



Biogenic compound removal from municipal wastewater – modeling and optimization

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ABSTRACT

The goal of study is to investigate the efficiency and biokinetics of intermittent cycle aerobic-anaerobic with granular activated carbon-bed reactor (ICAAGACR) in the removal of biogenic compounds from wastewater. 1-year period of study took place using an ICAAGACR with effective volume of 4 L on wastewater. Growth changes of microorganisms were identified by measuring mixed liquor volatile suspended solids (MLVSS) and chemical oxygen demand (COD) variations equivalent to the substrate in the range of 5–500 mg/L. With the data obtained from the experimental conditions, the k_0 and K_s values and the kinetic coefficients of growth of microorganisms were determined according to the changes in MLVSS and COD using the ASM1 model and the developed relationships of the Monod model.

Keywords: Organic compounds removal; Bio-kinetic coefficients; Monod model; Optimization

1. Introduction

In the past, the municipal wastewater treatment plants were mainly used for removing organic pollutants, suspended matter and microbial contaminants. New stringent standards emphasize the separation of nutrients, heavy metals and priority pollutants [1,2]. Over the years, as the toxic effect of different compounds was properly identified, restrictions on their concentrations in wastewater entering

the environment and the receiving waters were imposed. Nitrogen and phosphorus compounds in the aqueous environment [3] are an example of such compounds. They are important pollutants of municipal wastewater which are not eliminated by conventional treatment, but they require advanced systems for removal [4]. Phosphorus is introduced into municipal wastewater from sources such as water, human waste, industrial and commercial uses, artificial detergents and household cleaners. Industrial waste

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(outside the city) and landfill leachate sites [5] are other sources of phosphorus.

Nitrogen in municipal wastewater is often found in the form of organic nitrogen (40%) and ammonia (60%), which is mainly due to protein metabolism in humans [3,6] (as about 16% of the structural proteins of plants and animals forms nitrogen [7]).

The most important problems of the discharge of wastewater containing nitrogen and phosphorus into the environment are algae growth, accelerating the phenomenon of eutrophication, toxicity of free ammonia for fish and many other aquatic organisms, reducing dissolved oxygen, increasing the taste and odor in water, which can create many dangers for the ecosystem and aquatic life [8–11]. The health effects of nitrite and nitrate in the human body are the introduction of nitrite into the bloodstream and the development of methemoglobinemia in children [12,13]. Nitrite reactions with other stable nitrogen compounds (such as amines and second and third type amides in nutrient) also cause nitrosamine compounds which are carcinogenic [14–17].

There are several methods to remove nutrients from the wastewater, including physical, chemical, biological and combined methods. The choice of each method depends on the environmental factors such as the existing pollutants, the purification quality required, and in particular the economic aspect of the process [18]. Physical and chemical methods are costly. The biological system is more suitable because of its efficiency compared with other methods, simple and inexpensive in terms of its application and compatibility with the environment [19]. In the recent years, the use of biofilms in the biological treatment process has increased and biofilm systems with submerged biological filters having fixed media been replaced by a suspended growth system. In the integrated growth bed process, the microorganisms responsible for the conversion of organic or nutritious substances are attached a substrate of neutral materials. The organic substances and nutrients are removed from the wastewater stream that passes through a suspended growth bed called the biological layer. The materials used in the integrated growth bed include rock, sand, wood chips, a wide range of plastics and other artificial materials [18].

Activated carbon is one of the most used chemicals in adsorption processes [20]. Due to its porosity, specific surface and high absorption capacity, this medium is used the most to remove pollutants from water and wastewater. Also, activated carbon is suitable for removing antibiotics and pesticides from wastewater [21]. Nowadays, in order to increase the efficiency of removing pollutants, granular activated carbon is considered a suitable bed for growth of microorganisms. The biological systems that use biological activated carbon granules have a high efficiency compared with physical absorption [22–26] and can eliminate compounds such as soluble organic matter, phenol, aniline, removal of heavy metals (copper, lead, cadmium and bivalent nickel) and aromatic materials.

In this study, according to unique properties of granular activated carbon in comparison with other media, the use of granular activated carbon as a medium for the bed of the studied system was selected for removal of nutrients from municipal wastewater. On the other hand, since we are

dealing with a multivariate system in this study, the most important technique in optimization and economics is the experiment design method and response surface methodology (RSM). Also, the ASM1 model and the ASIM software were employed to model the biological process, and the results were compared with the kinetic coefficients obtained from the Monod model and experimental results. This relationship is the most important mathematical equation for examining the growth rate of microorganisms and the utilization of substrate in a biological environment of growth. The main art of these models is that by identifying the main processes, the most important reactions are expressed in the form of a matrix. The advantage of using this matrix form is to allow easy and quick identification of the fate of each component in the system and follow up of all interactions between system components. In order to determine the parameters of the Monod model, the biomass sample was grown in a pilot laboratory aeration vessel under different conditions of substrate concentration and the kinetic coefficients were determined using the three mathematical equations to be extracted from the main Monod equation. Then the kinetic coefficients of the experimental results were calculated including the specific growth rate of the microorganisms (Y), half-velocity constant (K_s), the maximum specific growth rate of the microorganisms (k) and the concentration of the limiting substrate for growth (k_d). k and K_s are empirical coefficients to the Monod equation. They will differ between species and based on the ambient environmental conditions.

2. Materials and methods

2.1. Experimental set up

In order to achieve the research objectives, an intermittent cycle extended activated system (ICEAS) with a useful volume of 4 L was initially used [27]. In the ICEAS reactor, the granular activated carbon was added in three volumes of 20%, 35% and 50% of the reactor volume to become an intermittent cycle aerobic-anaerobic with granular activated carbon-bed reactor (ICAAGACR). The characteristics of the used granular activated carbon are presented in Table 1.

In this research, after the hydraulic test on the reactor to start the system, primary effluent was used as feed and sludge of municipal wastewater treatment plant as a seed. The Plexiglas bioreactor has an internal diameter of 8 cm and a total height of 110 cm with a useful volume of 4 L

Table 1
Media characteristics

Media characteristics	Amount
Total surface area (m ² /g)	900
Approximate length (mm)	3
Approximate diameter (mm)	1
Density (g /cm ³)	1.3
Media volume at 20% filling (mL)	800
Media volume at 35% filling (mL)	1,400
Media volume at 50% filling (mL)	2,000

and a total volume of 5.5 L. An automatic drainage system was installed at a height of 60 cm from the bottom of the reactor (75% of the total volume) for discharge. Therefore, the hydraulic residence time (HRT) was calculated based on 1 L as an interchangeable useful volume. In each cycle, about 1 L of fresh liquid was drained and the same amount of fresh wastewater was introduced into the reactor, which affected the rate of dilution of the material inside the bioreactor. After the stability of the system, the reactor with HRT of 12 h (computed as the sum of the phase of mixing -anaerobic-, aerobic phase and sedimentation phase), began to be exploited. A peristaltic pump (LabF6, Shenchen, China) provided a continuous supply of wastewater into the system and a circulator device mixed the reactor content. The required air volume was supplied by a blower (centrifugal air suction blower C4-73, Xingyi Co., China) and four air bubbles placed at the bottom of the column. The schematic of the reactor used in this study is presented in Fig. 1.

2.2. Design of experiments

RSM is a collection of useful statistical and computational methods for analyzing the effect of several independent variables on system performance, which is an important application in design and process optimization [28]. In the present study, after the microorganisms were adapted to the existing conditions, the design of the experiments was carried out using Design-Expert version 7 software by

RSM. The central composite design was widely used for fitting a second-order model. By using this method, modeling is possible and it requires only a minimum number of experiments. It is not necessary in the modeling procedure to know the detailed reaction mechanism since the mathematical model is empirical. Generally, the CCD consists of a 2^n factorial runs with $2n$ axial runs and nc center runs (six replicates). These designs consist of a 2^n factorial or fractional (coded to the usual ± 1 notation) augmented by $2n$ axial points, and nc center points. Each variable is investigated at two levels. Meanwhile, as the number of factors, n , increases, the number of runs for a complete replicate of the design increases rapidly. In this case, main effects and interactions may be estimated by fractional factorial designs running only a minimum number of experiments. Individual second-order effects cannot be estimated separately by 2^n factorial designs. Therefore, the central composite design was employed in this study. The responses and the corresponding parameters are modeled and optimized using ANOVA to estimate the statistical parameters by means of response surface methods.

