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# Co-occurrence of simultaneous carbon and nitrogen removal and in-situ sludge separation in a novel single unit combined airlift bioreactor (CALBR) for milk processing wastewater treatment

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ABSTRACT

Combined anaerobic-aerobic bioreactors offer considerable potential for treating different industrial wastewaters. However, difficulty in maintaining a stable coexistence of anaerobic, anoxic, and aerobic microbial communities and the necessity of space-consuming external settlers remain as crucial drawbacks associated with these systems. In this study, an innovative single stage combined airlift bioreactor (CALBR) comprising multiple bioreactors (anaerobic/anoxic/aerobic) and an internal settler (a rotating spiral separator for in-situ separation of biomass) was exploited to achieve simultaneous carbon and nitrogen removal from milk processing wastewater (MPW). Results demonstrated that the anaerobic pre-treatment zone made a substantial contribution towards COD and TN removal. Successful biomass separation and high effluent quality revealed that the rotating spiral separator can be used as a coupled clarifier to sustain higher biomass concentration through boosting the sludge compressibility and settleability which further upgrades the overall treatment bioprocess. sCOD removal, TN removal, SVI, and effluent turbidity of >96%, >75%, <90 mL/g, and <10 NTU were reached, respectively under optimum condition. The outcomes suggested that the novel CALBR is a promising technology which has a great potential to be transferred into practical implementation for high-rate combined biological wastewater treatment and sludge separation.

## 1. Introduction

Recent human activity in industrial and agricultural sectors and climate change have caused massive wastewater volume and water resource depletion. Polluted water may cause widespread environmental issues if not properly treated [64]. Different wastewater types have been treated using different methods. The most common treatment methods are coagulation and flocculation [69], membrane separation [52], biological [68], adsorption [54], and photocatalytic membrane filtration [65]. Additionally, natural treatment systems handle municipal and industrial wastewaters cost-effectively. These systems have lower reactor volume output than mechanical treatment systems because to their longer retention period [13,59].

The global dairy sector is a major industrial sector producing one of the largest industrial effluent streams annually [31]. A significant amount of waterborne liquid waste is produced during the routine operation of a dairy manufacturing factory (milk processing, heating and cooling, cleaning, and sanitation [47]. Although the volume and composition of milk processing wastewater are quite variable, it is generally considered as an intermittent waste stream characterized by a high level of chemical oxygen demand (COD), biological oxygen demand (BOD), nutrients, and total suspended solids (TSS) [10,41]. Thus, the unsafe disposal of milk processing wastewater into the environment poses serious threats to the soil and water quality. Wetland

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(nature-based process), physico-chemical, and biological (aerobic and anaerobic) treatment methods have been used to meet discharge standards [3]. Among all, biological treatment processes (divided into aerobic and anaerobic processes) methods are the most favorable approaches adopted for treating dairy industry wastewaters on account of their high biodegradability [16]. Aerobic treatment processes are land-intensive and require high energy input, whereas anaerobic technologies are not able to remove nitrogen and therefore need to go under post-treatment to fulfill the discharge standards [5].

The consecutive combination of aerobic and anaerobic processes may compensate for the drawbacks of each individual treatment method and enhance the overall process performance ([22]; Kolev Slavov, 2017). Considering the benefits of airlift bioreactors (ALBRs), these integrated systems have attracted great interests in the field of wastewater treatment. The specific structure of ALBRs makes them capable to incorporate anaerobic, anoxic, and aerobic zones within a single reactor, and thus different types of microorganisms can coexist to achieve simultaneous carbon and nutrient removal [12]. Under continuous feeding and intermittent discharge (CFID) regime in an ALBR where the anaerobic condition was temporarily induced at the beginning of each run, 98%, 85%, and 78% of soluble COD (sCOD), total nitrogen (TN), and total phosphorous (TP) were removed, respectively at the optimum hydraulic retention time (HRT) (10 h) and air flow rate (AFR) (3 $L_{air}$/min) [7]. However, the evidence from our previous study points towards the idea that the separation of anaerobic and anoxic zones through mounting an anaerobic section preceding the anoxic zone rather than temporary airlift induced anaerobic phase may improve the continuous-flow ALBR performance [43]. Besides, steady-state continuous-flow reactors are more practical for application in large wastewater treatment plants receiving high influent flow owing to their advantages over sequencing batch reactor (SBR) and CFID systems including lower installation cost, easier operation, and better monitoring, maintenance, and control [2,63].

In addition to the specific configuration of ALBRs, maintaining sludge retention time (SRT) at moderately long time is an extra benefit for effective nitrogen removal through simultaneous nitrification and denitrification (SND) since the growth rate of microorganisms responsible for nitrification is very slow [1,25]. Introducing a membrane device into the airlift structure could be an attractive strategy to withhold the suspended biomass within the bioreactor, leading to a prolonged SRT and enhanced nitrogen removal efficiency via SND process [42]. However, the application of membrane bioreactors is still practically not feasible due to membrane fouling which refers to the accumulation of unwanted materials, such as solids, microorganisms, or organic compounds, on the surface or within the pores of the membrane. This accumulation can lead to a decrease in membrane performance, reduced filtration efficiency, and increased energy consumption [11,32,40]. Moreover, employing conventional continuous-flow ALBRs in activated sludge wastewater treatment systems imposes a requirement for a subsequent solid-liquid separation in large secondary settling tanks [33,56]. Therefore, there is a great demand to simplify the process and reduce the costs in industrial applications. Implementing an internal settling device within the bioreactor may be considered as a promising alternative approach to membrane filtration improving overall nitrogen removal and circumventing the need for large secondary clarifiers. Earlier, Zhang et al. developed a hybrid airlift reactor (HALR) integrating ALBR and an internal clarifier comprising of fixed inclined plates at the upper part of bioreactor to promote SND process and separate sludge from the treated synthetic wastewater. Based on their results, both SND process and sludge settling were successfully carried out in the HALR and an average COD and TN removal of over 90% and 76% were achieved in steady state condition, respectively [66]. Further, a modified airlift reactor (MALR) was proposed in the study of Liu et al., in which a sedimentation tank containing inclined plates was integrated into the down-comer section of the internal loop of a rectangular external/internal loop airlift design. The experimental data indicated that the MALR removed 94.5%, 78.6%, and 94.5% of COD, TN, and TP, respectively from municipal wastewater [39]. Nonetheless, since both abovementioned researches used static and motionless inclined plates in their settling zone, the sludge rising phenomenon may occur due to prolonged sludge storage on the plates resulting in bubble formation caused by organic compounds decomposition and consequent uncontrolled sludge scape in certain conditions [30]. Furthermore, these previous studies were limited to investigating the treatment capacity of the bioreactor regarding COD and nutrient removal and did not evaluate the effectiveness of the internal settling zone in overall bioreactor performance in terms of sludge settling properties and effluent turbidity.

To address these gaps, in this study, the aim was to assess a novel combined airlift bioreactor (CALBR) merging multiple bioreactors (anaerobic/anoxic/aerobic) and an internal rotating spiral separator with refreshing effects within a single unite and operating in continuous regime to accomplish highly efficient carbon and nitrogen removal and achieve a high-quality effluent. The performance of the CALBR in terms of carbon and nitrogen removal and effluent turbidity was determined under three independent operating variables, HRT (8–16 h), AFR (2.5–4.5 $L_{air}$/min), and chemical oxygen demand to nitrogen ratio (C/N) (4–6.7) to treat milk processing wastewater. In addition, the effect of mixed liquor suspended solids (MLSS) concentration, feed distribution, P concentration, attached biomass, and different substrate on the reactor stability and performance was investigated under optimum conditions.

## 2. Materials and methods

### 2.1. Wastewater characteristics

The milk processing plant in Bistoon dairy manufacturing (Kermanshah, Iran) produces about 80 tons of pasteurized milk and generates a large amount of wastewater each day. The produced effluent (Flow: 320–800 $m^3$/d, COD: 1500–2000 mg/L, and BOD: 1050–1400 mg/L) was collected, filtered, and immediately refrigerated at 4 C to prevent any physico-chemical and biological changes in the feed matrix characteristics. The wastewater used in this study was prepared daily by adding nutrients (ammonium chloride and potassium dihydrogen phosphate) to the stock solution in order to compensate for nitrogen and phosphorous deficiency and maintain different influent C:N:P ratios (100:15:1, 100:20:1, and 100:25:1). Table 1 presents the composition and characteristics variation of the influent.

### 2.2. Bioreactor set-up and process description

The schematic diagram and picture of the novel CALBR equipped with an internal rotating settling separator are shown in Fig. 1 and Plate 1, respectively. The transparent Plexiglass bioreactor column was fabricated with an inner diameter of 10 cm, a height of 100 cm, and a total working volume of 7.58 L. The working volumes of anaerobic, anoxic, and aerobic zones were 1, 5.01, and 1.57 L, respectively. The single stage CALBR comprises three zones: pretreatment zone, reaction zone, and settling zone. The anaerobic pretreatment zone is mounted at the lowest part of the column and connected to the reaction zone via a

**Table 1**
Influent characteristics.

| Parameters | Unit | C/N = 4 | C/N = 5 | C/N = 6.66 |
|---|---|---|---|---|
| **tCOD** | **mg/L** | 1200–1210 | 1200–1210 | 1200–1210 |
| **sCOD** | **mg/L** | 980–1100 | 980–1100 | 980–1100 |
| **$BOD_5$** | **mg/L** | 686–770 | 686–770 | 686–770 |
| **Org-N** | **mg/L** | 40–50 | 40–50 | 40–50 |
| **N-$NH_4^+$** | **mg/L** | 205–207 | 151–156 | 99–106 |
| **N-$NO_3^-$** | **mg/L** | 1–4 | 1–4 | 1–4 |
| **TN** | **mg/L** | 248–259 | 192–210 | 147–153 |
| **TP** | **mg/L** | 9.8–10.1 | 9.8–10.1 | 9.8–10.1 |
| **pH** | - | 7.53–7.73 | 7.53–7.73 | 7.53–7.73 |

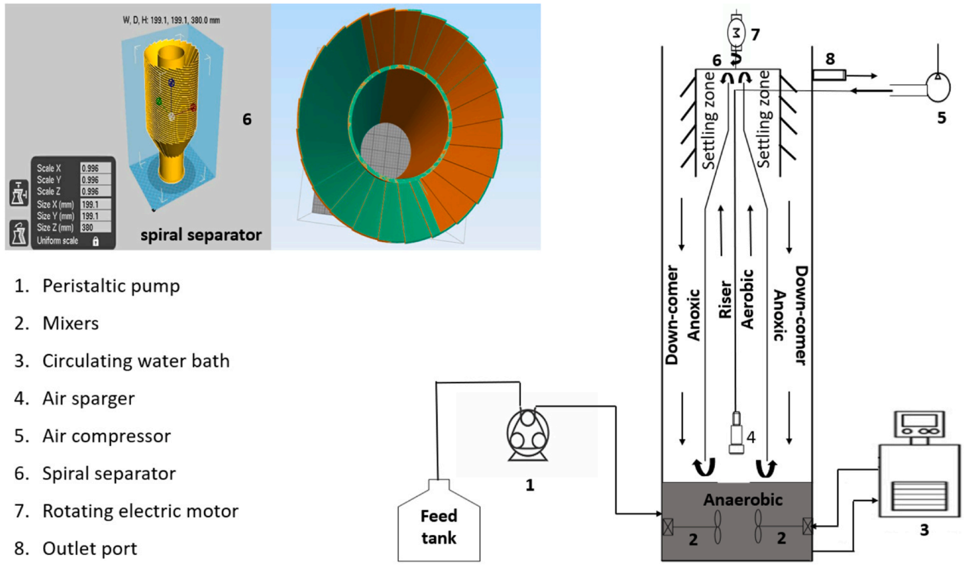


**Fig. 1.** The schematic diagram of the CALBR.

disk with a centric hole. The wastewater solution was fed continuously to the anaerobic chamber using a peristaltic pump. Efficient mixing and favorable temperature (mesophilic condition) were achieved in the anaerobic mixed liquor by employing two mechanical mixers and a heating element linked to a circulating water bath, respectively. A vertical cylindrical steel draft tube with 5 cm inner diameter and 80 cm height is submerged in the main column discriminating the reaction zone into two sections, riser and down-comer. Compressed air was sparged into the inner tube through a perforated pipe sparger placed in the upward flow area, namely, riser. The annule area between the riser and the main column is called the down-comer. The aeration intensity inside the riser was regulated and set on the required value manually by an airflow meter. The rising air bubbles in the inner tube flows upward and the gas hold-up leads to a difference in density or hydrostatic pressure between the riser and the annule zone. This difference acts as driving force which pulls the mixed liquor down in the downcomer inducing the fluid circulation. For the purpose of withholding the activated sludge within the bioreactor, a rotating spiral separator (a compact gravimetry settlement device) is placed at the upper part of the riser area, settling zone. In Fig. 1, the rotating spiral separator is shown in different views. This settling device is composed of a spiral plate pack including 6 spiral plates with the distance between each plate is 0.5 cm with a slope of 35° (patent-protected) [51]. The inner draft tube enters and passes up the central core of the settling device at the upper part of the riser area. The effluent flows along the inlet pipe and down the central core which acts as a baffle before flowing up through the plate pack. Suspended sludge, which is heavier than the liquid, settles onto the plates. The treated water passes up through the plate pack and leaves the separator via the outlet launder while the settled solids (sludge flocs) on the plates aggregate and form a coalesced sludge. The accumulated sludge then slides down the plates to the annular gap between the plate pack and the reactor wall and then into the bottom of the bioreactor. The plate pack is continuously rotated with the aid of a shaft and an electrical motor, which increases the relative velocity of the settling sludge particles on the plates and improves biosolids removal efficiency. The rotation also assists the movement of the settled sludge off the plates and prevents the annular space between the plates from being clogged due to sludge blockage. Moreover, gentle shearing action in the annulus gap between the plates may contribute to the sludge thickening. The treated effluent was withdrawn continuously through an outlet port located at 1 cm above the settling zone.

