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Process optimization of CNP removal from industrial soft drink wastewater in a single up flow A2O with continuous feed and intermittent discharge regime

Amir Nouri and Ali Akbar Zinatizadeh

ABSTRACT

Simultaneous removal of carbon and nutrients (CNP) in a single laboratory-scale bioreactor is advantageous in terms of reactor volume and energy consumption. In this study, an innovative up-flow anaerobic/anoxic/aerobic (A2O) single bioreactor with continuous feed and intermittent discharge (CFID) regime equipped with a movable aerator in the reactor height for simultaneous removal of CNP from soft drinks wastewater was successfully designed, fabricated and operated. The effects of four independent variables, i.e. hydraulic retention time (HRT), aerator height, biomass concentration and nitrogen/soluble chemical oxygen demand (N/sCOD) ratio at three levels in the range of 4–8 h, 37–55.5 cm, 4,000–6,000^{-1}, and 0.05–0.2, respectively, on eight process responses were investigated. The central composite design (CCD) and response surface methodology (RSM) were applied to design the experimental conditions, model the obtained data, and optimize the process. The bioreactor provides three conditions with different dissolved oxygen (DO) (anaerobic, anoxic and aerobic) in a single bioreactor by placing the aerator in the middle of the reactor. As a result, the maximum sCOD, total nitrogen (TN) and total phosphorus (TP) removal were about 100, 92 and 41%, respectively. The optimum region obtained was an HRT of 5–11 h, a mixed liquor suspended solids (MLSS) concentration of 4,000–4,700 mgL^{-1}, and an aerator height of 46.25 cm, at the N/sCOD ratio of 0.1.

Key words | A2O single bioreactor, biological nutrients removal, CFID regime, wastewater treatment

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INTRODUCTION

In the past two decades, integrated bioreactors have been investigated for wastewater treatment because this bioreactor produces less sludge with a smaller footprint. These integrated bioreactors are efficient, affordable and have a smaller land area requirement than the separated anaerobic-aerobic bioreactors (Moosavi *et al.* 2004; Tartakovsky *et al.* 2005; Zhang *et al.* 2005).

Recently, studies have been published on nitrogen and phosphorus removal via simultaneous nitrification–denitrification (SND) in the single bioreactors. Asadi *et al.* (2012) studied simultaneous carbon and nutrients (CNP) removal in a single up-flow aerobic/anoxic sludge bed bioreactor. Amini *et al.* (2013) evaluated the performance of an up-flow anaerobic–aerobic sludge blanket reactor for the treatment of dairy wastewater. Yang *et al.* (2010) reported 93.5, 82.6, 95.6 and 84.1% removal efficiencies of soluble chemical oxygen demand (sCOD), total nitrogen (TN), ammonia (NH_4^+-N) and total phosphorus (TP), respectively, in a sequencing batch moving bed membrane bioreactor. Other bioreactors examined are a moving bed sequencing batch reactor integrated with intermittent aeration strategy (Lim *et al.* 2012), moving bed sequencing batch reactors (Lim *et al.* 2011), fixed bed sequencing batch reactor (Rahimi *et al.* 2011), moving bed biofilm bioreactor (Zinatizadeh & Ghaytooli 2015), single ultrasound augmented upflow anaerobic/aerobic/anoxic bioreactor (Rezaee *et al.* 2015), continuous feed and intermittent discharge (CFID) airlift bioreactor (Asadi *et al.* 2016).

In the present study, a single laboratory-scale up flow anaerobic/anoxic/aerobic (A2O) reactor equipped with a movable aerator in the reactor depth was designed and operated to provide anaerobic, anoxic and aerobic conditions for removing CNP from soft drink wastewater. In this study, in addition to the examination of the bioreactor removing nutrients (N and P) and carbon, a comprehensive and constructive process modelling using response surface methodology

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(RSM) is exhibited in detail. In order to analyze the process, four important independent variables, viz. hydraulic retention time (HRT), mixed liquor suspended solids (MLSS), nitrogen/soluble chemical oxygen demand (N/sCOD) ratio and aerator height and eight dependent parameters as process responses were studied. The interactions among the variables as well as their direct impact on the process were measured and discussed. Using the experimental data obtained and the models developed, optimum conditions were determined.

MATERIALS AND METHODS

Preparation of wastewater and seed

A wastewater sample was prepared with soft drink industrial wastewater, ammonium chloride and potassium hydrogen phosphate as carbon, nitrogen and phosphorus sources, respectively. The wastewater composition is shown in Table 1. The soft drink wastewater was taken from a working industrial soft drink factory (Zamzam Co., Kermanshah, Iran) every three days. NH_4Cl and KH_2PO_4 were used to adjust different C/N/P ratios after characterization of the raw wastewater. The samples were stored in a refrigerator at 4 °C to prevent any changes in the matrix of samples. The sludge seed was taken from a working municipal wastewater treatment plant, Kermanshah, Iran. The volatile suspended solids (VSS) content of the seed sample was $6,000 \pm 500$ mgL^{-1} with VSS/SS ratio of about 0.8.

Bioreactor

Bioreactor configuration

Figure 1(a) illustrates the schematic diagram of the up-flow anaerobic/anoxic/aerobic (A2O) single bioreactor used in this study. The glass bioreactor column was manufactured with a total volume of 3.5 L, a liquid height of 120 cm and an internal diameter of 6 cm. An automatic control valve was mounted in the middle of column, in order to attain the intermittent discharge. HRT was calculated based on 1.75 L of feeding volume (the volume which is filled and discharged). The reactor was aerated with an air bubble diffuser connected with an air blower. The bubble diffuser was set at the middle of the reactor. To achieve an adequate mixing in the area without aeration, three fine mixers were inserted on the body of the bioreactor. Two other mixers used for creating of sludge blanket and sludge mixing in the reactor middle.

Table 1 | Characteristics of the wastewater used

Parameters	Unit	Amount
sCOD	(mgL^{-1})	900–1,200
BOD_5	(mgL^{-1})	600–950
TN	(mgL^{-1})	50–210
TP	(mgL^{-1})	39–43
pH	–	6.8–7.2

Reactor design

Based on an earlier test, the effect of the aerator height on the reactor performance was determined as in the range of 37–55.5 cm. This range was considered because in values smaller than 37 cm, an anoxic zone is not created and at values higher than 55.5 cm, an anaerobic zone is developed at the beginning of the cycle. In the bioreactor, the volume of the anoxic zone was almost constant (about 750–800 ml), created at the bottom of the column. A sludge blanket layer is formed between the anoxic and aerobic zones by adjusting the mixers mounted on the reactor body at the lower part of the system. It is noted that an increase in the aerator height did not show any influence on the volume of the anoxic zone. The volume ratio of the anoxic zone/aerobic zone was in the range of 0.21–0.23.