Basically this optimization process involves three major steps, which are, performing the statistically designed experiments, estimating the coefficients in a mathematical model and predicting the response and checking the adequacy of the model [29]. Three independent variables including aeration time, mixing time (without aeration) and the percentage of granular activated carbon were considered in three levels experiments, as shown in Table 2.

2.3. Operation of reactor

At the startup stage, the microbial seed from the return pipe and the raw wastewater from the entrance to the municipal wastewater treatment plant were introduced into the reactor. This stage was repeated in order to achieve a stable state and system reliance. Consequently, the biofilm formation period on the surface of granular activated carbon and reaching a steady state lasted more than 2 months. After the hydraulic test was carried out on the reactor, the raw wastewater was continuously introduced from the bottom of the reactor and the distilled out saliva was dissipated at the end of each stage. The system started with the anaerobic phase, followed by aeration, settling and drainage cycles. The duration of each of the above steps was controlled using an automatic timer. In all operation cycles, the sedimentation time was 30 min and the discharge time was 4 min. The amount of O_2 was varied 2.5–5 mg/L according to the concentration of input pollutant (especially ammonium and organic matter), and it was measured contentiously using (dissolved oxygen online meter [mode: DOG – 2092, Abnie – Paidar – Sabz company, Iran]). It should be noted that in the most of the operation time, the O_2 concentration was between 2.5 and 3.5 mg/L, but when the removal rate increased (organic matter decreased), the O_2 concentration for a short time reached 5 mg/L. Regarding how the operation of system was conducted, that is, the presence of alternating current in the system and the aeration of the system after the anaerobic stage, the pH fluctuations were not significant and were controlled in the range of 6.8–7.2. The mixed liquor suspended solids (MLSS) system was

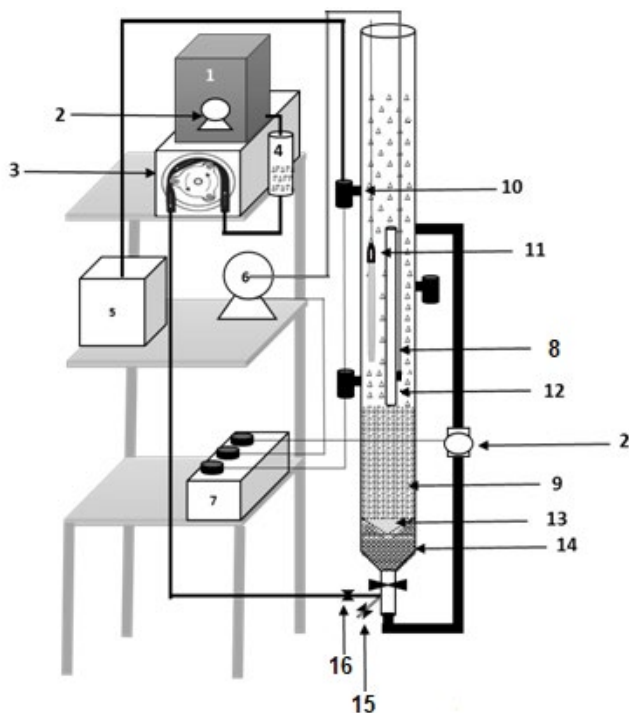


Fig. 1. Schematic of the ICAAGACR. (1) Feed tank, (2) circulator pump, (3) peristaltic pump, (4) filter, (5) effluent tank, (6) air pump, (7) timer, (8) thermometer, (9) zeolite, (10) discharge pump, (11) heater, (12) air lift, (13) plastic mesh, (14) glass beads, (15) drain valve, (16) influent valve.

Table 2
Actual and coded values of independent variables

Parameter name	Symbol	Down factorial point (-1)	Center point (0)	Up factorial point (1)
Mixing time, min	A	30	60	90
Aerobic retention time, h	B	2	4	6
Media fill percentage, %	C	20	35	50

kept constant as 4,000 mg/L regardless of the microbial film clinging to activated carbon.

2.4. Sampling process

To determine the system efficiency, sampling was done at each loading step from three points of input, output and contents inside the reactor (40 cm height from the bottom of the reactor). It should be noted that, in order to achieve a stable condition in sampling, three times repetition of each stage was done. Also, to determine the amount of NO₃⁻, NO₂⁻, TKN, NH₄-N and TP were applied 4500-NO₃⁻ E reduction cadmium, Chlorimetric Method 4500-NO₂⁻ B, Macro-Kjeldahl Method 4500-N_{org}-B, NH₃ HMethod4500-Nesslerization and Method 4500-P Dchloride Stannous methods, respectively [30].

2.5. Monod model for growth of microorganisms

In this study, the process of removing organic compounds is based on the A₂O system, which is a combination of anaerobic, anoxic and aerobic processes. The aerobic process was modeled using ASM1 and the data obtained during the 1-year period. In accordance with the obtained data, the temperature of the model was set at 20°C [31]. The ASM1 model contains 13 components that should be extracted based on the experimental data [32]. In this study, in order to simplify the model, some parameters such as growth of autotrophic bacteria, which can be neglected in comparison with the growth of heterotrophic bacteria in aeration tank, were removed. For this purpose, the data in Table 3, which is simplified by the main table in ASM1, were used to construct the model. Although the parameters of non-degradable solution (S_i) and non-degradable suspended matters (X_i) have no

coefficient, their values in wastewater samples are important in chemical oxygen demand (COD) and volatile suspended solids (VSS) measurements of sample outputs. The three main processes in the aeration tank include aerobic growth of heterotrophs, death of heterotrophs and a hydrolysis process, the components of which are determined by stoichiometric coefficients and process rate. In this table, Y_H is the efficiency of production of the heterotrophic biomass and f_p is the non-biodegradable section of the biomass.

As an example, the equation for the variation of the fast degradable soluble material in the matrix form of the ASM1 model is in accordance with Eq. (1) [31].

$$S_s = \frac{1}{Y_H} \mu_H \left(\frac{S_s}{S_s + K_s} \right) X_H + k_h \left(\frac{\frac{X_s}{X_H}}{K_x + \frac{X_s}{X_H}} \right) X_H \tag{1}$$

where K_s is half saturation constant of biomass heterotrophs, μ_H is maximum growth rate of biomass heterotrophs, k_h is maximum hydrolysis rate and K_x is semi-rate constant of organic compound degradable. To calibrate the results of aerobic process modeling, all model parameters must be estimated. The ASIM software (version 4) was used to estimate the model parameters. In this software, first, different parameters are selected with a basic guess at 1-year period and finally the best parameters with the least error are selected. The relationship between the model components and the measured values of the experimental pilot is in accordance with Eqs. (2)–(4) [33].

$$VSS = X_H + X_s + X_i + X_p \tag{2}$$

Table 3
Parameters, kinetic coefficients and process rate ASM1 simplified for use in this research

j	i	S _i	S _s	X _i	X _s	X _H	X _p	Process rate, ρ _j (ML ⁻³ T ⁻¹)
	Process							
1	Aerobic growth of heterotrophs		-1/Y _H			1		$-\frac{1}{Y_H} \mu_H \left(\frac{S_s}{S_s + K_s} \right) X_H$
2	Deaths of heterotrophs				1-f _p	-1	f _p	$b_H X_H$
3	Hydrolysis		1		-1			$k_h \left(\frac{\frac{X_s}{X_H}}{K_x + \frac{X_s}{X_H}} \right) X_H$

$$\text{COD} = X_i + X_s + S_i + S_s \quad (3)$$

$$\text{SCOD} = S_i + S_s \quad (4)$$

where X_p is non-degradable suspended COD due to cell destruction.

3. Results and discussion

Based on the experiment design, analysis and optimization of parameters under consideration were demarcated 20 runs. Table 4 displays the number of experiments and results of the system for removal of nitrogen and phosphorus compounds.