### 2.3. Operational strategy

The activated sludge inoculum was taken from the aeration unit of a continuously operated municipal wastewater treatment plant in Kermanshah with a treatment capacity of 6000 $m^3$/d. The seed had a MLSS content of 11000 mg/L which was diluted to 5000–6000 mg/L and acclimated to the influent solution. The bioreactor was seeded with activated sludge (MLSS of 5000–6000 mg/L) and fed continuously from the bottom part (anaerobic zone) for two weeks under HRT and AFR of 12 h and 3.5 $L_{air}$/min to get acclimated to the milk processing wastewater (C:N:P of 100:5:1). After start-up period, the CALBR was operated continuously for 10 months based on the experimental conditions presented in Table 3. MLSS concentration in the reaction zone was maintained at approximately 5000 mg/L and the temperature was kept at 40 ℃ ± 2 ℃ (mesophilic) and 20 ℃ ± 2 ℃ (room temperature) for the pretreatment and reaction zone, respectively. SRT was controlled and maintained at about 20 days during the whole operational period by manual removal of the surplus anoxic and anaerobic biomass periodically through valves mounted at the middle of reaction zone and bottom of the anaerobic zone, respectively.

Three independent variables, i.e., HRT (8, 12, and 14 h), AFR (2.5, 3.5, and 4.5 $L_{air}$/min) and C/N ratio (4, 5, and 6.7) were studied to investigate the bioreactor performance in simultaneous organic COD and nitrogen removal. HRT was adjusted by varying the feeding flow rate and AFR was set at required values by controlling the blower aeration intensity. Appropriate amounts of ammonium chloride were added to the feed solution to achieve certain C/N ratios. Each experimental run lasted two weeks to establish a well-adapted microbiome and reliable steady-state responses. Dissolved oxygen (DO) concentrations and oxidation-reduction potential (ORP) values demonstrated a distinct difference between the pretreatment (DO: 0 mg/L; ORP: +100 to −307 mv), riser (DO: 3.5–6 mg/L; ORP: +240 to +446 mv), and downcomer (DO: 0.2–0.5 mg/L; ORP: +56 to +104 mv) sections. The observed uneven distribution of DO and ORP provides a strong support the well-organized co-occurring of anaerobic, anoxic, and aerobic phases in a single bioreactor. The combination of different redox conditions within the CALBR promotes microbial community diversity which can enhance the organic biodegradation as well as SND process performance. The spiral separator was rotating at an optimum speed of 5 rpm (according to a preliminary experiment) during the entire bioreactor operation to reach an effective in situ separation of suspended sludge from the treated effluent. In doing so, it is hypothesized that the longer SRT is

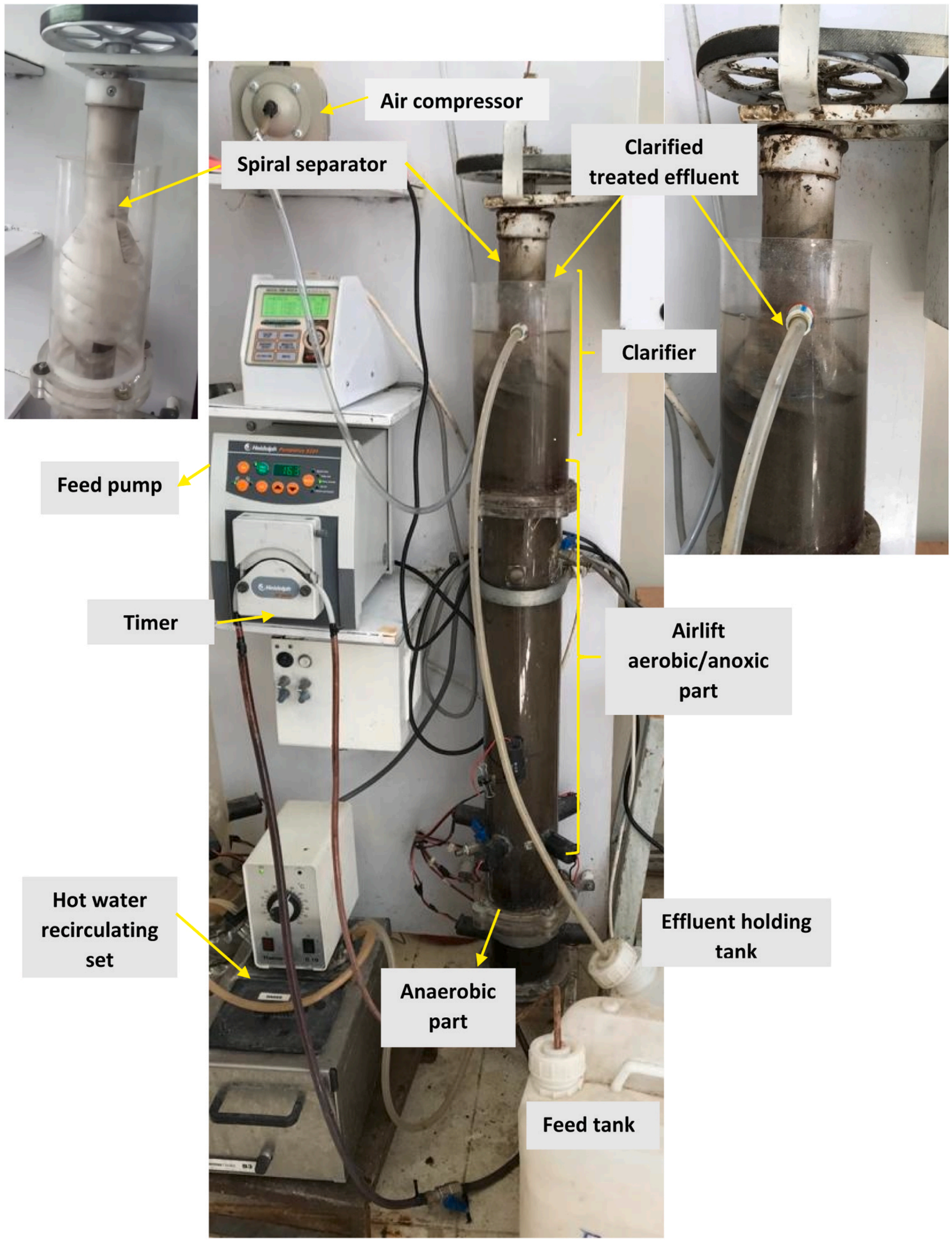


**Plate 1.** . Laboratory-scale experimental set up used in this study.

achieved at a relatively shorter HRT improving the SND nitrogen removal and effluent quality compared to conventional airlift bioreactors. In the final stage, the bioreactor was operated under optimum condition to examine the effect of MLSS concentration, feed distribution, influent P concentration, hybrid biomass, and wastewater characteristics on the CALBR performance.

### *2.4. Sampling and analytical methods*

Influent, mixed liquor, and effluent samples were withdrawn daily, filtered through a 0.45 μm pore size polyvinylidene (PVDF) membrane filter, and immediately analyzed following the procedures described in Standard Methods [53]. Five-day biological oxygen demand ($BOD_5$) tests were performed using a WWT OxiTOP IS manometric respirometric system (Wissenschaftlich-Technische-Werkstatten GmbH, Weilheim, Germany). The residual total and soluble chemical oxygen demand (tCOD and sCOD) were quantified by closed reflux colorimetric method. Colorimetric determination technique was used to calculate the concentration of Orthophosphate ($PO_4^{3-}$-P) and nitrite ($NO_2^-$-N) in the samples. The absorbance of COD, $NO_2^-$-N, and $PO_4^{3-}$P was measured at 600, 540, and 690 nm, respectively employing a visible (Model Jenway 6320D) spectrophotometer. The concentration of $NO_3^-$-N was determined by subtracting the absorbance at 275 nm from the absorbance at 220 nm for the samples and blanks (UV-Visible spectrophotometer, 8453, Agilent Technologies, United States). A TKN meter (vapodest10, C. Gerhardt GmbH & Co, Germany) was used to evaluate $NH4^{+}$-N and organic-N concentrations via a basic digestion and distillation method. pH was measured by a Sentek Glass electrode connected to a digital Jenway 3510 pH meter (Barloworld Scientific Ltd. T/As Jenway, Essex UK). Air flow rate in the raiser was controlled with a glass block style air flowmeter with flow controller (Model 101325 Pa). DO and ORP of the bulk liquid were monitored using portable DO meter (WTW DO Cell OX 330, Germany) and Sentix® pH/ORP meter (WTW pH 3110 SET 2, Germany) electrodes, respectively. Effluent turbidity measurement was carried out with a portable turbidimeter model 2100 P (Hach Co., USA). MLSS concentration and sludge volume index (SVI) values were determined on a daily basis to calculate the amount of biomass that needed to be discharged and assess the sludge settleability. In the case of tests with the focus on the biosolids concentration (MLSS and SVI) the sampling and analysis were conducted in duplicate to minimize the deviation induced by different biosolids content in the samples.

### *2.5. Experimental design*

Factorial experimental designs are widely used to improve the performance by minimizing the systematic errors through experimental errors estimation and optimizing the efficacy of multiple independent variables on the process [6]. In this report, version 11.1.1.0 of Design-Expert® (Stat-Ease Inc., Minneapolis, USA) using the central composite design (CCD) and the response surface methodology (RSM) was employed to minimize the number of trials, analyze the synchronous impacts of variables and data interdependency, and optimize the bioprocess performance. It is well documented that various parameters including configuration design, wastewater composition, and operational variables impact the performance of airlift bioreactors. Operating variables are easily manageable and can be adjusted and modified appropriately to fulfill the specific requirements. HRT, the ratio of the bioreactor volume to the influent flow rate, denotes the length of time that the biomass and substrate remain in the bioreactor. HRT is linked to the growth rates of different bacteria and the complexity of the organic compounds. Organic loading rate (OLR) is one of the most fundamental features of continuous flow bioreactors which increases at lower HRTs. The lower the HRT, the smaller the bioreactor size and the lower the capital cost. However, at short HRTs, the system may fail because a high content of active biomass is washed out from the bioreactor. Besides, the organic molecules may not be hydrolyzed and degraded effectively at a very low HRT. Therefore, it is essential to select an adequate and optimal HRT so that a high-quality effluent can be achieved at a minimized treatment period and space requirements. The overall performance of airlift reactors is affected by the fluid circulation velocity which crucially depends on the gas holdup. Gas holdup, as a major factor for the proper design and scale up, is profoundly influenced by the air injection rate or AFR. The influent C/N is another key parameter that may considerably control the SND process and influence the bioreactor performance in terms of TN removal [36]. Hence, HRT (8–16 h), AFR (2.5–4.5 $L_{air}$/min), and the influent C/N (4–6.7) were selected among the most critical factors which may affect the performance of the bioreactor in simultaneous COD and nitrogen removal. sCOD removal, TN removal, SVI, and effluent turbidity were monitored as the main process responses. The relation and combined effect of the three chosen independent variables, HRT (A), AFR (B), and C/N (C), was analyzed statistically using RSM with CCD. The experimental ranges of the variables at 3 corresponding levels with the coded values of −1, 0, and +1 are given in Table 2. 20 experimental runs (including 8 factorial points, 6 axial points, 1 center point, and 5 replications of the center point) with different combination of A, B, and C were carried out in the basis of factorial design: $N = 2^n + 2n + n_c$ (Table 3).

Where N, n, and nc denote the total number of trials, number of independent variables, and number of the center point replications, respectively.

