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Application of response surface methodology (RSM) for analyzing and modeling of nitrification process using sequencing batch reactors

Seyyed Alireza Mousavi[a,*], Shaliza Ibrahim[b]

[a]*Department of Environmental Health Engineering, Kermanshah University of Medical Sciences, Kermanshah, Iran, Tel. +98 9188336569; email: seyyedarm@yahoo.com*
[b]*Faculty of Engineering, Department of Civil Engineering, University of Malaya, 50603 Kuala Lumpur, Malaysia, Tel. +98 9188336569; email: shaliza@um.edu.my*

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ABSTRACT

In this study, three parallel laboratory-scale sequencing batch reactors were operated to investigate the nitrification efficiency as a function of high concentration of ammonium, without and with the interaction of chemical oxygen demand (COD). In order to study the effects of COD and ammonium concentrations on nitrification rate, the experiments were conducted based on a central composite design and response surface methodology was used to analyze the achieved data. The experiments were conducted at different COD concentrations (0, 250, and 500 mg L^{-1}) and NH_4^+-N concentrations (200, 600, and 1,000 mg L^{-1}) in 13 runs. The five suggested mathematical models using the analysis of variance by Design-Expert software were applied to predict the response values. The nitrification rate decreased from 0.5 to 0.364 g NH_4^+-N L^{-1}g VSS^{-1}, when the COD concentration increased from 0 to 500 mg L^{-1}. This study contributed to a better understanding of the function of COD concentration in the system with high concentration of ammonium.

Keywords: Ammonium; Sequencing batch reactor; Nitrification; Response surface methodology

1. Introduction

The high-strength ammonium wastewaters originate from different sources such as human excretions, agricultural wastes, and industrial effluents. Uncontrolled discharges of wastewater containing high concentration of ammonium (e.g. sludge-rejected water) can be noxious for aquatic living, cause oxygen depletion and eutrophication in receiving water, and affect chlorine disinfection efficiency [1]. Rejected waters, which originate from sludge anaerobic digestion, contain a high concentration of ammonium (NH_4^+-N: 558–1,301 mg L^{-1}) and a low concentration of chemical oxygen demand (COD: 300–600 mg L^{-1}) [2]. Different physicochemical and biological processes have been applied to treat wastewaters containing nitrogen components. The biological processes, which are more effective and relatively cost-effective have been widely implemented in comparison to the physicochemical processes [1,3].

Biological nitrification as a key process has been applied to remove ammonium from wastewaters by employing of mix culture of autotrophic nitrifying bacteria [4,5]. The process is affected by various factors such as ammonium and nitrite concentrations,

*Corresponding author.

dissolved oxygen, organic loading rate, micro-organism population including the ratio of heterotrophic bacteria to autotrophic bacteria, sludge age, temperature, pH, and hydraulic loading rate [4,6,7]. Amongst them, the influent of COD/N ratio as a critical pertinent factor directly influences the population growth of both the autotrophic nitrifying and heterotrophic bacteria in a sequencing batch reactor [8–10].

Many studies have been conducted to investigate the effect of organic materials on the rate of nitrification, which have reported that the activity of nitrifying bacteria was inhibited by the raise of COD concentration in the presence of heterotrophic bacteria, because the maximum growth rate and yield of heterotrophic bacteria are more than autotrophic nitrifying bacteria about five times and two to three times, respectively [4,11,12]. Carrera et al. [3] observed an exponential decline of nitrification rate from (0.14 ± 0.02) to (0.029 ± 0.004) g NH_4^+-N gVSS^{-1} d^{-1} when the influent COD/N ratio increased from (0.71 ± 0.05) to (3.4 ± 0.3) g COD/g N, respectively [3]. The results of nitrification in submerged filters by Zhu and Chen showed at the C/N ratio of 1–2 the rate of nitrification decreased by 70% in comparison to a pure nitrification system (C/N = 0) [13]. Okabe et al. [14] found that a COD/N ratio of 1.5 has unfavorable effects on nitrification rate in a biofilm system. A distinct reactor for nitrification may enhance the potential of nitrification, but it increases the initial capital and maintenance costs of the entire system. Moreover, reject water contains weakly biodegradable substances, which may allow a simultaneous nitrification and COD removal in a single reactor.

In this study, the influence of the COD and ammonium concentration was evaluated on a nitrification process from a high-strength ammonium wastewater through Design-Expert software. It is expected that the achieved results due to focus on optimizing and modeling via analysis of variance (ANOVA) would be valuable to better understand the effect of variables (concentration of COD and ammonium) on the nitrification performance, which have not been considered in the open literature.

2. Material and methods

2.1. Microbial culture and artificial wastewater

The activated sludge with a mixed culture of bacteria was originated from an urban wastewater treatment plant (WWTP) in Pantai Dalam, Kuala Lumpur, Malaysia.