Chemical analysis

The NH_4-N, nitrate and nitrite, concentrations of COD, TP, sludge volume index (SVI) and MLSS were determined by using *Standard Methods* (APHA 2005). NH_4-N was determined by TKN meter Gerhardt model (Vapodest 10, Germany). For sCOD, a colorimetric method with closed reflux method was developed. Spectrophotometer (DR 5000, Hach, Jenway, USA) at 600 nm was used to measure the absorbance of COD samples. Turbidity was measured by a turbidity meter model 2100 P (Hach Co., USA). The dissolved oxygen (DO) concentration in wastewater was determined using a DO probe. The DO meter was supplied by WTW DO Cell OX 330, electro DO probe, Germany.

Bioreactor operation procedure

The soft drink wastewater continuously entered into the bioreactor from the bottom of the column, but the treated effluent was intermittently removed from the middle of column at the end of each cycle. Each operating cycle

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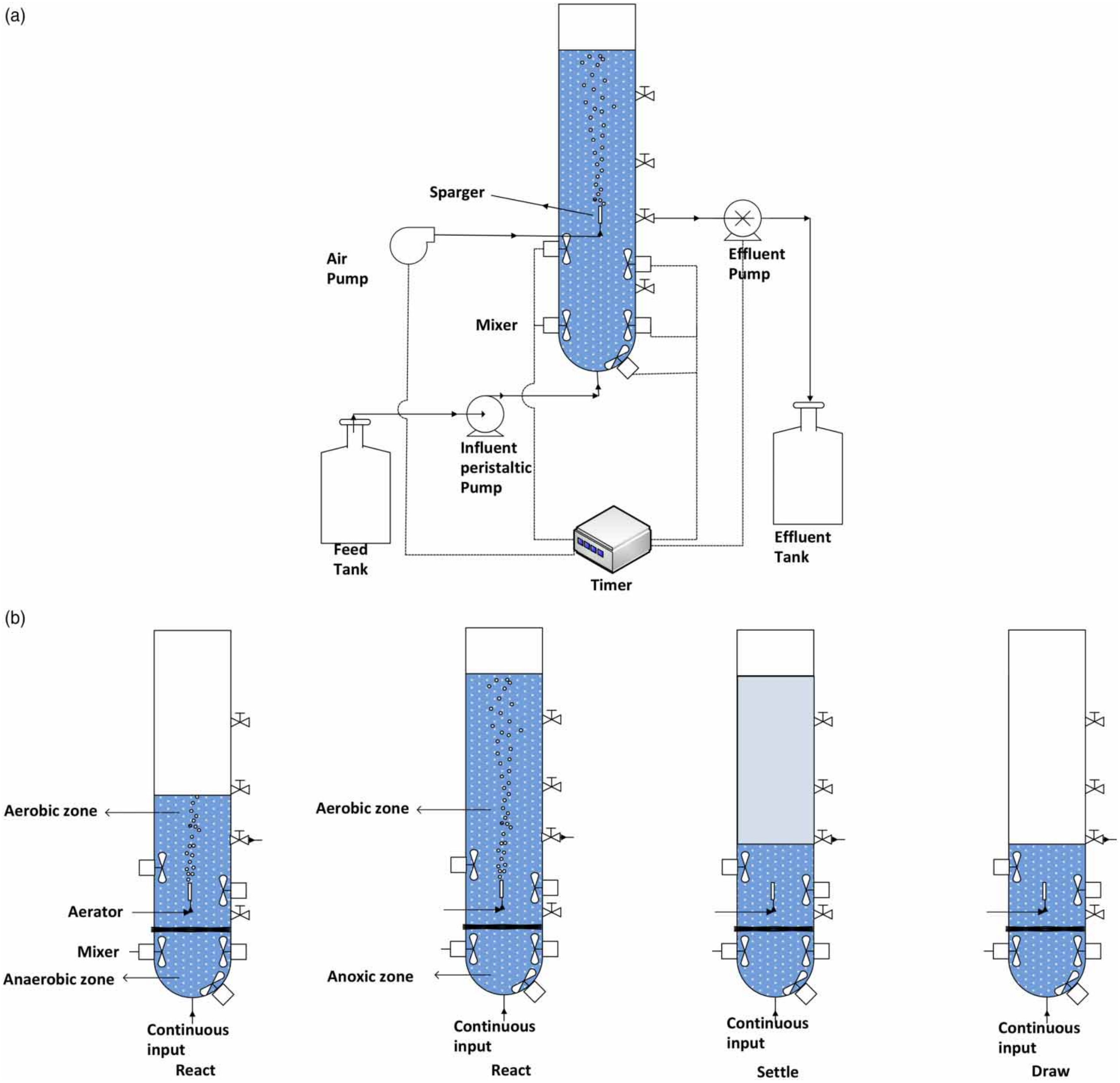

Figure 1 | (a) Schematic diagram of the UAAASB used and (b) the laboratory-scale A2O used in this study at different operating steps.

comprised anaerobic, anoxic and aerobic phases (react), sedimentation and discharge (Figure 1). At the beginning of each cycle, an anaerobic zone is created while feeding continues. The length of the anaerobic phase depends on the HRT and aerator height, e.g. 1.25 h for an HRT of 8 h and aerator height of 46.25 cm. After this time, the anaerobic zone turns to the anoxic zone. The mixers and aerator were continuously working during the bioreactor operation. The settling time for all runs was 25 minutes. Excess sludge was periodically withdrawn through the bottom valve. The A2O single bioreactor was operated under room temperature ($25 \pm 2\,^{\circ}$C). The A2O single bioreactor was continuously operated for 6 months in a CFID mode. In the first stage, bioreactor start-up, the bioreactor was operated at HRT, MLSS, N/COD ratio and aerator height of 8 h, 6,000 $\mathrm{mgL^{-1}}$, 12.5 and 46.25 cm, respectively. This was continued to 14 days, for the adaptation of the sludge to the wastewater. During the operation period (166 days), the effects of HRT, aerator height, biomass concentration and N/COD ratio on reactor performance were investigated.

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Each experiment was monitored by the quality and processes parameters. After achieving a stable performance, the process was continued for 5 HRT to ensure the steady state condition. The mixers and aerator were continuously working during the bioreactor operation. To investigate the effect of the variables on the reactor performance, the bioreactor was operated according to conditions which were designed by Design Expert Software (DOE) (Stat-Ease Inc., version 7.0) as presented in the following section. The settling time for all runs was 25 minutes.