**Table 2**
Operating variables with symbols and range and levels for CCD.

| Variables | Range and levels | | |
|---|---|---|---|
| | -1 | 0 | +1 |
| **HRT, h** | 8 | 12 | 16 |
| **AFR, $L_{air}$/min** | 2.5 | 3.5 | 4.5 |
| **C/N** | 4 | 5.35 | 6.7 |

## 3. Results and discussion

### *3.1. Statistical analysis*

The data obtained from the experimental runs was analyzed and fitted to a polynomial model (Eq. 1) to describe the bioprocess behavior and bioreactor performance through correlating the deign factors and responses.

$$Y = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_{ii} X_i^2 + \beta_{jj} X_j^2 + \beta_{ij} X_i X_j + \ldots \quad (1)$$

Where Y is the predicted response, and $X_i$ and $X_j$ are independent variables, and $\beta_0$ , $\beta_i$ and $\beta_j$, $\beta_{ii}$ and $\beta_{jj}$, and $\beta_{ij}$ represent the regression coefficients including the intercept, linear coefficients, quadratic coefficients, and interaction coefficient, respectively. The predicted models were evaluated applying the analysis of variance (ANOVA) based on Fisher's statistical test to interpret the relationship between the three independent variables and responses and estimate the impact and significance of each model term. The insignificant model terms were rejected and the ANOVA output for the modified models is summarized in Table 4. From the ANOVA results, all models were significant confirmed by very low values obtained for the probability (<0.0001, >F-value) [14]. The model P-values were greater than 0.05 (critical alpha value) indicating a statistically insignificant lack of fit between models and data. The regression coefficient ($R^2$) was in a satisfactory agreement with the adjusted $R^2$ value for all models authenticating that the predicted models fit well. The adequate precision parameter quantifies the signal-to-noise-ratio (SNR or S/N) by comparing the range of predicted values to the mean prediction error. Since, the desirable value for adequate precision is equal or greater than 4, the adequate precision values obtained in this study indicated the noise is negligible for all models. The relative standard deviation, namely coefficient of variation (CV), offers the relative comparison of data points variability and dispersion around the mean. In this investigation, the value of CV was low in all models (3.65–13%) suggesting that the deviations between the empirical and predicted data are low which provides additional support for the relatively high accuracy and reliability of the experimental results [35]. 3D surface plots were constructed to visualize the interactions among the independent variables at different levels and their impacts on the obtained responses.

### *3.2. The performance of CALBR*

#### *3.2.1. sCOD removal*

To reach an eco-friendly effluent, it is essential to effectively biodegrade and mineralize the organic constituents of milk processing wastewater (MPW). Thus, in this study, sCOD removal was selected as a response and monitored to assess the potential of bioreactor to treat MPW. Table 4 presents the ANOVA results for sCOD removal efficiency. Based on the Table, a reduced quadratic model was used to describe the sCOD removal efficiency variations in response to variables. As can be seen, A, B, AB, BC, and $C^2$ are the significant model terms affecting the sCOD removal efficiency. The interactive effect of A (HRT) and B (AFR) on sCOD removal is illustrated with three dimensions in Fig. 2. It is immediately obvious that the sCOD removal is more sensitive to HRT rather than AFR. From the Fig., during all operational conditions, the bioreactor demonstrated a remarkable ability in sCOD removal (more than 80%). The high sCOD removal efficiency is chiefly due to the high $BOD_5$ to COD ratio (0.8) of milk processing wastewater [44]. Another plausible reason for the high sCOD removal even at the minimum HRT is the specific characteristics of airlift configuration improving the oxygen transfer performance. Considering the amount of tCOD and sCOD presented in Table 1, readily biodegradable or soluble COD (sCOD) and slowly biodegradable or hydrolysable COD represent around 83.33% and 16.67% of tCOD. The lowest value for COD removal was obtained at the minimum HRT which can be attributed to the presence of slowly

**Table 3**
Experimental conditions and the obtained results.

| | | Variables | Responses | | | | |
|---|---|---|---|---|---|---|---|
| Run | Factor 1 A: HRT (h) | Factor 2 B: AFR (Lair/min) | Factor 3 C: C/N | sCOD removal (%) | TN removal (%) | SVI (mL/g) | Effluent turbidity (NTU) |
| **1** | 8 | 3.5 | 5.35 | 100 | 47.67 | 93 | 11 |
| **2** | 12 | 3.5 | 5.35 | 98.43 | 84.45 | 72 | 7 |
| **3** | 12 | 2.5 | 5.35 | 91.76 | 35.4 | 76 | 8 |
| **4** | 16 | 4.5 | 4 | 95.53 | 85.03 | 38 | 4 |
| **5** | 16 | 4.5 | 6.7 | 100 | 77.85 | 37 | 3 |
| **6** | 8 | 2.5 | 4 | 81.95 | 46.99 | 95 | 12 |
| **7** | 8 | 4.5 | 4 | 86.73 | 73.8 | 89 | 9 |
| **8** | 12 | 3.5 | 5.35 | 99.5 | 82.5 | 73 | 9 |
| **9** | 12 | 3.5 | 5.35 | 96.3 | 77.95 | 68 | 8 |
| **10** | 12 | 3.5 | 6.7 | 95.18 | 87.5 | 66 | 6 |
| **11** | 8 | 2.5 | 6.7 | 74 | 47.67 | 92 | 10 |
| **12** | 12 | 3.5 | 5.35 | 98.3 | 78.4 | 68.7 | 7 |
| **13** | | | | | | | |
| **14** | | | | | | | |
| **15** | | | | | | | |
| **16** | | | | | | | |
| **17** | | | | | | | |
| **18** | | | | | | | |
| **19** | | | | | | | |
| **20** | 8 | | | | | | |
| 16 | | | | | | | |
| 16 | | | | | | | |
| 12 | | | | | | | |
| 12 | | | | | | | |
| 16 | | | | | | | |
| 12 | | | | | | | |
| 12 | 4.5 | | | | | | |
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| 2.5 | | | | | | | |
| 3.5 | | | | | | | |
| 4.5 | | | | | | | |
| 3.5 | | | | | | | |
| 3.5 | | | | | | | |
| 3.5 | 6.7 | | | | | | |
| 6.7 | | | | | | | |
| 4 | | | | | | | |
| 5.35 | | | | | | | |
| 5.35 | | | | | | | |
| 5.35 | | | | | | | |
| 5.35 | | | | | | | |
| 4 | 98.44 | | | | | | |
| 99.82 | | | | | | | |

(*continued on next page*)

**Table 3** (*continued*)

| | | Variables | Responses | | | | |
|---|---|---|---|---|---|---|---|
| Run | Factor 1 A: HRT (h) | Factor 2 B: AFR (Lair/min) | Factor 3 C: C/N | sCOD removal (%) | TN removal (%) | SVI (mL/g) | Effluent turbidity (NTU) |
| 100 | | | | | | | |
| 94.7 | | | | | | | |
| 97.82 | | | | | | | |
| 100 | | | | | | | |
| 93.7 | | | | | | | |
| 97.6 | 84.17 | | | | | | |
| 79.58 | | | | | | | |
| 68.1 | | | | | | | |
| 80.5 | | | | | | | |
| 75.36 | | | | | | | |
| 76.26 | | | | | | | |
| 70.91 | | | | | | | |
| 76.6 | 87 | | | | | | |
| 43 | | | | | | | |
| 44 | | | | | | | |
| 71 | | | | | | | |
| 59 | | | | | | | |
| 40 | | | | | | | |
| 74 | | | | | | | |
| 67 | 8 | | | | | | |
| 6 | | | | | | | |
| 5 | | | | | | | |
| 9 | | | | | | | |
| 6 | | | | | | | |
| 3 | | | | | | | |
| 8 | | | | | | | |
| 7 | | | | | | | |

**Table 4**
ANOVA results for the regression equations obtained for the studied responses.

| Response | Modified equations with significant terms | Probability | $R^2$ | Adj. $R^2$ | Adeq. Pricesion | S.D | C.V. % | PRESS | Probability for lack of fit |
|---|---|---|---|---|---|---|---|---|---|
| **COD Removal** | 97.37+5.42 A+ 3.10 B-4.19 AB+ 3.04 BC-4.77 $B^2$ | <0.0001 | 0.8097 | 0.7418 | 13.6416 | 3.47 | 3.65 | 433.00 | 0.1205 |
| **TN removal** | 74.05 + 8.65 A + 11.58 B - 6.01 AB – 15.55 $B^2$ + 11.12 $C^2$ | <0.0001 | 0.7546 | 0.6669 | 8.8631 | 8.66 | 12.06 | 2123.45 | 0.0515 |
| **Effluent turbidity** | 7.30–2.90 A - 1.10 B – 0.40 | <0.0001 | 0.8717 | 0.8476 | 20.7418 | 0.9487 | 13.00 | 21.32 | 0.4551 |

biodegradable organic matter such as fats and proteins in dairy effluents [45]. As was foreseeable, increasing in HRT from 8 to 16 h (in accordance with (anaerobic HRTs of 1.05–2.11 h) caused an increase in the response. One reasonable conclusion might be the biodegradation of carbohydrates, fats, and proteins via hydrolysis and acidification under mesophilic conditions in the anaerobic zone. Convincing evidence from some studies has shown that the anaerobic biodegradation and hydrolysis of proteins in dairy effluents speeds up the overall biological treatment [57,60].

The average values for pH and ORP in the anaerobic zone were in the

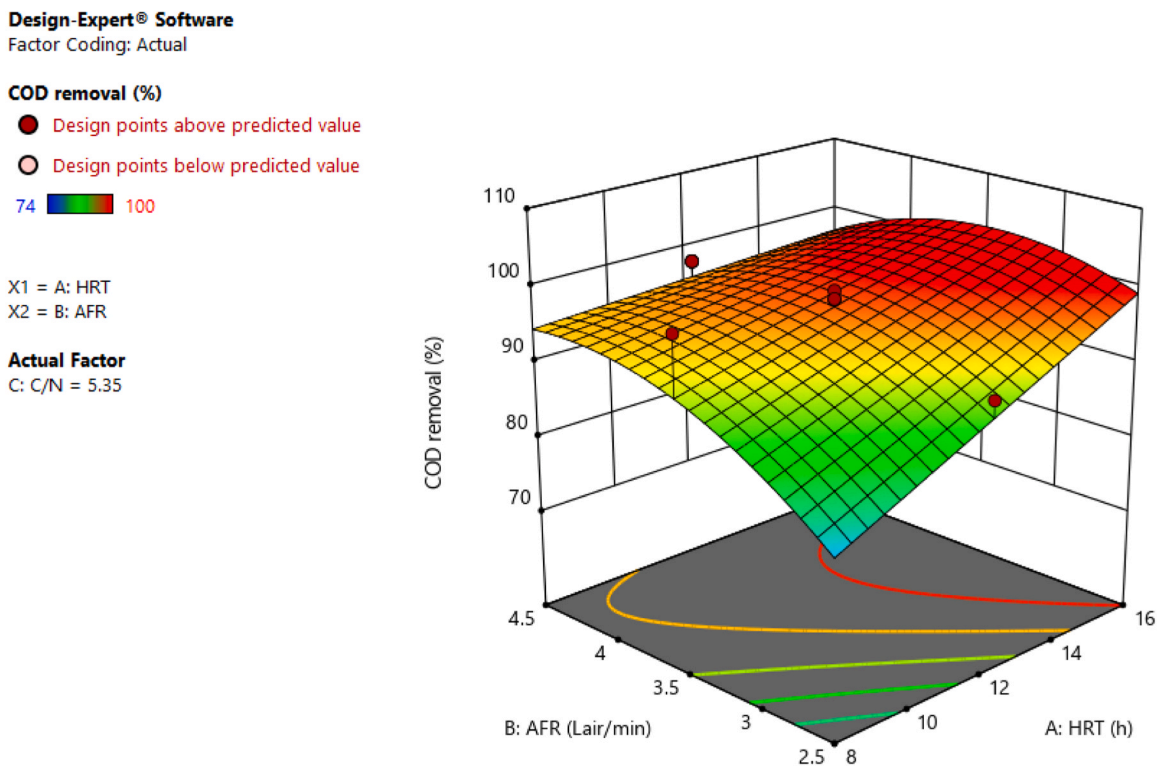


**Fig. 2.** 3D response surface plot for COD removal efficiency as a function of HRT and AFR at constant C/N (5.35).

ranges of 5.93–6.5, and −180 to −446 mv, respectively. These results are consistent with the desirable pH and ORP ranges reported for hydrolysis and acidification [18,37,58], reflecting the importance of higher hydrolysis and acidification time (higher anaerobic HRT) as a contributory factor in sCOD removal.

As seen in Fig. 2, AFR also indicated an increase in the response. However, a declining trend was observed in sCOD removal at the maximum HRT (16 h) with increasing AFR from 3.5 to 4.5 $L_{air}$/min. This could be due to the soluble microbial products (SMPs) synthesis and excretion caused by high oxidation potential at the highest airflow rates and lowest organic loading rates [27].

