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Research article

Enhancing nitrogen removal from wastewater in a low C/N ratio using an air-lift bio-electrochemical reactor (ALBER)

Amir Nouri[a], Ali Akbar Zinatizadeh[a,b,c,*], Sirus Zinadini[a,b], Mark Van Loosdrecht[d]

[a] *Department of Applied Chemistry, Faculty of Chemistry, Razi University, Kermanshah, Iran*
[b] *Environmental Pollution and Engineering Group, Environmental Research Center (ERC), Razi University, Kermanshah, Iran*
[c] *Australian Centre for Water and Environmental Biotechnology (ACWEB, formerly AWMC), Gehrmann Building, The University of Queensland, St. Lucia, 4072, Brisbane, Australia*
[d] *Department of Biotechnology, Delft University of Technology, Julianalaan 67, 2628 BC, Delft, the Netherlands*

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ABSTRACT

This study focuses on the development of an air-lift bio-electrochemical reactor (ALBER) with a continuous feeding regime. The objective is to enhance nitrogen removal from synthetic wastewater with a low carbon-to-nitrogen (C/N) ratio. The chemical oxygen demand (COD) and total nitrogen (TN) of the influent wastewater were 500 and 200 mg/L, respectively. The effect of four independent variables, i.e., temperature, hydraulic retention time (HRT), $N-NH_4^+/TN$ ratio and current density in the range of 16–32 °C, 6–12 h, 25–75%, and 2–10 A/m^2, respectively, at three levels on the bio–electrochemical reactor performance were investigated during the bio–electrochemical reactor operation. The Face Center Cube (FCC) of response surface methodology (RSM) was used for design of experiments and model of obtained data. The ALBER achieved the maximum TN removal of 73% (146 mg/l) using external voltage and zeolite/plastic medium at temperature of 16 °C, HRT of 6 h, current density of 2 A/m^2 and $N-NH_4^+/TN$ ratio of 75%. The results indicated that shortening the HRT from 12 to 6 h, reducing the temperature from 32 °C to 24 °C, increasing the current density from 2 to 6 A/m^2 and the reduction of nitrate concentration caused an increase in the TN removal. The results indicated that the performance of air-lift bio-electrochemical for nitrogen removal could be attributed to autotrophic denitrification (AD) and simultaneous nitrification/denitrification (SND). The research findings suggest that the ALBER should be further studied for potential use in treating industrial wastewater at low temperatures.

1. Introduction

Nitrification process is the bio-oxidation of NH_4^+ (ammonium) to NO_2^- (nitrite) and further to NO_3^- (nitrate). Denitrification process is the bio-reduction of nitrite and nitrate to nitrous oxide and nitrogen (Guerrero et al., 2016). Simultaneous nitrification and denitrification (SND) process is a microbial treatment process for total nitrogen removal from sewage. SND process integrates the activities of denitrifying and nitrifying bacteria in one tank (Guerrero et al., 2016). Some disadvantages of the SND process are high energy costs (Cecen, 1996), slow growth rate of the bacteria involved in the SND process, and high sensibility to low concentrations of nitrates, nitrites and sulfurs (Zubair et al., 2020). That is the reason why the research for improvements of the existing processes has led to the suggestion of using zeolite as an exchanger for ammonium ion and a microbial support medium (Collison and Grismer, 2018; Yapsakli et al., 2017). During the last two decades, numerous researches have also been performed about the effect of zeolite on anammox (Collison and Grismer, 2018; Yapsakli et al., 2017), partial nitrification (Zhang et al., 2022; Chen et al., 2018; Chen et al., 2023), nitrification process (Zhang et al., 2022), and SND (Wen et al., 2023). In general, all these studies showed that the zeolite improved the performance of anammox, partial nitrification and nitrification processes, with an increase in ammonium removal from 6% to 1100% (Montalvo et al., 2020). Zeolite in nitrification process has been used as biofilm carrier and ammonium ion (NH_4^+) exchanger (Montalvo et al., 2020). Studies have shown that nitrifying bacteria can regenerate cation exchange capacity of the zeolite (Montalvo et al., 2020).

In the past years, several researches have been done to enhance denitrification process for wastewater treatment with a low chemical oxygen demand (COD) to nitrogen (COD/N) ratio, which commonly

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* Corresponding author. Department of Applied Chemistry, Faculty of Chemistry, Razi University, Kermanshah, Iran.
E-mail addresses: zinatizadeh@razi.ac.ir, zinatizadeh@gmail.com (A.A. Zinatizadeh).

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include the use of cheap alternative carbon sources (Hang et al., 2016), and incorporating heterotrophic and autotrophic denitrification technology (Park et al., 2015). Researchers have struggled to accelerate the biological treatment by detection ways to supply better contact between the microorganisms and the nitrate (Moosavi et al., 2004; Ghafari et al., 2008). There has been focus on biofilm methods as they are appropriate for denitrification process as a result of synergistic relationship among various microorganisms in the aggregate. Fixed-bed reactors are more successful compared to fluidized bed and suspended reactors due to smaller reactor volumes, simple operation, capacity to handle shock loading, less sludge, and greater stability (Moosavi et al., 2004). Other methods namely rotating biological contactors (RBC), packed bed, sequencing batch biofilm (Song et al., 2021), bio-electrochemical system (BES) (Moosavi et al., 2004; Ghafari et al., 2008), and membrane biofilm techniques (Visvanathan, 2019) have also been studied.

The BES is a hybrid system that combines both electrochemical and biological processes for wastewater treatment with the energy generation. The advantages of bio-electrochemical system such as low investment costs, capacity for wastewater treatment with a low C/N ratio, low need to add reduced organic compounds, and use of a small reactor create a promising method to integrate with other biological processes to transcend some of the disadvantages of the biological process (organic residues and slow process) (Srikanth et al., 2018; Sun et al., 2020).

In the past years, the BES has been extensively used for wastewater treatment was regarded as a hopeful nitrogen removal process (Kelly and He, 2014; Zhang et al., 2013). The electron is capable to be transferred between the electrode and bacteria by nanowire surrounding the exoelectrogenic bacteria in the BES system. In the study carry outed by application of a bio-electrode, the denitrification rate in the bio-electrode was 76% higher than typical biofilm (Jiang et al., 2018). Cathodic exoelectrogens in the bio-cathode take electrons from the cathode surface, and afterwards electrons transfer to reducible substances such as nitrite (Wang et al., 2013). The ammonia removal process in the BES can be indicated as follows (Liang et al., 2019; Yang et al., 2019):

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+ \tag{Eq. 1}$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \tag{Eq. 2}$$

$$NO_2^- + 3e^- + 4H^+ \rightarrow 0.5N_2 + 2H_2O \tag{Eq. 3}$$

$$NO_3^- + 2e^- + 2H^+ \rightarrow NO_2^- + H_2O \tag{Eq. 4}$$

$$1.5CH_3COO^- + 4NO_2^- + 0.5H_2O \rightarrow 2N_2 + 5.5\ OH^- + 3CO_2 \tag{Eq. 5}$$

$$2.5CH_3COO^- + 4NO_3^- \rightarrow 2N_2 + 6.5\ OH^- + 5CO_2 + 0.5H_2O \tag{Eq. 6}$$

In nitrification process, the nitrifying bacteria contribute to the oxidation of ammonia to nitrite (Eq. (1)) and further to nitrate (Eq. (2)). Denitrification can occur through autotrophic or heterotrophic pathways, depends on the bacteria and the compounds that are accessible in the ecosystem (Vidal et al., 2002). In bio-electrochemical systems with a low C/N ratio, autotrophic bacteria can be overcome and responsible for the nitrogen compounds removal with electrode (cathode) as the electron donor; while for wastewaters with a high C/N ratio, both autotrophic and heterotrophic bacteria coexist and together eliminate nitrogen compounds (Huang et al., 2013). In bio-electrochemical denitrification, autotrophic denitrifying bacteria are capable of electron-accepting from a cathode electrode (Gregory et al., 2004). On the cathode, autotrophic denitrification process is a type of respiratory process including several consecutive reductions (Eq. (3) & (4)) (Liang et al., 2019). According to previous studies, Eq. (3) was the dominant reaction involved (Liang et al., 2019). The denitrifying heterotrophic bacteria use the oxygen present in the nitrite (NO_2^-) and nitrate (NO_3^-) to oxidize the acetate (CH_3COO^-) (Eqs. (5) and (6)) (Nouri and Zinatizadeh, 2018; Fu et al., 2009).