Experimental design and mathematical model

Variables evaluation

Nitrogen removal by sequential nitrification and denitrification in the biological reactors depends on a multitude of variables. Among these, eight significant factors that affect the nitrogen removal in various systems are biomass concentration, HRT, N/sCOD ratio, internal recirculation from the aerobic unit to the anoxic zone, flow rate of the returned activated sludge (RAS), biomass concentration, anoxic zone volume, pH and temperature (Akhbari *et al.* 2011). In this study, four important variables (HRT (4, 8, 12 h), MLSS concentration (4,000, 5,000, 6,000 $\mathrm{mgL^{-1}}$), aerator height (37, 46.25, 55.5 cm) and N/sCOD ratio (5, 12.5, 20)) from the above mentioned list were selected. HRT is an important operating variable as it is directly related to bioreactor volume which is required for completion of the treatment process. The optimum amount of the biomass concentration was also explored as a process factor, as it acts as a biocatalyst in the system and affects the reaction rate. To have simultaneous CN removal in the single bioreactor providing an anoxic zone is of vital importance. So, in this study, aerator height as a physical variable was selected as another factor that indicates the anoxic condition. The COD/N ratio influent should be high enough to denitrify all nitrates arising in the nitrification process. However, in the present study, the N/sCOD ratio was chosen as another process variable to be in the range of 50/1,000 to 200/1,000. Selection of the range of the variables was based on the obtained results from preliminary studies. It must be noted that all discussions are based on actual values of the responses. The settling time for all runs was 25 minutes.

Experimental design

Statistical design of the experiments and data analysis was carried out by Design Expert Software (version 7.0). In this study, four independent effective variables were selected in the experiment design. The range and levels of the variables in coded and actual units are given in Table 2. The four variables were considered at three levels. In the basis of the factorial design, 30 experiments (including eight axial points, 16 factorial points, one center point and five replications of the center point) were designed. sCOD removal efficiency, TN removal efficiency, effluent nitrate, effluent nitrite, effluent ammonium, TP removal efficiency, effluent turbidity and SVI were measured or calculated as response in the bioreactor. The experimental conditions and obtained results are shown in Table 3. The responses are an average of several measurements during five HRT to ensure the steady state condition.

Mathematical modeling

The coefficients of the polynomial model were computed utilizing the following equation (Salemi 2005).

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

where, i, j and ß are the linear, quadratic and regression coefficients, respectively. P value with 95% confidence level was considered to evaluate the effectiveness of the model terms.

Statistical analysis

As COD, TP and TN removal are the significant criteria to appraise the performance of a bioreactor for removal of CNP from wastewaters, these parameters were modeled using DOE in this study. The regression equation obtained was applied to predict the system performance in the different operating variables. In order to coordinate the obtained data, various degrees of polynomial models were exerted. The response data were analyzed by default in the DOE. Polynomial models were chosen for each response based

Table 2 | Experimental range and levels of the independent variables

	Range and levels		
Variables	−1	0	+1
HRT, h	4	8	12
MLSS, $\mathrm{mgL^{-1}}$	4,000	5,000	6,000
N/sCOD, %	5	12.5	20
Aerator height, cm	37	46.25	55.5

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Table 3 | Experimental conditions and results

	Variables						**Responses**						
Run	**Factor 1 (A) HRT, (h)**	**Factor 2 (B) MLSS, (mgL^{-1})**	**Factor 3 (C) Aerator height (cm)**	**Factor 4 (D) N/sCOD (%)**	**sCOD removal, (%)**	**TCOD removal, (%)**	**TN removal, (%)**	**Effluent N-NO$_3$, (mgL^{-1})**	**Effluent N-NO$_2$, (mgL^{-1})**	**Denitrification rate, (g/l·d)**	**TP removal, (%)**	**Effluent turbidity, (NTU)**	**SVI, (ml/g)**
1	4	4,000	37	20	92.70 ± 1.40	91.53 ± 1.40	23.38 ± 4.6	1.1	0	0.0666	40.63 ± 2.6	12	71
2	4	6,000	55.5	20	87.15 ± 1.52	84.8 ± 1.72	15.29 ± 4.3	4.17	0.08	0.0047	32.05 ± 2.1	21	150
3	4	4,000	55.5	20	91.40 ± 1.48	85.76 ± 1.69	20.45 ± 4.5	1.5	0	0.0723	35.40 ± 2.5	48	38
4	4	5,000	46.25	12.5	100.00 ± 0.0	99.41 ± 0.0	34.24 ± 4.6	0	0	0.0115	27.94 ± 1.7	6	76
5	4	6,000	37	20	89.00 ± 1.52	88.00 ± 1.52	19.26 ± 4.1	8.5	0	0.0151	40.46 ± 2.8	3	120
6	4	6,000	37	5	82.80 ± 1.43	82.45 ± 1.43	75.01 ± 3.5	3.3	0	0.0160	30.27 ± 2.9	4	146
7	4	4,000	37	5	98.68 ± 1.45	98.00 ± 1.45	84.93 ± 4.7	0.8	0	0.0166	31.56 ± 3.1	8	66
8	4	4,000	55.5	5	93.42 ± 1.47	87.94 ± 1.47	70.32 ± 5.1	2.1	0	0.0000	2HR7.45 ± 1.8	60	35
9	4	6,000	55.5	5	87.15 ± 1.51	84.73 ± 1.51	72.00 ± 4.5	3.6	0	0.0900	37.14 ± 2.6	23	136
10	8	6,000	46.25	12.5	92.9 ± 1.530	92.74 ± 1.530	45.19 ± 3.7	3.28	0	0.1070	26.06 ± 2.6	2	133
11	8	5,000	46.25	12.5	100.00 ± 0.0	99.61 ± 0.3	51.10 ± 4.6	0	0	0.0958	25.36 ± 2.4	4	89
12	8	5,000	46.25	20	100.00 ± 0.0	99.61 ± 0.3	31.07 ± 4.5	0	0	0.0945	27.07 ± 1.9	4	82.5
13	8	5,000	55.5	12.5	100.00 ± 0.0	97.58 ± 1.0	38.76 ± 3.9	2.4	0	0.0704	27.41 ± 2.3	22	97
14	8	5,000	46.25	12.5	97.00 ± 1.49	96.6 ± 1.49	45.00 ± 4.0	0.1	0	0.0901	23.00 ± 2.6	5	94
15	8	5,000	46.25	12.5	96.50 ± 1.51	96.04 ± 1.51	51.11 ± 5.2	0	0	0.0973	26.00 ± 2.3	3	86
16	8	5,000	46.25	12.5	99.00 ± 1.00	98.69 ± 1.00	51.00 ± 4.6	0.15	0	0.1077	26.80 ± 1.5	4	90
17	8	4,000	46.25	12.5	98.05 ± 1.56	97.05 ± 1.56	60.79 ± 3.8	1	0	0.1152	30.69 ± 2.8	9	46
18	8	5,000	46.25	12.5	99.00 ± 0.80	98.72 ± 0.80	52.50 ± 4.9	0.05	0	0.0943	25.40 ± 2.1	4	91.5
19	8	5,000	46.25	12.5	99.20 ± 0.60	99.00 ± 0.60	51.50 ± 3.7	0	0	0.0945	23.80 ± 2.9	5	89.7
20	8	5,000	46.25	5	94.85 ± 1.73	93.76 ± 1.73	87.64 ± 5.6	0	0	0.0793	26.36 ± 1.8	11	90
21	8	5,000	37	12.5	89.95 ± 1.44	89.56 ± 1.44	32.77 ± 4.6	6.25	0	0.0494	25.06 ± 2.4	4	90
22	12	4,000	55.5	5	100.00 ± 0.0	96.2 ± 1.74	83.07 ± 3.5	0	0	0.0518	23.70 ± 2.1	39	42
23	12	6,000	55.5	20	90.20 ± 1.47	88.59 ± 1.53	28.21 ± 4.3	3.9	0	0.0843	26.09 ± 1.8	18	135
24	12	4,000	37	20	100.00 ± 0.0	99.08 ± 0.51	26.88 ± 4.7	1.32	0	0.0627	27.72 ± 2.6	9	42
25	12	6,000	37	5	92.05 ± 1.80	91.78 ± 1.78	67.54 ± 4.4	1.85	0	0.0318	26.31 ± 1.3	3	82
26	12	6,000	37	20	90.60 ± 1.43	90.39 ± 1.43	16.35 ± 3.9	0.5	0	0.0347	23.65 ± 2.1	2	131
27	12	4,000	37	5	100.00 ± 0.0	98.88 ± 1.08	84.09 ± 4.4	1.85	0	0.0553	19.17 ± 2.2	10	38
28	12	4,000	55.5	20	100.00 ± 0.0	96.11 ± 1.80	31.13 ± 5.1	0.08	0	0.0939	30.61 ± 1.6	35	52
29	12	5,000	46.25	12.5	100.00 ± 0.0	99.16 ± 0.70	34.99 ± 4.3	0	0	0.0501	25.98 ± 3.1	9	143
30	12	6,000	55.5	5	92.67 ± 1.58	90.16 ± 1.44	90.99 ± 4.1	0.22	0	0.0787	28.19 ± 2.6	27	132

