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TOTAL PHOSPHORUS AND TOTAL KJELDAHL NITROGEN REMOVAL USING AN AEROBIC GRANULAR SLUDGE PROCESS. CASE STUDIES ANN AND RSM MODELING

Removing nutrients from wastewater is essential because high concentrations in aquatic systems lead to severe eutrophication problems, the most common impairment of surface waters such as lakes and oceans. Total phosphorus (*TP*) and total Kjeldahl nitrogen (*TKN*) were removed from mixed wastewater using an aerobic granular sludge process in a sequencing batch reactor (AGS-SBR). An artificial neural network (ANN) and response surface methodology (RSM) were applied to evaluate the main parameters of the process. For *TKN* removal, only cycle time (*CT*) (0.0475) was a significant variable, achieving removal efficiencies of up to 81%. In *TP* case removal, two parameters, *VER* and *AR*, were substantial for this process, completing elimination efficiencies of around 40%. On comparing the models with statistical indices, ANN coupled with the moth-flame optimization algorithm (ANN-MFO) demonstrated higher performance with an adjusted R2 (0.9866) for the case of *TP* removal and (0.9519) for *TKN* removal.

1. INTRODU*CT*ION

Removal of nutrients from wastewater is essential because high concentrations in aquatic systems lead to severe problems of eutrophication, the most common impairment of surface waters such as lakes and oceans [1]. In this regard, ever since aerobic

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granular sludge (AGS) technology was first reported in 1991, the formation mechanisms and application of aerobic granular sludge have become significant topics in the field of water treatment research due to its being a breakthrough technology with tremendous potential to become the new standard for aerobic treatment of wastewater [2]. On the other hand, the conventional activated sludge (CAS) method commonly faces some operational challenges: its performance is compromised due to low sludge settling capability, byproduct generation, long sludge retention time, and the large surface areas required for installing tanks for proper nitrogen treatment [3].

Compared with CAS, AGS exhibits excellent settling capability, high biomass retention, simultaneous nutrient removal, lower area requirement, strong resistance to organic loading rate, lower energy expenditure during the process, a small foo*model*rint, and spectacular biomass biodiversity [3, 4]. All these promising features are rooted in the phenomenal structure of aerobic granules [5]. Aerobic granules are dense, self-immobilized, and multi-species microbial aggregates cultivated mainly in bubble-column sequencing batch reactors (SBRs) [5]. To improve, promote, and standardize their practical application for wastewater treatment, researchers worldwide extensively investigated the fundamentals of AGS systems to understand their performance capacities, microbial interrelationships, and structural properties [6]. Therefore, many studies specifically focused on identifying the effect of operating factors that influence AGS processes, such as organic loading rate (*OLR*), pH, dissolved oxygen (*DO*), volumetric exchange ratio (*VER*), temperature, aeration rate (*AR*), cycle time (*CT*), and the ratio of chemical oxygen demand to nitrogen (*COD*/*N*), among other [7, 8]. Since the operating factors applied are closely tied to AGS performance, these processes may be identified as complex and largely unpredictable systems. In this regard, a mathematical simulation model that encompasses all the influential factors could be invaluable as an evaluation tool to aid in the design, operation, and optimization of the system at large scales [4, 6]. Moreover, mathematical modeling has proven to be very useful in studying complex processes, such as AGS systems [6].

Artificial intelligence such as artificial neural networks (ANN), has undergone significant development, allowing the simulation of highly non-linear biological systems with outstanding results [4]. Traditional statistical models have limitations that do not allow them to simulate the system under study; artificial intelligence models, on the other hand, do not have those limitations [4, 9]. Nonetheless, there needs to be more research reported on artificial intelligence to predict the performance of an AGS system on wastewater management [4, 6]. One of the main difficulties of simulating highly non-linear systems such as AGS is the complicated relationship between biological, physical, and chemical activities [9].