3.1. Properties of input raw wastewater to the system

The properties of the raw sewage input to the system were measured and reported in Table 5. The characteristics of nourishing compounds of the studied raw sewage were classified as the middle one based on the classification of urban sewages [34].

The results of different analyses on the system in removal of azotic compounds and total phosphorus are tabulated in Table 3.

3.2. Removal of organic nitrogen

With reference to the relatively high level of organic nitrogen in urban sewage, the assessment of influential

parameters on efficiency of its removal is highly demanded. Results indicated the utmost efficiency (97.5%) in the removal of organic nitrogen was obtained at the mixing time 30 min, 6 h aeration and 50% of medium fill. The efficiency in the removal of organic nitrogen improved as the percentage of media and aeration time increased (Fig. 2a).

According to the results, with increasing blending time, organic nitrogen were digested, and transformed into mineral nitrogen compounds during ammonification. Increasing aeration time from 30 to 90 min lifted the efficiency of organic nitrogen removal almost 20%. Moreover, the efficiency reached a peak at an aeration time of 6 h and a 50% filling of medium (Fig. 2a). The observed significant efficiency of removal in 50% of medium can be associated

Table 5
Properties of input raw wastewater

Parameter	Concentration (mg/L)
COD	365 ± 23.07
sCOD	110 ± 9.3
BOD	162.42 ± 21.91
TKN	51.67 ± 3.99
NH ₄ -N	31.54 ± 2.8
N-Org	20.12 ± 1.77
TN	51.70 ± 3.99
TP	9.42 ± 0.81
Alkalinity	309.11 ± 30

Table 4
Central composite design (CCD) design matrix for three variables

Run	A	B	C	Removal efficiency (%)					Outputs (mg/L)	
				TKN	NH ₃ -N	N-Org	TN	TP	NO ₃	NO ₂
1	30	2	20	59.6	58.3	61.5	45.8	25.4	21.87	0.27
2	30	2	50	88.2	87.5	89.2	83.5	36.9	13	0
3	30	6	20	91.9	92.5	91	81.9	33.7	10.26	0.09
4	30	6	50	97	96.7	97.5	89.8	47.6	13.3	0
5	90	2	20	58.1	55.7	61.8	46.6	29.8	19.46	0.19
6	90	2	50	90.8	90.4	91.5	84.5	41.6	13.2	0
7	90	6	20	93.1	93.9	91.9	84.8	36.9	8.9	0.05
8	90	6	50	96.8	96.8	96.9	95	50.6	14.73	0
9	60	4	20	79.2	78.5	80.4	71.2	27.1	11.65	0.11
10	60	4	50	94.4	93.5	95.7	87	60.7	13	0
11	60	2	35	68.2	66.9	70.3	54.8	33.2	14.73	0.42
12	60	6	35	96.4	96.2	96.7	87.6	42.3	10.57	0.06
13	30	4	35	79.9	80.1	79.6	66.7	29.5	19.58	0.17
14	90	4	35	85.9	85.3	86.7	72.4	33.9	17.51	0.23
15	60	4	35	82.5	82.1	82.9	69.3	30.4	17.03	0.18
16	60	4	35	80.8	80.3	81.7	66.9	29.7	17.52	0.52
17	60	4	35	79.4	80.5	77.6	68.4	32.9	14.73	0.13
18	60	4	35	82	84.8	77.5	68.8	27.4	14.73	0.22
19	60	4	35	78.3	76.1	81.8	67.2	26.8	14.73	0.33
20	60	4	35	78.9	75.5	84.7	69.1	29.8	14.73	0.14

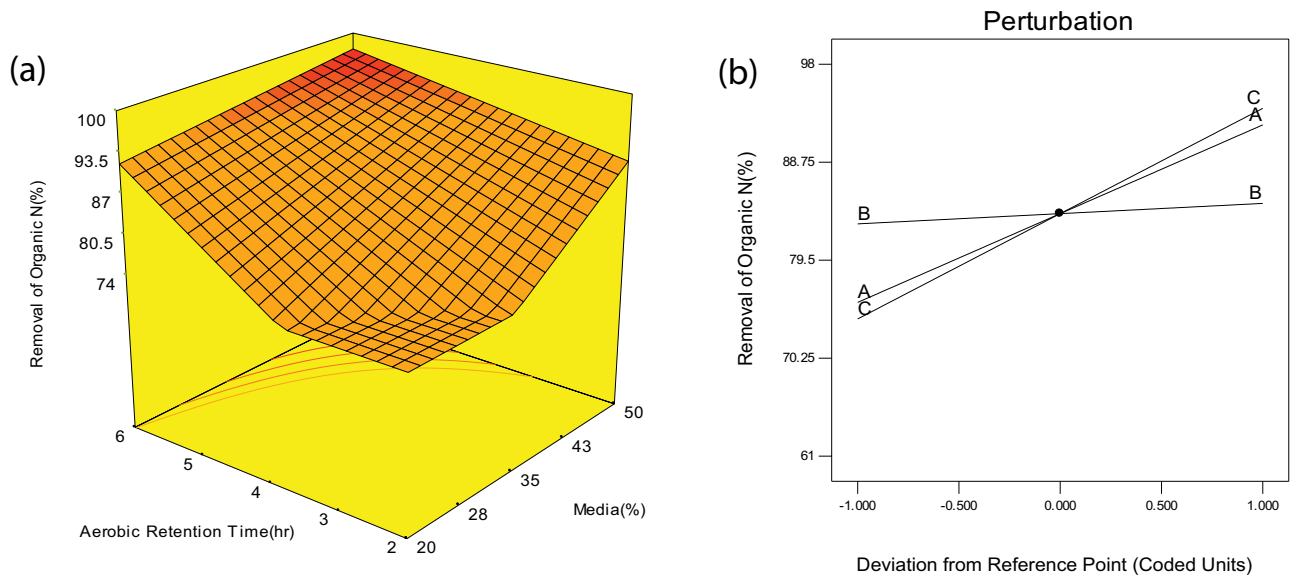


Fig. 2. (a) 3D plan of removal efficiency of organic nitrogen at maximum blending time, (b) deviation from the central point in removal efficiency of organic.

with the high capacity of activated carbon in physical adsorption of organic compounds (due to its high porosity as suitable foundation for growth along with the activity of microorganisms emitting nitrogen) [35–38]. Based on Fig. 2b, in comparison with the mixing time, parameters of aeration time and percentage of medium filling are more effective on the removal of organic nitrogen.

The results of Imai et al. [39] showed that microorganism-attached activated carbon fluidized bed system is able to remove 60% of the nitrogenous compounds from the waste leachate [39].

Bao et al. [40] used the autoclaved aerated concrete particles (AACP) and commercially available ceramsite as media in biological aerated filters (BAFs) in order to simultaneously remove phosphorus and nitrogen. The results indicated removal efficiency of TN with AACP and CAC were obtained 45.96% and 15.64%, respectively [40].

The study of Hwang and Weng [41] indicated that the type of media filter influence on TN removal so that the efficiency removal of TN was obtained 64.3% and 60% for the oyster shell filter and the bio-ball filter, respectively.

3.3. Removal of ammonia nitrogen

With regard to the results, the level of response (removal efficiency) raised as aeration time and medium percentage increased. The efficiency in removal of ammonia at 6 h of aeration and 50% of medium was more than 96%. According to the maximum efficiency in removal of ammonia with addition of aeration time and medium percentage, there was a negligible change in the efficiency as aeration time increased from 2 to 4 h at 20%–35% of medium filling (Fig. 3a). This little reduction in ammonia in this step may be associated with transformation of organic nitrogen into ammonia in the system replaced by the emitted ammonia from the system. In other words, this points to the process of ammonification in this stage of application. At the maximum condition of

mixing (90 min) and 50% of medium filling, the removal efficiency of ammonia sharply rose by increasing the aeration time to 6 and leads to an aerobic process of nitrification. As the mixing time increased from 30 to 90 min, the increment in the efficiency was merely 5%. A slight reduction in ammonia under anaerobic condition of mixing was related to its consumption by anaerobic bacteria in the system. Based on the gradient presented in Fig. 3b, aeration time in comparison with mixing time was more influential on removal efficiency of ammonia since, in addition to aerobic nature of nitrification in transformation of ammonia into nitrite and nitrate, hydraulic retention time was also effective in this regard. Due to the population increment of the attached microorganisms as a result of the medium filling increase and high physical adsorption of ammonia nitrogen (caused by granular activated carbon), the maximum efficiency in removal of ammonia occurred at 50% of medium filling [7]. Moreover, using granular activated carbon in biological systems, along with adsorption of toxic and deterrent materials to the nitrifying microorganisms, improves the removal of ammonia in the system [35,36,42].

