodegradability at optimum conditions. In general, the high capacity of the bioreactors in treatment of such wastewaters was due to occurrence of local micro-anaerobic zones and the increased microbial population at low HRT (10 h) as a result of the effective entrapment of the sludge with the aid of installed baffles and packing media.

3.7. The impact of the feeding location on the bioreactors performance at the optimum conditions

In this part, the impact of two feeding locations, the top of the anoxic zone and the anaerobic zone, was evaluated on the bioreactor performance for TCOD, TN, phosphorus removal efficiencies and effluent turbidity treating the MPW. To be noted, the bioreactors performance previously investigated was based on feeding from the top of the anoxic zone. The obtained outcomes were denoted in Table 4. As can be seen from the Table, there was no inscrutable change in the TCOD removal efficiency by varing the feeding location showing the high oxidation potential of the bioreactors regardless the feeding location. However, the feeding from the anaerobic zone gave rise to a decrement in TN removal efficincy than that of the feeding from the anoxic zone. While, the phosphorus removal was enhaned once feeding was conducted from the anaerobic zone. These results were in the relation with the accessibility of the functional bacterial species to the soluble biodegradable COD (sbCOD) that's a key parameter for denitrifieres and PAOs to do their job. Asadi et al., studied the impact of two feeding locations (from the riser and downcomer) at the optimal citcumstances (HRT and AFR of 10 h and 2 L/min, respectively) in an airlift bioreactor with continous feed and intermitent discharge (CFID) regime treating the MPW (Asadi et al., 2017). The authors declared the feeding from the riser distrupted the proper balance of aerobic and anaerobic zones due to the lack of enough carbon source. So that, the aerobic zone was enhanced while the anaerobic zone was reduced compared to when the bioreactor was fed from the downcommer. The authors reported the TN and TP removal efficiencies dropped from 83 to 76 %, and 54 to 8 %, respectively, by changing the feeding location from downcomer to riser. However, the significant change was not reported for sCOD removal efficiency. In this present study, the changes in TN and phosphorus removal efficiencies were not as much as what reported by Asadi et al., ascribed to the unique design of the bioreactor and efficient manupulation created in anoxic zone with the physical barriers to reduce the circulation rate (via the increase in the hydrulic resistance) between the various zones to provide the right conditions for PAOs, denitrifiers and anammox bacteria. The efflunt turbidity was not affected significantly by the feeding location.

3.8. Microbial community at the optimum conditions

Microbial communities residing in the DCAL-A2O bioreactor with three different configurations, i.e., ordinary, baffled, and hybrid, were identified using PCR tests. In this regard, the entire sludge of the three bioreactors was mixed in a separate container and a sample was taken for the PCR analysis.

The obtained outcomes for the three bioreactors are depicted in Fig. 8 and summarized in Table 5. Successful detection of various bacterial species was determined based on the presence of bands observed in the Figure. The results indicated that manipulation of the anoxic zone influenced the bacterial community.

From the PCR tests, the co-existence of functional bacteria straints responsible for CNP removal was detectable synchronously in a single unit. Furthermore, the occurrence of nitrifiers (AOB and NOB), denitrifiers, GAOs and anammox bacteria confirmed the nitrogen removal was carried out via various pathways i.e. SND, simultaneous partial nitrification and denitrification (SPND) and anammox. From the Table, all the three bioreactors indicated the similar AOB and NOB bacteria species since the aerobic conditions were similar for the bioreactors. The presence of denitrifying bacteria (Pseudomonas) was verified nicely in the bioreactors disclosing the incorporation of the SND mechanism in nitrogen removal. The more PAOs bacteria straints were observed in the baffled DCAL-A2O bioreactor revealing the positive effect of dead