This paper aims to improve nitrogen removal efficiency in low C/N ratio wastewaters by designing an innovative air-lift bio-electrochemical reactor (ALBER). The development of such bio-electrochemical reactors could provide a cost-effective and efficient system. This study utilized the ALBER to explore the potential of integrating zeolite and airlift technology in a BES for nitrogen removal. Zeolite was employed as a substrate to enhance the rate of nitrification at low temperatures. Airlift technology was employed to increase reactor volume yield and reduce treatment costs. To enhance the removal of total nitrogen (TN), an external electrical current was applied. This study aimed to evaluate the impact of four independent factors, namely temperature, hydraulic retention time (HRT), $N - NH_4^+/TN$ ratio, and current density, on the performance of CNP removal in a bio-electrochemical reactor.

2. Materials and methods

2.1. CharacteristicsF of inoculum and synthetic wastewater

The bioreactor was inoculated with 2 L of activated sludge taken from an industrial wastewater treatment plant in Kermanshah, Iran. The influent synthetic wastewater was freshly prepared daily with potassium hydrogen phosphate, ammonium chloride, sodium nitrate and sodium acetate as phosphorus, NH_4^+/NO_3^- and dissolved organic carbon sources, respectively. To adjust the wastewater conductivity (μ), NaCl solution (0.05% w/w) was used. The wastewater conductivity (μ) in all experiments was adjusted to be about 3.7 mS/cm. Sodium bicarbonate was added for providing alkalinity. The characteristic of synthetic wastewater is shown in Table 1.

2.2. Reactor configuration

The schematic diagram of the air-lift bio-electrochemical reactor (ALBER) is shown in Fig. 1. The air-lift bio-electrochemical reactor consisted of an air-lift bioreactor and a settling tank with volumes of 4.9 and 3.2 L, respectively, with the total volume of bio-electrochemical reactor of 8.1 L. The liquid height in the ALBER was equal to 37 cm. The internal diameter of the outer column and the inner column were 15, and 5.5 cm, respectively. The aeration diffuser was set at the bottom of the reactor. HRT was computed based on 4.9 L of feeding volume. Graphite sheets (2500 cm^2) were pretreated first and then used as cathode (Chen et al., 2015). The electrode (graphite sheet), bio-cathode, was placed on the outer surface of the inner column and inner surface of the outer column. The zeolite-plastic media, in hollow cylindrical shape with a thickness of 2 cm, was held inside the inner column as shown in Fig. 1. The ALBER was aerated with an air blower. A peristaltic pump was used to feed and a centrifuge pump was used in order to returning the effluent sludge to the ALBER. In the anoxic zone, four fine mixers were used to achieve an adequate mixing.

2.3. Reactor operation

The synthetic wastewater was continuously entered into the system from the bottom of the bioreactor. Excess sludge was intermittently drawn off from the bottom of the settling tank. The bio–electrochemical reactor at a constant air flow rate of 4 l/min was continuously aerated

Table 1
Characteristics of synthetic wastewater used.

Parameters	Unit	Amount
COD	(mg/l)	500
TN	(mg/l)	200
TP	(mg/l)	10
NaCl	(mg/l)	500
$NaHCO_3$	(mg/l)	500
pH	–	7–7.5

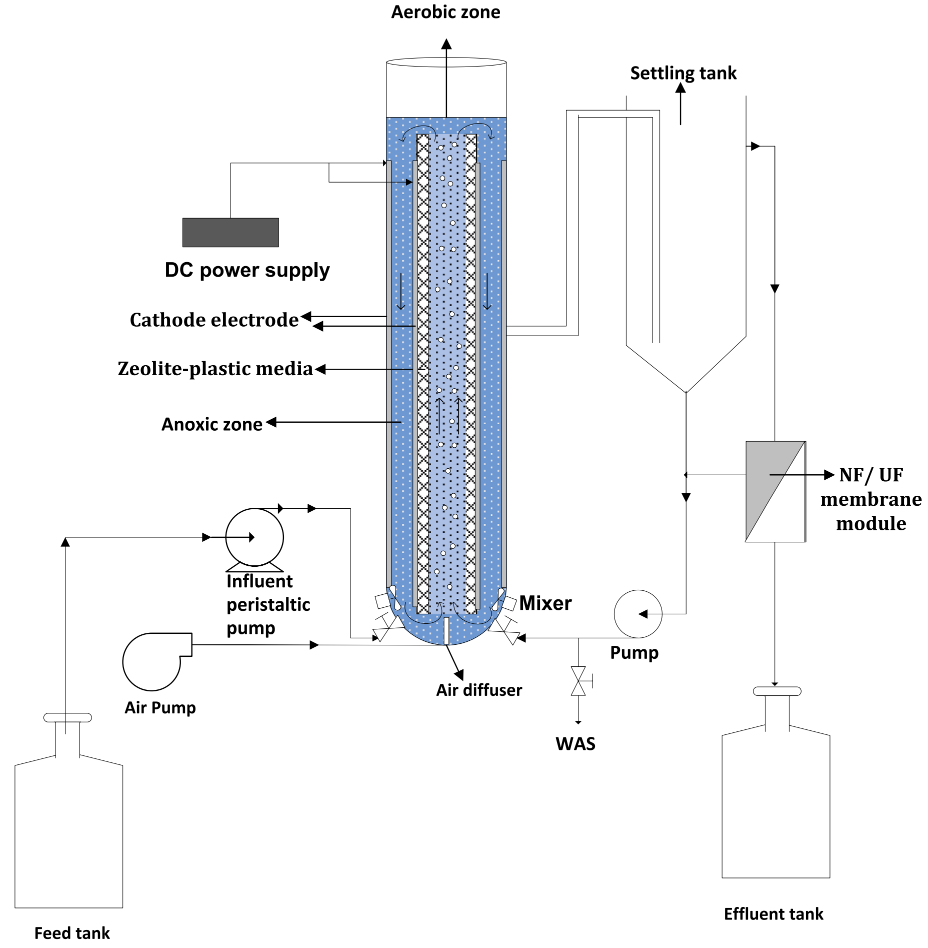


Fig. 1. The schematic diagram of the air-lift bio-electrochemical reactor with an externally applied voltage.