The results of Muhamad et al. [43] showed that granular activated carbon sequencing batch biofilm reactor (GAC-SBBR) system is able to remove 100% of $\text{NH}_3\text{-N}$ in condition of aeration rate of $3.2 \text{ m}^3/\text{min}$ and HRT of 1 d. The study of Ren et al. [44] showed that the highest removal efficiency (55.48%) of $\text{NH}_4^+\text{-N}$ was obtained at 80 cm height of media and 40 L/h aeration. Results indicated the supply of DO and distribution of organic matter are two important parameters in removal of $\text{NH}_4^+\text{-N}$ [44].

The study of Holloway and Soares [45] showed the media fill ratio influence the removal efficiency of NH_4^+ . So that the performance of submerged aerated filter had the highest NH_4^+ removal efficiencies 60% and 66% at 100% and 50% media fill ratio, respectively. While, the removal efficiency was decreased to 60% at 100% media fill ratio. Results demonstrated that organic loading rate has remarkable effect

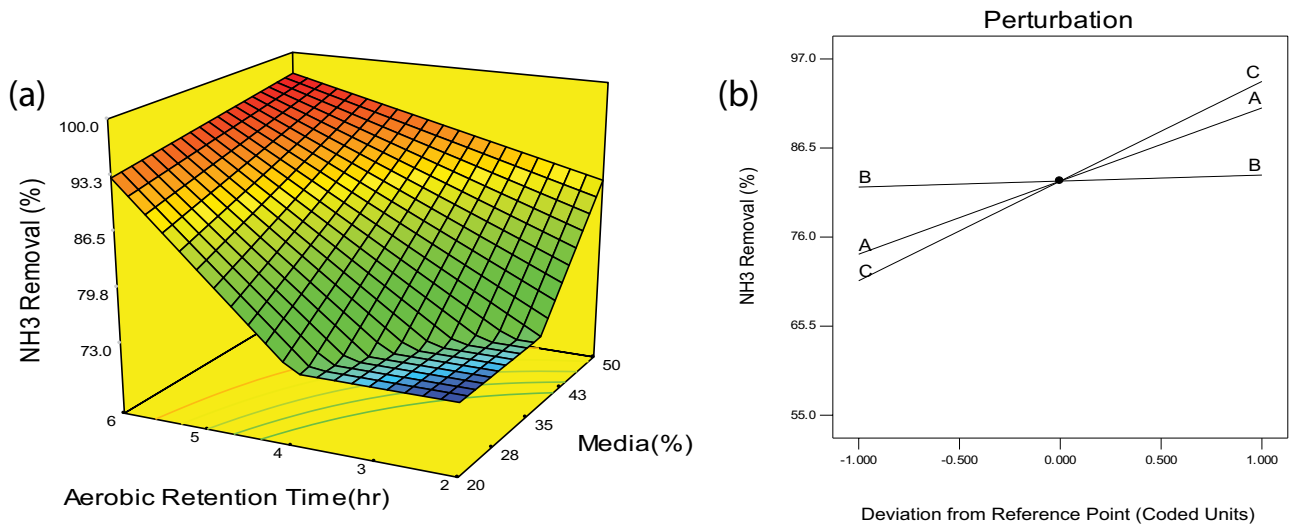


Fig. 3. (a) 3D plan of removal efficiency of ammonia nitrogen at the maximum anaerobic time, (b) deviation from the central point in removal of ammonia nitrogen.

on NH₄⁺ removal so that the maximum efficiency obtained at 0.42 kg m² d⁻¹ of organic loading rate [45].

3.4. Removal of nitrite and nitrate

In the biologic removal of nitrogen compounds, nitrite and nitrate are inter-reaction compounds which are omitted via denitrification process. At the maximum time of mixing (90 min), by increasing the percentage of medium filling; (i) from 20% to 35%, output nitrite increased; and (ii) from 35% to 50% of filling, nitrite experienced a significant reduction (Fig. 4a). The observed decrement in output nitrite in 20% compared with 35% of medium filling was due to less amount nitrogen microorganisms and uncompleted nitrification in the system. In the other words, the nitrogen in the

output sewage is found in the shape of organic nitrogen and ammonia. As the percentage of medium filling increased to 50%, the nitrogen microorganism as well as the consumption of nitrite rose. At the maximum time of aeration and mixing, the decrement in the output nitrite was due to increasing HRT and complete nitrification in the system. This observation was due to this fact that the nitrifier bacteria (as a result of their high rate of growth and not competing with heterotrophic bacteria), with increasing hydraulic retention time and decreasing BOD, overcame the heterotrophic bacteria and grew [46].

According to the results, the output nitrate followed a similar trend to the output nitrite so that the minimum amount of the output nitrate in the maximum time of aeration coincided with maximum time of mixing and medium

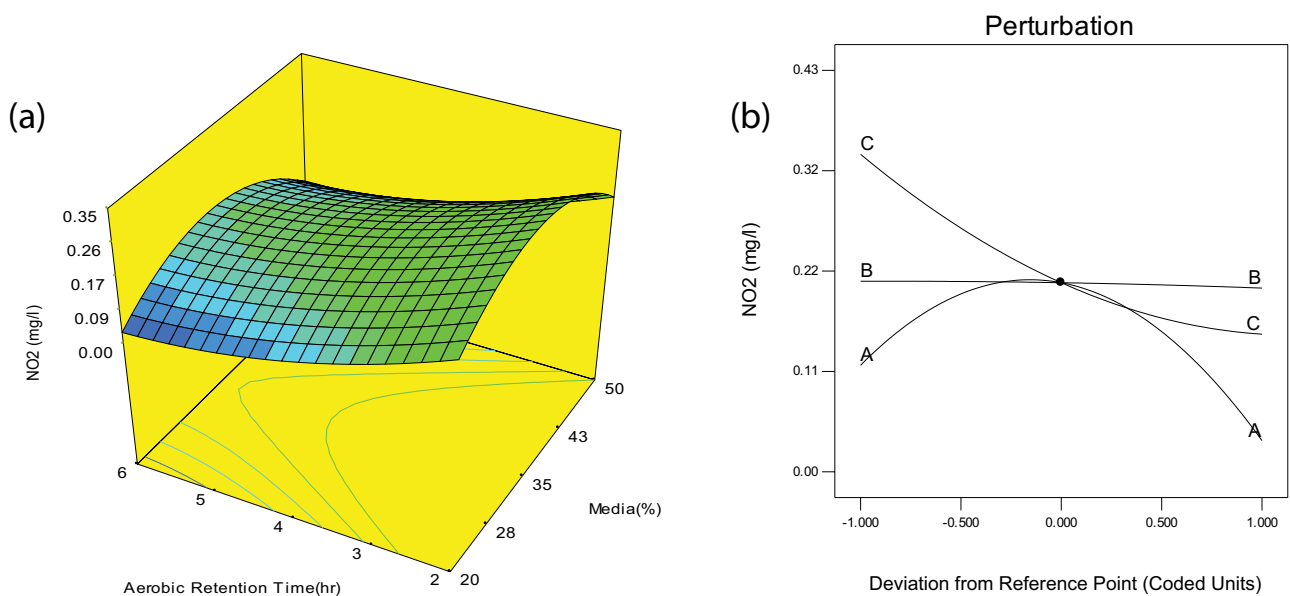


Fig. 4. (a) 3D plan of output nitrite at the maximum anaerobic time, (b) deviation from the central point in output nitrite.