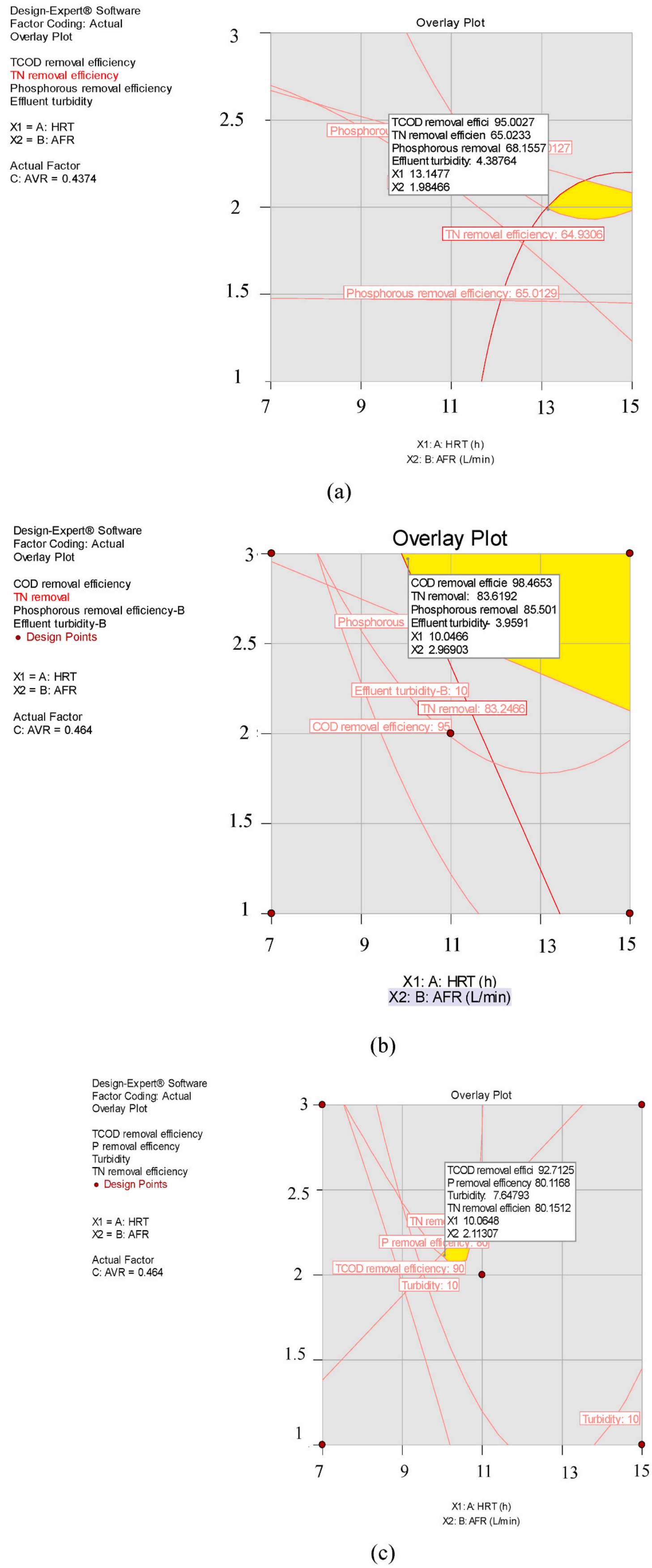

Fig. 7. Overlay plots visualized for (a) ordinary DCAL-A2O bioreactor, (b) baffled DCAL-A2O bioreactor, and (c) hybrid DCAL-A2O bioreactor at the optimum region with more strict conditions.

Table 3
The influence of the wastewater characteristics (MPW, SDW and SOW) on the process performance of the three DCAL-A2O bioreactors under optimum conditions obtained while treating MPW.

Type of wastewater	TCOD removal efficiency, %	TN removal efficiency, %	Phosphorous removal efficiency, %	Effluent turbidity, NTU
Ordinary DCAL-A2O bioreactor (Optimum conditions: HRT of 13 h, AFR of 2 L/min & AVR of 0.437)				
MPW	94.8	59.5	68.2	8
SDW	97.5	37.3	73.4	6
SOW	90.8	48.7	22.6	16
Baffled DCAL-A2O bioreactor (Optimum conditions: HRT of 10 h, AFR of 3 L/min & AVR of 0.464)				
MPW	96.8	90.0	92.0	8.0
SDW	97.4	73.0	97.0	6.0
SOW	93.8	80.0	30.0	20.0
Hybrid DCAL-A2O bioreactor (Optimum conditions: HRT of 10 h, AFR of 2 L/min & AVR of 0.464)				
MPW	96.8	92.0	90	9.0
SDW	97.7	80.0	93.0	12.0
SOW	94	85.0	24.0	14.0

Table 4
The impact of the feeding location on the performance of the three DCAL-A2O bioreactors at the optimum conditions obtained while treating the MPW.