with an air blower. The bio–electrochemical reactor temperature was adjusted by an aquarium heater. During the bio–electrochemical reactor operation, the mixers were periodically working. The ALBER was continuously operated for 8 months in a continuous mode with an external applied voltage of 0.6 V. In the bio-electrochemical reactor start-up, the first stage, the bio-electrochemical reactor was operated at temperature, hydraulic retention time (HRT), $N-NH_4^+/TN$ ratio and current density of 25 °C, 9 h, 50% and 6 A/m^2, respectively. The first stage was continued to 2 months for the compatibility of the sludge to the wastewater and biofilm formation on the cathode electrode and zeolite/plastic medium. The effect of four independent variables (i.e., temperature, HRT, $N-NH_4^+/TN$ ratio and current density in the range of 16–32 °C, 6–12 h, 25–75%, and 2–10 A/m^2, respectively at three levels) were investigated on the ALBER performance during the system operation (180 days). The range of variables was selected based on the results of preliminary tests and relevant literatures (Yapsakli et al., 2017; Zhang et al., 2022). The BES performance was assessed by monitoring the process and quality parameters. Each experiment was continued for 4 HRT after achieving a stable performance to confirm the steady state condition. The responses are average of several times measurements during 4 HRTs to ensure the steady state condition.

2.4. Experimental design and mathematical modeling

To study the effect of the specified variables at three levels on the ALBER performance, the system was operated under experimental conditions which were designed by Design Expert Software (DOE) (Stat-Ease Inc., version 7.0). Table 2 represents the actual and coded levels of the variables and experimental conditions. In the basis of the face center cube (FCC) of response surface methodology (RSM) 30 experiments (including one center point, 16 factorial points, eight axial points, and five replications of the center point) were designed. Based on four independent variables, thirty experimental runs were designed by FCC according to Eq. (7), comprising sixteen factorial runs (n^2), eight axial runs (2n), and six replicated center runs (n_c). The center point provides a wonderful estimation of data reproducibility and experimental error.

$$N = n^2 + 2n + n_c = 16 + 8 + 6 = 30 \quad \text{(Eq. 7)}$$

where, N is the total number of experimental runs, n is the number of independent variables, and n_c is number of center runs.

The polynomial model coefficients are calculated using Eq. (8).

$$Y = ß_0 + ß_i X_i + ß_j X_j + ß_{ii} X_i^2 + ß_{jj} X_j^2 + ß_{ij} X_i X_J + \dots \quad \text{(Eq. 8)}$$

The coefficients ß, i, and j are the regression, linear, and quadratic coefficients, respectively. To measure the effectiveness of the model terms, P value with 95% assurance level is considered (Singh et al., 2019).

2.5. Statistical analysis

Since COD, TN and TP removal are the important criteria to evaluate the bioreactor performance for CNP from wastewaters, these responses were modeled using DOE. The regression equation derived from various operating variables was used to predict the performance of ALBER. For each variable, the polynomial equations in the design space are depend on coded values (−1, 0, +1) to predict the responses. The obtained results were evaluated with various statistical parameters such as coeffi-

Table 2
Experimental range, levels and conditions of the independent variables.

Variables	Range and levels		
	−1	0	+1
HRT, h	6	9	12
Temperature, °C	16	24	32
Current density, $\frac{A}{m^2}$	2	6	10
$\frac{N-NH_4^+}{TN}$ ratio,%	25	50	75

Experimental conditions

Run No.	**Factor 1 (A) HRT, (h)**	**Factor 2 (B) current density,($\frac{A}{m^2}$)**	**Factor 3 (C) temperature, (°C)**	**Factor 4 (D) $\frac{N-NH_4^+}{TN}$ ratio, (%)**
1	9	10	24	50
2	6	10	32	75
3	9	2	24	50
4	9	6	24	75
5	6	10	16	25
6	6	10	16	75
7	12	2	16	25
8	9	6	24	50
9	9	6	24	50
10	9	6	24	50
11	9	6	24	25
12	12	2	32	25
13	12	2	16	75
14	6	2	32	75
15	12	6	24	50
16	9	6	16	50
17	6	2	16	75
18	6	2	16	25
19	6	6	24	50
20	6	10	32	25
21	12	10	32	25
22	12	10	32	75
23	6	6	24	50
24	9	6	32	50
25	9	6	24	50
26	9	6	24	50
27	12	10	16	25
28	12	2	32	75
29	6	2	32	25
30	12	10	16	75

cient of variance (CV), standard deviation (SD), adequate precision (AP), adjusted determination of coefficient (Adj. R^2), determination coefficient (R^2), degree of freedom (DF), F-value, and p-value to investigate the statistical compatibility of the model terms. CV illustrates the precision and experimental reliability (Dawood and Li, 2013). The agreement level of the model data with experimental data was assessed by predicted vs. actual values plot. The three dimensional (3D) RSM plot were used to determine the optimal condition of responses by the diverse independent variables. The adequate precision (AP) higher than 4 illustrates sufficient model conception (Mason and Hess, 2003; Neubauer, 2012). The adequate precision is utilized for comparing the range of the predicted amounts to average predicted error at design point (Mason and Hess, 2003; Neubauer, 2012). The R squared (R^2) coefficient calculates the proportion of total variation in the response which illustrates the ratio of the residual sum of squares (RSS) to the sum of squared total (SST) and varies from 0 to 1. For desirable and satisfactory quadratic model equation of the R-squared value close to 1 is chiefly preferred (Birjandi et al., 2013). For each response, polynomial models were selected depend on the appropriateness with real data, considering R-squared (R^2) as the important criterion. P-value, probability value, describes the statistical fitness of the modified model whereas, f-value ascertain the ratio of two variances in responses. For a model term to be significant, probability value for lack of fit test with a confidence level of 95 % should be higher than 0.05 (typically $\geq$0.05), and probability value for the model terms should be less than 0.05 (typically $\leq$0.05) (Singh et al., 2019). In order to fit the obtained data, different forms of polynomial models were applied. The response data by default in the DOE were analyzed. Hydraulic retention time (HRT), current density, temperature, and $N-NH_4^+/TN$ ratio in the regression equations are demonstrated with A, B, C and D, respectively.

2.6. *Chemical analysis*

Concentrations of mixed liquor suspended solids (MLSS), chemical oxygen demand (COD), NH_4–N, nitrite, nitrate, and TP were determined by using standard methods (APHA, 2005). COD was determined following colorimetric method with closed reflux. Concentrations of TP and nitrite were determined following colorimetric method. Spectrophotometer (DR 5000, Hach, Jenway, USA) was utilized to determine the absorbance of COD, nitrite and TP samples. NH_4–N was measured by total Kjeldahl nitrogen (TKN) meter, (Gerhardt model, Vapodest 10, Germany). The pH was determined by a pH meter, (model pH 211, Hanna, Italy). Turbidity was determined by a turbidity meter, (model 2100 P, Hach Co., USA). The conductivity was determined by a conductivity meter, (model 4320, Jenway., England). Air flow rate was measured by an air flow meter model 101325Pa.

3. Results and discussion

3.1. *Statistical analysis*

For the three responses (COD, TN and TP removal), the results of the analysis of variance (ANOVA) are shown in Table 4. As different responses were investigated, various forms of polynomial models for data fitting were used (Table 4). The regression equations achieved are displayed in Table 4. The model terms in the regression equations are after removal of nonsignificant variables and the interaction between variables. As shown, all model terms were significant at 99% confidence level. From the data analysis, the model terms were very significant with very small probability values (<0.0001). The lack-of-fit F-tests were used for the fitted models' adequacy. The lack of Fit F-value was not significant ($\geq$0.05). Adequate precision for all models was found to be desirable (>4). Correspondingly, low values of the CV (coefficient of variation) indicated favorable reliability of the experiments and precision. Detailed analysis of the models in the following sections is presented.