The response surface method (RSM) is a mathematical tool for modeling and experimental design. This method has been employed for modeling physical and biological systems to allow an estimation of the main parameters surrounding the processes with a limited number of experiments [10]. The present study aims at developing an

RSM and ANN for modeling highly non-linear biological systems such as AGS in sequential batch reactors (SBR) at bench-scale, to define the main parameters in removal efficiencies of *TKN* and *TP* from mixed wastewater using aerobic granular sludge system.

2. MATERIAL AND METHODS

Reactor operation. The SBR with a total volume of 12 dm^3 (internal diameter of 14 cm and height of 90 cm) shown in Fig. 1 had a working volume of 4.5 dm^3. The reactor was equipped with air pumps (Elite-801 and Elite-802, Hagen group®, USA) to keep the *DO* higher than 2 mg/dm^3. The reactor operated at room temperature between 23 and 31 °C, with volumetric exchange ratios of 50, 67, and 75%.

Fig. 1. View of equipment for SBR operation

Cultivation of granules was carried out exclusively under aerobic conditions. These experiments were conducted in the installations of an industrial WW*TP* located in the Cuernavaca Valley in Morelos, Mexico. The seed inoculum, of municipal origin, was adapted to the characteristics of the feed wastewater.

Subsequently, stable aerobic granulation was developed to apply the established operational configurations. In the experiments, three influential operating parameters were varied according to the experimental design in situ, with measurements being divided into successive stages. The preceding was ordered to evaluate the AGS performance on organic contaminant removal under different operating conditions. The experimental setup, using a Box-Behnken design (BBD), provided a total of 17 experiments. These operating combinations were applied to the AGS-SBR. The total operating period for operational combinations was about 87 days.

Wastewater source and seed sludge. The wastewater samples were collected from the primary settling tank of the installation, where the experimental study was carried out (Table 1). The seed sludge for the formation of aerobic granulation and experiments were taken from a municipal WW*TP* and an industrial WW*TP* located in the Cuernavaca Valley.

Table 1

Composition of mixed industrial /municipal wastewater used in this study

Parameter [mg/dm^3]	Average
COD	2.316±1.287
TSS	569±1.146
BOD	709±122.7
TP	24.4±7.2
TN	85.1±37.02
pH[a]	7.32±0.37

[a]pH units; $n = 45$.

Analysis methods and data collection. The input variables in the model formulation were total phosphorus (*TP*), total Kjeldahl nitrogen (*TKN*), nitrates, chemical oxygen demand (*COD*), total suspended solids (*TSS*), volumetric sludge index (*SVI*), temperature (*T*), pH, *DO*, and conductivity. The data were obtained through measurement of each parameter throughout the operating period. The *TKN* data were acquired with a Büchi Kjelmaster K-375 instrument. Nitrate nitrogen (NO_3-N) was measured following the 8,039 HACH colorimetric method. *TP* was determined using a colorimetric method (10,127 HACH). *SVI* was analyzed based on standard methods [11]. *COD* was measured according to NMX-AA-030/2-SCFI-2011. *DO* was monitored every 24 h with a dissolved oxygen meter dissolved oxygen meter YSI model 58. Temperature, pH, and conductivity were measured in situ with an OAKlon PC540 potentiometer. The behavior of the AGS and the general performance of the system were monitored.

To verify the measurement quality for the parameters of mixed wastewater, the following were used: *VER*, %, *AR*, dm^3/min, and *CT*, day [6, 13, 14]. The following equation was used to obtain the removal efficiencies for *TKN* and *TP*

$$R = \frac{Y_0 - Y}{Y_0} \times 100\% \tag{1}$$

where R represents the removal efficiency, Y_0 is the initial measurement, and Y is the final *TP* or *TKN* measurement.

Response surface methodology modeling. Table 2 shows the design matrix with three main factors. One factor had three levels where *VER* ranged from 50 to 75% (A), *AR* from 2.5 to 3.5 dm^3/min (B), and *CT* from 1.8 to 3.2 day (C). The response variables were *TP*

(Y_1) and *TKN* (Y_2) removals using a BBD. Design-Expert software 10.0.1 (Stat-Ease, Inc., Minneapolis, MN, USA) was used for all statistical analysis and 3D plotting.