Feeding location	TCOD removal efficiency, %	TN removal efficiency, %	Phosphorous removal efficiency, %	Effluent turbidity, NTU
Ordinary DCAL-A2O bioreactor (Optimum conditions: HRT of 13 h, AFR of 2 L/min and AVR of 0.437)				
Top of anoxic zone	94.8	59.5	68.2	8
Anaerobic zone	94.6	42.6	75.8	7
Baffled DCAL-A2O bioreactor (Optimum conditions: HRT of 10 h, AFR of 3 L/min and AVR of 0.464)				
Top of anoxic zone	96.8	90	92	15
Anaerobic zone	96	80	96	10
Hybrid DCAL-A2O bioreactor (Optimum conditions: HRT of 10 h, AFR of 2 L/min and AVR of 0.464)				
Top of anoxic zone	97.0	92.0	90.0	9.0
Anaerobic zone	96.7	80.0	94.0	10.0

regions created by the installed baffles in the growth of diverse PAOs. While, the more DPAOs were found in the ordinary bioreactor and attached growth in the hybrid bioreactor verifying the activity of PAOs in the anoxic conditions under optimum conditions. DPAOs contribute in the nitrogen and phosphorous removal (Wang et al., 2015). More importantly, two anammox bacteria (Candidatus Brocadia and Candidatus kuenenia) were detected in the baffled and hybrid DCAL-A2O bioreactors showing the occurrence of the more convenient anaerobic conditions by means of the manupulated anoxic zone compared to the ordinary bioreactor without any manupulation (with one detectable anammox bacteria strain i.e. Candidatus Brocadia). Although GAOs have a similar metabolism with PAOs, they lack the ability to release and uptake phosphorus (Metcalf and Eddy, 2013). However, according to the literature they can play a role in nitrogen removal through the simultaneous partial nitrification and denitrification (SPND) process (Yuan et al., 2020).

However, the more GAOs were reported for the hybrid bioreactor owing to the additional anoxic zones appeared inside the biofilm. In summary, the obtained findings all are confirming the effective role of the manupulation in the anoxic zone on the bioreactor performance especially in the case of the hybrid bioreactor. It worth mentioning difference in the process performance of the bioreactors, in particular as NP removal, might come back to the abundance of various functional bacterial population remarkably enhanced by the manupulated anoxic zone.

3.9. Economic evaluation

The operating cost evaluation of the airlift A2O bioreactors under different operating conditions was conducted at a 7-day steady state condition for each experimental run generalized for 1 m^3. It was forecasted the proposed bioreactors would indeed be affordable as investment cost and technical monitoring due to the integrated configuration embedded into a single unit. In addition, in this unique design, the existence of airlift force resulted in reducing the number of mixers, required certainly for convenient agitation of the anoxic and anaerobic chambers in conventional systems, and eliminating the recycling pumps.

The aeration energy (AE) was computed using various mass transfer coefficients ($K_{La,i,}$ 1/d), obtained from the computational fluid dynamics (CFD) studies, and the volume reference (V_{ref}; 1333 m^3). In this study, an effort has been made to avoid excessive length in the text and provide a more comprehensive comparative perspective by presenting the data specifically obtained under optimum conditions. From the calculations, the consumed energy for aeration was achieved to be in the range of 1.3–2.5 kwh/d in the whole experiments that is much lower than those reported by other researchers owing to the special design of the airlift A2O bioreactor (Abyar et al., 2018b; Zhou et al., 2015). The cost of the AE was estimated to be in the range of 0.1–0.2 €/m^3 (1.284–2.513 kwh/d) that is in agreement with 0.3 €/m^3 (4.42 kwh/d) and 0.39 €/m^3 obtained by Abyar et al. (at optimum conditions of HRT of 8 h, aeration volume of 65 %, COD/N of 100:14) and Gil et al., respectively (Abyar et al., 2018b; Gil et al., 2010). The mixing energy (ME) gained in this study was the same as that was obtained by Abyar et al. i.e. 0.12 kwh/d for treating 1 m^3 of wastewater. This value equaled to benchmark simulation model no. 1 (BMS1) where the average mixing energy of 240 kWh/d was achieved for a 2000 m^3 anoxic reactor (Alex et al., 2008). The functionality of the bioreactors in terms of the sludge production (SP) was determined through the measure of concentration and flow rate of waste sludge. From the obtained results, less surplus sludge (0.37, 0.27 and 0.18 kg/d for the ordinary, baffled and hybrid DCAL-A2O bioreactors, respectively) was produced for the operated bioreactors compared to the findings reported by Abyar et al. (0.86 kg/d) at optimum conditions. The calculated results at optimum conditions are presented in Table 6.