The synthetically produced contaminated water used in all experimental works based on the data in Table 1 contain of deionized water, $(NH_4)_2SO_4$ as nitrogen source for nitrification processes, $NaHCO_3$ and glucose as carbon source, KH_2PO_4 and K_2HPO_4 as phosphorous source, and 1 ml trace elements per liter. The synthetic wastewater was used as fresh or stored in a cold room at temperature below 4℃. The feed temperature increased to 25℃ before input to the sequencing batch reactors (SBRs) by a water bath.

2.2. Experimental setup

Experiments in this study were conducted in three laboratory-scale SBRs (Fig. 1) with a working volume of 5 L, and headspace of 1 L was provided to prevent any solid loss generally caused by foaming. The reactors, namely R1, R2, and R3 were inoculated with an initial mixed liquor suspended solid (MLSS) concentration of 2 gL^{-1}. Oxygen was supplied by three air pumps (HAILEA, ACO-9820, China) through air diffusers at the bottom of the reactors and dissolved oxygen (DO) was monitored by installed sensor (METTLER TOLEDO, O_2-sensor, Switzerland) in reactors, which was connected to controller to maintain higher than 3 mg O_2/L by adjusting the air flow rate. The pH was measured with a pH meter (METTLER TOLEDO, pH sensor, Switzerland) and adjusted between 7.3 and 7.9 by automatic injection of acid (H_2SO_4; 1 N) or alkaline (NaOH; 1 N) solution, respectively.

Table 1
Synthetic wastewater composition [15]

	Value (mg L^{-1})
Constituent	
NH_4^-N	1,000
$NaHCO_3$	3,000
KH_2PO_4	200
$MgSO_4$	60
Glucose	0, 500
pH[a]	7.6 ± 0.3
Trace elements[b]	
EDTA	10
$ZnSO_4·7H_2O$	2.2
$COCl_2·6H_2O$	3.2
$MnCl_2·4H_2O$	10.2
$CuSO_4·5H_2O$	0.22
$(NH_4)_6Mo_7O_{24}·4H_2O$	2.2
$CaCl_2·2H_2O$	1.1
$FeSO_4·7H_2O$	10
H_3BO_3	0.3
$NiSO_4·6H_2O$	1

[a]No unit.
[b]Composition of trace element solution (1 mL L^{-1}).

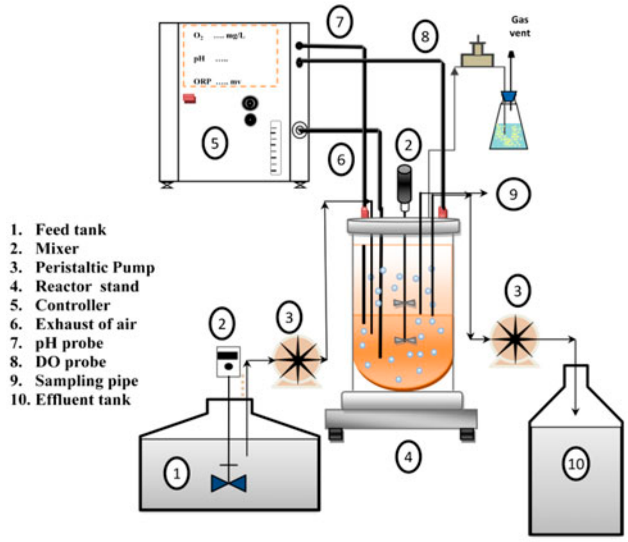

Fig. 1. Schematic diagram of the experimental apparatus.

Mechanical stirrer (200 rpm) and fine air bubbles agitated the medium throughout the reaction time in the three reactors. The reactors were equipped with a thermostatic jacket, and a thermostatic bath was used to maintain the temperature at 30 ± 0.5˚C. The reactors (R1, R2, and R3) are run according to operational conditions (Table 2) at 12 h cycles with sequencing stages of 5 min filling, 11 h reaction time, 50 min settling, and 5 min decanting. At the end of each settling phase, 50% of the reactor contents were discharged and replaced with new feed. The reactors steadily were operated in each run for 5 d at a steady-state condition.

2.3. Sampling and analytical methods

The samples were analyzed immediately after filtering (Syringe Filter Unit, 0.2 μm) or were stored at cold temperature (4˚C) prior to the analysis. The samples for the determination of ammonium, nitrate, and nitrite concentration were analyzed using an advanced compact ion chromatograph IC 861 (Metrohm® Ltd., Herisau, Switzerland) and guard column according to the method applied by Mousavi et al. [15]. Temperature, pH, and DO were monitored continuously online. In addition, the COD, MLSS, mixed liquor volatile suspended solids, and other experimental tests were determined using standard methods [16]. The repetition of analysis for all samples was considered when an error higher than 5% was observed in the samples during the experiments.