The values of COD removal rate were calculated and plotted at different experimental runs (Fig. 3). The results show that the COD removal rate increased (from 0.19 to 0.39 g COD removed/L.d) as the HRT was extended from 8 hours to 16 hours. This increase in COD removal rate with longer HRTs aligns with the abovementioned findings, where extended contact time between the organic pollutants and the microbial community in the bioreactor allows for more efficient degradation and removal of contaminants. The higher COD removal rates observed at longer HRTs can be attributed to enhanced biodegradation processes, such as hydrolysis and acidification, which are crucial for breaking down complex organic compounds into simpler forms that

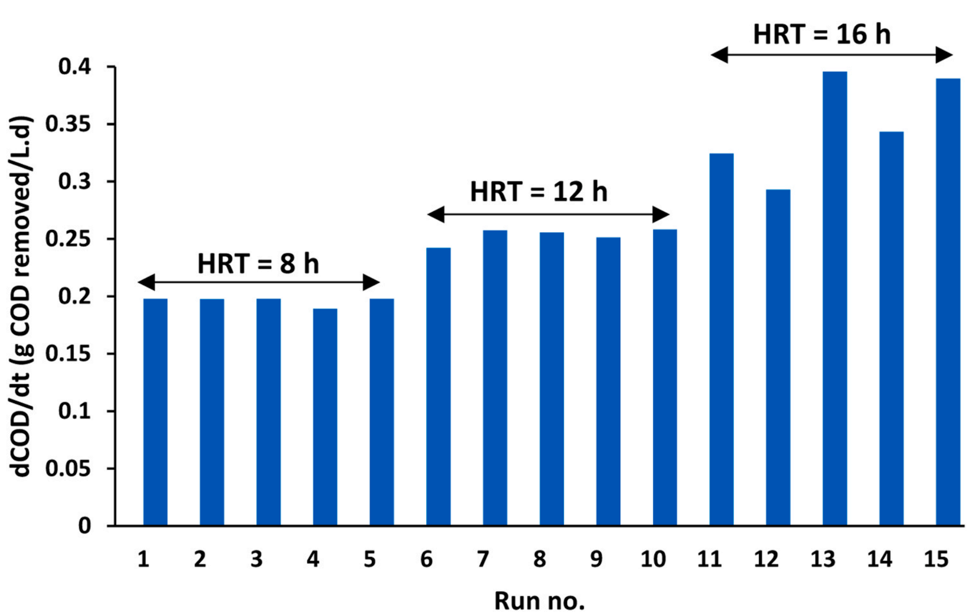


**Fig. 3.** COD removal rate at different experimental runs.

are more readily biodegradable. Furthermore, the significant impact of HRT on COD removal efficiency, as highlighted in your study, underscores the importance of optimizing operational parameters, such as HRT, to achieve optimal treatment performance.

Fig. 4 depicts the pH profiles at different stages within the bioreactor for all experimental runs. The pH values of the MPW flowed into the bioreactor slightly fluctuated between 7.53 and 7.73 resulting from minor alterations in the quality or quantity of influent. As the wastewater inflows to the anaerobic zone, pH level moves down to an average value of 6.2. The bioconversion of MPW lactose to lactic acid under anaerobic condition could be the probable explanation for this considerable drop in pH [15,55]. Following the pretreatment zone, the pH level was raised to 7.02–7.5 during the whole aerobic/anoxic phase in the reaction zone. Finally, effluent pH values ranged between 7.1 and 7.61 which is in good agreement with the acceptable pH range to freshwater ecosystems. The influential role of anaerobic zone in biodegradation is further supported by calculating carbonate and volatile fatty acid alkalinity under anaerobic and aerobic/anoxic phases (Fig. 5). In general, when comparing anaerobic phase to aerobic/anoxic phase, the alkalinity values observed are obviously lower for the anaerobic condition. This may be ascribed to the formation of VFAs and carbon dioxide in the first stage of anaerobic digestion (acid phase). Fig. 5 shows that VFAs as well as carbonate have incorporated in the alkalinity value in anaerobic phase. In contrast, under aerobic/anoxic condition, the carbonate alkalinity was the main contributor to the total alkalinity. The higher levels of VFA alkalinity in the anaerobic phase along with relatively notable variations between total alkalinity values at different stages may be attributable to the solubilization of organic matter and its subsequent VFAs formation reducing alkalinity. These data support the hypothesis that the integration of an anaerobic phase at the lower part of airlift bioreactor can facilitates the overall COD removal.

*3.2.2. TN removal*

TN removal efficiency varied between 35.4% and 87.5% at various combinations of interactive variables. According to Table 4, A, B, AB, $B^2$, and $C^2$ were the most significant model terms affecting TN removal efficiency in the bioreactor ($p < 0.005$). Both HRT and AFR had positive effects on TN removal, and their cross product had a negative impact on it, divulged by positive sign of A and B, and negative value of AB in the reduced quadratic model. The second-order effect of AFR ($B^2$) and C/N ($C^2$), demonstrated negative and positive effects on TN removal, respectively. Fig. 6a depicts the three-dimensional plot of TN removal efficiency as a function of HRT and AFR at C/N value of 5.35. As shown, the TN removal in the bioreactor had a similar trend at different C/N ratios. Obviously, TN removal was more sensitive to AFR values than those of HRT and with the increasing AFR from 2.5 to 4.5, the removal efficiency was markedly improved. Since the higher AFR increases the oxygen retention and provides higher DO concentration in the riser, the nitrification rate is expected to increase. Increasing OLR restricts nitrification rate [49] indicating inhibited nitrification at lower HRTs. As in the case of HRT of 8 h, when AFR increased from 2.5 to 4.5, the average nitrification rate and TN removal efficiency were increased from 0.067 to 0.084 g $NH_4^+$-N/L.d and 55.5–47.33–78.98%, respectively. Thus, the positive effect of increasing AFR on the nitrification rate and TN removal at the lowest HRT (highest OLR) lend a strong support to the restricted nitrification. TN removal efficiency was increased as the HRT was increased from 8 to 16 h (corresponding to OLRs of 0.39–0.19 gCOD/L.d). On the one hand, these results point to the enhanced nitrification process at lower OLRs (higher HRTs). Although, on the other hand, it is generally believed that carbon source deficiency at higher HRTs inhibits denitrification process and decreases the nitrogen removal efficiency through SND. Regarding the HRT values of 8, 12, and 16 h, denitrification rates of 0.057, 0.053, and 0.045 gN/L.d were obtained, respectively. Nevertheless, the increasing trend of TN removal efficiency observed here could be an indication of proper and well-adjusted aerobic and anoxic phases in the airlift design boosting the SND process. As ANOVA results put in, AB (HRT-AFR) is the only significant 2-level interactive term with a decreasing impact on the response. The combined effect of HRT and AFR at C/N of 5.35 is visualized in the interaction plots (Fig. 6b-c). The non-parallel lines and the cross-over interaction reveal the existence of a significant interaction between A and B which conform to ANOVA data. AFR and HRT were found to cause higher removal efficiency at the high level. The relation between TN removal efficiency and increasing HRT was slightly curved at a fixed level of AFR (Fig. 6b), while TN removal grew with a pronounced curvilinear form by increasing AFR at a fixed level of HRT (Fig. 6c). These results also agree with the TN removal findings discussed above. From Fig. 6a, it is apparent that increasing AFR from 2.5 to 4.5 $L_{air}$/min increased TN removal efficiency even at the lower coded hydraulic retention time (A:-1), which was 8 h, by more than twofold (from 32% to 68%). This proves that higher oxygen retention may compensate for the negative impact of high OLR on TN removal. In order to divulge the mechanisms by which nitrogen is removed, the amount of nitrogen removed through assimilation (cell synthesis) and denitrification process was measured at different modes of operation.

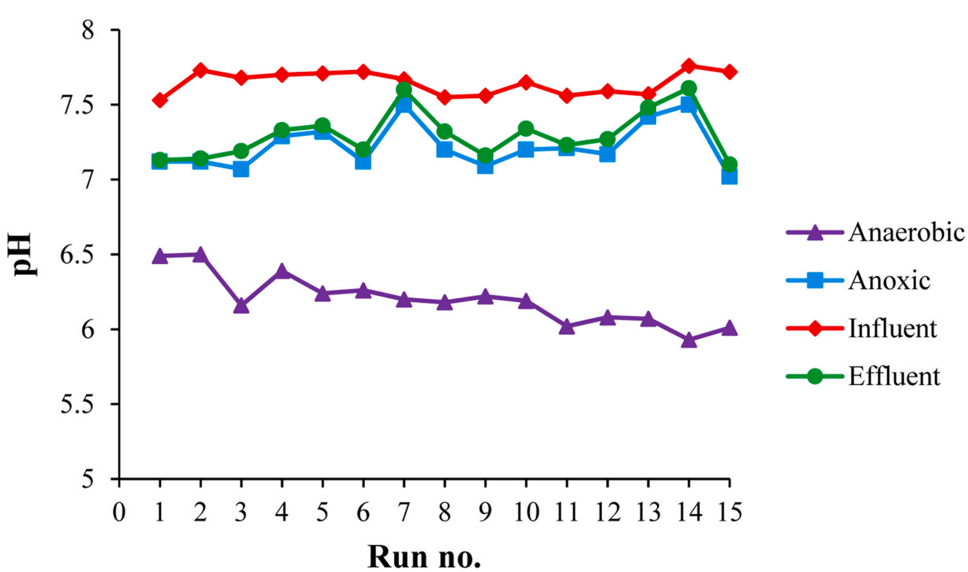


**Fig. 4.** pH profiles at different stages within the bioreactor.

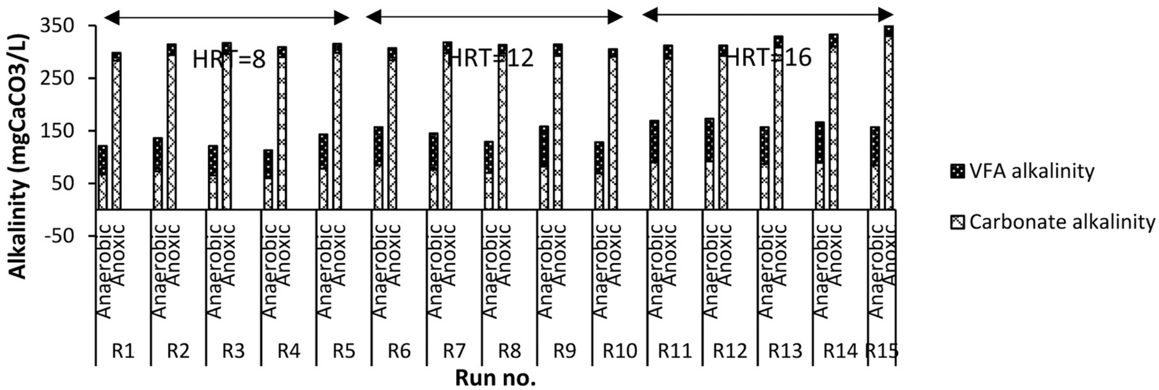


**Fig. 5.** VFA and carbonate alkalinity at different experimental runs.

Fig. 7 displays the TN removal rate (dTN/dt) at different experimental runs. As it is obvious, TN removal rates showed a clear trend of increasing with higher AFR values, especially evident at longer HRTs. The data indicates that increasing AFR from 2.5 to 4.5 $L_{air}$/min led to a marked improvement in TN removal rate, where the highest value (0.063 g TN removed/L.d) and lowest value (0.028 g TN removed/L.d) of TN removal rate obtained at AFR values of 4.5 $L_{air}$/min (HRT of 16 h) and 2.5 $L_{air}$/min (HRT of 8 h), respectively. This observation highlights the positive impact of higher oxygen retention and increased DO concentration on nitrification rates. The results also demonstrate the importance of balancing AFR and HRT to optimize TN removal, with higher AFR values showing a more significant effect on TN removal compared to HRT values. Additionally, the observed trend in TN removal is in accordance with previous findings regarding the relationship between AFR, HRT, and TN removal rates, further supporting the validity of the abovementioned results. Furthermore, the comparison of TN removal rates at different AFR values and HRTs reveals the dynamic nature of nitrogen removal processes in the bioreactor. The data exhibited that TN removal rate increases as HRT is extended from 8 to 16 hours, indicating enhanced nitrification processes at lower OLRs (higher HRTs). The significant contribution of denitrification to TN removal, even at high HRTs, suggests the effectiveness of the SND process in nitrogen removal. By presenting a comprehensive analysis of TN removal efficiencies and mechanisms, along with the impact of AFR and HRT on nitrogen removal efficiency, this study provides a solid foundation for understanding and optimizing nitrogen removal in airlift bioreactor systems. These findings not only contribute to the existing knowledge in the field but also offer valuable insights for improving the design and operation of bioreactors for enhanced TN removal.