The pumping energy (PE) was reduced considerably in comparison with the data declared by BMS1, contributed to the elimination of the recycling pumps owing to the special design of the airlift A2O bioreactor. In fact, the required sludge and produced nitrate were circulated between the anaerobic and anoxic zones, respectively, with the aid of the airlift force and dual internal circulation. Therefore, the pumping energy (PE) was related to only the discharge of the surplus sludge. Abyar et al. obtained the pumping energy as high as 0.118 kwh/d while operating an anaerobic-anoxic-oxic (A2O) bioreactor treating meat processing wastewater at optimum conditions owing to the use of both sludge recycling and discharge pumps (Abyar et al., 2018b). Vivekanandan et al. could successfully decrease the pumping energy to 0.05 kwh/d using the predetermined set-point to monitor the activated sludge process (Vivekanandan et al., 2017). The average value of the pumping energy in BMS1 model was gained to be nearly 0.1 kWh/d. However, the effluent quality (EQ) reported in the case of ordinary DCAL-A2O bioreactor (4.1 kg/d) at optimum conditions was much

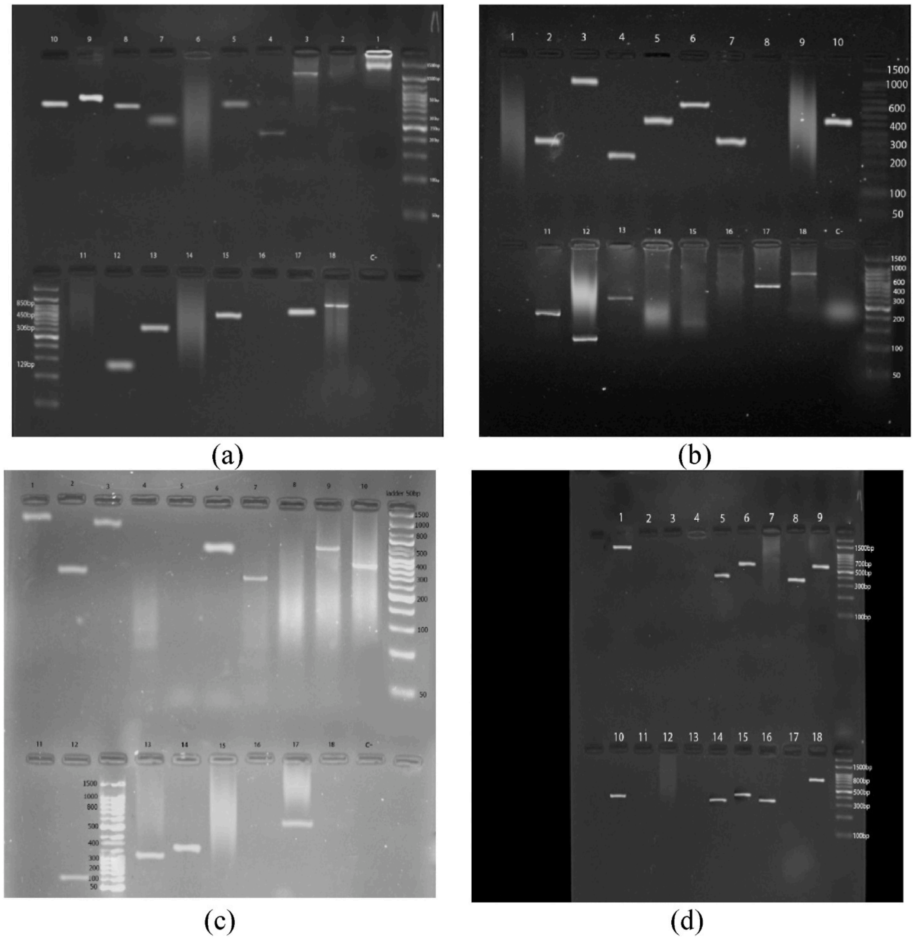

Fig. 8. The PCR results obtained from (a) ordinary DCAL-A2O bioreactor; (b) baffled DCAL-A2O bioreactor; (c) suspended sludge of hybrid DCAL-A2O bioreactor; and (d) biofilm of hybrid DCAL-A2O bioreactor.

Table 5
The various bacterial species identified in the DCAL-A2O bioreactor with three different configurations (ordinary, baffled and hybrid).

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

lower than that of findings released by Abyar et al. (0.43 kg/d) (Abyar et al., 2018b). The decrease in the effluent quality of ordinary bioreactor was in the relation with the inefficiency of the anoxic and anaerobic zones due to high internal circulation rate created by the airlift force and the high influent loading that was out of the bioreactor capacity. In a study carried out by Gabarron et al., the effluent quality was declined to 19 % through the decrease in the internal recirculation from 6Q to 1Q in order to decrease the pumping energy (Gabarrón et al., 2015). Given that the denitrification process is of significant importance for wastewater treatment plant, it is stated the reduction in the pumping energy and the effluent nitrate concentration is impossible with the reduction in the internal recirculation (Abyar et al., 2018b). However, this possibility

Table 6
The results obtained for the operating cost evaluation of the ordinary, baffled and hybrid DCAL-A2O bioreactors under optimum operating conditions obtained while treating MPW (The obtained results were generalized to 1 m^3).

Type of bioreactor	Operating variables			Economic cost evaluation parameters					
	HRT, h	AFR, L/min	AVR	ME, kwh/d	PE, kwh/d	SP, kg/d	AE, kwh/d	EQ, kg/d	CPI, €/d
Ordinary	13	2	0.437	0.12	0.002	0.375	2.4	4.079	0.809
Baffled	10	3	0.464	0.12	0.002	0.27	3.1	1.272	0.450
Hybrid	10	2	0.464	0.12	0.002	0.176	2.4	1.160	0.368