percentage (Fig. 5a). Compared with the first 30 min, the increment in the output nitrate at the anaerobic mixing time of 60 min overcomes the phosphate bacteria and the shortage of organic materials for the denitrifier bacteria in the regeneration of nitrate. This process was also demonstrated by oxidation–reduction potential (Fig. 5b). One of the other effective parameters on removal of nitrate was the percentage of medium filling for forming biofilms of denitrifier microorganisms. As a result of high porosity and increasing cell retention time, the population of nitrogen microorganisms rose in the high percentage of medium, thereby growing the reduction of nitrate. Based on the outcomes, the use of activated carbon can be assumed as suitable foundation for growing nitrogen microorganisms [47–49]. At 50% of medium filling, the minimum amount of output nitrate was achieved in the maximum time of aeration and blending. Increasing blending time and the nonexistence of soluble oxygen in the system provided the appropriate circumstances for activities of denitrifier bacteria, and the process of denitrification was completely accomplished. Moreover, the decrease of output nitrate in the maximum time of aeration was due to the reduction of ammonia nitrogen in the system and due to the use of nitrate as a nitrogen source in assimilation process (cell synthesis). The amount of nitrogen stored in the biomass is 0.12 gN/g biomass (Metcalf and Eddy [50]). Based on the amount of daily biomass mass in the maximum COD removal, the amount of nitrogen stored in the biomass is about 38 mgN/d.

Kjeldahl nitrogen, which includes organic and ammonia nitrogen, at the maximum blending time (without aeration), had a high removal efficiency when increasing the aeration time and the medium percentage (Fig. 6a). The maximum removal efficiency of Kjeldahl nitrogen was achieved at the maximum amount of the parameters since the hydraulic retention time rose due to increase in blending and aeration

time. Organic and ammonia nitrogen were eliminated from the system via the complete process of nitrification. Based on the fact that nitrogen forms 12% of dry weight of microorganisms, increasing the medium percentage lead to the increase of microorganism growth rate, thereby increasing nitrogen consumption in the system. Based on the gradient of Kjeldahl nitrogen removal efficiency (presented in Fig. 6b), the aeration time, medium percentage and blending time (without aeration) are the most influential parameters, respectively. In this regard, the use of granular activated carbon in biological systems adsorbing heavy metals (zinc, copper, cadmium, etc.) was mentioned in the relevant literature to prevent poisoning of microorganisms and to provide suitable conditions for growth of nitrifier microorganisms having low growth rate [51].

The total numbers in nitrite-oxidizing bacteria (NOB) compared with ammonium-oxidizing bacteria is expected to be even further lowered in systems where simultaneous nitrification/denitrification is taking place. Therefore, most part of ammonium will be transformed to nitrate. On the other hand, denitrification can be occurred in the attached flock to the media. This process results in NO_3^- concentration to be half of ammonium concentration which is expected to completely transform to nitrate. Also, Both nitrite and nitrate can be used as electron acceptor by denitrifying bacteria to generate nitrogen gas. If denitrification takes place mainly over nitrite, NOB would have to compete for nitrite with denitrifying organisms [52].

The results of García-Martínez et al. [53] showed that continuous up-flow stirred packed bed reactor containing biological sludge carbonaceous material system is able to remove 99% of nitrate in condition of space times of 2 min and HRT of 6 min. Also, the study of Ren et al. [44] revealed that the highest removal of nitrate (94.85%) was obtained at 0–10 cm height of media. On the other hand, results

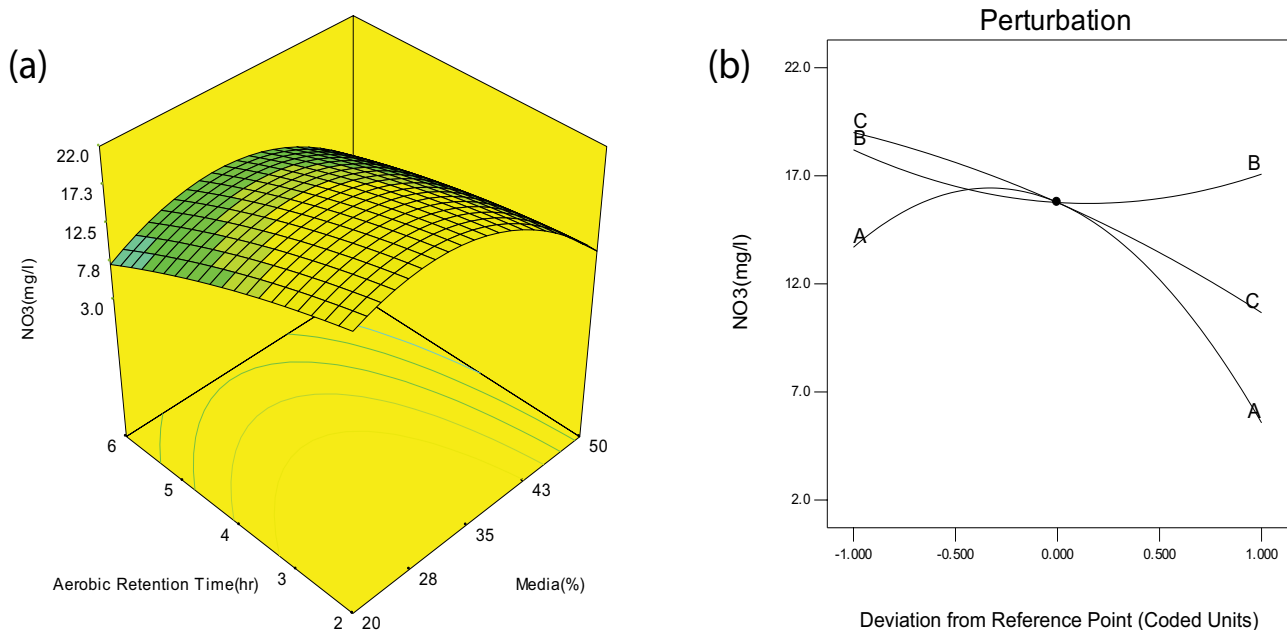


Fig. 5. (a) 3D plan of output nitrate at the maximum anaerobic time, (b) deviation from the central point in output nitrate.

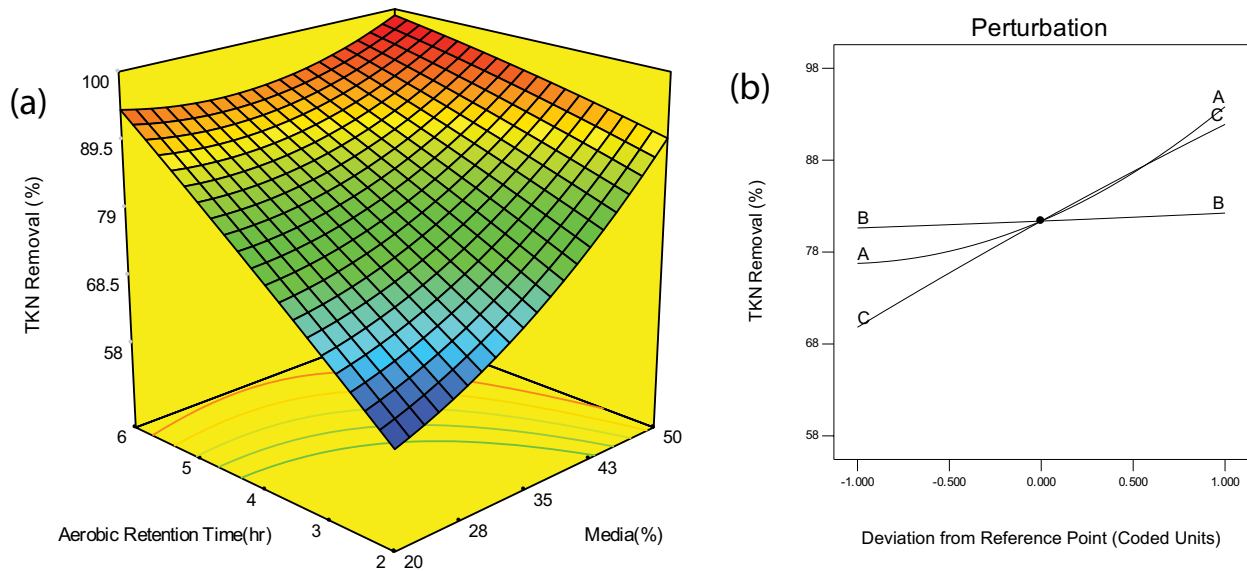


Fig. 6. (a) 3D plan of efficiency of Kjeldahl nitrogen removal at the maximum anaerobic time, (b) deviation from the central point in efficiency of Kjeldahl nitrogen removal.

illustrated the inverse relationship between nitrate removal and height of media, so that the nitrate removal reached 70% at 90 L/h aeration rate [44].