3.2. *Process analysis and modeling*

Performance of the ALBER, as a multivariable system, was analyzed and modeled by altering 4 independent variables and monitoring 8 inter related responses. Table 3 represents the obtained results and experimental conditions. TN removal efficiency, effluent ammonium, effluent nitrite, effluent nitrate, TP removal efficiency, COD removal efficiency, and effluent turbidity were calculated or measured as response in the ALBER.

3.2.1. *COD removal*

In Table 4, the ANOVA values are displayed for COD removal efficiency. From the ANOVA results, A (HRT), B (current density), D ($N-NH_4^+/TN$ ratio), AD, CD, A^2, C^2, ABC, ACD, and A^2 C were significant model terms explained COD removal efficiency as a function of the variables. Another model terms in the regression equations were not significant (with a large p-value (>0.05)). The values of R^2 and adjusted R^2 for COD removal are 0.8939, and 0.8380, respectively, which indicated that the unfailing relation between the independent and dependent variables. As can be seen in Table 4, the parameters A, B, A^2, and A^2 C had a positive influence, while, D, AD, CD, C^2, ABC, and ACD had a negative influence on the response. The adequate precision calculated for COD removal was found to be 15.427, which indicated that the

Table 3
Experimental conditions and results.

Run No.	Variables				Responses							
	Factor 1 (A) HRT, (h)	Factor 2 (B) current density, ($\frac{A}{m^2}$)	Factor 3 (C) temperature, (°C)	Factor 4 (D) $\frac{N-NH_4^+}{TN}$ ratio,(%)	COD removal, (%)	TN removal, (%)	Effluent $N-NH_4^+$, (mg/l)	Effluent N–NO_3, (mg/l)	Effluent N–NO_2, (mg/l)	Denitrification rate, (g/l.d)	TP removal, (%)	Effluent Turbidity, NTU))
1	9	10	24	50	69.2	36.16	25	81	21.68	0.0265	50.5	15
2	6	10	32	75	69.2	49.69	62	30	8.62	0.2953	20.6	16
3	9	2	24	50	64.6	24.6	24	109	17.8	0.0351	29	7
4	9	6	24	75	69.2	53.5	24	48	21	0.1813	31	6
5	6	10	16	25	63.8	45.045	0	92	17.91	0.2418	22.9	11
6	6	10	16	75	62.6	56.48	49	23	15.04	0.3387	23.5	9
7	12	2	16	25	67	17.455	14	140.5	10.59	0.0511	7.4	16
8	9	6	24	50	71.1	33.25	29	82	22.5	0.0673	33	6
9	9	6	24	50	71.5	28.3	35	87	21.4	0.0323	35	7
10	9	6	24	50	67.5	42.5	16	76	23	0.1289	29	7
11	9	6	24	25	72.6	26	3	137	8	0.1311	2.2	11
12	12	2	32	25	77.2	19.445	7	147	7.11	0.0563	8.9	14
13	12	2	16	75	65	20.41	99	40	20.18	0.0678	5.7	18
14	6	2	32	75	62.6	46.6	74	23	9.8	0.2896	17.2	13
15	12	6	24	50	73.2	49.69	2	86	12.62	0.1698	12	12
16	9	6	16	50	63.2	19.695	44	95	21.61	0.0681	11.4	8
17	6	2	16	75	61.8	73.29	23	15	15.42	0.4728	23.5	9
18	6	2	16	25	63.6	18.765	4	146	16.47	0.0207	23.5	11
19	9	6	24	50	70.4	39.5	20	83	18	0.1078	32	6
20	6	10	32	25	70	24.64	2	133	15.72	0.1217	15.5	16
21	12	10	32	25	77.8	22.81	1	140	13.38	0.0761	6.2	22
22	12	10	32	75	64.6	39.64	68	41	11.72	0.1389	8	20
23	6	6	24	50	68.6	60.165	0	41	38.67	0.2397	48.6	14
24	9	6	32	50	62.6	34.1	7	91	23.95	0.1984	2.9	12
25	9	6	24	50	68.4	36.8	22	84	20.4	0.0966	30	5
26	9	6	24	50	69.1	39.8	19	78	23.4	0.1123	31	6
27	12	10	16	25	67.8	10.755	16	151	11.49	0.0206	9.26	17
28	12	2	32	75	65.2	29.78	75	51	14.44	0.0783	16	16
29	6	2	32	25	63.6	25.5	0	137	12	0.1331	14.3	15
30	12	10	16	75	66.6	35.415	69	41.5	18.67	0.1373	1.8	14

Table 4
ANOVA results for the equations of the Design Expert 7.0.0 for studied responses.

Response	Modified Equations with significant terms	Probability	R^2	Adj.R^2	Adeq. precision	S.D	CV	PRESS	Probability for lack of fit
COD removal	$+69.16+2.14\,A+1.17\,B-2.03\,D-1.47\,AD-1.30\,CD+2.75\,A^2-5.25\,C^2-0.90\,ABC-1.45\,ACD+2.00\,A^2C$	<0.0001	0.8939	0.8380	15.427	1.69	2.5	131.33	0.4593
TN removal	$+37.92-8.60\,A+10.80\,D+4.67\,AC-3.58\,AD+16.09\,A^2-8.46\,B^2-11.94\,C^2+4.21\,ABD$	<0.0001	0.8634	0.8113	16.321	6.33	17.91	1892.73	0.3024
TP removal	$+29.73-18.30\,A+10.75\,B+14.40\,D+2.55\,AC+13.21\,B^2-19.39\,C^2-9.94\,D^2-11.30\,A^2B-13.88\,A^2D+12.19\,AB^2$	<0.0001	0.9486	0.9216	22.533	3.65	18.19	3209.97	0.0816

A: HRT, B: current density, C: temperature, D: $\frac{N-NH_4^+}{TN}$ ratio, PRESS: predicted residual error sum of squares, CV: coefficient of variation, SD: standard deviation, Adeq. Precision: Adequate precision, Adj. R2: adjusted R2, R2: determination coefficient.

modified model can be applied within a precise array for designing. The CV for COD removal was found to be 2.5, which illustrated that reliability and precision of the model term was very good. The statistical analysis for COD removal confirmed that the p-value (probability value) was less than 0.0001 and F-value was noted to be 16.00. Results indicate that the modified model for COD removal is significant and reliable.

The effects of temperature, HRT, $N-NH_4^+/TN$ ratio and current density on the COD removal efficiency in the ALBER are displayed in Fig. 2. It is clearly shown that decrease in $N-NH_4^+/TN$ ratio from 75 to 25 % (increase in $N-NO_3^-/TN$ ratio from 25 to 75 %) increased the removal of COD because the denitrifiers and bacteria consuming COD are heterotrophic. From the Figure, increase in current density from 2 to 10 A/m^2 had a positive influence on the COD removal. From the Figure, a decreasing trend in the response was observed by decreasing HRT in the design space. From the Figure, The COD removal first increased with increase in the temperature up to 24 °C, however after temperature 24 °C, COD removal decreased with increase in the temperature. The effect of temperature on removal efficiency of COD was in compliance with the research carried out by Li and Chen (2018). The maximum value of COD removal efficiency was 77.8% when the temperature, HRT, $N-NH_4^+/TN$ ratio and current density were 32 °C, 12 h, 25%, and 10 A/m^2, respectively. Recent studies have indicated that activity of electrochemically active biofilms, better cell growth rate (Liu et al., 2011) and the dominance of electrogenic bacteria, such as Geobacter sulfurreducens, (Trinh et al., 2009) at the ideal temperatures near 30 °C.