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on the proportionality with real data, considering R-squared (R^2) as the significant criterion. The polynomial equations are based on coded values (-1, 0, $+1$) to predict the responses in the design space for each variable. In the regression equations, HRT, MLSS, aerator height and N/sCOD ratio are represented with A, B, C and D, respectively.

Chemical analysis

The NH_4-N, nitrate and nitrite, concentrations of COD, TP, sludge volume index (SVI) and MLSS were determined by using *Standard Methods* (APHA 2005). NH_4-N was determined by total Kjeldahl nitrogen (TKN) meter Gerhardt model (Vapodest 10, Germany). For sCOD, a colorimetric method with closed reflux method was developed. Spectrophotometer (DR 5000, Hach, Jenway, USA) at 600 nm was used to measure the absorbance of COD samples. Turbidity was measured by a turbidity meter model 2100 P (Hach Co., USA). The DO concentration in wastewater was determined using a DO probe. The DO meter was supplied by WTW DO Cell OX 330, electro DO probe, Germany.

RESULTS AND DISCUSSION

Statistical analysis

Table 4 summarizes the analysis of variance (ANOVA) results for the three responses (sCOD, TN and TP removal) as a function of the variables studied. As various responses were investigated in this study, different degree polynomial models were used for data fitting (Table 4). The regression equations obtained are presented in Table 4. The model terms in the equations are after elimination of insignificant variables and their interactions. According to the statistical analysis, the models were highly significant with very low probability values (<0.0001). It was shown that the model terms of independent variables were significant at the 99% confidence level. The models' adequacy was tested through lack-of-fit F-tests. The lack of fit F-statistic was not statistically significant, as the *P*-values were greater than 0.05. Adequate precision is a measure of the range in predicted response relative to its associated error or, in other words, a signal-to-noise ratio that was found to be desirable for all models (>4). Simultaneously, low values of the coefficient of variation (CV) (1.59–8.90%) indicated good precision and reliability of the experiments. Detailed analysis of the models is presented in the following sections.

Process analysis and modeling

sCOD removal

In this study, the particulate COD (pCOD) content of the wastewater was negligible. So, in order to appraise the bioreactor performance, sCOD removal was opted for as a response. The ANOVA values for sCOD removal efficiency are displayed in Table 4. The importance of each coefficient was specified by F-value and *P*-value. B (MLSS), C (Aerator height) and D (N/sCOD), B^2, C^2, ABD, A^2C, A^2D and AB^2

Table 4 | ANOVA results for the equations of the Design Expert (ver. 6.0.7) for studied responses

Response	Modified equations with significant terms	Probability (*P*-value)	R^2	Adj.R^2	Adeq. precision	S.D.	CV	PRESS	F-value	Probability for lack of fit
sCOD removal	$+98.50 - 3.84B + 5.02C + 2.57D-2.50B^2 - 3.02C^2 - 1.16ABD - 5.29A^2C - 2.89A^2D + 2.66AB^2$	<0.0001	0.9200	0.8840	16.126	1.69	1.78	140.20	25.57	0.3271
TN removal	$+47.78 + 2.71A - 3.08B - 27.98D + 3.94AC + 2.66BC - 10.39A^2 + 7.71B^2–9.03C^2 + 14.17D^2$	<0.0001	0.9795	0.9702	31.920	4.05	8.23	848.64	105.99	0.1390
TP removal	$+25.85–4.00A + 1.88D + 1.44AC-1.98BD + 4.03 B^2 + 4.03B^2$	<0.0001	0.7880	0.7439	16.544	2.51	8.90	258.32	17.84	0.0742

A: HRT, B: MLSS, C: aerator height, D: N/sCOD ratio , R^2: determination coefficient, Adj. R^2: adjusted R^2, Adeq. precision: adequate precision, SD: standard deviation, CV: coefficient of variation, PRESS: predicted residual error sum of squares.

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were significant model terms. Other model terms are not significant (with a probability value larger than 0.05).

The regression equation presented in Table 4 illustrates an experimental model in terms of symbolic factors for the sCOD removal. The influent sCOD concentration was constant at about 1,000 mgL^{-1} throughout the experiments. Figure 2 represents the efficacies of the variables on the COD removal efficiency in the system. It is clearly shown that an increase in MLSS concentration from 4,000 to 6,000 mgL^{-1} caused a decrease in the removal of sCOD at high values of HRTs (from 98 to 92%), which could be related to soluble microbial products (SMPs) and endogenous respiration (Barkerm & Stuckey 1999; Ahmed *et al.* 2007).