Table 2

Levels of independent variables

Factor	Parameter	Coded variables		
		–1	0	1
A	*VER*, %	50	67	75
B	*AR*, dm^3/min	2.5	3	3.5
C	*CT*, day	1.8	2.5	3.2

The second-order polynomial function obtained by correlating the three independent variables by predicting the response values is as follows

$$Y = b_0 + \sum_{i=1}^{k} b_i X_i + \sum_{i=1}^{k} b_{ii} X_i^2 + \sum_{i=1}^{k}\sum_{j=1}^{k} b_{ij} X_i X_j + \varepsilon \qquad (2)$$

where Y represents response *TP* and *TKN* removal efficiencies, %, b_0 is the intercept value, b_i, $i = 1, 2, 3, \ldots, n$, represents the first model coefficients for X_i, b_{ii} represents the quadratic coefficients of X_i, b_{ij} are the correlation coefficients for X_i and X_j, and ε is the random error.

Neural network modeling and optimization. The MATLAB R2021a software (Mathworks, Inc., Natick, MA, USA) and neural network toolbox were used to operate the different architectures proposed (*TP* and *TKN* removal). A moth flame optimizer algorithm (MFO) coupled with ANN was used to optimize the models [15] (Table 2). The proposed architecture for the ANN models (Fig. 2) was built by applying an input layer with three neurons *VER* (A), *AR* (B), and *CT* (C), a hidden layer, and an output layer with one neuron (*TP* or *TKN*).

To increase the performance of the ANN, the system was taken as the objective function, which was then minimized using a metaheuristic algorithm. The metaheuristic MFO algorithm was used to adjust the weights of the internal layers of the ANN [10]. Three groups were formed: a training group (70%), a validation group (15%), and a test group (15%), to find the most efficient number of neurons in the hidden layer. Iterative tests were used to evaluate groups of 4, 6, 8, 10, and 12 hidden neurons and in assessing the performance of each simulation, the mean square error (MSE) was determined. All the data presented were normalized using equation [16].

$$X_{\text{norm}} = 0.8\left(\frac{x_i - x_{\min}}{x_{\max} - x_{\min}}\right) + 0.1 \qquad (3)$$

where x_i represents the current value that is being normalized, $x_{\min}$ is the minimum value of the data set, and $x_{\max}$ is the maximum value of the data set.

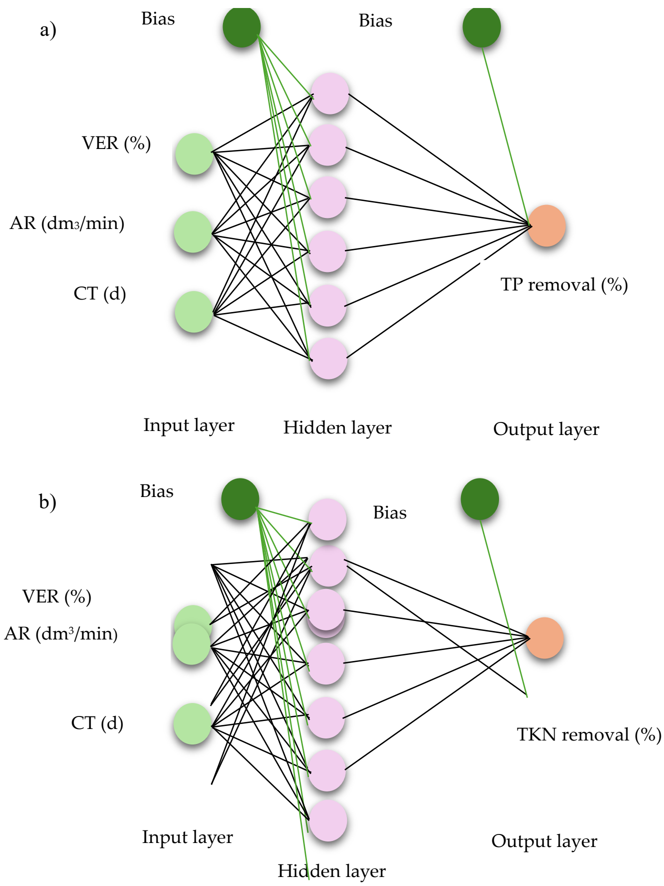

Fig. 2. Architecture for: a) ANN *TP* removal, b) ANN *TKN* removal

Evaluation of the developed mathematical models. To delimit the operation of the models, statistical indicators were used to evaluate the performance of the models presented in this work. The indicators were: the correlation coefficient (R), the coefficient of determination (R^2), adjusted R^2, *MSE*, root mean squared error (RMSE), mean absolute error (*MAE*), and average absolute deviation (*AAD*) [10].