Field emission scanning electron microscopy (FESEM) system (AURIGA® the new Cross Beam® Workstation (FIB-SEM) from Carl Zeiss NTS) with a computer system at a magnification capacity ranging from 5 to 30 kV folds was used to observe the morphology of bacteria according to method applied by Mousavi et al. [15].

2.4. Experimental design and mathematical modeling

The experimental design was conducted using the statistical method of factorial design of experiments (DOE). The method has the ability to eliminate errors systematically with an estimate of the experiment, minimize the number of experiments, and determine an empirical model based on the experiments performed. A common method in optimizing multi-factors is through the individual optimization of a

Table 2
Operational conditions of the SBRs

Parameters	R1	R2	R3
Temperature (˚C)	30 ± 0.5	30 ± 0.5	30 ± 0.5
DO (mg L^{-1})	>3	>3	>3
Cycle (h)	12	12	12
HRT (h)	24	24	24
MLSS (mg L^{-1})	2,000	2,000	2,000
MLVSS (mg L^{-1})	1,650	1,650	1,650
pH	7.6 ± 0.3	7.6 ± 0.3	7.6 ± 0.3
NH_4^+-N (mg L^{-1})	200, 600, 1,000	200,600, 1,000	200, 600, 1,000
COD (mg L^{-1})	0	250	500
SRT (d)	20	20	20
Qin (L/d)	5	5	5
Volumetric exchange rate (%)	50%	50%	50%

factor while the other factors are kept constant. This common method, however, is time-consuming, ignores the interactions among variables, and is usually unable to attain the optimum result. Therefore, researchers have proposed some statistical techniques to determine the effects of independent factors and their interactive influences to overcome pertinent limitations [17,18]. Among such techniques, the response surface methodology (RSM) is a collection of statistical and mathematical techniques that are practical in the analysis and modeling of problems. The responses can be affected by a number of variables, and the aim is to optimize these responses. In addition, the interaction of feasible, effective factors and the efficiency of the bioelectrochemical system can be appraised using RSM at a limited number of designed experiments [19,20].

The central composite design (CCD) is the standard RSM, which allows the use of second-degree polynomial in the estimation of relationships between the independent and dependent variables. CCD also provides information on the interaction between variables based on the dependent variable [15,20]. For this reason, RSM using the CCD was selected via Design-Expert (version 8.0.0) software in building an empirical model and statistical analysis based on the objectives of this study, which include the optimization of operating conditions and the study on the interactive effects of experimental factors.

The statistical method of factorial DOE (version 8.0.0) software was applied to evaluate the effect of independent variables, namely COD (factor A) and ammonium (factor B) concentration, and their interactive influences on nitrification performance. The reasonable range of nitrogen (Actual Value = 200, 600, 1,000 mg NH_4^+-N/L) and COD (Actual Value = 0, 250, 500 mg L^{-1}) was selected according to the real concentration of COD and ammonium concentration in reject water that the coded value term was used to represent the independent variables at three levels, which consist of −1 (low level), 0 (Central), and +1 (high level). As shown in Table 3, the experimental conditions for the nitrification process from synthetic wastewater based on CCD with a factorial matrix of 13 steady-states runs (the reactors steadily was operated in each run for 5 d) were designed in nine factorial points and five experimental runs were approved as center points (600 mg NL^{-1} – 250 mg $CODL^{-1}$). In order to perform a comprehensive analysis of the nitrification process, some dependent parameters such as ammonium removal, nitrate and nitrite production, and NO_2^--N/(NO_3^--N + NO_2^--N) ratio were evaluated as results of variables interaction (Table 3).

After accomplishing the experiments at a set value of independent variables (NH_4^+-N and COD), the experimental data according to Table 4 were used to develop empirical models based on actual factors (AF) and coded factors (CF), using ANOVA via the Design-Expert software. The significance of the variables was recognized based on the confidence levels above 95% ($p < 0.05$) in the polynomial model. The quadratic model based on Eq. (1) was used to estimate the coefficients of the statistical model [19].

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i x_i + \sum_{i=1}^{k} \beta_{ii} x_i^2 + \sum_{i<j}^{k} \sum \beta_{ij} x_i x_j + e \qquad (1)$$

where i represents linear coefficient, j stands for the quadratic coefficients, β is the regression coefficient, x represents independent variables, k is the number of studied and optimized factors in the experiment, and e is the random error.