It is obviously shown that the bioreactor had a good performance at different conditions (sCOD removal > 90%). One reason could be high biodegradability of the wastewater used. Figure 2(b) and 2(c) illustrate the effects of aerator height, MLSS and N/sCOD on the response. It is clearly shown that increase in aerator height from 37 to 55 cm increased the removal of sCOD. The maximum sCOD removal efficiency was about >99%, which was achieved at MLSS concentrations of 5000 (Run Nos. 11, 12, 13 and 29) and 4,000 mgL^{-1} (Run Nos. 22, 24, 27 and 28). The COD removal efficiency was in agreement with the research works carried out by Asadi *et al.* (2016). In this work, about 100% COD removal efficiency was obtained in a CFID airlift bioreactor from soft drink wastewater with an HRT of 12 h. Sheldon & Erdogan (2016) obtained 93% COD removal efficiency at an HRT of 12 h and COD removal loading of 11 kg COD m^{-3} d^{-1} in a pilot scale anaerobic expanded granular sludge bed treating soft drink wastewater.

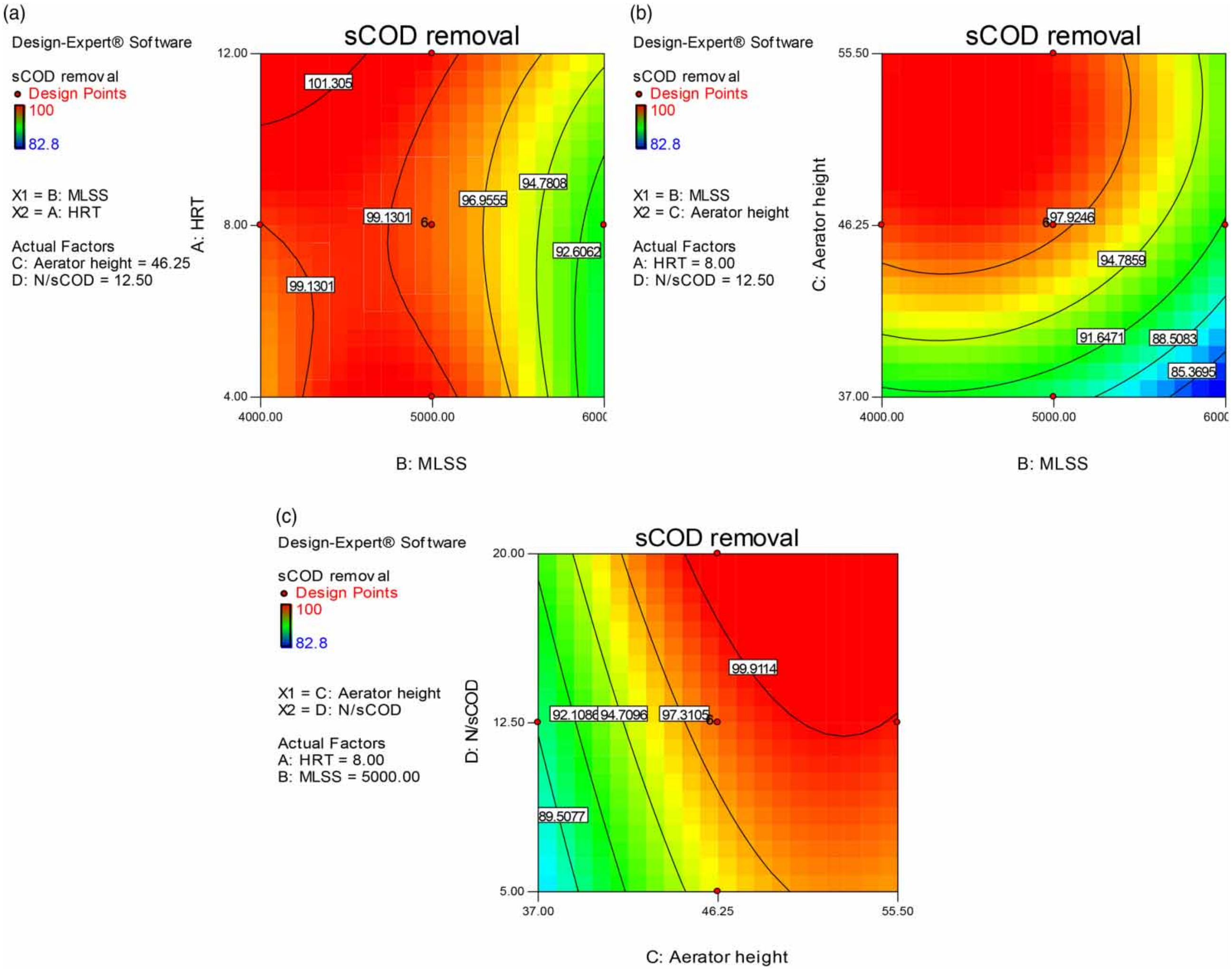

Figure 2 | Response surface plots for sCOD removal efficiency as a function of (a) MLSS and HRT, (b) MLSS and aerator height, (c) N/sCOD, while the other two variables remained constant at their middle value.

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Nitrogen removal

TN removal. Nitrogen removal was obtained by sequential nitrification and denitrification. In the nitrification process, *Nitrosomonas* bacteria (autotrophic nitrifying bacteria) oxidize ammonia nitrogen to nitrite and then *Nitrobacter* oxidizes nitrite to nitrate in the aeration zone. In the denitrification process, heterotrophic denitrifying bacteria reduce nitrite and/or nitrate to nitrogen gas in the anoxic zone (Fu *et al.* 2009a, 2009b). In this study, the two zones for nitrogen removal were provided in a single reactor by adjusting the aerator height in the system. From the ANOVA results (Table 4), A, B, D, AC, BC, A^2, B^2, C^2, D^2 were significant model terms describing TN removal efficiency as a function of the variables (Table 4). The influence of the variables on TN removal efficiency is shown in Figure 3. At an MLSS concentration of 4,000 mgL^{-1}, the portion of assimilation is increased, which related to increased cell growth rate at a high F/M ratio (Song *et al.* 2009). As the MLSS concentration and HRT are increased, the section of denitrified nitrogen is increased. From Figure 3, the minimum TN removal (15%) obtained was an HRT of 4 h, an MLSS concentration of 6,000 mgL^{-1}, and an aerator height of 55.5 cm, at the N/sCOD ratio of 20%. In the other side, the maximum TN removal (92%) achieved was an HRT of 8 h, an MLSS concentration of 4,000 mgL^{-1}, and an aerator height of 46.25 cm, at the N/sCOD ratio of 12.5%. At an aerator height of 55.5 cm, the oxidation potential in the bioreactor was decreased, which caused a restriction in the nitrification process. Increase in the denitrification rate