came true using development of the airlift regime-based bioreactor configuration. The effluent quality of the baffled and hybrid DCAL-A2O bioreactors was obtained to be 1.27 and 1.16 kg/d, respectively.

As an ultimate conclusion, the operating cost estimated for the ordinary airlift A2O bioreactor (0.77 €/d) was higher than those declared by Abyar et al. (0.556 €/d) and other investigators (Abyar et al., 2018b; Vivekanandan et al., 2017). This is related to the deterioration of the effluent quality, in spite of the decrease in other energy sources compared to the reported work. While, the operating cost was reduced to 0.41 and 0.33 €/d after manipulation of the anoxic zone with baffle and packing media led to the improvement in effluent quality as a result of the enhanced nutrient removal. In addition, such sever reduction in the consumption of energy sources (pumping energy & aeration energy) was in association with the unique design of the proposed bioreactor which resulted in removing additional equipment and simplifying the technical operation.

3.10. Determination of selected DCAL-A2O bioreactor

As a brief conclusion, based on the obtained results, the hybrid dual internal circulation airlift A2O (DCAL-A2O) bioreactor was introduced as the selected bioreactor from the perspective of satisfactory bioprocess performance, easy technical operation and proper hydraulic regime. The ordinary dual internal circulation airlift A2O bioreactor in spite of the having easy technical operation revealed the poorer bioprocess performance in terms of TN and phosphorus removal efficiencies owing to the weakness in the anoxic and anaerobic zones compared to two other bioreactors. On the other hand, although the baffled dual internal circulation airlift A2O bioreactor showed the superior bioprocess performance similar with the hybrid dual internal circulation airlift A2O bioreactor, its operation was not as easy as the hybrid one. This drawback was related to the high number of and the long length of installed baffles in the anoxic zone that led to disrupting the internal circulation and creating channeling phenomenon at long term operation. So that, in some cases, the entrapped sludge needed to be circulated manually. Furthermore, high retention of the sludge in the anoxic and anaerobic zones deteriorated the sludge settling properties. Besides, the presence of the extra installed baffles created the many dead zones over two other bioreactors resulting in the reduction in the actual HRT. In general, this kind of airlift A2O bioreactor configuration required special monitoring in comparison with the others. While, the hybrid bioreactor benefits the attached growth leading to the increase in the microbial population and microbial diversity. It effectively maintains local micro-anaerobic and anoxic zones without causing inadequate sludge entrapment. These characteristics boost the efficiency of the bioprocess and improve sludge properties, making the hybrid DCAL-A2O bioreactor a cost-effective choice. The decisive evaluation of the chosen configuration, compared to the other three, is evident in Tables 2, 5 and 6.