3. Result and discussion

3.1. Development of mathematical models and data analysis

The results of the nitrification of high ammonium concentrations were appraised based on the CCD (Table 3). Different degree polynomial models have been developed by using Design-Expert 8.0.0. The empirical models in terms of coded (standardized equation) and actual values (unstandardized equation) were confirmed in Table 4 with more than 0.95 model significance. The CFs for the effluent concentration of ammonium as first response represent a coefficient for COD (58.65), which is 1.17 times less than the coefficient for NH_4^+-N concentration (69.02). This finding proves that nitrogen contributed an effective function in the effluent concentration of ammonium. The CFs for the percentage removal of ammonium as a second response represent a coefficient for COD (11.09), which is 14.59 times less than the coefficient for NH_4^+-N (0.76). This finding indicates that COD contributed a less effective function in ammonium removal in the system. The Model F-value was 90.29, implying that the model is significant and that there is only a 0.01% chance that this large "Model F-value"[1] could occur due to noise. Values of "Prob. > F"[2] less

[1]An F-test is any statistical test in which the test statistic has an F-distribution under the null hypothesis. It is most often used when comparing statistical models that have been fitted to a data-set.

[2]Probability of seeing the observed F-value if the null hypothesis is true (there is no factor effect).

Table 3
Results of experiments according to CCD for three-level factorial of variables (COD, and NH_4^+-N)

Run	Code	Reactor	Factor A:COD (mg L^{-1})	Factor B:NH_4^+-N (mg L^{-1})	COD/N	NH_4^+-N (mg L^{-1})	NH_4^+-N (% Removal)	NO_2^--N (mg L^{-1})	NO_3^--N (mg L^{-1})	NO_2^--N/(NO_2^--N + NO_3^--N)
1	(+1)	R3	500	200	2.5	58.64	70.68	21.08	116.64	0.15
2	(0)	R2	250	200	1.25	27.72	86.14	10.45	150.61	0.06
3	(0)	R2	250	600	0.41	79.57	86.73	257	13	0.95
4	(0)	R2	250	600	0.41	67.4	88.76	267	10.35	0.96
5	(0)	R2	250	600	0.41	61.7	89.71	262.9	27.35	0.91
6	(+1)	R3	500	1,000	0.5	259.6	74.04	631	15.18	0.98
7	(−1)	R1	0	1,000	0	88.65	91.13	791	20.33	0.97
8	(0)	R2	250	600	0.41	69.8	88.36	264	19.8	0.93
9	(−1)	R1	0	600	0	44.8	92.53	263	30.23	0.90
10	(+1)	R3	500	600	0.83	172.9	69.5	224.7	9.19	0.96
11	(−1)	R1	0	200	0	5.76	97.12	2.47	184.01	0.01
12	(0)	R2	250	600	0.41	76.1	87.31	273.9	16.89	0.94
13	(0)	R2	250	1,000	0.25	158	84.2	732.3	17.3	0.98

than 0.05 point out that the model terms are significant and that A, B, and AB are significant model terms [17,18]. As noted in Table 4, the "Probability for Lack of Fit (LOF) *F*-value"[3] was used to determine the adequacy of the model. In addition, the value of LOF *F*-value 4.39 for ammonium removal as a response implies that the LOF is not significant relative to pure error. Thus, there is a 9.3% chance that a "LOF *F*-value" this large could occur due to noise. Nonsignificant LOF is desirable, and "Prob. > *F*" greater than 0.1 indicates that the model terms are not significant [17,19]. The "Pred *R*-Squared"[4] of 0.984 is in reasonable agreement with the "Adj *R*-Squared"[5] of 0.973. The "Adeq Precision"[6] measures the signal-to-noise ratio, wherein a ratio greater than four is desirable [17,20]. The achieved ratio of 33.92 was 8.48 times greater than the requirement of the model and indicates an adequate signal. The developed models displayed relatively high coefficients of determination (R^2) (0.984, 0.971, 0.998, 0.994, and 0.998), indicating good prediction of responses.

3.2. Effects of substrate concentration (COD and ammonium) on nitrification process

As mentioned, different parameters (e.g. temperature, DO, and SRT) could affect nitrification rate; amongst them, the study of COD (factor A) and ammonium (factor B) concentration was object when other parameters were maintained constant. The performance of nitrification process in three SBRs was evaluated under different COD/N ratios (0, 0.25, 0.41, 0.5, 0.83, 1.25, and 2.5). The nitrification efficiency of R1 (without COD) was examined and compared with the outcomes of reactors R2 and R3 (with COD interaction) to appraise the effects of COD on the system. Under a TN dose of 200 mg NH_4^+-NL^{-1}, the ammonium removal rate significantly decreased with increasing of the COD/N ratio. The achieved results in this study as compared with previous studies on activated sludge systems and biofilters showed a similar relationship between COD/N and nitrification rate [3,4].

The response surface analysis in Fig. 2(a) and (b) shows the effect of the operational parameters, namely, concentration of glucose and ammonium, on the efficiency of ammonium removal during the nitrification process. As shown in Table 3, the percentage of ammonium removal was 97% by nitrification, with a high accumulation of nitrate when the COD (A) and ammonium (B) were applied at low values of 0 and 200 mg L^{-1}, respectively. Fig. 2 shows the effects of COD value on nitrification rates in R1, R2, and R3. The inhibitory effect of organic matter on the nitrification rate is obvious. As COD increased from 0 to

[3]This is the variation of the data around the fitted model. If the model does not fit the data well, this will be significant. LOF values often give experimenters concern.
[4]A measure of the amount of variation in new data explained by the model.
[5]A measure of the amount of variation around the mean explained by the model, adjusted for the number of terms in the model.
[6]This is a signal to noise ratio. It compares the range of the predicted values at the design points to the average prediction error. Ratios greater than four indicate adequate model discrimination.