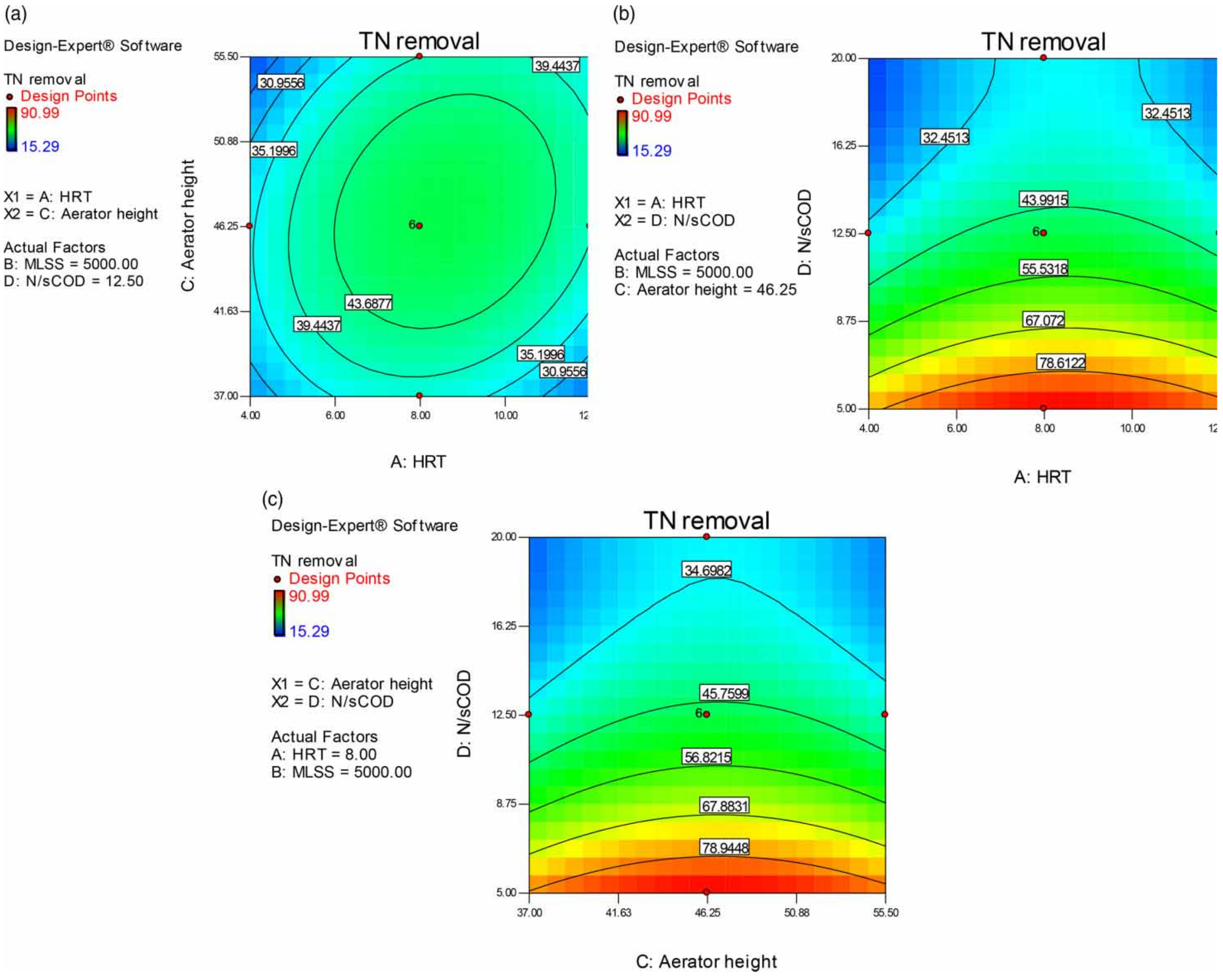

Figure 3 | Response surface plots for TN removal efficiency as a function of (a) aerator height and HRT, (b) N/sCOD and HRT, (c) N/sCOD and aerator height, while the other two variables remained constant at their middle value.

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with increasing the aerator height from 55.5 to 46.25 cm at constant values of HRT and MLSS concentration was good evidence to prove that the nitrification process was a limiting step for TN removal (Run Nos. 11 and 13). This result demonstrates a good balance between anoxic and aerobic zones for TN removal at 46.5 cm aerator height.

At different amounts of HRT, the maximum TN removed due to the denitrification process was obtained at an aerator height of 46.25 cm (Run No. 17). This might be due to the good balance between aerobic and anoxic conditions. At HRTs of 4–12 h, with increase in the N/sCOD ratio from 0.05 to 0.2, the quantity of TN removed was increased. Similar results have been reported by other researchers (Sobieszuk & Szewczyk 2006; Zhao *et al.* 2008; Fu *et al.* 2009a, 2009b). In a similar research work, 60% TN removal was obtained at an HRT of 8 h with soft drink wastewater as feed (Merchuk & Garcia Camacho 2010). Kuehl (2000) reported that 90% TN removal efficiency was obtained in a vertical submerged membrane bioreactor at an HRT of 6 h and an influent TN concentration of 30 mgL^{-1}. Asadi and her coworkers (2016) have obtained 72% TN removal efficiency in a CFID airlift bioreactor using soft drink wastewater. The maximum denitrification rate obtained in the reactor was 0.115 $gNL^{-1} d^{-1}$ (Run No. 17, at an MLSS concentration of 4,000 mgL^{-1}, N/sCOD of 12.5%, aerator height of 46.25 cm and HRT of 8 h). DO is the significant factor that influences the rates of the nitrification and denitrification processes. High DO concentration is desirable for the nitrifying bacteria but disadvantageous for the denitrifying bacteria; so, in order to attain the SND process in a single tank, the DO concentration should be controlled at a specific level. To determine the effect of DO on the system performance in terms of nutrient removal, an experiment with the different DO concentrations was performed. For TN removal, the optimum DO concentration was found to be in the range of 0.1 ± 0.1 $mg\,L^{-1}$ in the anoxic zone and 3 ± 0.5 $mg\,L^{-1}$ in the aerobic zone, which was similar to the reported data in the literature (Lin *et al.* 2009).

Fate of nitrogen fractionation. In the present study, influent and effluent nitrogen fractionation at the experimental conditions designed (Table 3) were measured to assess the nitrification and denitrification processes. Effluent NO_2^- concentration was very low in all the experiments, illustrating that the nitrification process is complete. The effluent NO_3^- concentration was also low throughout the experiment, indicating a favored condition for the denitrification process. The maximum amount for the effluent NO_3^- concentration was obtained to be 8.5 mgL^{-1} at low HRT (4 h) (Run No. 5), while the NO_3^- concentration was reduced to 0.5 mgL^{-1} (Run No. 26) (at 6,000 mgL^{-1}, 0.2 and 37 cm of MLSS, N/sCOD and aerator height, respectively). The most effective factor in the effluent nitrate concentration was HRT.

Phosphorus removal

There would be a possibility to develop probable anaerobic micro environments in the non-aerated zone based on the range studied for the variables (HRT, MLSS, N/sCOD, and aerator height). Therefore, in order to justify the claim assumed, phosphorus removal was selected as a response. From the ANOVA results, A, D, AC, BD, and B^2 were significant model terms (Table 4). HRT and MLSS were determined as the most influential factors for phosphorus removal efficiency in the experimental conditions designed.