4. Conclusions

The present study, generally, deals with the synchronous CNP removal in an innovative one-stage dual internal circulation airlift A2O (DCAL-A2O) bioreactor in three new configurations provided by manipulation in anoxic zone. The three configurations of the airlift bioreactor nominated as ordinary DCAL-A2O bioreactor (no manipulation), baffled DCAL-A2O bioreactor (manipulation by baffle) and hybrid DCAL-A2O bioreactor (manipulation using packing media) were designed and constructed. The anoxic zone was manipulated in order to enhance the nutrients removal processes via reduction in circulation rate and effective entrapment of the sludge in anoxic and anaerobic zones. The airlift A2O bioreactor in three configurations was operated in parallel to concomitantly remove carbon and nutrients from the milk processing wastewater (MPW) under varying operating circumstances. According to the obtained results, the optimum region for the ordinary DCAL-A2O bioreactor was identified at an HRT, AFR and AVR of 13 h, 2 L/min, and 0.437, respectively. Under these conditions, the removal efficiencies were 94.5% for TCOD, 59.6% for TN (116.2 mg/L), and 62.2% for phosphorus (37.3 mg/L), with an effluent turbidity of 8 NTU. In the baffled airlift A2O bioreactor, optimal conditions were achieved at HRT, AFR and AVR of 10 h, 3 L/min, and AVR of 0.464, respectively, leading to the highest removal efficiencies for TCOD, TN, and phosphorus, as well as reduced effluent turbidity corresponding to 96.8%, 90% (175.5 mg/L), 92% (55.2 mg/L), and 8 NTU. The hybrid airlift A2O bioreactor demonstrated superior performance under its optimal conditions obtained at HRT, AFR and AVR of 10 h, 2 L/min, and 0.464, respectively. It achieved removal efficiencies of 97.0% for TCOD, 92% for TN (179.4 mg/L), and 90% for phosphorus (50.33 mg/L), with an effluent turbidity of 9 NTU. The impact of wastewater biodegradability and the feeding location on simultaneous CNP removal was evaluated under these optimum conditions for each airlift A2O bioreactor. From the outcomes, the SDW-fed bioreactors exhibited better function in phosphorus elimination due to their high content of readily biodegradable carbon, which is essential for PAOs. While, the bioreactors operated with two other wastewaters i.e. MPW and SOW revealed better nitrogen removal efficiencies incorporated to the presence of the adequate carbon source for denitrifiers. Nonetheless, poor phosphorus removal efficiency was reported for SOW-fed bioreactor ascribed to the high original phosphorus concentration of the pertinent wastewater. Furthermore, the effect of two feeding locations, from the anoxic zone and anaerobic zone, was explored on the process performance of three bioreactors treating the MPW under optimum conditions obtained for each bioreactor. According to the achieved findings, the feeding from the anoxic zone enhanced the nitrogen removal, while, the phosphorus removal efficiency was improved once the bioreactors were fed from the anaerobic zone. These results were in the relation with the accessibility of the functional bacteria i.e. dentrifiers and PAOs responsible for nitrogen and phosphorus removal, respectively, to readily biodegradable organic matters. The PCR tests also confirmed the symbiosis of variant bacterial species confirming the appreence of anaerobic, anoixc and aerobic zones in a single unit. More importantly, the growth of dentrifiers, DPAOs, GAOs, anammox bacteria verified various memchanisms contributed in the nitrogen removal as SND, SPND and anammox. The superior functionality of the baffled and hybrid DCAL-A2O bioreactors might be related to high abundance of the functional microbial community as a result of the manipulated anoxic zones created the ensured anaerobic and anoxic environments compared to the ordinary bioreactor. However, the hybrid bioreactor was chosen as optimum configuration due simplicity in operation, reduction in oxygen consumption (2 mg/L as DO vesus 3 mg/L), the increase in microbial population and their biodiversity due to the hybrid growth (biofilm and suspended sludge) amoung other configurations. According to the process and economic evaluation (0.368 €/d versus 0.809 and 0.450 €/d for ordinary and baffled bioreactors respectively) conducted for the three bioreactors, the hybrid DCAL-A2O bioreactor was chosen as the optimum bioreactor.