Table 4
Developed models and ANOVA results using Design-Expert 8.0.0 for studied responses

Response	Final model with significant terms coded factors (CF), and actual factors (AF)	Probability	R^2	Adj. R^2	Adeq. Precision	SD	CV	PRESS	Probability for lack of fit
Eff. Concentration of NH_4^+-N (mg L^{-1})	CF: = + 74.73 + 58.65*A + 69.02*B + 29.52*A*B + 24.60*A^2 + 8.61*B^2 AF: = + 0.77276 − 0.13925*A + 0.034218*B + 2.95175E − 004*A*B + 3.93531E − 004*A^2 + 5.37856E − 005*B^2	0.0001	0.984	0.9738	33.92	11.08	12.31	5231.14	0.093
Percentage removal of NH_4^+-N (mg L^{-1})	CF: +87.45–11.09*A − 0.76*B + 2.34*A*B − 4.63*A^2 − 0.48*B^2 AF: +97.48454 − 0.021331*A − 4.15955E − 003*B + 2.33750E − 005*A*B − 7.41352E − 005*A^2 − 2.99030E − 006*B^2	0.0001	0.971	0.95	20.87	1.89	2.23	143.54	0.0851
Eff. Concentration of NO_2^--N (mg L^{-1})	CF: +263.43–29.95*A + 353.38*B − 44.65*A*B − 15.74 * A^2 + 111.78*B^2 AF: −67.90499 + 0.27405*A + 0.15671*B − 4.46525E − 004*A*B − 2.51848E − 004*A^2 + 6.98653E − 004*B^2	0.0001	0.99	0.998	112.57	10.41	3.38	6103.25	0.0706
Eff. Concentration of NO_3^--N (mg L^{-1})	CF: +17.77 – 15.59*A − 66.41*B + 15.56*A*B + 1.20*A^2 + 65.44*B^2 AF: +304.74915 – 0.16527*A − 0.69571*B + 1.55550E − 004*A*B + 1.91283E − 005*A^2 + 4.09003E − 004*B^2	0.0001	0.994	0.989	43.5	6.03	12.43	1063.8	0.6395
Ratio of NO_2^--N/ (NO_2^--N+NO_3^--N)	CF: +0.94 + 0.034*A + 0.45*B − 0.035*A*B − 8.868E − 004*A^2 − 0.41*B^2 AF: 0.74503 + 3.51265E − 004*A + 4.27618E − 003*B − 3.45633E − 007*A*B − 1.41892E − 008*A^2 − 2.55495E − 006*B^2	0.0001	0.998	0.997	78.91	0.018	2.42	5.803E − 003	0.8523

Notes: A: glucose, B: ammonium, 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.