The effects of the variables on TP removal efficiency are displayed in Figure 4. From Figure 4(a), at the highest amount of HRT (12 h), TP removal efficiency showed a decreasing trend with a decrease in the aerator height, indicating a limitation in the anaerobic zone. As can be seen in Figure 4(b), at MLSS concentrations of 4,000–6,000 mgL^{-1} with decreasing HRT from 12 to 4 h, TP removal was increased. The highest amount of TP removal efficiency was about 40.63%, which was determined at the highest F/M ratio (1.5 g COD/g VSS) and the lowest amount of MLSS, HRT and aerator height (4,000 mgL^{-1}, 4 h, 20% and 37 cm, respectively). Asadi *et al.* (2016) reported 60% TP removal efficiency in a laboratory-scale CFID airlift bioreactor fed by soft drink wastewater.

Sludge volume index

In the present study, SVI was determined at the experimental conditions designed, that the data indicate in Figure 5. As shown in this figure, the range of changes in the SVI was from 35 to 150 ml/g. The minimum amount of SVI (35 ml/g) obtained was an HRT of 4 h, an MLSS concentration of 4,000 mgL^{-1}, and an aerator height of 55.5 cm, at the N/sCOD ratio of 5%. The maximum SVI amount achieved was 150 ml/g when the MLSS concentration, aerator height, HRT and N/sCOD ratio were 6,000 mgL^{-1}, 55.5 cm, 4 h, and 20% respectively. From this figure, the most important factor for SVI was determined to be MLSS. It was observed that the response increased with an increasing amount of MLSS concentration from 4,000 to 6,000 mgL^{-1}, which may depend on a low F/M ratio that led to excessive growth of filamentous bacteria (Farizoglu *et al.* 2007).

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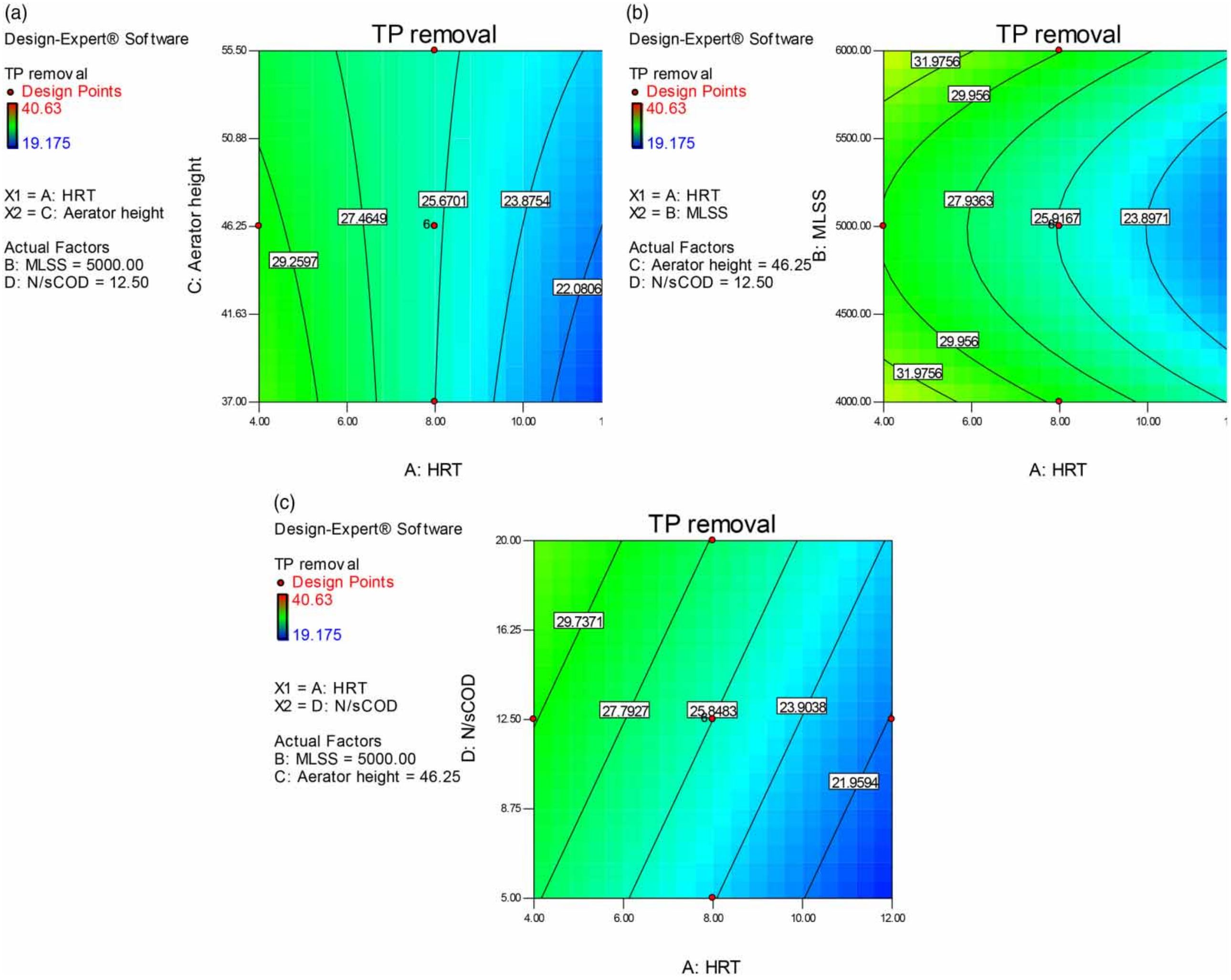

Figure 4 | Response surface plots for TP removal efficiency as a function of (a) HRT and aerator height, (b) HRT and MLSS (c) HRT and N/sCOD, while the other two variables remained constant at their middle value.