3.2.2. Nitrogen removal

As can be seen in Table 4, A, D, AC, AD, A^2, B^2, C^2, and ABD were significant model terms explained TN removal efficiency. Another model terms in the regression equations were not significant (with a

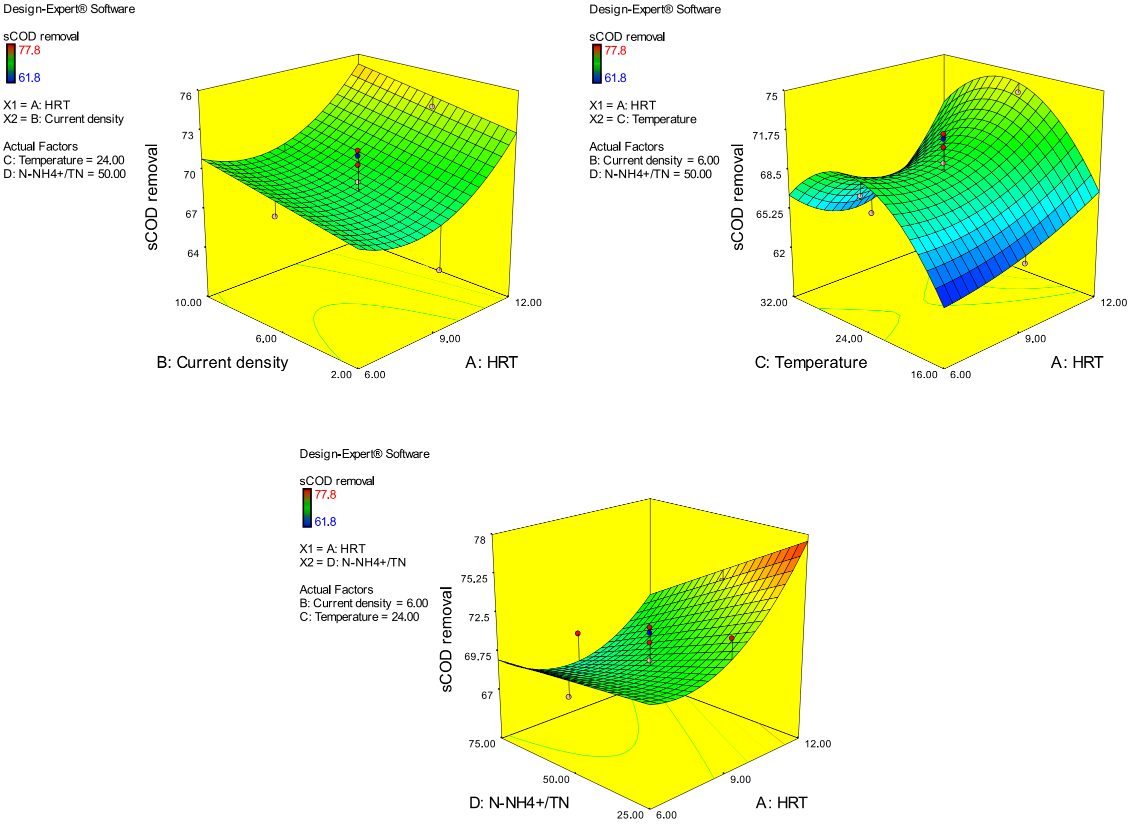


Fig. 2. Response surface plots for the removal efficiency of COD.

large p-value (>0.05)). The values of R^2 and adjusted R^2 for TN removal are 0.8634, and 0.8113, respectively, which, indicated that a reliable relation between the independent and dependent variables. As can be seen in Table 4, the parameters D, AC, A^2, and ABD had a positive influence, while A, AD, B^2, and C^2 had a negative impact on the response. The statistical analysis for TN removal confirmed that the p-value was less than 0.0001 and F-value was noted to be 16.59. The adequate precision calculated for TN removal was determined to be 16.321, indicating that the modified model can be effectively used for design purposes. Results indicate that the modified model for TN removal is well suited.

The effects of the independent variables on the removal efficiency of TN in the ALBER are displayed in Fig. 3. It is clearly shown in Fig. 3 that shortening the HRT from 12 h to 6 h and reducing the temperature from 32 °C to 24 °C caused an increase in the TN removal, which related to increased F/M ratio that led to more growth of bacteria (Song et al., 2009). It should be noted that an increase in the feed to microorganism ratio resulting from heightened feed loading rate, leads to an elevation in the microbial growth rate. However, the biomass concentration within the reactor, which experiences intermittent discharge, remains constant. The observed relationship between temperature and the removal efficiency of total nitrogen (TN) aligns with findings reported in other investigations. According to the findings of Rusmana I., there was a positive correlation between temperature and the maximum specific growth rate of denitrifying bacteria. The researchers observed that the highest growth rate was achieved at a temperature of 26 °C. This observation helps to explain the limited impact of temperature changes on denitrification performance when the temperature rises from 26 °C to 32 °C. In a previous study conducted by Meng et al. (2019), it was shown that dropping the temperature from 35 °C to 27 °C in a low carbon-to-nitrogen (C/N) ratio environment resulted in an enhanced removal of total nitrogen (TN). In our investigation, we observed that when the temperature climbed from 24 °C to 32 °C, the concentration of nitrate increased while the removal of total nitrogen reduced. These observations were made while keeping the current density, ($N-NH_4^+/TN$ ratio, and hydraulic retention time (HRT) unchanged in Run No. 8 & 24 (Table 3). The denitrifying bacteria, similar to BOD oxidizing bacteria, exhibit heterotrophic characteristics. Therefore, the observed phenomenon of denitrifying bacteria being influenced by temperature may not just be attributed to temperature alone, but also to the potential restriction in the amount of available carbon supply. Based on the aforementioned observations, it can be posited that the F/M ratio has an impact on the denitrification process via the augmentation of carbon source enrichment. As can be seen in Fig. 3, TN removal was increased with the current density from 2 to 6 A/m^2, indicating the dominate reactions of Eqs. (5) and (6) (Liang et al., 2019), however after current density 6 A/m^2, TN removal decreased with increase in density current, which could be related to decreased thickness of the biofilm on the bio-electrode surface. The results indicated a good balance between biofilm thickness, current density and TN removal at current density 6 A/m^2. From the Figure, TN removal increased with increasing the $N-NH_4^+/TN$ ratio from 25 to 75 %, which could be related to Eqs. (3) and (5). The maximum TN removal (73.29%) was obtained a temperature of 16 °C, an HRT of 6 h, a current density of 2 A/m^2 and a $N-NH_4^+/TN$ ratio of 75%. By the same token, the minimum TN removal (10.75%) was obtained a temperature of 16 °C, an HRT of 12 h, a current density of 10 A/m^2 and a $N-NH_4^+/TN$ ratio of 25%. To evaluate nitrification and denitrification processes, the fate of nitrogen contents was determined at different operating conditions and the results are shown in Fig. 4. From the Figure, TN removal with increasing the concentration of influent nitrate from 50 to 150 mg/l was decreased, demonstrating low