3.5. Removal of phosphorus

In the studied system, unlike nitrogen compounds, the best removal efficiency of total phosphorus was observed at aeration time of 4 h and blending time of 60 min (Fig. 7a). The removal efficiency of phosphorus had a considerable increase at 50% of medium filling. Furthermore, at 50% of medium filling, the removal efficiency of phosphorus increased from 39.36% to 53% as blending time rose from 30 to 90 min (Fig. 7b).

Related to the need of successive aerobic and anaerobic steps in biological removal of phosphorus having source of adequate carbon for growth of polyphosphate microorganisms is mandatory and thus, ICAAGACR is one of the modern biological processes of removal of nourishing materials with continuous input of raw sewage [54].

In the present study, notwithstanding high removal efficiency of nitrogen compounds within 6 h of aeration, the removal efficiency of phosphorus was reduced as a result of aggregation of nitrate during complete process of nitrification and overcoming denitrifier bacteria in competing phosphorous bacteria so as to absorb organic materials. Therefore, in 50% of medium, the best removal efficiency of phosphorus took place at 4 h of aeration (Table 5). Similarly, in a research by Nair and Ahammed [55] on removal of total phosphorus from urban sewage, an increment in an aeration time from 4 to 6 h increased the removal efficiency of phosphorus from 26% to 52.2%.

In addition, the assessment of effects of mixing time and medium percentage in optimum conditions of aeration (4 h) indicated a high influence of medium percentage on the best removal efficiency of phosphorus, compared with blending time (Fig. 7c). As filling percentage of activated carbon

increased and anaerobic conditions improved, polyphosphate bacteria became dominant and adsorbed more phosphorus in aerobic conditions.

Escapa et al. [56] carried out a study where an ICEAS reactor was used to remove phosphorus from urban sewage. The best removal efficiency of phosphorus was 55.9% with 16 h of hydraulic retention time. In this research, by adding a bed of granular activated carbon at 5.5 h of hydraulic retention time, the removal efficiency of phosphorus increased to 60.7% [56]. Moreover, Hussain et al. [7] acquired a removal efficiency of 70% for removing orthophosphate from urban sewage using a granular active-carbon adsorbent.

Li et al. [57] achieved a phosphate removal of 36%–61.1% using an activated ceramic as a biologic bed at 4–10 h of hydraulic retention time. In this study, at 3 h of hydraulic retention time, a removal of 36.9% was reported which indicates the superiority of granular active carbon compared with the activated ceramic in growth and activities of polyphosphate microorganism. Thus, according to the literature and the present results, granular activated carbon is not only a suitable bed for growth of polyphosphate microorganism but also an intense adsorbent for phosphorus removal [57].

The study of Wang et al. [58] showed that the traditional BAF only achieved the phosphorus removal efficiency of 9.3%. In the study of Ren et al. [44], the TP removal efficiency oscillated as the filter material increased, the removal efficiency reached the maximum amount at high aeration rates. The results of this study confirmed that the TP removal was increased at 0–20 cm height of the filter media layer, the removal decreased at 20–40 cm height of the media and afterward removal efficiency of TP was increased at 40–80 cm height of media in 90 L/h aeration rate [44].

3.6. Statistical analysis

Central composite design (CCD) was utilized for finding connections between variables and responses of the process.

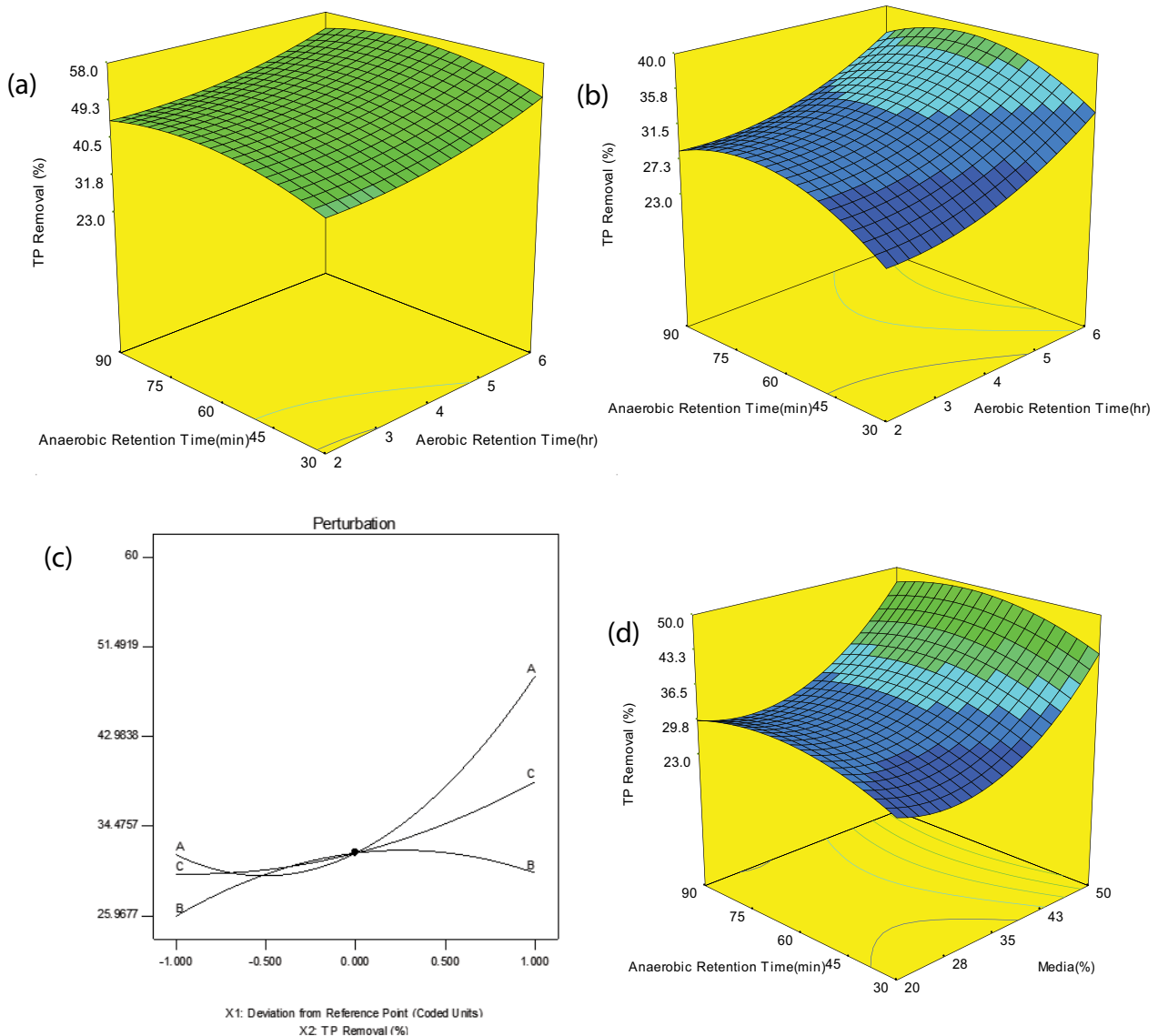


Fig. 7. (a) 3D plan of removal efficiency of TP under the condition of 35%, (b) 50%, (c) maximum aeration, (d) deviation from the central point in removal efficiency of TP.