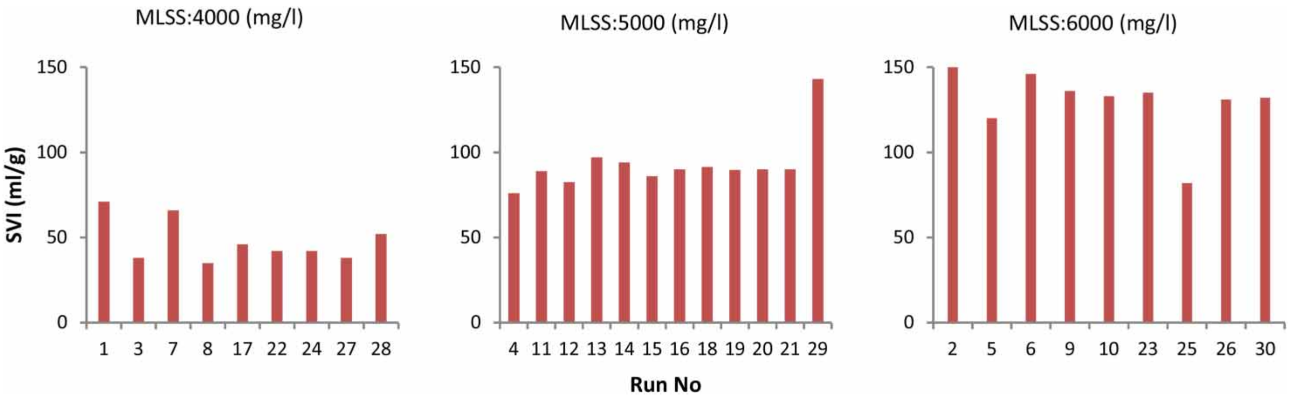

Figure 5 | SVI of sludge under different operating conditions.

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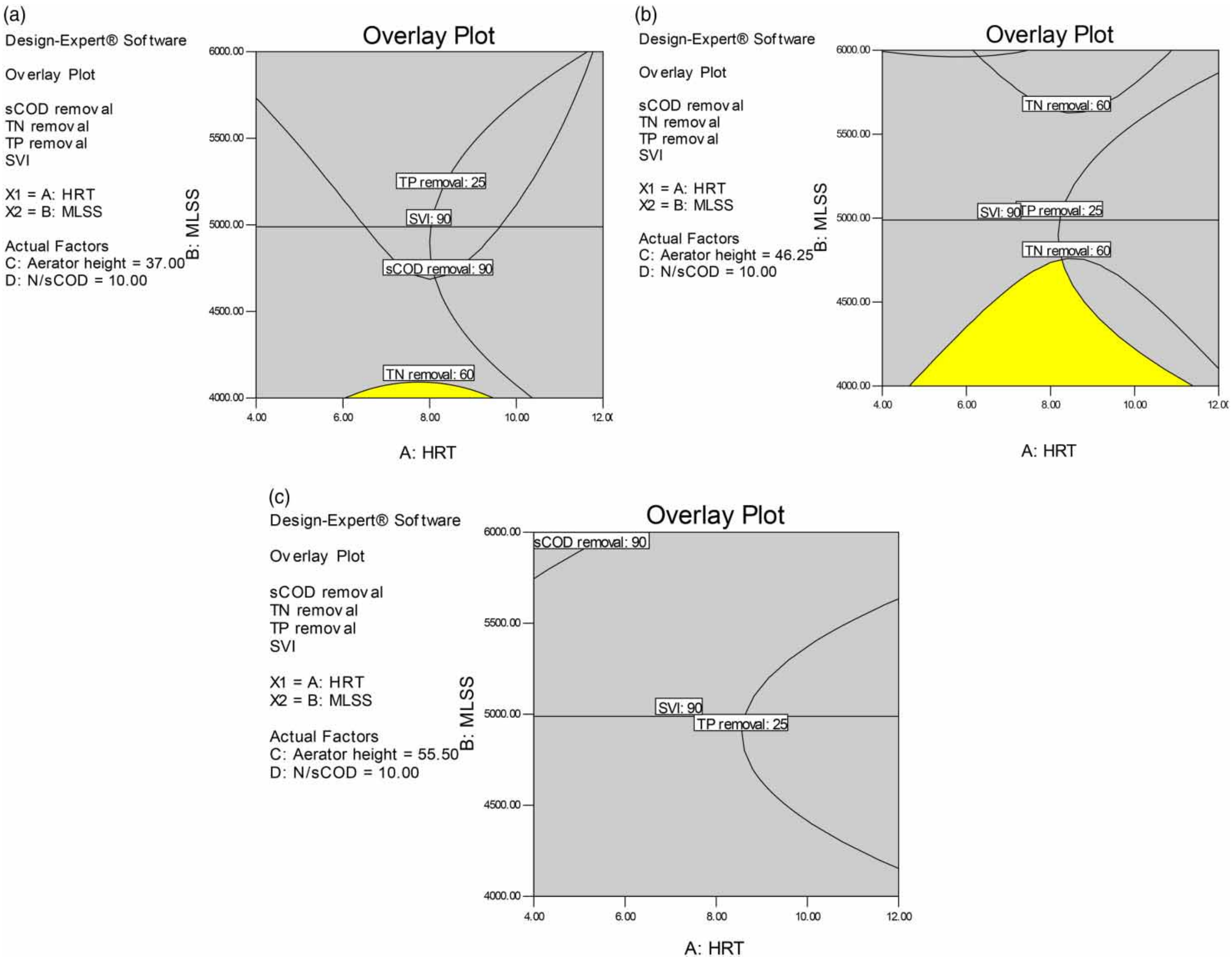

Figure 6 | Overlay plot for the optimal region as a function of MLSS and HRT at different aerator heights, (a) 37, (b) 46.25, (c) 55.5 cm.

Process optimization

Figure 6(a)–6(c) display the graphical optimization that represents the region of feasible response amounts (colored portion) in the factors space. Based on four critical responses (TN removal, sCOD removal, TP removal and SVI), received from Table 5, located the optimum regions. From the figure, an aerator height of 46.25 cm illustrates the optimal area because of a good balance among anoxic (for denitrification) and aerobic (for COD removal and nitrification) zones. MLSS concentrations higher than 5,000 mgL^{-1} restrict the optimum area at any aerator height. Figure 6(a)–6(c) compare the optimum region obtained at three different aerator heights. As seen in the figures, at an aerator height of 37 cm the region is enclosed in the MLSS range 4,000–4,200 mgL^{-1} and HRT of 6–10 h, while at the middle value of the variable the region was spread to an MLSS of 4,000–4,700 mgL^{-1} and HRT of 5–11 h at the aerator height of 46.25 cm and N/sCOD ratio of 10%. From the figure, the optimum region could not be achieved at the aerator height of 55.5 cm and N/sCOD ratio of 10%.

Table 5 | The optimization criteria for the responses studied

Response	**Limit**	**Unit**
sCOD removal	$\geq$90	%
TN removal	$\geq$60	%
TP removal	$\geq$25	%
SVI	$\leq$100	mgL^{-1}

CONCLUSION

An innovative continuous-fed up-flow anaerobic/anoxic/aerobic (A2O) single bioreactor with intermittent discharge

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mode equipped with a movable aerator in the reactor height for simultaneous removal of CNP from a soft drink wastewater was successfully designed, fabricated and operated. The RSM results demonstrated the effects of the operating variables as well as their interactive effects on the response. The HRT and aerator height were determined to be the most effective operational factors on the system performance for removing the nutrients. As a conclusion, the innovation is a promising method as a high rate SND process in a single bioreactor with easy operation.

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