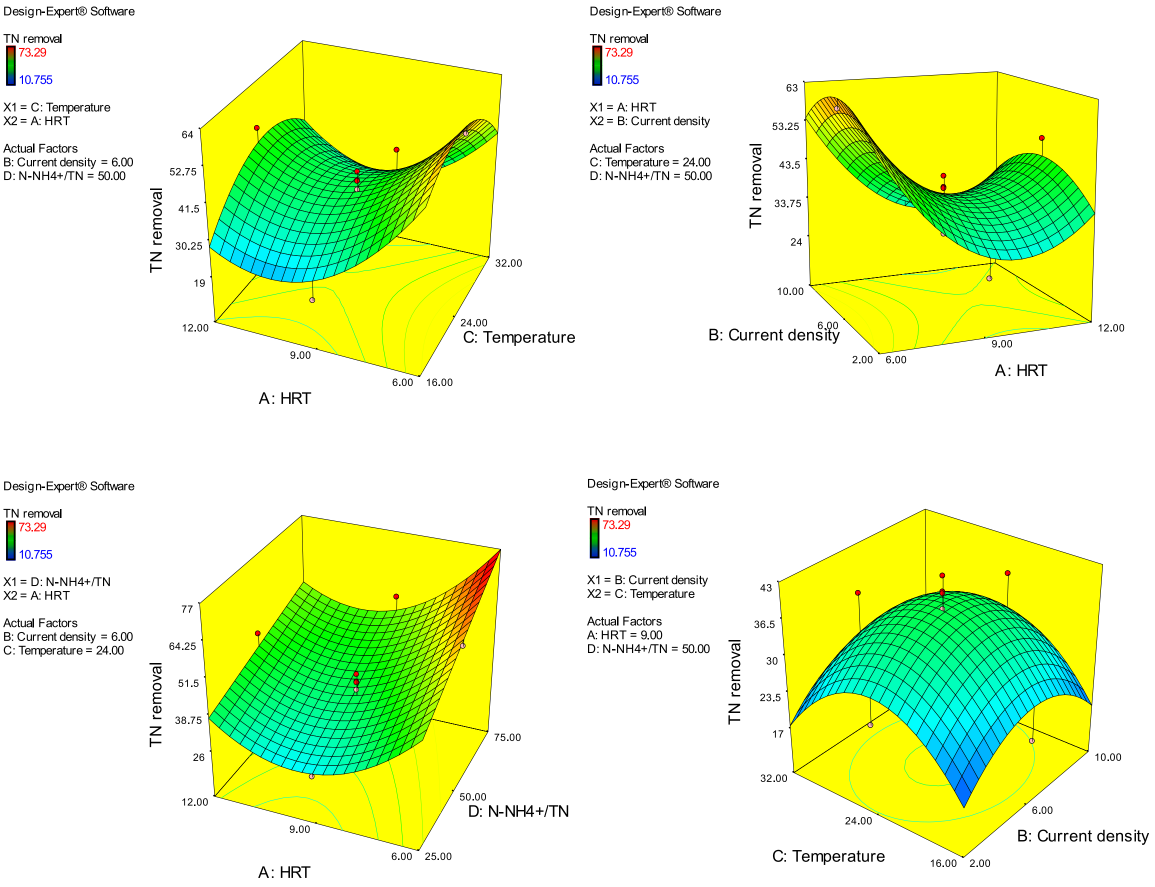
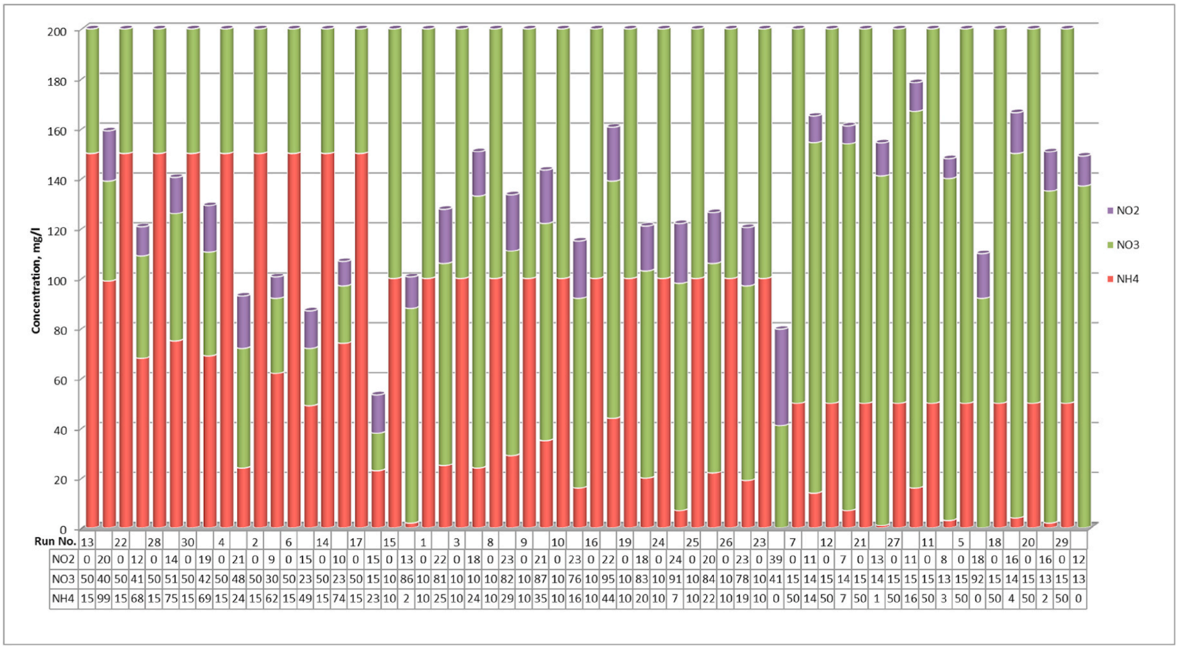


Fig. 3. Response surface plots for the removal efficiency of TN.



Run No.	13		22		28		30		4		2		6		14		17		15		1		3		8		9		10		16		19		24		25		26		23		7		12		21		27		11		5		18		20		29	
NO2	0	20	0	12	0	14	0	19	0	21	0	9	0	15	0	10	0	15	0	13	0	22	0	18	0	23	0	21	0	23	0	22	0	18	0	24	0	20	0	23	0	39	0	11	0	7	0	13	0	11	0	8	0	18	0	16	0	16	0	12
NO3	50	40	50	41	50	51	50	42	50	48	50	30	50	23	50	23	50	15	10	86	10	81	10	10	10	82	10	87	10	76	10	95	10	83	10	91	10	84	10	78	10	41	15	14	15	14	15	14	15	15	15	13	15	92	15	14	15	13	15	13
NH4	15	99	15	68	15	75	15	69	15	24	15	62	15	49	15	74	15	23	10	2	10	25	10	24	10	29	10	35	10	16	10	44	10	20	10	7	10	22	10	19	10	0	50	14	50	7	50	1	50	16	50	3	50	0	50	4	50	2	50	0

Fig. 4. Nitrogen fractionation in the influent and effluent at various operating conditions studied.

capacity of the ALBER for denitrification process. The range of changes in the concentration of effluent nitrite was from 7.11 to 38.67 mg/l that indicating high capacity of the ALBER for partial nitrification processes.