3. RESULTS

Two empirical models were determined for analysis of the behavior between the process variables and the responses, giving the following equations for *TP* (Y_1) and *TKN* (Y_2) removal, respectively

$$Y_1(\%) = 25.21 + 1.41A + 1.53B - 0.65C + 0.072AB - 3.09AC$$
$$- 0.63BC + 8.58A^2 + 11.24B^2 + 3.09C^2 \quad (4)$$

$$Y_2(\%) = 80.16 - 0.19A - 1.56B - 3.17C - 9.92AB - 11.88AC$$
$$+ 5.02BC - 1.90A^2 - 9.60B^2 - 12.83C^2 \quad (5)$$

Table 3

Box Behnken design: observed and predicted values for *TP* and *TKN* removal

No.	Experimental	Predicted ANN-MFO	Predicted RSM	Experimental	Predicted ANN-MFO	Predicted RSM
	TP			*TKN*		
1	44.99	41.59	42.35	80.15	81.59	80.16
2	25.21	25.21	25.21	58.88	53.12	56.99
3	25.21	25.21	25.21	54.27	54.39	68.79
4	32.25	32.25	40.73	53.47	53.13	58.02
5	40.14	40.14	42.05	80.15	81.59	80.16
6	47.63	47.44	51.06	78.03	85.72	77.21
7	39.80	39.80	42.03	80.15	81.59	80.16
8	25.21	25.21	25.21	56.10	57.69	54.32
9	40.14	40.14	31.73	49.31	50.92	51.1
10	25.21	25.21	25.21	52.81	53.13	62.07
11	25.21	25.21	25.21	76.59	76.24	68.41
12	35.34	35.34	38.03	79.10	79.09	79.95
13	42.48	42.48	39.79	72.00	71.97	67.48
14	49.77	49.77	47.99	58.57	56.95	60.49
15	35.32	35.32	33.03	80.15	81.59	80.16
16	42.57	44.89	39.00	80.15	81.59	80.16
17	35.34	35.34	37.99	78.03	69.08	62.45

Table 3 shows the results of 17 experiments on wastewater in a facility that treats industrial and municipal effluents by applying three-level-three factors through BBD.

3.1. EVALUATION OF THE PREDICTIVE PERFORMANCE OF PROPOSED MODELS

Table 4 shows the results of the analysis of variance (ANOVA) for *TP* (a) and *TKN* (b). This analysis establishes, following the linear factors in the case of *TP* removal, that the quadratic parameters for *VER* and *AR* with a lower *p*-value (<0.05) are determinants for the modeling in the removal of this pollutant from mixed wastewater [10]. For the modeling of *TKN* removal, the *CT* parameter was also very influential, since it gives a *p*-value of 0.0475. In addition, the *VER* parameter gave values above 0.05 for the *p*-value in both variables.

Table 1

Analysis of variance (ANOVA) for *TP* and *TKN*

Source	*TP*					*TKN*				
	Sum of squares	*df*	Mean square	*F*-value	*p*-value	Sum of squares	*df*	Mean square	*F*-value	*p*-value
Model	1039.24	9	115.47	8.25	0.005	2340.24	9	260.03	18.68	0.0004
A-*VER*	15.95	1	15.95	1.14	0.3212	0.29	1	0.29	0.021	0.8888
B-*AR*	18.67	1	18.67	1.33	0.2860	19.55	1	19.55	1.40	0.2747
C-*CT*	3.43	1	3.43	0.25	0.6357	80.18	1	80.18	5.76	0.0475
A^2	309.97	1	309.97	22.15	0.0022	15.19	1	15.19	1.09	0.3310
B^2	531.78	1	531.78	38.00	0.0005	388.44	1	388.44	27.90	0.0011
C^2	40.22		40.22	2.87	0.1338	692.80		692.80	49.77	0.0002
Residual error	97.97	7	14.00			97.44	7	13.92		
Lack of fit	79.21	3	32.66			97.44	3	13.92		
Pure error	0.00	4	0.00			0.00	4	0.00		
C_{or} total	1137.21	16				2437.68	16			