The amount of nitrogen removal by each mechanism together with the effluent nitrate concentration are shown in Fig. 8. On balance, more nitrogen content was assimilated into biomass at lower values of HRT (8 and 12 h) in comparison to the maximum HRT (16 h). Conversely, increasing HRT from 8 to 16 h at AFR of 2.5 $L_{air}$/min was accompanied by a sharp rise in TN removal from 117.41 to 156.44 mg/L through denitrification process (run no: 2,14). Since the results closely match the TN removal data, SND proved to be the dominant mechanism accounting for nitrogen removal in the bioreactor. The significant contribution of denitrification to TN removal even at high HRTs may prompt speculation that the solubilization of slowly biodegradable fraction of influent COD under anaerobic condition might dispense a sufficient carbon source for denitrification. The nitrate concentration in the effluent ranged from 0 to 7.2 mg/L providing conclusive support for a properly balanced and efficient anoxic condition established in the downcomer by virtue of specific geometry of airlift bioreactor. Moreover, coupling settling process with the biological reaction may have contributed to the enhancement of TN removal through prolonging SRT which benefits the slow growing nitrifiers.[66] TN in the influent is mainly composed of ammonia and organic nitrogen (Table 1). The ammonification of organic nitrogen takes place under both anaerobic and aerobic conditions; however, this process proceeds rapidly in the latter case [29]. According to Fig. 8., the concentration of organic nitrogen in the effluent was decreased considerably with increasing HRT from 8 to 16 h. The extended contact time between air and mixed liquor, created by effective continuous aeration in the airlift structure, may be the possible explanation for the reduction in the organic nitrogen content through microbial ammonification and its further oxidation to nitrate. This finding is in line with the upward trend observed in the effluent nitrate concentration.

### 3.2.3. *Effluent turbidity*

Effluent turbidity originates from suspended mineral and organic particles and may cause environmental damage in terms of both saftey and aesthetic. Turbidity is monitored in real-time and therefore can be employed as an alternative indicator for time-consuming TSS measurement. The turbidity of the treated effluent was measured during different experimental runs to evaluate the clarification performance of the bioreactor. As shown in Table 4, only terms A and B had significant decreasing effects on the effluent turbidity. The impact intensity of these two variables on the response was HRT >AFR in order. Fig. 9 depicts the 3D response surface plot to elucidate the variation in the tendency of the effluent turbidity at different HRTs and AFRs and C/N of 5.35. Effluent turbidity ranged from 3 to 12 NTU at different operational conditions (Table 3). From the data provided, it is quite clear that effluent turbidity was decreased drastically with the increase of HRT from 8 to 16 h corresponding to OLRs of 0.39–0.19 gCOD/L.d, respectively. The deteriorating effect of low HRT (high OLR) on the effluent turbidity might be due to the fact that the contact time between microbial community and the substrate is not sufficient enough which expose the active biomass to a great shock. DO concentration plays an important role in controlling the effluent quality [61]. High DO concentration is found to promote the formation of more stable and denser flocs in the airlift bioreactor [8]. DO concentration increased in the bioreactor with raising AFR, as expected. As shown in the figure, AFR demonstrated a decreasing effect on the effluent turbidity. It is reported that sludge flocs are more prone to disintegration at high AFRs resulting in the excretion of microbial coloids and solutes to the bulk liquid and a more turbid effluent [42]. This is contrastive to the findings of this study, where the lowest level of effluent turbidity (3 NTU) was achieved at the condition with the maximum HRT and AFR of 16 h 4.5 $L_{air}$/min, respectively. The high homogenous turbulence flow regime provided by airlift configuration is the most likely explanation for the formation of denser flocs with good settling properties at higher AFRs.

### 3.2.4. *Sludge characteristics*

Most of the settling problems in activated sludge systems stem from sludge bulking phenomenon induced by either overgrowth of filamentous bacteria (filamentous bulking) or excessive production of EPS

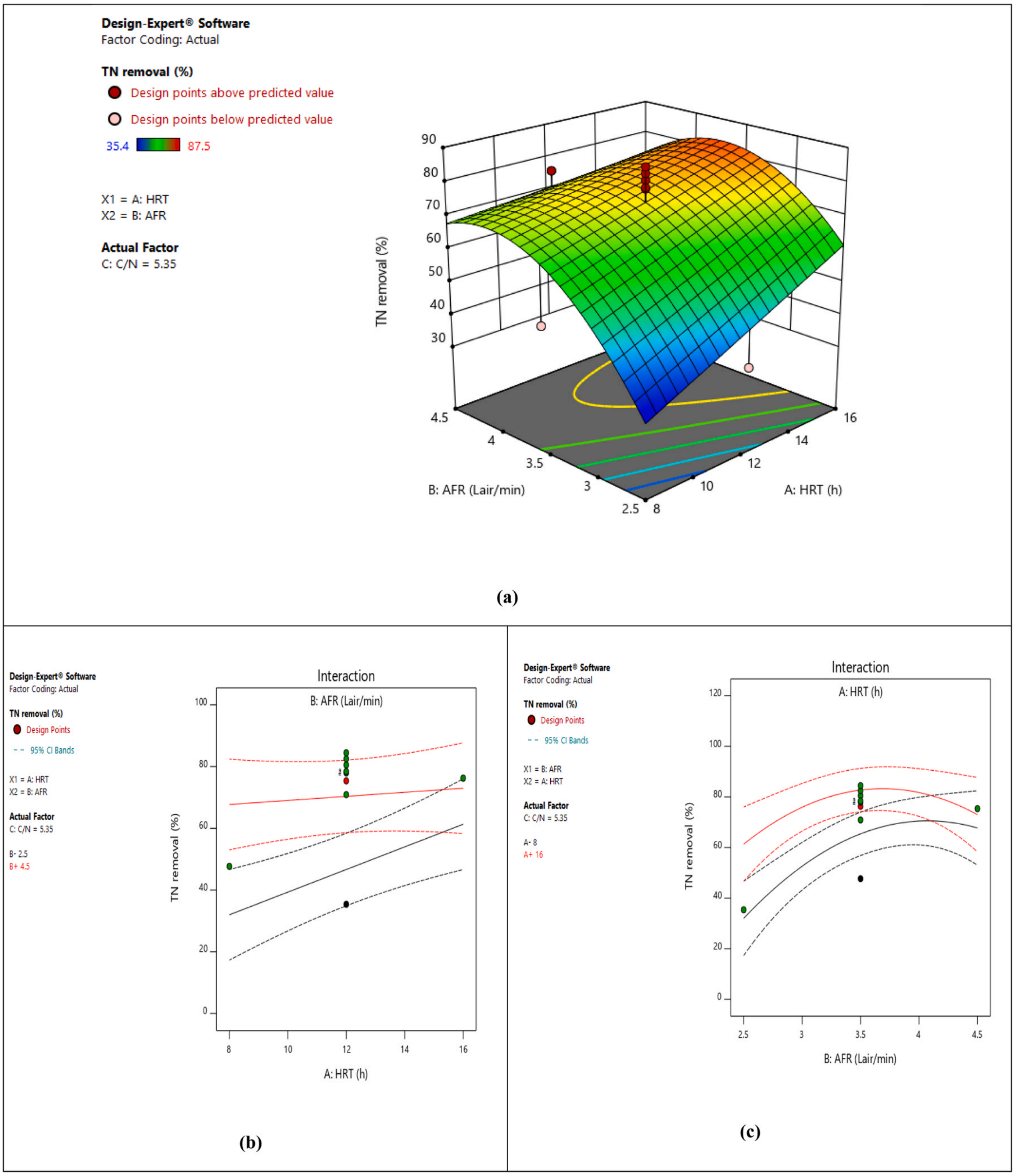


**Fig. 6.** . 3D response surface plot for TN removal efficiency as a function of HRT and AFR at constant C/N (5.35) (a), and interactive effect of AVR and HRT on the response (b-c).

(viscous bulking) [48]. SVI is frequently used to evaluate sludge bulking and quantify settling characteristics of biomass in the mixed liquor [23]. Therefore, $SVI_{30}$ values were monitored at different operating conditions to assess the settleability of suspended biomass in the bioreactor. According to Fig. 10, increasing HRT from 8 to 16 led to a noticeable decrease in SVI representing a distinct improvement in sludge settleability. The high amount of EPS produced at higher OLRs (higher F/M ratios) is probably responsible for viscous bulking and poor sludge settling at low HRTs [50]. There is a close relationship between low AFR and low DO concentration. It is claimed that low DO concentration develops excessive growth of filamentous bacteria and deteriorate SVI and flocs settling [24]. In the current study, the highest SVI (95 mL/g) and the lowest SVI (37 mL/g) were observed at the AFR of 2.5 $L_{air}$/min (with HRT of 8 h) and 4.5 $L_{air}$/min (with HRT of 16 h), respectively. These results indicate that the limited oxygen supply at lower AFRs may provide favorable condition for domination of filamentous bacteria, and that the effect is more pronounced at higher OLRs.

Asadi et al. reported that anoxic phase is restricted as a result of transition from CFID to continuous flow regime in the airlift bioreactor causing balance disruption in microbial diversity [9]. This is presumably because of heterotrophic bacteria outcompetition by faster growing filamentous bacteria [28]. However, from the figure, SVI values were lower than 100 mL/g at all experimental runs which demonstrates the activated sludge in the bioreactor falls into well-settling sludge category (SVI < 100 mL/g) [20]. This underlines the contribution of the compact gravity settling devise to the compact and thick sludge with improved settling characteristics. In contrast to the conventional secondary clarifiers with fixed inclined plates, the spiral separator proposed in this

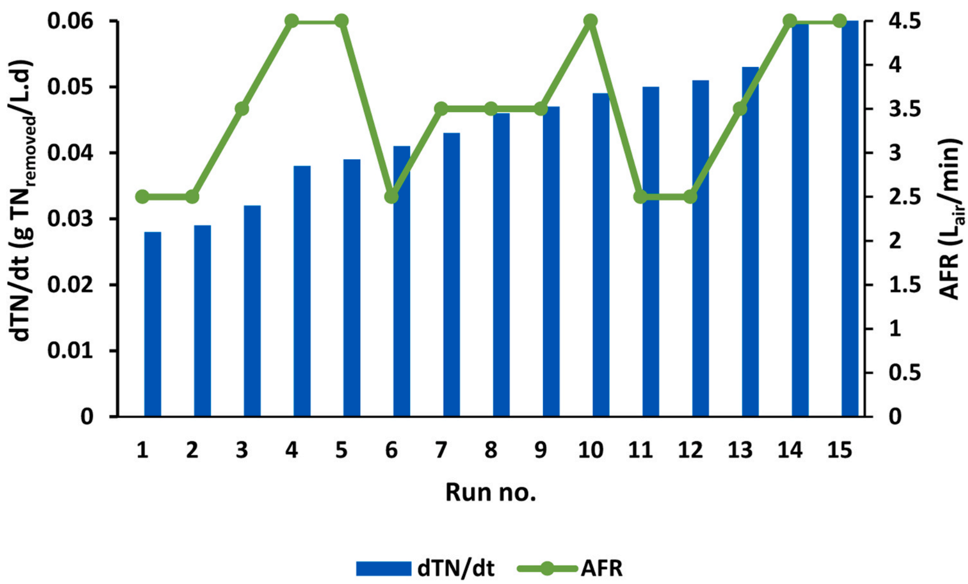


**Fig. 7.** TN removal rate at different experimental runs.

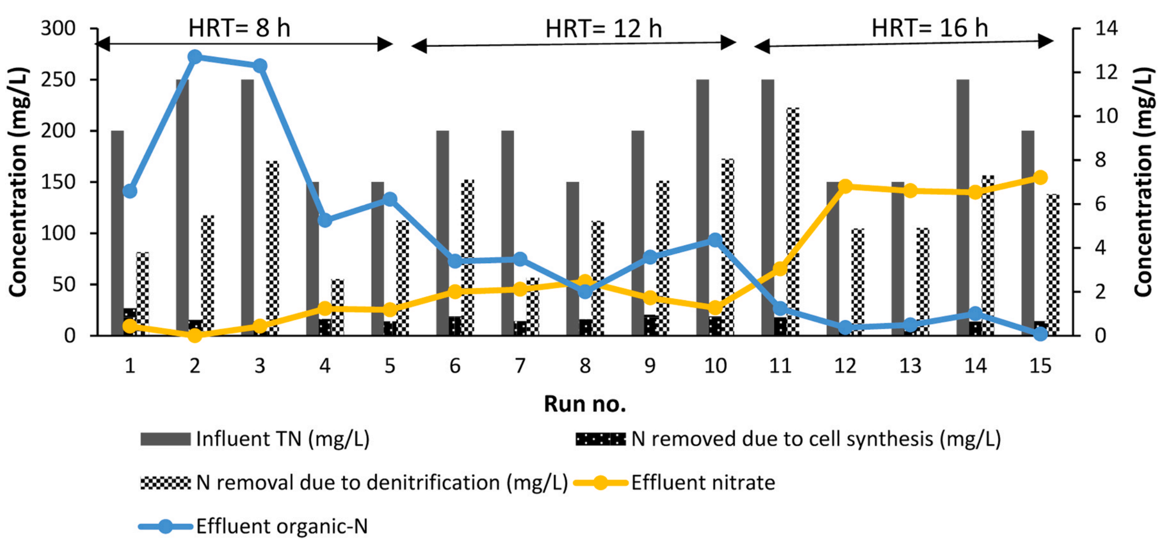


**Fig. 8.** The nitrogen contents and contribution of different reactions to TN removal under different operating conditions.

study exerts a refreshing effect on the sludge due to its rotation. This distinctive feature minimizes the probability of sludge rising phenomenon by preventing bubble formation and consequent uncontrolled sludge scape in certain conditions. In fact, gas bubbles are detached from the biosolids and avoid sludge rising thanks to the rotation. To draw a more detailed comparison of sludge settling behavior, the variation in supernatant/sludge interface height with time was monitored at different experimental runs. Fig. 11 presents the profile of sludge height versus time for the runs with the lowest (a) and highest (b) SVI values. As can be seen, the downward trend was relatively linear for the condition with the highest SVI and the interface height was decreased from 17.4 to 14.4 cm after 60 min resulting in a settling velocity of 0.032 m/h. Whereas, the interface height for the mixed liquor with the lowest SVI underwent an exponential decay with time and was reduced more rapidly from 17.4 to 4.6 cm after 60 min representing a sludge settling velocity of 0.128 m/h. These findings correlate favorably with the SVI and turbidity data discussed earlier. The average settling velocity of the system was 0.13 m/h. Therefore, it can be concluded that the bioreactor operation at a properly adjusted HRT and AFR value will positively affect the sludge settling properties and enhance the system clarification performance.