The maximum value of effluent nitrite concentration (38.67 mg/l) was obtained a temperature of 24 °C, an HRT of 6 h, a current density of 6 A/m^2 and a $N-NH_4^+/TN$ ratio of 50%. By the same token, the minimum value of effluent nitrite concentration (7.11 mg/l) was obtained a temperature of 32 °C, an HRT of 12 h, a current density of 2 A/ m^2 and $N-NH_4^+/TN$ ratio of 25%. The accumulation of nitrite in the ALBER was in compliance with the research carried out by Liang and her coworkers (2019). However, the accumulation of nitrite is a challenge for bio-electrochemical denitrification process. The results indicated that the performance of ALBER for nitrogen removal could be attributed to autotrophic denitrification (AD) and simultaneous nitrification/denitrification (SND).

3.2.3. *Denitrification rate*

In the present study, denitrification rates were calculated at the operating conditions studied, that the data illustrate in Fig. 5. As can be seen in Table 3, the range of changes in the denitrification rates was from 0.0206 to 0.4728 g/l.d. The maximum value of denitrification rate (0.4728 gN/l.d) was achieved a temperature of 16 °C, an HRT of 6 h, a current density of 2 $A/m^2$and a$N-NH_4^+/TN$ ratio of 75% (Run No. 17). In a similar research work, Ding et al. (2018) reported denitrification rate of bio-cathode denitrification was around 0.102 (g N) / (l.d) with sodium acetate as carbon source. At different temperatures, the denitrification rate increased with increasing the $N-NH_4^+/TN$ ratio from 25 to 75 % at constant values of current density and HRT (Run Nos. 2&20, 4&11, 5&6, 7&13, 12&28, 17&18, 14&29, 21&22 and 27&30). This might be due to Eq. (3) as the dominant reaction involved (Liang et al., 2019).

3.2.4. *Phosphorus removal*

ANOVA for the TP removal in Table 4 showed that A, B, D, AC, B^2, C^2, D^2, A^2B, A^2D, and AB^2 were significant model terms. Other model terms were in the regression equations not significant (with a large p-value (>0.05)). The values of R^2 and adjusted R^2 for TP removal are 0.9486, and 0.9216, respectively, which indicated that the unfailing relation between the independent and dependent variables. As can be seen in Table 4, the parameters B, D, AC, B^2, and AB^2 had a positive effect, while, A, C^2, D^2, A^2B, and A^2D showed a negative influence on the response. The statistical analysis for TP removal confirmed that the p-value (probability value) was less than 0.0001 and F-value was noted to be 35.09. The adequate precision calculated for TP removal was found to be 22.533, which indicated that the modified model can be utilized within a precise array for designing. Results indicate that the modified model for TP removal is significant and reliable. From the model presented in Table 4, the most effective factor for TP removal in the experimental conditions designed was determined to be HRT.

The effects of temperature, HRT, $N-NH_4^+/TN$ ratio and current density on the removal efficiency of TP in the ALBER are shown in Fig. 6. The results presented in Fig. 6 showed that a decrease in HRT from 12 to 6 h at temperature of 16–32 °C caused an increase in the TP removal, which could be related to increased cell growth rate (Nouri and Zinatizadeh, 2018). From the Figure, the TP removal increased with the increase of the $N-NH_4^+/TN$ ratio from 25 to 75 % and current density from 2 to 10 A/m^2. The maximum TP removal (50.5%) was achieved at a temperature of 24 °C, an HRT of 9 h, a current density of 10 A/m^2 and a $N-NH_4^+/TN$ ratio of 50%. By the same token, the minimum TP removal (1.8%) was obtained a temperature of 16 °C, an HRT of 12 h, a current density of 10 A/m^2 and $N-NH_4^+/TN$ ratio of 75%.

3.2.5. *Effluent turbidity*

Fig. 7 represents the effluent turbidity at various operating conditions studied. As can be seen in the Figure, the range of changes in the effluent turbidity was from 5 to 22 NTU. The minimum value of effluent turbidity (5 NTU) was obtained a temperature of 24 °C, an HRT of 9 h, and a current density of 6 A/m^2, at the $N-NH_4^+/TN$ ratio of 50% (Run No. 25). By the same token, the maximum value of effluent turbidity (22 NTU) obtained was a temperature of 32 °C, an HRT of 12 h, and a current density of 10 A/m^2, at the $N-NH_4^+/TN$ ratio of 25%. It was observed that high current density (10 A/m^2) also showed a negative influence on the effluent turbidity. The effluent turbidity increased as the hydraulic retention time (HRT) increased from 9 to 12 h, as observed in the Figure. This increase in turbidity could be attributed to the initiation of microbial granulation (Kapdan and Oztekin, 2006).

3.3. *A comparative review on bioreactors*

Table 5 compares different bioreactors removing CN with the ALBER. This comparison shows the ALBER had more capability for N removal relative to the other examined air-lift bioreactors (no. 1 and 2) (Jiang et al., 2013; Lin et al., 2013). Heterotrophic nitrifying/denitrifying air-cathode microbial fuel cell (no. 3) indicated good performance removing 436 and 131 mg/l of COD and TN, respectively, under HRT of 5 d with acetate as the feed where the influent COD/N ratio was 3.48 (Yang et al., 2019). Single-chamber microbial fuel cell (no. 4) has been operated with COD/N ratio 4.27. At this MFC 520 and 133 mg/l of COD and nitrogen removed, respectively, at an HRT of 8 d with acetate as the feed (Yang et al., 2018). In the dual-chamber MEC (no. 5), acetate was used as substrate and 1896 and 488 mg/l of COD and TN removed, respectively, at HRT of 2.5 d and the voltage of 0.8 V (Yang et al., 2017). In this study, the ALBER is capable to eliminate 146 mg/l of TN at a voltage of 0.6 V, an HRT of 6 h with COD/N ratio of 2.5. It is noted that HRT value for the ALBER is 6 h. The comparison indicates that the ALBER can be considered as a bioreactor for C/N removal from wastewater with low COD/N ratio which reduces the complication of operation, energy consumption, and required volume.

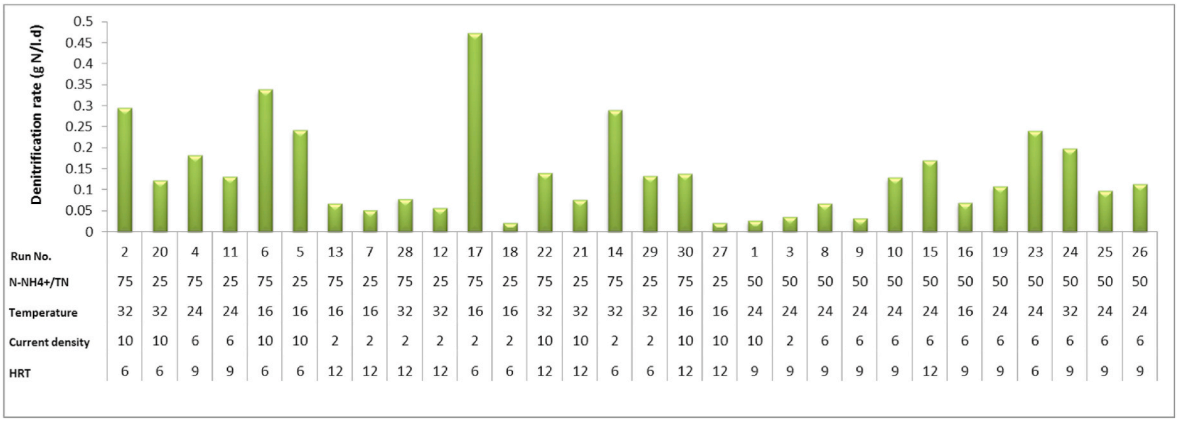


Fig. 5. Denitrification rates at various operating conditions studied.