As is seen from the table, the *VER* does not play such an essential role within the modeled system, which was also in line with other studies found in the literature [1, 17].

3.2. ARTIFICIAL NEURAL NETWORK MODELING

Results for *TP* removal using the ANN-MFO model give the accuracy obtained from 4 neurons in the hidden layer (*MSE* = 16.467), 6 neurons (*MSE* = 3.456), 8 neurons (*MSE* = 21.233), 10 neurons (*MSE* = 13.832) and 12 neurons (*MSE* = 14.562), and reveal that the most efficient architecture was the one using 6 neurons in the hidden layer (Fig. 3a). Also, the prediction plots show *R*-values of 1 for validation, 1 for testing, 1 for training, and 0.99255 for the whole data set. Results for *TKN* modeling for 4 neurons in the hidden layer (*MSE* = 14.620), 6 neurons (*MSE* = 8.912), 8 neurons (*MSE* = 5.481), 10 neurons (*MSE* = 13.708) and 12 neurons (*MSE* = 18.332), showing that the most significant architecture was the one using 8 neurons in the hidden layer (Fig. 3b). Prediction plots, meanwhile, show *R*-values of 0.9699 for validation, 1 for testing, 0.9868 for training, and 0.9659 for the whole data set [10].

3.3. POLLUTION REMOVAL EFFICIENCY

This work evaluated three different conditions of *AR* (2.5, 3, 3.5 dm^3/min), *CT* (1.8, 2.5, 2.3 d), and *VER* (50, 67, 75%) in a reactor at bench-scale. These configurations were modeled to obtain three-dimensional plots as shown in Fig. 4.