Most industrial sectors are often only equipped with conventional wastewater treatment plants. Residues from treated effluents and sludge from conventional processes (activated sludge) and anaerobic digestion processes can still pose environmental hazards, and the incapacity of anaerobic processes to remove nitrogen and their extensive retention times will remain as challenges to conventional biological systems for the efficient treatment of industrial wastewater. In addition, the settleability performance of the biological process is a key indicator of the efficient operation of the treatment plant. In most commonly used biological wastewater treatment processes an external settler is employed to allow a good biosolid-liquid separation and assure adequate effluent quality. This necessity of space-consuming settlers is a critical drawback for the practical and economic operation of the bioreactors in industrial sectors. Therefore, there is a need to provide a compact integrated biological system to remove organic carbonaceous and nutrients compounds simultaneously and produce a high-quality effluent in an environmentally friendly, simple, and cost-effective manner. This innovative research had its focus on combining different redox conditions (anaerobic/anoxic/aerobic) in a single bioreactor together with employing an

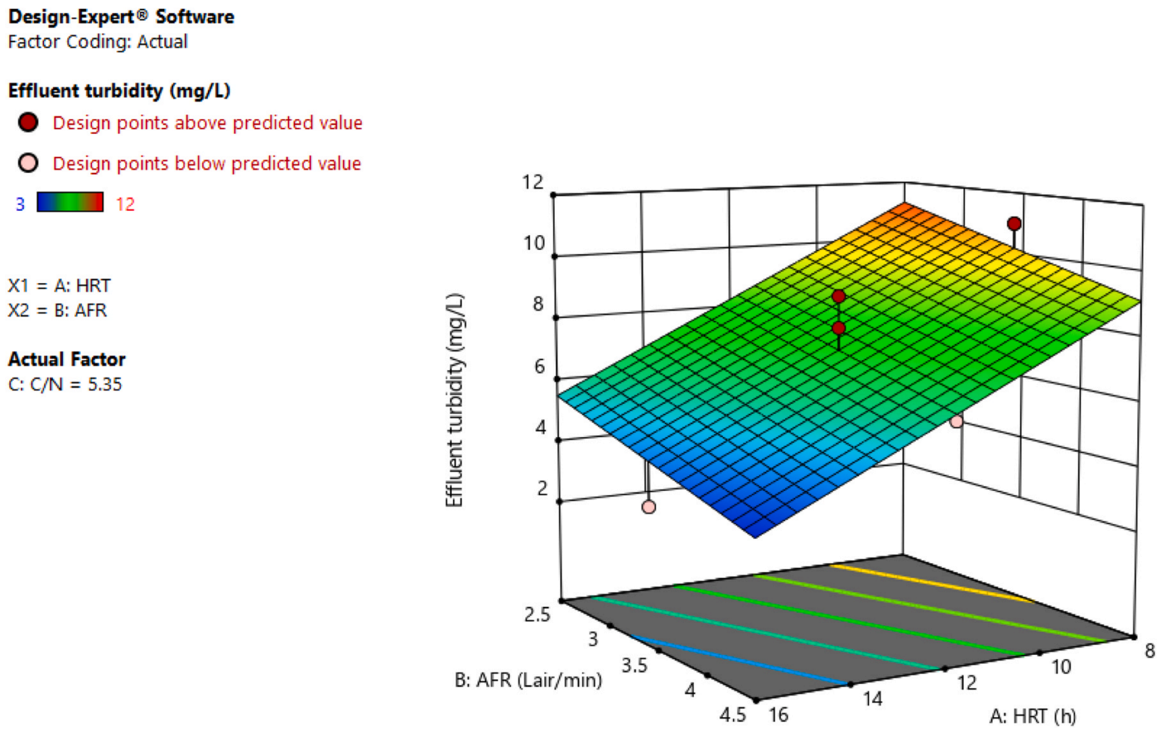


**Fig. 9.** . 3D response surface plot for effluent turbidity as a function of HRT and AFR at constant CN (5.35).

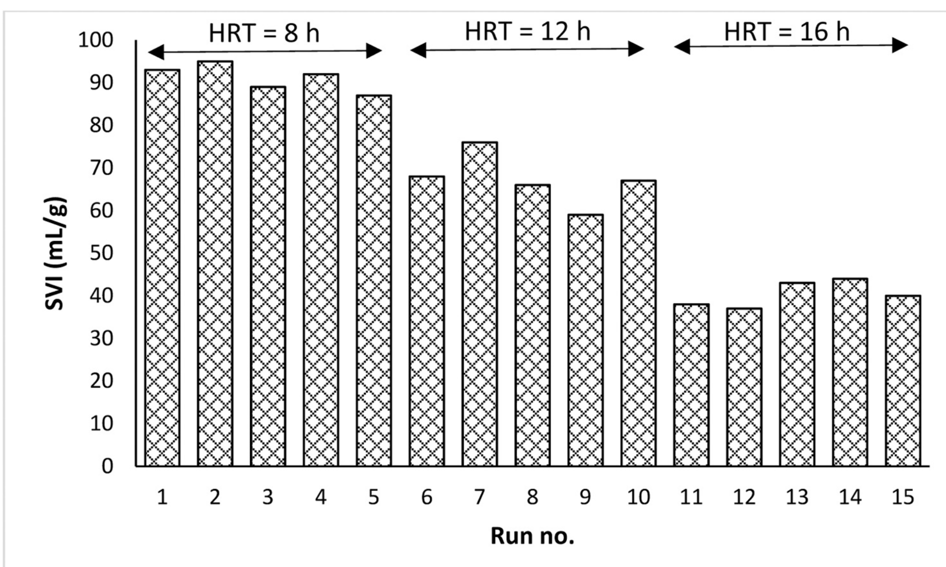


**Fig. 10.** Sludge volume index (SVI) under different operating conditions.

in-situ rotating spiral separator as a pragmatic solution to enhance biodegradation and the efficiency of total nitrogen removal from an industrial wastewater and result in economic benefits due to removing the need for secondary clarifier, keeping the SRT at a relatively long time, and lower construction costs. Therefore, a novel modified airlift bioreactor (CALBR) integrating biological treatment and sludge separation in a single structure was developed to treat MPW, which is one of the largest effluents amongst numerous industrial sectors wastewaters. As elaborately discussed in previous sections, the establishment of a hydraulic separation between anaerobic and aerobic/anoxic zones allows for concurrent solubilization of slowly biodegradable compounds and enhanced SND process. Furthermore, the results observed in the present section indicated that the upper coupled clarification zone prevents the escape of the active biomass, thus ensuring an adequate SRT to accomplish the SND as well as improving the effluent quality.

### 3.3. *Process optimization and verification*

When dealing with multiple responses, it is essential to discover regions where the outcomes concurrently meet the demanding quality. The optimal condition for independent operating variables, in which COD and TN removal are maximized and effluent turbidity is minimized, was determined using the optimization feature of RSM by Design-Expert® software (version 11). The contours of each main response are

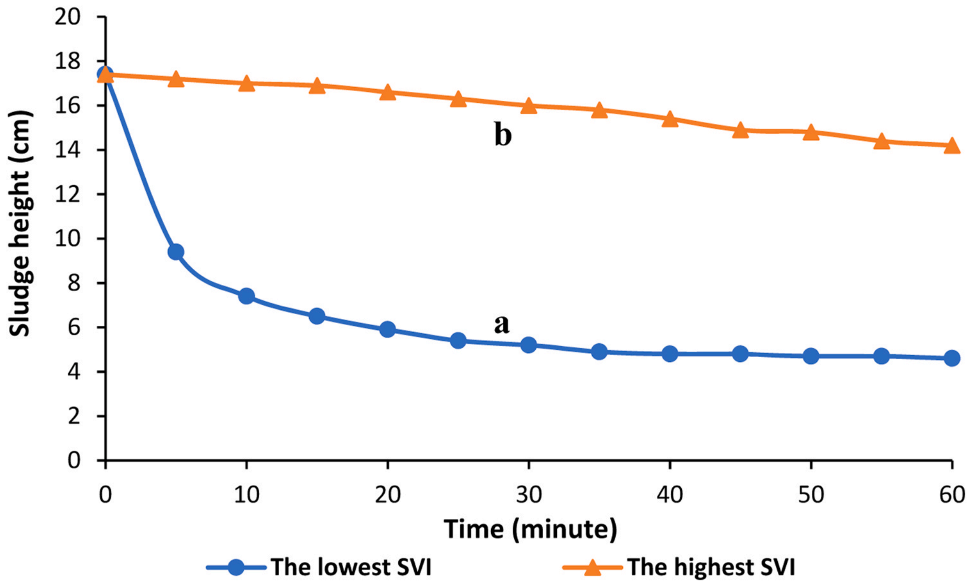


**Fig. 11.** The profile of sludge height versus time for the runs with the lowest (a) and highest (b) SVI values.

superimposed on one contour plot (overlay plot) to visualize the zone that matches the offered criteria. The overlay plot presented in Fig. 12 provides the graphical optimization of operating conditions at C/N of 3.5. The area which meets the proposed criteria (covering sCOD removal > 96%, TN removal > 75%, effluent turbidity < 10 NTU, and SVI < 90 mL/g) is yellow shaded, while the region that does not satisfy the criteria is gray shaded. According to the plot, the optimum region was identified at HRT values between 11.1 and 16 h and AFR values between 2.95 and 4.4 $L_{air}$/min. Three confirmation tests were performed at operating conditions selected randomly from the optimum region. The selected experimental conditions for HRT, AFR, and C/N, the actual experimental and predicted response values, and the standard deviation for each response are summarized in Table 5. The validation results indicated that there is a satisfactory agreement between the experimental and predicted data suggesting that the developed models are fairly accurate.

*Performance study under optimized operating condition*

*3.3.1. Effect of different MLSS concentrations on the bioreactor performance*

MLSS concentration is one of the critical control parameters in

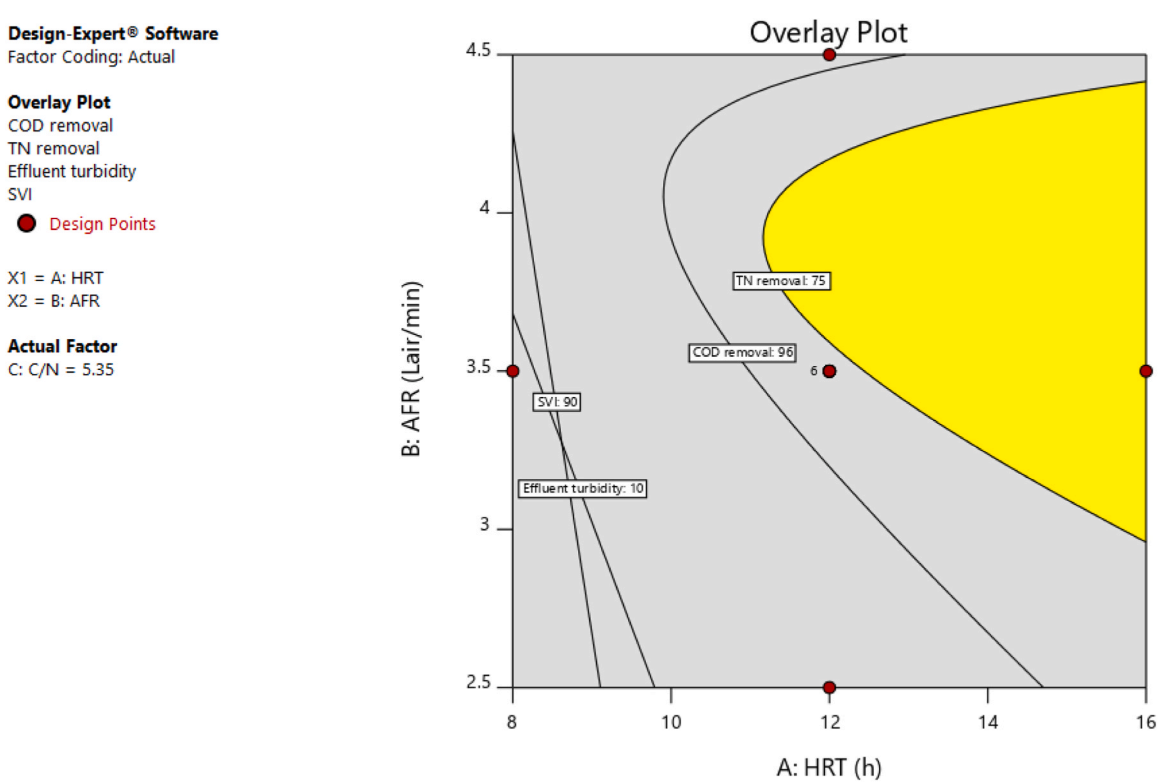


**Fig. 12.** Overlay plot for the optimal region.

**Table 5**
Verification experiment results at optimum operating conditions.

| Conditions | | Response | | | |
|---|---|---|---|---|---|
| | | COD removal (%) | TN removal (%) | Effluent turbidity (NTU) | SVI (mL/g) |
| HRT= 14 h | Experimental value | 100 | 79.83 | 8 | 57 |
| AFR = 3.5 $L_{air}$/min | Model values | 100 | 78.56 | 5.8 | 54.54 |
| C/N = 5.35 | Standard deviation | 0 | 0.89 | 1.55 | 1.74 |
| HRT= 15 h | Experimental value | 98 | 78.56 | 5 | 50 |
| AFR = 4 $L_{air}$/min | Model values | 100 | 80.35 | 4.5 | 46.4 |
| C/N = 5.35 | Standard deviation | 1.41 | 1.26 | 0.35 | 2.55 |