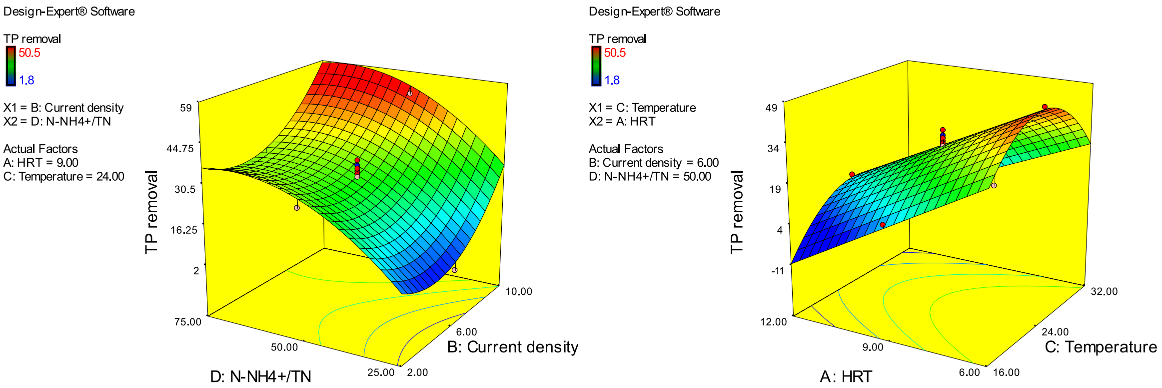
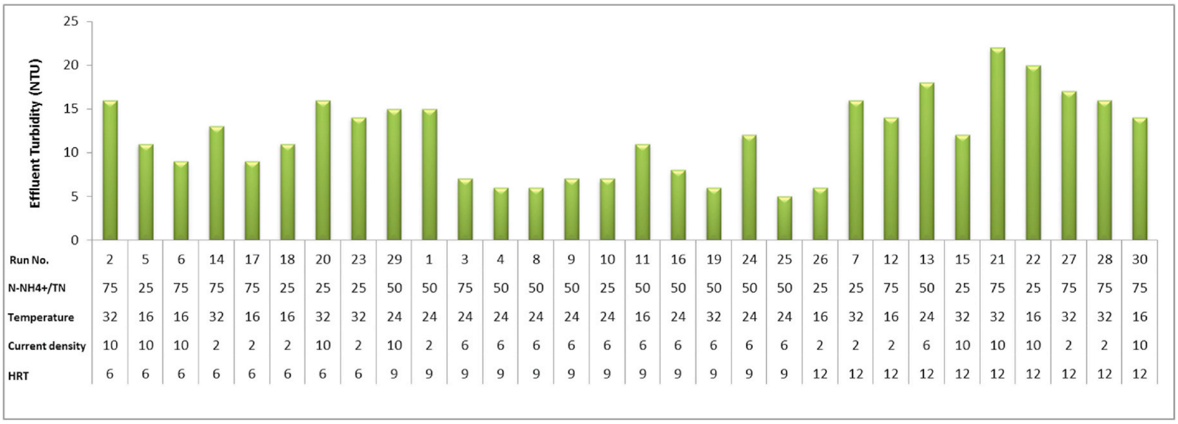


Fig. 6. Response surface plots for the removal efficiency of TP.



Run No.	2	5	6	14	17	18	20	23	29	1	3	4	8	9	10	11	16	19	24	25	26	7	12	13	15	21	22	27	28	30
N-NH4+/TN	75	25	75	75	75	25	25	25	50	50	75	50	50	50	25	50	50	50	50	50	25	25	75	50	25	75	25	75	75	75
Temperature	32	16	16	32	16	16	32	32	24	24	24	24	24	24	24	16	24	32	24	24	16	32	16	24	32	32	16	32	32	16
Current density	10	10	10	2	2	2	10	2	10	2	6	6	6	6	6	6	6	6	6	6	2	2	2	6	10	10	10	2	2	10
HRT	6	6	6	6	6	6	6	6	9	9	9	9	9	9	9	9	9	9	9	9	12	12	12	12	12	12	12	12	12	12

Fig. 7. Effluent turbidity at various operating conditions studied.

Table 5
Performance of different bioreactors.

No.	System	Removal Process	Feed	Influent COD, mg/l	Influent TN, mg/l	COD removed, mg/l	TN removed, mg/l	HRT/ Voltage
1	Airlift loop reactor under a limited filamentous bulking state	SND	Acetate	300	40	273	37	11 h
2	Airlift intermittent circulation membrane bioreactor	SND	Glucose	650	87	626	72	22 h
3	Heterotrophic nitrifying/denitrifying air-cathode microbial fuel cell	SND	Acetate	492	141	436	131	5 d
4	Single-chamber microbial fuel cell	SND- Aerobic denitrification	Acetate	573	134	520	133	8 d
5	Dual-chamber MEC	Nitritation-anammox	Acetate	2000	500	1896	488	2.5 d; 0.8 v
6	Air-lift bio-electrochemical reactor (This study)	SND-AD	Acetate	500	200	310	146	6 h; 0.6 v

AD: autotrophic denitrification, SND:**simultaneous nitrification–nenitrification**.

4. Conclusions

In this study, a bio-electrochemical reactor employing an air-lift configuration and operating under a continuous feed regime was designed and investigated. The objective was to enhance the removal of nitrogen from synthetic wastewater characterized by a low carbon-to-nitrogen (C/N) ratio, while considering various temperature conditions. The utilization of bio-electrodes within the anoxic zone, coupled with the implementation of zeolite/plastic medium in the aerobic zone, has been observed to enhance the efficiency of nitrogen removal processes. The significance of temperature and hydraulic retention time (HRT) in relation to COD removal was established. The significance of all independent variables in the experimental conditions devised was established as crucial factors for the removal of nitrogen. The HRT was identified as the primary influential factor for the removal of TP in the experimental conditions. The COD, TN, and TP removal rates reached a maximum of approximately 77.8%, 73.29%, and 50.5%, respectively. The denitrification rate reached its highest value of 0.473 gN/l.d under specific conditions, including a temperature of 16 °C, a HRT of 6 h, a current density of 2 A/m^2, and a ratio of ammonium nitrogen (N–NH_4^+) to total nitrogen (TN) of 75% (as observed in Run No. 17). The findings of the study demonstrated a favorable equilibrium among biofilm thickness, current density, and total nitrogen (TN) removal when the current density was set at 6 A/m^2. The obtained results provide compelling evidence that warrants additional research on the potential applications of ALBER in the treatment of industrial wastewater.

Credit author statement

Amir Nouri: Conceptualization, Methodology, Writing. Ali Akbar Zinatizadeh: Supervision, Designing, Reviewing and Editing. Sirus Zinadini: Reviewing and Editing. Mark van Loosdrecht: Reviewing and 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

No data was used for the research described in the article.

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