CRediT authorship contribution statement

Zahra Rahimi: Writing – original draft, Validation, Software, Methodology, Conceptualization. **Ali Akbar Zinatizadeh:** Writing – review & editing, Supervision, Software, Methodology, Investigation. **Sirus Zinadini:** Writing – review & editing, Project administration. **Mark van Loosdrecht:** Project administration. **Damien J. Batstone:** Writing – review & editing.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jclepro.2024.144303.

Data availability

Data will be made available on request.

References

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

Abyar, H., Younesi, H., Bahramifar, N., Zinatizadeh, A.A., 2018b. Modeling the kinetics and cost performance index of high rate up-flow anaerobic, anoxic, oxic bioreactor for meat wastewater treatment. Water Environ. J. 32, 637–649. https://doi.org/10.1111/wej.12362.

Adav, S.S., Lee, D.J., Lai, J.Y., 2010. Enhanced biological denitrification of high concentration of nitrite with supplementary carbon source. Appl. Microbiol. Biotechnol. 85, 773–778. https://doi.org/10.1007/s00253-009-2265-4.

Alex, J., Benedetti, L., Copp, J., Gernaey, K.V., Eppsson, U., Nopens, I., Pons, M.N., Rieger, L., Rosen, C., Steyer, J.P., Vanrolleghem, P., Winkler, S., 2008. Benchmark simulation model no. 1 (BSM1). Prepared by the IWA Taskgroup on Benchmarking of Control Stategies for WWTPs.

Alimahmoodi, M., Yerushalmi, L., Mulligan, C.N., 2013. Simultaneous removal of carbon, nitrogen and phosphorus in a multi-zone wastewater treatment system. J. Chem. Technol. Biotechnol. 88, 1136–1143. https://doi.org/10.1002/jctb.3953.

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

Asadi, A., Zinatizadeh, A.A., van Loosdrecht, M., 2017. Effects of operational models (batch, continuous and CFID modes) on the performance of a single A2O airlift bioreactor for treatment of milk processing wastewater. Chem. Eng. Res. Des. 125, 471–482. https://doi.org/10.1016/j.cherd.2017.08.004.

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

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

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

Asadi, A., Zinatizadeh, A.A., Van Loosdrecht, M., Younesi, H., 2016. Nitrogen removal by ANAMMOX and simultaneous nitrification-denitrification (SND) processes in a novel single airlift bioreactor. RSC Adv. 6, 74367–74371. https://doi.org/10.1039/c6ra11174b.

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

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

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

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

Cao, W., Mehrvar, M., 2011. Slaughterhouse wastewater treatment by combined anaerobic baffled reactor and UV/H_2O_2 processes. Chem. Eng. Res. Des. 89, 1136–1143. https://doi.org/10.1016/j.cherd.2010.12.001.

Chen, F.Y., Liu, Y.Q., Tay, J.H., Ning, P., 2013. Alternating anoxic/oxic condition combined with step-feeding mode for nitrogen removal in granular sequencing batch reactors (GSBRs). Sep. Purif. Technol. 105, 63–68. https://doi.org/10.1016/j.seppur.2012.12.015.

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

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

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

Di Bella, G., Torregrossa, M., 2013. Simultaneous nitrogen and organic carbon removal in aerobic granular sludge reactors operated with high dissolved oxygen concentration. Bioresour. Technol. 142, 706–713. https://doi.org/10.1016/j.biortech.2013.05.060.