activated sludge treatment systems which has a direct impact on the treatment efficiency and effluent quality. Therefore, the performance of bioreactor under various MLSS concentrations (3000, 5000, and 7000 mg/L) was evaluated at a selected condition within the optimum region (HRT=14 h, AFR= 3.5, and C/N =5.35). The results for sCOD and TN removal, effluent turbidity, and SVI at different MLSS concentrations are compared in Fig. 13 a-d. From the Fig. 13a, the lowest sCOD removal efficiency was achieved in MLSS concentration of 3000 mg/L. Increasing MLSS concentration from 3000 to 5000 mg/L increased sCOD removal efficiency from 79.7% to 100%. It thus can be reasonably concluded that the insufficient amount of active microbial biomass within the bioreactor accounts for ineffective biodegradation of organic content at MLSS of 3000 mg/L. sCOD removal efficiency was reduced to 84% with a further increase in MLSS concentration from 5000 to 7000 mg/L. The observed decrease in sCOD removal might be attributed to the reduced microbial activity caused by low F/M ratio under high MLSS concentration. Fig. 13b showed that TN removal efficiency was poor at the lowest level of MLSS concentration indicating that active microorganisms were not present in sufficient amounts to perform nitrification and denitrification effectively. As expected, increasing the MLSS from 3000 to 5000 improved TN removal efficiency from 62.98% to 79.83%. With a further rise in the MLSS concentration from 5000 to 7000 mg/L, TN removal efficiency was decreased to 76.85. As is well known, the reduction in F/M ratio can restrict the carbon source accessibility to microorganisms in the anoxic phase [38,46]. However, the slight difference in TN removal efficiency values for MLSS concentrations of 5000 and 7000 mg/L observed here points to an acceptable balance between aerobic and anoxic conditions provided by airlift configuration. Fig. 13c and d represent changes in SVI and effluent turbidity at different MLSS concentrations under optimum condition, respectively. As shown, the highest SVI (95 mL/g) and effluent turbidity (23 NTU) values were observed at the lowest (3000 mg/L) and highest (7000 mg/L) MLSS concentrations corresponding to the maximum and minimum F/M ratios, respectively. It can be hypothesized that the

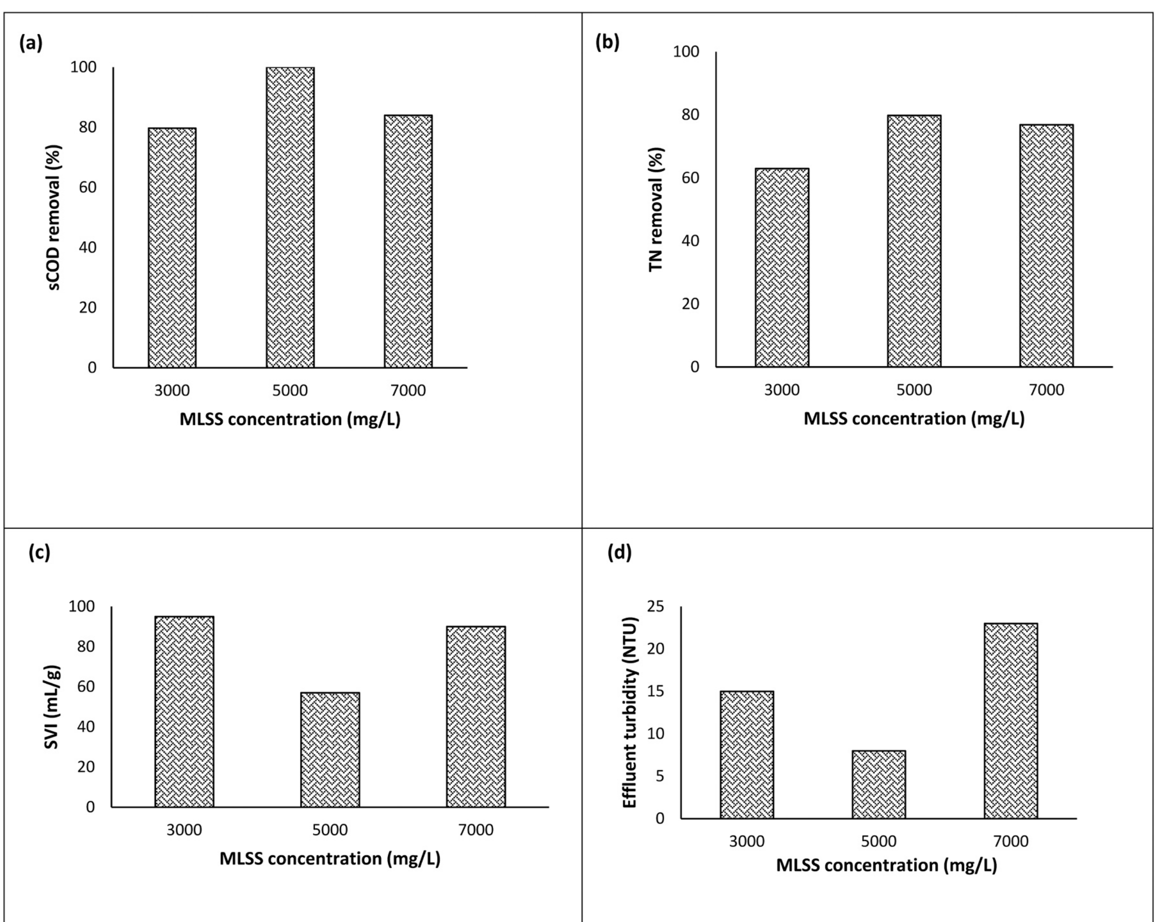


**Fig. 13.** Performance of the bioreactor at different MLSS concentrations, (a) sCOD removal efficiency, (b) TN removal efficiency, (c) SVI, and (d) effluent turbidity.

balance between bacterial growth and decay rates is negatively affected under both feast and famine conditions which results in flocculation of looser flocs and poor sludge settleability [17,21].

### 3.3.2. Effect of anaerobic feeding on the bioreactor performance

The bioreactor performance in terms of tCOD, TN, effluent nitrate, and effluent turbidity under different influent flow distribution ratios into anaerobic and anoxic zones is presented in Table 6. From the results, effluent turbidity was stable at all distribution ratios and did not exceed more than 9 NTU, while tCOD and TN removal efficiencies were decreased with increasing the portion of feed flow introduced into the anoxic zone. The complete degradation of tCOD at 100% anaerobic feeding demonstrated that employing anaerobic pre-treatment prior to the aerobic oxidation stage can be a potential solution to improve biodegradability and overall COD removal through hydrolysis of complex (slowly biodegradable) organic compounds. Feeding into the downcomer (anoxic phase) is expected to enhance SND process and overall nitrogen removal thanks to the presence of enough carbon source for denitrification. Contrary to expectations, introducing greater portions of feeding flow into the anoxic zone created a negative impact on TN removal efficiency. TN removal efficiency was declined from 79.83% to 58% by switching the feed distribution from fully anaerobic fed to fully anoxic fed mode. In contrast, the effluent nitrate concentration increased from 5.3 to 7.32 mg/L confirming a lower denitrification capacity in a fully anoxic fed system. The slowly biodegradable fraction of organics in the feed substrate, slowly biodegradable COD (sbCOD), must be effectively hydrolyzed to be further utilized as the denitrification carbon source [26]. Thus, it can be reasonably assumed that a considerable fraction of tCOD in the MPW is slowly biodegradable and the rate of denitrification process would be much lower when the bioreactor is forced to use sbCOD as carbon source. This observation indicates that for a wastewater containing a medium to high content of sbCOD, an anaerobic pre-treatment stage in which sbCOD is hydrolyzed and converted into readily biodegradable COD (rbCOD) can increase denitrification and improve TN removal efficiency.

### 3.3.3. Effect of different influent TP concentrations on the bioreactor performance

Since the raw MPW used in this study was P deficient, the average phosphate concentration in the influent was set on 10 mg/L at all experimental conditions to support microbial growth. The specific design of the bioreactor in the current study appears to provide required anaerobic and aerobic conditions in different zones for PAOs enrichment and proliferation. Therefore, in order to assess the capability of the bioreactor in P removal, the system was operated at optimum conditions (HRT = 14 h, AFR = 3.5 $L_{air}$/min, and C/N = 5.35) with different influent TP concentrations of 10, 20 and 30 mg/L. As it is observed in Table 7, sCOD removal efficiency was unaffected by the influent TP concentration and achieved 100%. Also, increasing TP concentration displayed no significant effect on the effluent nitrate concentration and TN removal efficiency. However, an increase in the influent TP concentration from 10 to 30 mg/L, exhibited a decrease in TP removal efficiency from 79.83% to 69.27%. The PAOs capacity for aerobic p-uptake depends on the amount of PHB synthesized and stored during anaerobic P-release. The higher the synthesized PHB quantity, the higher the aerobic P-uptake capacity [67]. Given that the average influent tCOD and rbCOD (VFAs) concentrations were constant, the amount of $PO_4^{3-}$-P released and PHB stored is expected to be stable. This points to a constant maximum P-uptake capacity under aerobic phase regardless of influent TP concentration. Taken together, the TP removal results would seem to suggest that at the highest C/P ratio (100), the amount of phosphate released was lower than the PAOs maximum P-uptake capacity due to the lower influent TP concentration (10 mg/L). Thus, the $PO_4^{3-}$-P released in the mesophilic anaerobic condition was taken up completely in the following aerobic condition. While, when C/P ratio was decreased to 50 and 33.33 (corresponding to influent TP concentrations of 20 and 30 mg/L, respectively), the higher amounts of phosphate released in the anaerobic zone might exceed the maximum P-uptake capacity of PAOs. Hence, the amount of PHB stored in PAOs may be insufficient to absorb all of the $PO_4^{3-}$-P content in the mixed liquor. Consequently, incomplete P-uptake could eventually lead to a reduction in the final TP removal efficiency. Although, overall, it is worth noting that more than 88% of the influent TP was removed under optimum condition for all initial TP concentrations. The effectiveness of bioreactor in TP removal can be attributed to a well-balanced and practical arrangement of anaerobic and aerobic phases. In fact, this result support the idea that the strict anaerobic bio-environment (rich in VFAs) at the bottom of the bioreactor and the subsequent aerobic condition in the riser favors P-release and P-uptake by PAOs, respectively in a single system.