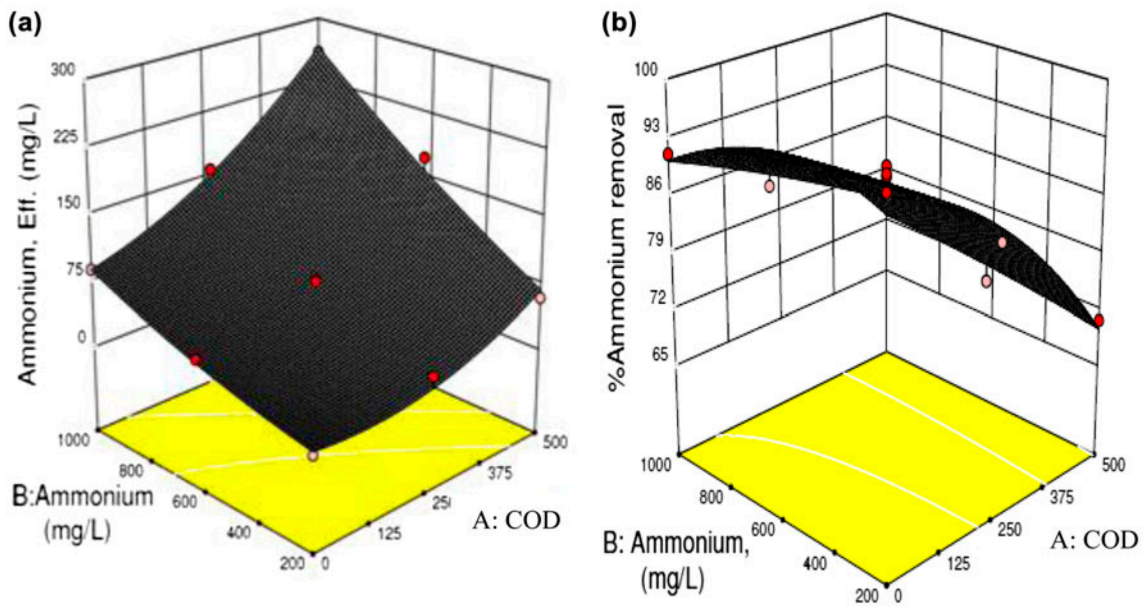

Fig. 2. The response surface plot (a) effluent concentration, and (b) percentage removal of NH_4^+-N; represent the effect of "COD" and "ammonium" on nitrification efficiency.

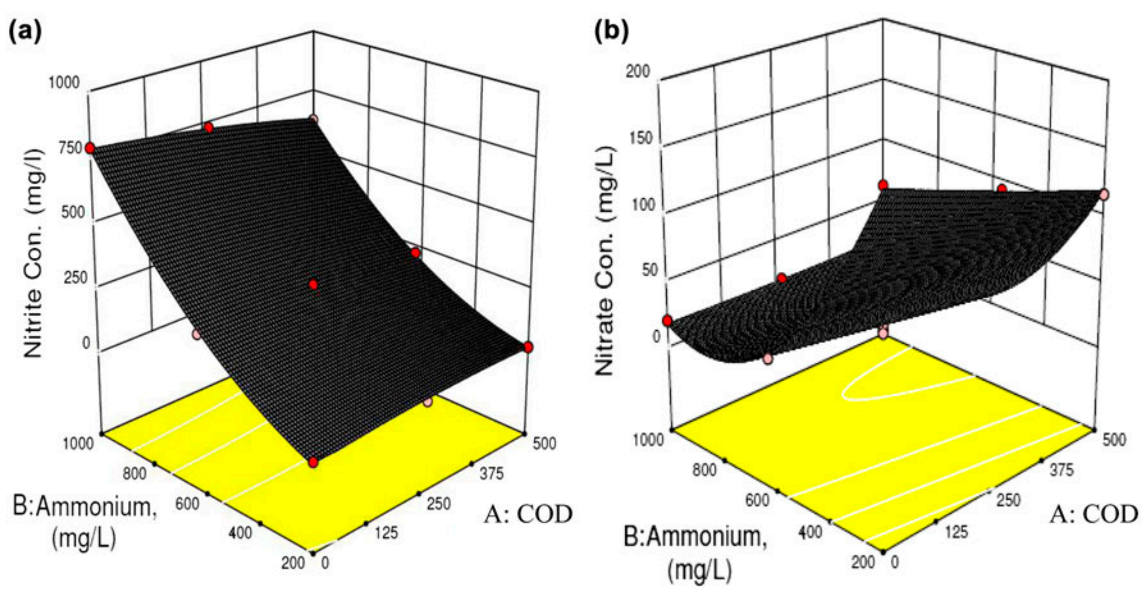

Fig. 3. The response contour plot of produced nitrite (a) and nitrate (b) representing the effect of "COD" and "ammonium" on nitrification process.

250 mg L^{-1} (COD/N = 1.25), the nitrification rate decreased with increasing COD/N ratio. However, at higher COD values (500 mg L^{-1}), the percentage removal of ammonium (30%) decreased because of the high growth level of heterotrophic bacteria. This finding is in agreement with the results obtained by Carrera et al. and Ling and Chen [3,4]. The nitrification rate estimated according to Eq. (2) was approximately 0.5 g NH_4-N (L^{-1} gVSS^{-1}) for R1, 0.44 g NH_4-N (L^{-1} gVSS^{-1}) for R2, and 0.364 g NH_4^--N (L^{-1} gVSS^{-1}) R3, when ammonium was 200 mg L^{-1} and the COD/N ratio was raised from 0 to 2.5.

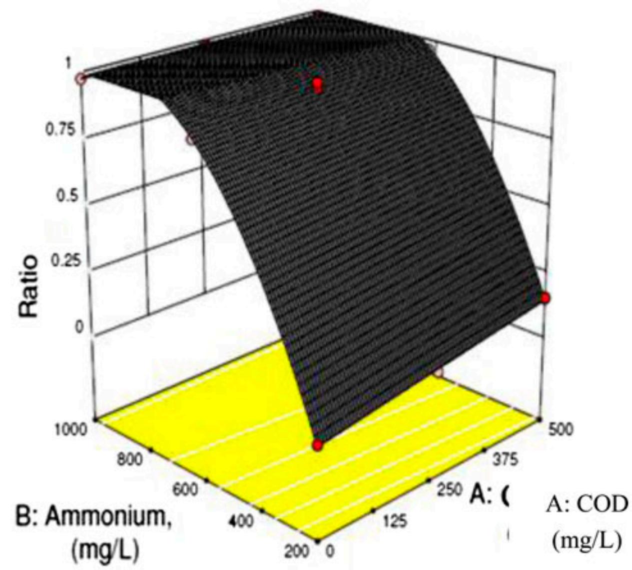

Fig. 4. The response contour plot of NO_2^--N/(NO_2^--N + NO_3^--N) ratio, representing the effect of "COD" and "ammonium" on nitrification process.