Models with coded factors are presented in Table 6 along with an analysis of variance (ANOVA) for the intended responses. In statistical analysis (ANOVA), the level of significance (p -value) was presented in each response in order to determine significance of models. The detected values of p -value for removal of nitrogen and phosphate compounds were ($p < 0.0001$) and ($p < 0.01$), respectively.

In this study, the amounts of accuracy were 7.49–23.60. The coefficient of determination based on the proposed model in removal of TKN, NH_4 , N-Org, TN, and TP was 0.97, 0.93, 0.91, 0.97 and 0.8, respectively. According to the outcomes of statistical analysis of ANOVA from software of Design-Expert, the amount of p -value used for determining significance of models in removal of all forms of nitrogen compounds and total phosphorus was $p < 0.05$, indicating the significance of the proposed model for nourishing materials removal [59–61]. A high coefficient of determination

(R^2) also authenticated the correlation of coefficient and proposed model. The measurement of model accuracy is for determining the experiment errors, and a ratio more than 4 units is a desired amount [60,62–64]. In this study, the calculated accuracy was 7.49–23.60 which obviously more than 4.

In regard to the outcomes of Design-Expert, blending time (without aeration) was detected as a parameter with the lowest effect on the removal of pollutant so that the least coefficient of model was allocated to this parameter. Moreover, the most effective factors on removal of nitrogen compounds were aeration time and medium percentage, respectively, and in removal of compounds of total phosphorus, the filling percentage of reactor with medium (loading of total solids) was more effective in comparison with aeration time (Table 6).

The results of experiments along with the outcomes of the proposed model under optimum conditions for removal

Table 6
Results of analysis of variance (ANOVA) for the studied response in Design-Expert

Removal efficiency	Significant equations for considered response of medium percentage, anaerobic and aerobic (A, B and C)	Model	R ²	Accuracy	p-value
TKN	+81.37 + 8.53A + 0.81B + 11.05C + 0.35AB - 6.56AC - 0.025BC + 3.92A ² + 0.060B ² - 0.52C ²	Quadratic	0.97	20.84	0.0001<
Lack-of-fit F value:	11.21				
NH ₄	+82.6 + 8.62A + 0.71B + 11.75C + 0.52AB - 7.11AC + 0.13BC	2FI	0.93	19.10	0.0001<
N-Org	+83.89 + 8.38A + 0.97B + 9.94C + 0.084AB - 5.72AC - 0.26BC	2FI	0.91	17.43	0.0001<
Lack-of-fit F value:	12.40				
TN	+69.22 + 11.22A + 1.14B + 12.82C - 0.17AB - 6.70AC + 0.31BC + 8.49A ² - 0.99B ² + 0.62C ²	Quadratic	0.97	23.60	0.0001<
Lack-of-fit F value:	6.72				
TP	+31.93 + 8.48A + 2.04B + 4.37C + 0.094AB + 0.49AC - 0.31BD + 8.31A ² - 3.93B ² + 2.40C ²	Quadratic	0.80	7.49	0.01<
Lack-of-fit F value:	8.14				

A: Mixing time (min).

B: Aeration time (h).

C: Media fill percentage (%).

of nourishing materials including standard deviation are presented in Table 7. Accordingly, the results of experiments were in good agreement with the outcomes of the proposed model.

3.7. Optimization of experimental conditions using RSM

The conditions were optimized according to the best combination of parameter levels that obtain maximum amounts for the studied response. The chosen criteria for optimization goal were 'maximize' for response (removal of TKN, NH₄, N-Org, TN and TP) and 'in range' for input parameters. Among 29 proposed solutions, the top 27 solutions were expressed with higher desirability. The distinguished optimal conditions were the mixing time (37.64–89.74 min), aeration time (3.49–5.61 h) and media fill percentage (25.54%–45.83%) with the peak desirability value 100%. The maximum percentage of TKN, NH₄, N-Org, TN and TP removal were 97.49%, 94.21%, 97.54%, 96.27% and 68.76%, respectively.

3.8. Determine the coefficients of the Monod equation

The rate of substrate consumption in the aerobic biological process was determined using the Monod equation (Eq. (5)) [65]. In this equation, coefficients can be calculated

by changing the use of organic substrate vs. the production of biomass.

$$r_{su} = \frac{k_0 X_s}{K_s + S} \quad (5)$$

where r_{su} is the rate of variation of the biomass concentration due to its consumption (g/m³ d), k_0 is maximum rate of consumption of substrate (g substrate/g microorganism. day), X_s is biomass concentration (g/m³), S is concentration of the substrate limiter growth in solution (g/m³) and K_s is half rate constant, concentration of substrate in half the maximum rate of substrate consumption (g/m³). To determine the K_s and k_0 , the main equation of the Monod equation should be linearized, and by determining the slope and intercept of line, these values is determined. In this research, three Lineweaver–Burk, Hanes and Hofstee models are used to linearize the main equation.

3.8.1. Lineweaver–Burk model

In this model, the main Monod equation can be expressed as Eq. (6) [66].

$$\frac{1}{k} = \frac{K_s}{k_0 S} + \frac{1}{k_0} \quad (6)$$

Table 7
Confirmation of test in optimum conditions

	Removal efficiency (%)					Outputs (mg/L)	
	TN	TKN	NH ₃ -N	N.Org	TP	NO ₃	NO ₂
Experimental	87	94.4	93.5	95.7	60.7	13	0
Model	89.5	94.15	91.5	92.55	49.06	5.6	0.03
Standard deviation	+2.5	-0.35	-2	-3.15	-11.64	-7.4	+0.03

Aeration time = 4 h, mixing time = 60 min, medium filling = 50%.

By drawing $1/k$ vs. $1/S$, a straight line is obtained. The slope of this line is K_s/k_0 and the intercept is $1/k_0$. Fig. 8 shows the changes in the concentration of the biomass and substrate input to the pilot based on Lineweaver–Burk model. As it can be seen, k_0 and K_s are obtained at 41.66 and 394.625 g/m^3 , respectively.

3.8.2. Hanes model

In this model, the main Monod equation can be expressed as Eq. (7) [67].

$$\frac{S}{k} = \frac{S}{k_0} + \frac{K_s}{k_0} \quad (7)$$

By drawing S/k vs. S a straight line is obtained. The slope of this line is $1/k_0$ and the intercept is K_s/k_0 . Fig. 9 shows the changes in the concentration of the biomass and substrate input to the pilot based on Hanes model. As it can be seen, k_0 and K_s are obtained at 3.267 and 3.813 g/m^3 , respectively.

3.8.3. Hofstee model

In this model, the main Monod equation is multiplied in $(K_s + S)/S$ and through simplification, Eq. (8) is obtained [68].

$$k = k_0 - K_s \left(\frac{K}{S} \right) \quad (8)$$

By drawing k vs. k/S , a straight line is obtained. The slope of this line is $-K_s$ and the intercept is k_0 . Fig. 10 shows the changes in the concentration of the biomass and substrate input to the pilot based on Hofstee model. As can be seen, k_0 and K_s are obtained at 3.231 and 86.23 g/m^3 , respectively.

3.9. Mathematical relations of kinetic coefficient

The pilot system was calculated using the data obtained from the experimental setup and by applying Eqs. (9) and (10) [69], related to the conventional activated sludge system. The entrance flow rate to the aeration tank is 2 L/min, the volume of the tank is 4 L, and HRT is 1.5 h.

$$\frac{1}{\text{SRT}} YU - k_d = \frac{Y(S_0 - S)}{\theta X} - k_d \quad (9)$$

$$\frac{\theta X}{S_0 - S} = \frac{K_s}{k_0 S} + \frac{1}{k_0} = \frac{1}{U} \quad (10)$$

where SRT is cell retention time (d), S_0 is concentration of input substrate (mg/L COD), S is concentration of output substrate (mg/L COD), U is rate of substrate consumption (mg COD/mg VSS) and θ is hydraulic retention time (d). Kinetic coefficient is determined by drawing U vs. $1/\text{SRT}$ and $1/S$ vs. $1/U$ diagrams. By linearizing the U vs. $1/\text{SRT}$ diagram, the intercept is k_d and slope is Y . For $1/S$ vs. $1/U$ diagram, the intercept is $1/k_0$ and slope is K_s/k_0 . As shown in Fig. 11, Y is 86.66 mg/mg, k_d is 0.245 d^{-1} , k_0 is 2.04 mgCOD/L and K_s is 226.02 $\text{g COD}\cdot\text{m}^{-3}$.