### 3.3.4. The effect of hybrid biomass and wastewater characteristics on the bioreactor performance under optimum condition

Hybrid systems, combined suspended and attached growth biomass in a same reactor, can be considered as an advantageous alternative for the traditional wastewater treatment systems. Therefore, the effect of using a hybrid biomass on the CALBR performance was examined under optimum condition. Kaldnes carriers (K2) with a diameter of 15 mm and a specific surface area of 360 $m^2/m^3$ were fixed onto the outer surface of the draft tube occupying approximately 6% of the downcomer area. The packing media were soaked in a 1.2% w/v sodium alginate solution before being placed within the bioreactor. The bioreactor was operated in a batch mode for 4 weeks and no excess sludge was withdrawn during this period. At the end of the start-up period (30 days) for biofilm development, the MLSS concentration was set on 5000 mg/L and the bioreactor was operated in a continuous mode under optimum condition. Since the wastewater characteristics (substrate biodegradability and nitrogen content) plays a significant role in bioreactor performance, the potential of the hybrid CALBR for carbon and nitrogen removal from fresh composting leachate was also evaluated at the optimum condition. The characteristics of the fresh composting leachate is presented in Table 8. The obtained data for sCOD removal, TN removal, effluent turbidity, and SVI is shown in Fig. 14 a-d. As can be seen, influent sCOD was removed completely in both CALBR and hybrid growth CALBR fed with MPW (HCALBR (MPW)). However, 95% of the influent sCOD was removed in the case of hybrid growth CALBR fed with composting

**Table 6**
The bioreactor performance under different influent flow distribution ratios into anaerobic and anoxic zones at optimum condition.

| Distribution strategy | tCOD removal (%) | TN removal (%) | Effluent nitrate (mg/L) | Effluent turbidity (NTU) |
|---|---|---|---|---|
| **$Q_{anaerobic}$= 100% Qt; $Q_{anoxic}$= 0** | 100 | 79.83 | 5.3 | 8 |
| **$Q_{anaeroobic}$= 50% Qt; $Q_{anoxic}$= 50% Qt** | 95.68 | 63.16 | 6.53 | 9 |
| **$Q_{anaerobic}$ = 0; $Q_{anoxic}$= 100% Qt** | 90 | 58 | 7.32 | 9 |

**Table 7**
The bioreactor performance under different influent TP concentrations at optimum condition.

| TP concentration | sCOD removal (%) | TN removal (%) | TP removal (%) | Effluent Nitrate (mg/L) |
|---|---|---|---|---|
| **10 mg/L** | 100 | 79.83 | 100 | 5.3 |
| **20 mg/L** | 100 | 79.05 | 94.12 | 6 |
| **30 mg/L** | 100 | 79.27 | 88.88 | 5.9 |

**Table 8**
Characteristics of composting leachate (CL).

| Parameters | Unit | Amount |
|---|---|---|
| **sCOD** | mg/L | 1000–1200 |
| **$BOD_5$** | mg/L | 350–420 |
| **Org-N** | mg/L | 15–20 |
| **N-$NH_4^+$** | mg/L | 172–179 |
| **N-$NO_3^-$** | mg/L | 6–8 |
| TP | mg/L | 8.5–10 |
| pH | - | 5.13–5.6 |

leachate (HCALBR (CL)). The $BOD_5$/COD ratio of MPW and CL are 0.7 and 0.35 respectively, proving that the higher biodegradability of MPW is the foremost reason for the better COD removal efficiency. When feeding with the MPW, the amount of nitrogen removed in the HCALBR (170 mgN/L) was more than that of CALBR (159.66 mgN/L) (Fig. 14b). Higher TN removal efficiency for HCALBR might have resulted from the enhancement of both nitrification and denitrification processes. The combination of suspended biomass with low sludge age and the attached biofilm with higher sludge age in the HCALBR allows fast-growing heterotrophs (responsible for BOD removal) to proliferate mainly in suspension whilst the slow-growing autotrophic nitrifiers grow on the packing media. Thus, the nitrification process in the HCALBR is expected to performed at much shorter SRTs in comparison to CALBR which increases the overall nitrogen removal capacity of the system. Moreover, the oxygen concentration gradients within the attached biofilm provides an enhanced anoxic condition (in addition to the anoxic zone created by airlift configuration) which contributes to increase SND performance through improving denitrification process. TN removal efficiency dropped from 85% to 75.3% due to shifting the feed substrate from MPW to CL in the HCALBR. Better denitrification was expected for CL, given that the decomposition rate of humic and fulvic acid is slower than carbohydrates, fats, and proteins in MPW providing sufficient carbon source for efficient denitrification. However, the reason for the observed decrease in TN removal is probably the low BOD/TN ratio of CL (1.75) which falls below the required level (≥3) for efficient nitrogen removal by nitrification and denitrification [62]. The effluent turbidity was lower than 10 NTU for the CALBR and HCALBR and both wastewaters (Fig. 14d). For the MPW, the HCALBR had a better effluent quality (effluent turbidity of 5 NTU) than the CALBR (effluent turbidity of 8 NTY). In the HCALBR, the overall SRT is higher due to the increase in the total biomass concentration (MLSS + Biofilm). As a result of longer SRT, the bioreactor operates at a lower overall F/M ratio leading to a slower microbial growth rate and more EPS production [4]. This could hypothetically improve the bioflocculation and sludge settleability resulting in a lower effluent turbidity [9,19,34]. This assumption was verified by the SVI data which was 57 mg/L in the CALBR, while a considerably lower SVI (19 mL/g) was achieved in the HCALBR. According to Fig. 14d, the effluent turbidity in the HCALBR fed with CL (9 NTU) was higher than that of fed with MPW (5 NTU). Nevertheless, the SVI was decreased from 19 to 13.7 by shifting the influent from MPW to CL. Therefore, as the sludge showed an excellent settleability, the higher

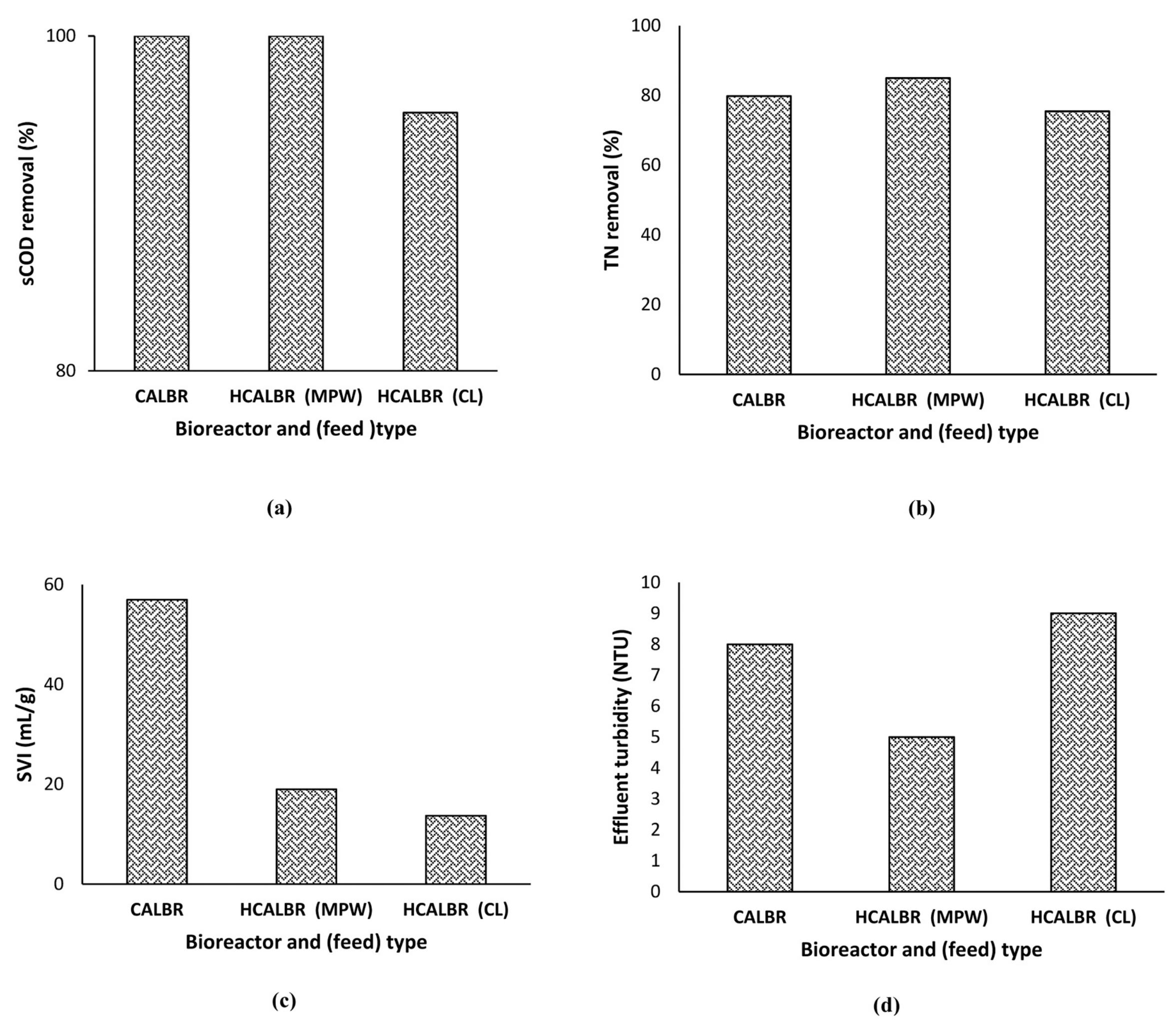


**Fig. 14.** Performance of the bioreactor with suspended and hybrid biomass fed by two different wastewaters; (a) sCOD removal efficiency, (b) TN removal efficiency, (c) SVI, and (d) effluent turbidity.

effluent turbidity in the CL-fed HCALBR can be ascribed to the residual slowly biodegradable (mainly particulate COD fraction) in the treated CL causing color and turbidity. Overall, the aforementioned results determined that HCALBR achieves similar or better performance compared to CALBR, where the combination of suspended and attached biomass improves the nitrogen removal efficiency and sludge settling characteristics. However, a longer anaerobic phase prior to the aerobic/anoxic phase is recommended for treating wastewaters with a low BOD/COD.

Overall, the innovative CALBR may serve as a feasible and reliable system in the field of biological wastewater treatment. The proposed technology offers significant advantages over conventional wastewater treatment systems such as high-performance and robust treatment system, consistent effluent quality, modular and flexible design, easy and rapid on-site implementation, eliminating the need for secondary clarifier, small footprint, and enhanced automation and continuous-flow process enabling simple and user-friendly operation. However, further research needs to be carried out in following areas to respond to challenges for practical application: (i) studying the performance of CALBR/HCALBR for enhanced TP removal, (ii) monitoring the long-term stability of the system, (iii) analyzing the microbial community and possible nitrogen removal pathways, (iv) investigating the effect of structural parameters including clarifier surface area, space between the plates, and the slope of inclination as well as proving economic and technical feasibility of the rotating spiral separator, and (v) evaluating the reliability, scalability and robustness of the proposed technology.

## 4. Conclusion

The operational data demonstrated that an average removal efficiency of 94.98% and 71.83% were successfully achieved for sCOD and TN, respectively, while maintaining a biomass with good settleability (with an average SVI of 67.63 mg/L) and low effluent turbidity (3–12 NTU). Different C/N ratios did not cause any noticeable effect on the TN removal efficiency denoting that the anaerobic biodegradation of organic substrate prior to aerobic/anoxic condition and enhanced suspended biomass retention within the bioreactor may improve the capability of airlift systems for SND. HRT and AFR were the main factors governing the bioprocess performance to achieve optimum operational conditions. The optimal region ranged from 11.1 h to 16 h for HRT and 2.95 $L_{air}$/min to 4.4 $L_{air}$/min for AFR. The CALBR performance was also investigated for different influent TP concentrations under optimum condition. TP removal efficiency was more than 88% and 18.82 and 26.65 mg/L of TP concentration were removed for the influent TP concentration of 20 and 30 mg/L, respectively. It is noted that only 10 mg/L of influent TP content is consumed though heterotrophic microbial assimilation, and therefore there is potential to enhance and optimize the TP removal of the CALBR. Finally, the behavior of the HCALBR, where hybrid biomass (suspended sludge plus attached biofilm) is employed, was studied for treating wastewaters with different $BOD_5$/COD ratios (0.7 and 0.35) at optimum condition. The CALBR containing hybrid biomass (HCALBR) exhibited a remarkable improvement in sludge settling properties supported by low SVI values of 19 and 13.7 mL/g compared to SVI of 57 mL/g for the CALBR. Although the HCALBR operated with MPW ($BOD_5$/COD of 0.7) showed a better performance in sCOD and TN removal, high removal efficiencies of 95.42 and 75.43% were also achieved for sCOD and TN, respectively in the HCALBR fed with CL ($BOD_5$/COD of 0.35). These observations highlighted that this modified high-rate bioreactor is capable of treating wastewaters containing slowly biodegradable organic matter and nitrogen. The good sludge settleability (SVI<100 mL/g) and low effluent turbidity (average value of 7.3 NTU) implicated that the internal rotating spiral separator can be used to eliminate the need for the large secondary clarifiers.

## CRediT authorship contribution statement

**Mahsa Mirghorayshi:** Writing – original draft, Methodology, Investigation, Conceptualization. **Ali Akbar Zinatizadeh:** Writing – review & editing, Supervision. **Mark C.M. van Loosdrecht:** Writing – review & editing. **M. T. Rayhani:** Writing – review & editing. **H. Bonakdari:** Writing – review & editing. **S. Moradi:** Writing – review & editing.

## Declaration of Competing Interest

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

## Data availability

Data will be made available on request.

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