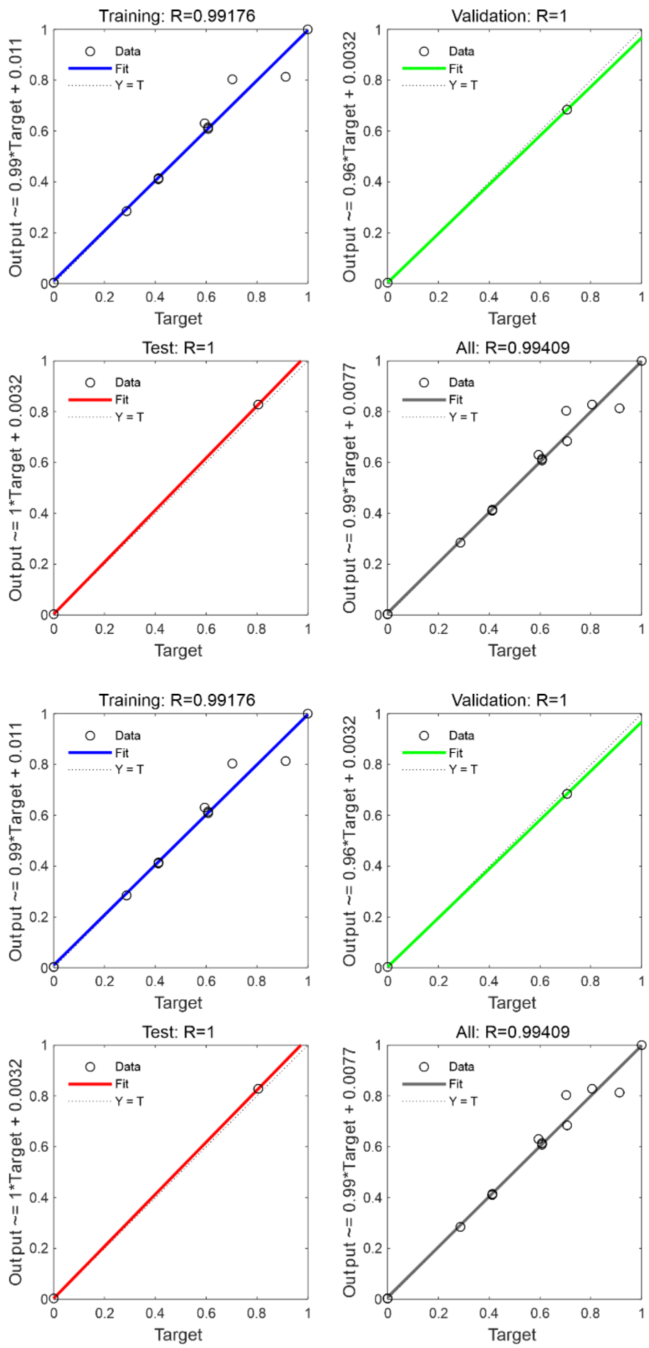

Fig. 3. Correlation plots for ANN-MFO model for
TP removal (four upper plots), and *TKN* removal (four lower plots)