Gabarrón, S., Dalmau, M., Porro, J., Rodriguez-Roda, I., Comas, J., 2015. Optimization of full-scale membrane bioreactors for wastewater treatment through a model-based approach. Chem. Eng. J. 267, 34–42. https://doi.org/10.1016/j.cej.2014.12.097.

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

Gil, J.A., Túa, L., Rueda, A., Montaño, B., Rodríguez, M., Prats, D., 2010. Monitoring and analysis of the energy cost of an MBR. Desalination 250, 997–1001. https://doi.org/10.1016/j.desal.2009.09.089.

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

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

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

Jia, Y., Zhou, M., Chen, Y., Hu, Y., Luo, J., 2020. Insight into short-cut of simultaneous nitrification and denitrification process in moving bed biofilm reactor: effects of carbon to nitrogen ratio. Chem. Eng. J. 400, 125905. https://doi.org/10.1016/j.cej.2020.125905.

Jimenez, J., Miller, M., Bott, C., Murthy, S., De Clippeleir, H., Wett, B., 2015. High-rate activated sludge system for carbon management - evaluation of crucial process mechanisms and design parameters. Water Res. 87, 476–482. https://doi.org/10.1016/j.watres.2015.07.032.

Li, J., Peng, Y., Zhang, L., Li, X., Zhang, Q., Yang, S., Gao, Y., Li, S., 2020. Improving efficiency and stability of anammox through sequentially coupling nitritation and denitritation in a single-stage bioreactor. Environ. Sci. Technol. 54, 10859–10867. https://doi.org/10.1021/acs.est.0c01314.

Li, X.Y., Yang, S.F., 2007. Influence of loosely bound extracellular polymeric substances (EPS) on the flocculation, sedimentation and dewaterability of activated sludge. Water Res. 41, 1022–1030. https://doi.org/10.1016/j.watres.2006.06.037.

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

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

Metcalf & Eddy, 2013. Wastewater Engineering: Treatment and Resource Recovery.

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

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

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

Mohammadhosseinpour, B., 2016. Nitrogen Removal by the Combined Partial Nitrification and Anammox Processes in the BioCAST Technology 97.

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

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

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

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

Saborimanesh, N., Walsh, D., Yerushalmi, L., Arriagada, E.C., Mulligan, C.N., 2019. Pilot-scale application of a single-stage hybrid airlift BioCAST bioreactor for treatment of ammonium from nitrite-limited wastewater by a partial nitrification/anammox process. Environ. Sci. Pollut. Res. 26, 25573–25582. https://doi.org/10.1007/s11356-019-05754-2.

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

Vivekanandan, B., Rao, A.S., Saravananae, R., 2017. Enhanced control of activated sludge process using predetermined set-points. Int. J. Environ. Sci. Technol. 14, 487–496. https://doi.org/10.1007/s13762-016-1157-2.

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

Wang, X., Wang, S., Xue, T., Li, B., Dai, X., Peng, Y., 2015. Treating low carbon/nitrogen (C/N) wastewater in simultaneous nitrification-endogenous denitrification and phosphorous removal (SNDPR) systems by strengthening anaerobic intracellular carbon storage. Water Res. 77, 191–200. https://doi.org/10.1016/j.watres.2015.03.019.

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

Zhang, M.L., Sheng, G.P., Yu, H.Q., 2008. Determination of proteins and carbohydrates in the effluents from wastewater treatment bioreactors using resonance light-scattering method. Water Res. 42, 3464–3472. https://doi.org/10.1016/j.watres.2008.04.010.

Zhou, Z., Shen, X., Jiang, L.M., Wu, Z., Wang, Z., Ren, W., Hu, D., 2015. Modeling of multimode anaerobic/anoxic/aerobic wastewater treatment process at low temperature for process optimization. Chem. Eng. J. 281, 644–650. https://doi.org/10.1016/j.cej.2015.07.017.

Zinatizadeh, A.A., Ghaytooli, E., 2015. Simultaneous nitrogen and carbon removal from wastewater at different operating conditions in a moving bed biofilm reactor (MBBR): process modeling and optimization. J. Taiwan Inst. Chem. Eng. 53, 98–111. https://doi.org/10.1016/j.jtice.2015.02.034.