$$\text{Nitrification rate} = \frac{Q_{in}([NH_4^+ - N]_{in} - [NH_4^+ - N]_{out})}{V_r[VSS]_r} \quad (2)$$

where

the influent flow rate is Q_{in} (L/d), the reactor working volume is Vr (L), and the VSS concentration in reactor is [VSS] r (gL^{-1}).

The highest glucose and ammonium concentrations showed lower ammonium removal efficiency. In this condition, however, the high concentration of NO_2^--N achieved and nitrate production dropped (Fig. 3). The partial nitrification occurred because of the presence of high ammonium concentration (1,000 mg N–NH_4^+-N/L), shorter retention time (HRT = 24 h), high temperature (T = 30°C), and presence of heterotrophic bacteria, yielding a high ratio of NO_2^--N/(NO_2^--N + NO_3^--N) (Fig. 4). The ratio of NO_2^--N/(NO_2^--N + NO_3^--N) was 0.97 when the efficiency removal of ammonium was at the lowest value in the R3. The ammonium concentration, as second variable (B), acts as an inhibiting factor at high value, causing nitrite accumulation and inhibiting the nitrification process [21–23].

3.3. Optimization of experimental conditions

To determine the optimum region for the studied variables, the responses that were used to check the SBRs performance in this study were modeled. The concentration of ammonium in effluent, ammonium

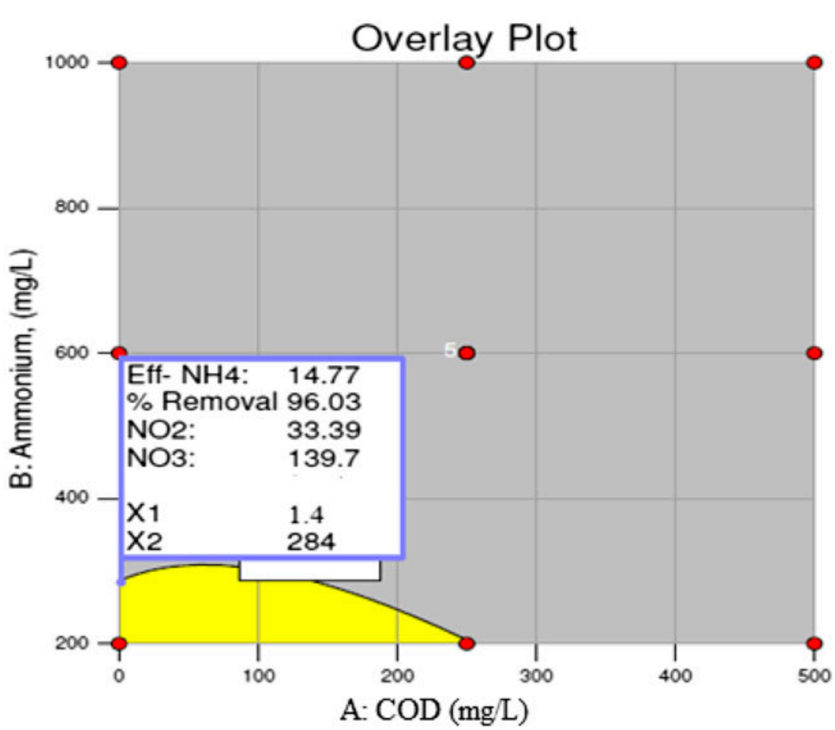

Fig. 5. Overlay plot for optimal area of effluent ammonium concentration at optimized COD and NH_4^+-N value.