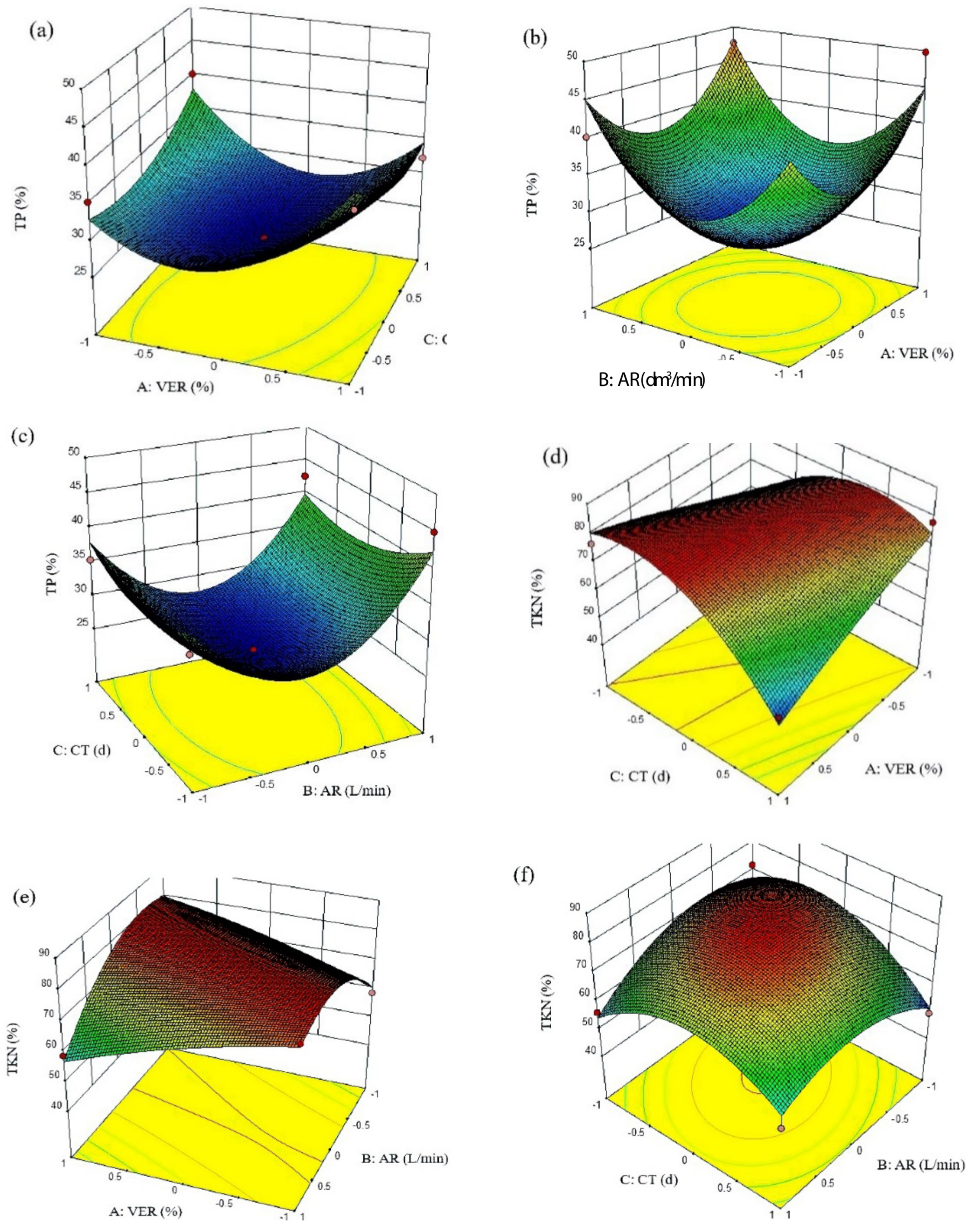

Fig 4. Three-dimensional response surface plots showing effects of variables and their interaction on reduction of *TP* and NTK removal effectiveness: a), d) *VER* vs. *CT* at *AR* = 3 dm^3/min, b), e) *AR* vs. *VER* at *CT* = 2.5 d, c), f) *AR* vs. *CT* at *VER* = 67%

These parameters are associated directly or indirectly with each other, defined according to treatment targets, properties of granules, and treated water quality. The model results showed that the operating configurations shown in Figs. 4a and 4d, where *VER* = 50%, and *CT* = 3.2 d, under an *AR* fixed at 3.2 dm^3/min, can achieve *TP* removal efficiency of 40%. However, *TKN* removal under these conditions achieves efficiencies of ca. 74%. Conversely, under an increase of *VER* to 75% and a decrease of *CT* to 1.8 day, *TKN* removal of more than 80% can be achieved (Fig. 4d). On the other hand, Figs. 4b and 4e show that a configuration of *CT* constant at 2.5 day combined with *AR* of 2.5 dm^3/min and *VER* = 75% achieved maximum *TP* and *TKN* removal efficiencies of 50 and 80%, respectively. However, if *VER* values are reduced under these operating conditions, the efficiencies of this parameter are negatively affected, only achieving *TP* and *TKN* removals of ca. 35 and 57%, respectively.

Furthermore, Figs. 4c and 4f show that by applying operational configurations where all the factors evaluated in this study are constant (*CT* = 2.5 day, *VER* = 67%, *AR* = 3 dm^3/min) the *TP* removal efficiency is lower at 15%, while *TKN* removal under these same conditions is higher at 70%. These conditions show inverse behavior on the removal efficiency of *TP* and *TKN* at constant values of the operating parameters. The results showed that by applying a $CT \geq 2.5$ day, the AGS system gives a better performance on *TP* removal, although *TKN* removal is affected. The highest *TKN* removals (ca. 70%) were obtained under a $CT \geq 2.5$ day, combined with values of $AR \geq 3$ dm^3/min and *VER* = 75% (Figs. 4d and 4e). Moreover, Fig. 4f showed that an $AR \leq 3$ dm^3/min setup with a *CT* = 1.8 day achieves *TKN* removals of up to 70%. These operating conditions were applied at constant *VER* equal to 67%.

4. DISCUSSION

4.1. EVALUATION OF THE PREDI*CT*IVE MODELS

The performance of the models developed in Table 5, RSM and ANN-MFO in the removal of *TP* and *TKN* from mixed wastewater by aerobic treatment, was determined by using the statistical equations that evaluate the coefficients *R*, R^2, adjusted R^2, *MSE*, *RMSE*, *MAE*, and *AAD* [10].