As previously mentioned, in order to model the biological process using ASM, the required data were obtained from an experimental pilot in a 1-year time period. A total of 97 replicates sampling were done. The range of changing of input and output parameters are presented in Table 5. As it can be observed, the average input COD is 365 mg/L and its removal efficiency is more than 86%. The mixed liquor volatile suspended solids (MLVSS) variations in the aeration tank were in the range of 2,034–2,467, and the average weekly data

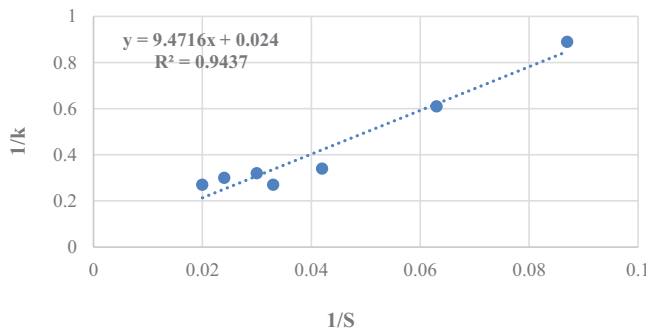


Fig. 8. Determination of kinetic growth coefficient of the microorganisms in the Monod equation, Lineweaver–Burk model.

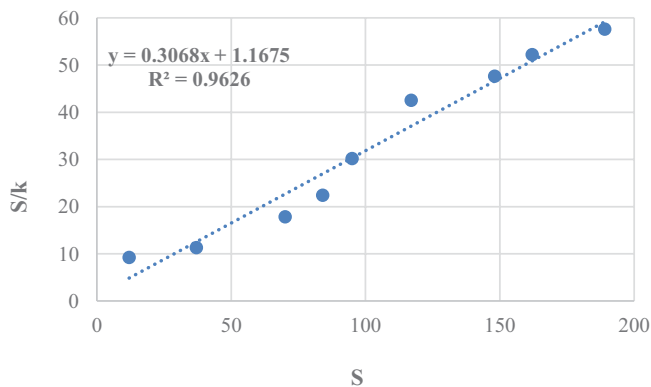


Fig. 9. Determination of kinetic growth coefficient of the microorganisms in the Monod equation, Hanes model.

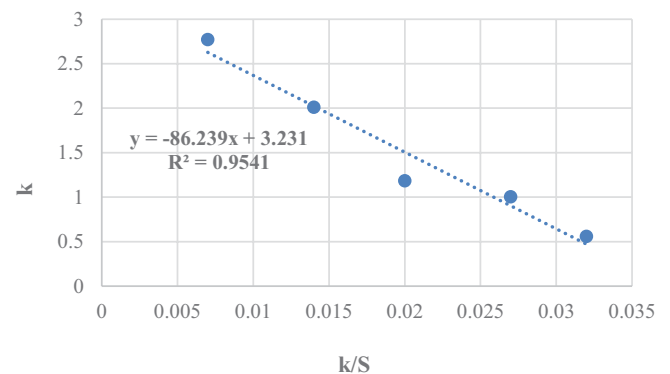


Fig. 10. Determination of kinetic growth coefficient of the microorganisms in the Monod equation, Hofstee model.

Table 8
Results of kinetic coefficients determined using the ASM1 model and its comparison with the results of the Monod model

Kinetic coefficient	ASM1 model	Results from experimental set-up			Recommended amount in IWA (in 20°C)	Recommended range by Metcalf–Eddy*
		Lineweaver-Burk model	Hanes model	Hofstee model		
K_s , g COD.m ⁻³	28.44	394.625	3.813	86.23	20	5–40
μ_H (k_0), 1/d	2.6	41.66	3.267	3.231	6	3–13.2
b_H (k_d), 1/d	0.051	–	–	–	0.062	0.06–0.2
Y_{Hf} gCOD X_H (gCOD SS) ⁻¹	0.42	–	–	–	0.67	0.3–0.5
f_p	0.05	–	–	–	0.08	0.08–0.2
k_h (g cell COD. d) ⁻¹	2.77	–	–	–	3	–
K_x (g cell COD. D)	0.027	–	–	–	0.03	–

*For heterotrophic bacteria (in 20°C).

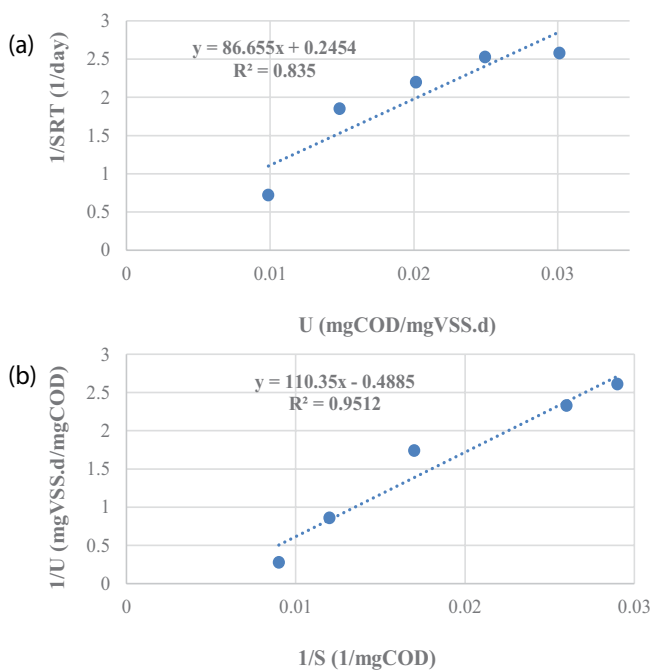


Fig. 11. Determination of the kinetic coefficients of growth of microorganisms.

were used in the input model for the software. To run the model, according to the VSS, COD and SCOD parameters, the appropriate range of process coefficients was estimated in the ASM1 model (Table 8). As you can see, in all cases the results of the model is within the recommended range of the International Water Association (IWA) [70]. Also, Table 8 presents the results of comparison of Monod and ASM models. As it can be seen, there is a good correlation between the kinetic coefficients of biological processes and the results of the study of the growth of microorganisms in the experimental pilot, the results of operating and the ASM1 model. The results of the ASM1 model for K_s of 28.44 gCOD m⁻³, μ_H of 2.6 d⁻¹, b_H of 0.051 d⁻¹ and Y_H of 0.42 g COD X_H (gCOD SS)⁻¹. On the other hand, the kinetic coefficients of growth of microorganisms are within the recommended range of references. In other words, the system's management in terms of

the efficiency of removal and the biological conditions of the process is in desirable conditions.

4. Conclusion

In this work, an ICAAGACR with effective volume of 4 L was used to grow microorganisms and a biofilm, commercial granular activated carbon which filled 20%–50% of the reactor volume was applied. The RSM results showed that aeration time was the most effective factor for removal of nitrogen compounds. The area of the maximum removal efficiency was achieved at aeration time of 6 h, blending time (without aeration) of 9 min and 50% filling of volume of reactor. The removal efficiency for TKN, NH₄, N-Org and TN was 96.8%, 96.8%, 96.9%, and 95%, respectively. The maximum removal efficiency of TP was 60.7% at aeration time of 4 h, blending time (without aeration) of 9 min and 50% filling of volume of reactor. The percentage of filling medium was the most important variable in the removal of total phosphorus.

Also, linearization of the Monod equation was determined using three different mathematical methods with proper accuracy of the maximum growth rate and the half-rate constant. The results showed that there is a good correlation between biological kinetics coefficients in laboratory conditions with the same coefficients in operating conditions. Furthermore, estimation of kinetic coefficients using the ASM1 model correlated strongly with the results of the Monod model and the determination of the kinetic coefficients of the operation of the experimental system.

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