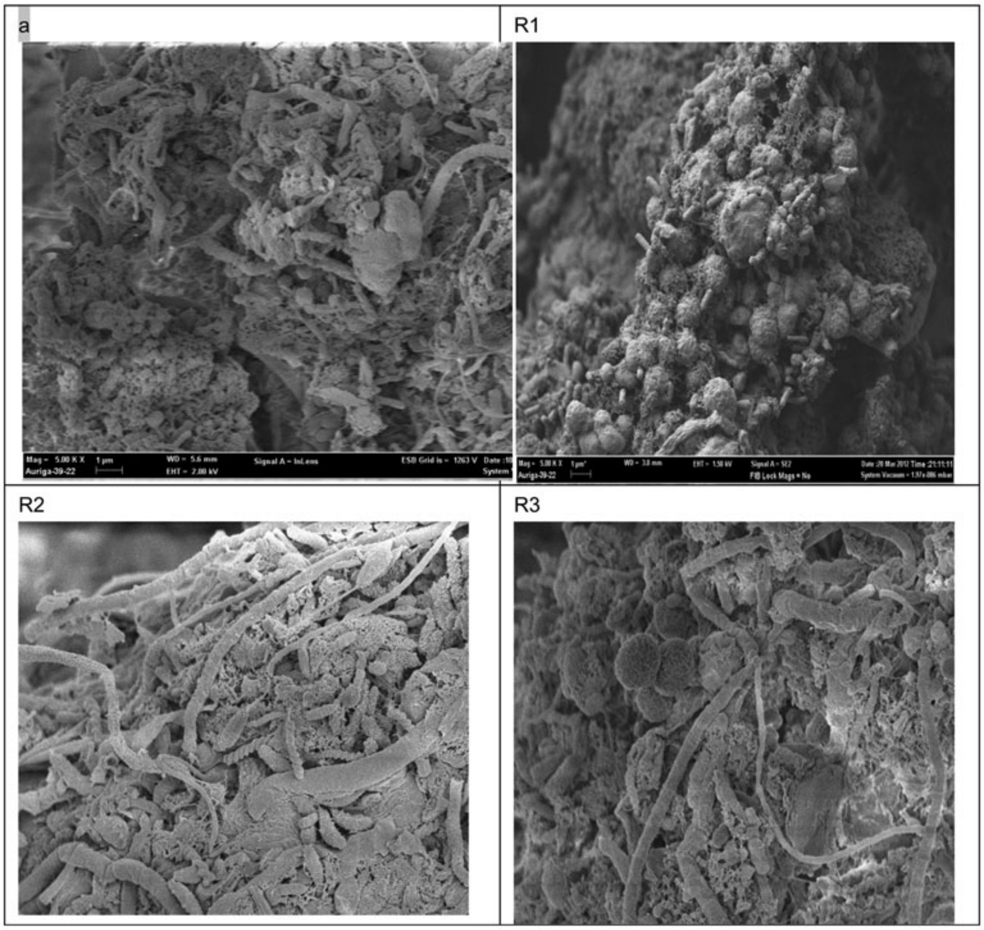

Fig. 6. FESEM observation of the micro-organisms in SBRs (a) first day for raw sludge and (R1), (R2), and (R3) after day 90Th.

percentage removal, nitrate and nitrite production, and NO_2^--N/ (NO_2^--N + NO_3^--N) ratio as responses were each optimized as a function of the studied variables (A: COD and B: Ammonium). Fig. 5 illustrates the overlay plot, where the highlighted spherical surface is the optimum area based on the concentration of ammonium in effluent for achieving the ammonium discharged standard (<15 mg L^{-1}). To confirm the dependability of the models' predictions, one point within the optimum region was chosen for implementation in verification experiments (10.4 mg NH_4^+-N/L in effluent with 95% removal). The obtained experimental results confirmed that the model was able to make a reasonably precise prediction for the optimum conditions, in terms of ammonium and COD concentrations. At the applied values of variables (COD: 50 mg L^{-1} and NH_4^+-N: 250 mg L^{-1}) the concentration of ammonium in the effluent was 14 ± 3.7 mg L^{-1}. Therefore, the obtained results verified that the model was able to make an acceptable prediction for the optimum conditions.

3.4. FESEM observation

The field emission scanning electron microscopy (FESEM) observation of the activated sludge in SBRs was conducted to observe the morphology of the seed sludge (day 0) and (day 90) from the reactor without COD. The image of the seed sludge according to Fig. 6(a) on day 0 shows that the biomass from the urban WWTP consists of straight rod, curved rod, and vibrio-shaped bacterial cells with different sizes of about 0.38–0.6 μm and 0.5–1.26 μm. FESEM observation of sludge on day 90 revealed an abundance of bacteria with different sizes (Fig. 6(b)) which were mentioned in previous studies [24–26].

4. Conclusion

Both COD and ammonium concentrations are important factors influencing nitrification rate. The results showed that nitrification performance was influenced by both the high ammonium concentrations

(1,000 mg L^{-1}), due to inhibition effects of ammonia on nitrifiers and the high COD/N ratio (2.5) due to heterotrophic bacteria grow. The developed models with high correlation based on the experimental results of the CCD and RSM were useful to understand the direct effect of ammonium and COD concentrations on the performance of nitrification process. The optimum operational conditions in order to have a maximum nitrification rate with more than 95% removal of ammonium was achieved when the NH_4^+-N and COD were 0–284 and 0–250 mg L^{-1}, respectively. The nitrite accumulation was observed throughout the experiments when the substrate concentration increased from 200 to 1,000 mgNH_4^+-N/L. This study contributed to a better understanding of the role of COD/N ratio in a system with high concentration of ammonium.

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