Table 5

Statistical indices for *TP* and *TKN* removal

Parameter	*TP*		*TKN*	
	ANN-MFO	RSM	ANN-MFO	RSM
R	0.9925	0.9069	0.9660	0.8534
R^2	0.9848	0.8146	0.9519	0.8269
Adjusted R^2	0.9866	0.7986	0.9566	0.8033
MSE	3.456	28.4085	11.2285	38.9639
RMSE	1.8590	5.3299	3.3509	6.2421
MAE	5.3523	9.5159	2.0935	9.8653
AAD	8.8023	21.5038	6.9959	26.1884

For *TP* removal, better operation was achieved using ANN-MFO compared to RSM for all the statistical parameters estimated. This modeling strategy produced a better performance of the ANN-MFO by obtaining an MSE of 3.456, compared to an error of 28.408 found in the RSM model. In the case of *TKN* removal, the RSM model gave a moderate performance, shown by analyzing the statistical indices and the relevance of the ANN-MFO model R^2 (0.9519) compared to RSM R^2 (0.8269). In addition, the ANN-MFO model shows lower error rates, indicating the superiority of the neural model over the RSM statistical model [18].

4.2. EFFECTS OF THE FACTORS ON TP AND TKN REMOVAL EFFICIENCY

The effect of different factors was evaluated in an aerobic granular system developed in-situ for mixed wastewater treatment. The removal efficiencies shown in Fig. 4 are also due to the predominant species developed under the diverse operational strategies applied to the system. The latter is thanks to selection pressures present in the biological medium such as the hydrodynamic shear strength in the water, which depends on the volume of liquid in the tank and the *AR* applied to that volume. Figures 4c and 4f show that the applications of operational strategies where lower values of *AR* and *CT* and high values of *VER* are established improves the removal efficiency of *TKN* in the AGS system. These results are similar to those reported by Priyanka et al [19], which evaluated simultaneous nitrification and denitrification-SBR. They reported that optimum *VER* (70%), *CT* (0.2 day), and intermittent minimum aeration through anaerobic/oxic/anoxic mode achieved a *TKN* removal efficiency of 89.6±1.1%.

Various studies reported the *TP* and *TKN* removal efficiencies achieved using different operating strategies and configuration mechanisms in AGS systems. Most studies obtained a removal efficiency higher than 80% under *AR* between 1.7 and 2.5 dm^3/min and a *VER* equal to 50% [20, 21]. In this regard, the operational factors evaluated in this study are essential for effective N removal in AGS systems. Meanwhile, the P removal efficiencies reported were limited, since only ca. 40% of the total studies reported *TP* removal values, which were less than 50% on average for mixed wastewater treatment [22, 23].

On the other hand, the low PT removal efficiency obtained in this study under any operating strategy applied may be due to the influence of other factors such as the *DO* concentration from high aeration velocity or short *CT*, which prevented the growth of phosphorus-accumulating organisms (PAOs) responsible for P degradation.

These organisms are inhibited since they need relatively high residence times and low *DO* concentrations to develop [24, 25]. The high *TKN* removal efficiency in comparison to *TP* was due to a very effective nitrification thanks to the long aerobic phase inside the granules. However, the limited anoxic phase applied did not achieve adequate denitrification due to the low growth rate of the denitrifying bacteria. This was corroborated by the nitrite and nitrate concentrations obtained in the experimental measurements. The AGS system in this study was operated under the anaerobic/oxic (A/O) mode where the oxic stage was more than 90% of the *CT* under high aeration rates in some of the applied configurations.

5. CONCLUSIONS

RSM modeling proved to have limitations in simulating the treatment of an aerobic granular sludge process for removing *TP* and NKT; since the statistical indices show a restricted performance for this study, this technique provides 3D graphs to interpret the influence of the main parameters for this process. Neural networks coupled with metaheuristic algorithms like MFO can effectively model highly nonlinear systems such as aerobic granulation systems for mixed wastewater treatment with an enhanced adjustment as the statistical method used. However, suitable operating ranges of *VER* need to be established according to the type of pollutant to be treated. Finally, the competition between heterotrophic and nitrifying bacteria resulted in a considerable decrease of nitrifying bacteria in the aerobic zone and, consequently, a lower *TKN* removal efficiency. Therefore, the behavior of the AGS process needs to be studied further when the A/O modes of operation are modified due to the operating velocities applied.

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