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Evaluation of nitrification and denitrification in an activated sludge process through mass balance using GPS-X software

Evaluación de la nitrificación y desnitrificación en un proceso de lodos activados a través del balance de masa usando el software GPS-X

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Abstract

During the biological treatment of the wastewater, chemical, and biological reactions occur under controlled conditions. The analysis of these reactions is complex due to the interaction developed by the microorganisms. The use of the mass balance represents a viable option to observe the behavior of the biological treatment of the wastewater. This work aimed to evaluate the degree of nitrification and denitrification in the activated sludge system of a wastewater treatment plant. The evaluation methodology was developed through the mass balance with the support of the GPS-X software. The mass balance of the biological treatment system under study showed that more nitrification is required in aerobic reactors and a greater volume of the anoxic zone for denitrification, since only 38% of the total nitrogen was removed. Alternatively, by simulating a new treatment model with the GPS-X software, it was possible to increase the total nitrogen removal efficiency from 38% to 81%. The parameters of alkalinity, pH, DO, T and SRT were very useful indicators to observe the nitrification and denitrification process in the activated sludge system.

Keywords: mass balance, nitrification, denitrification.

Resumen

Durante el tratamiento biológico de las aguas residuales, se producen reacciones químicas y biológicas bajo condiciones controladas. El análisis de dichas reacciones es complejo debido a la interacción desarrollada por los microorganismos. El uso del balance de masa representa una opción viable para observar el comportamiento del tratamiento biológico de las aguas residuales. El objetivo de este trabajo fue evaluar el grado de nitrificación y desnitrificación en el sistema de lodos activados de una planta de tratamiento de aguas residuales. La metodología de evaluación, se desarrolló a través del balance de masa con apoyo del software GPS-X. El balance de masa del sistema de tratamiento biológico en estudio, mostró que se requiere más nitrificación en reactores aerobios, y un mayor volumen de zona anóxica para la desnitrificación, ya que sólo se removió un 38% del nitrógeno total. Como alternativa, al simular un nuevo modelo de tratamiento con el software GPS-X, se logró aumentar la eficiencia de remoción del nitrógeno total del 38% al 81%. Los parámetros de alcalinidad, pH, OD, T y SRT fueron indicadores muy útiles para observar el proceso de nitrificación y desnitrificación en el sistema de lodos activados.

Palabras clave: balance de masa, nitrificación, desnitrificación.

1 Introduction

Some essential chemical reactions mediated by microorganisms in aquatic and soil environments involve nitrogen compounds. Currently, in wastewater treatment, particular interest has been placed on nitrogen removal due to the environmental and sanitary consequences of its discharge into water bodies, such as increased acidity, eutrophication, and toxicity of aquatic ecosystems, which affect the survival, growth, and reproductive capacity of some animals (Zhou *et al.,* 2023).

The growth of algae and lilies in an aquatic environment is favored by waters rich in nutrients, especially nitrogen, and phosphorus. The biomass of these species can double in a few weeks, which generates the appearance of large floating colonies (Guevara & Ramírez, 2015). The increase in the concentration of nitrogen and phosphorus provoke to the loss of oxygen and the mortality of some aquatic species (Espósito *et al.,* 2016). Dissolved oxygen concentrations in surface waters are governed by the balance between oxygen production (i.e. through photosynthesis), consumption (e.g. respiration and other chemical reactions that consume oxygen), and exchange with the atmosphere (Zhang *et al.,* 2010).

Although nitrogen is quantified and reported as such, during its journey through the biogeochemical cycle, it passes through various chemical species. According to Figure 1, total nitrogen (N_{TOTAL}) is equal to total Kjeldahl nitrogen (TKN) plus nitrites (NO_2^-) and nitrates (NO_3^-), and TKN is equal to organic nitrogen (N_{ORG}) plus ammoniacal nitrogen (NH_4^+ - NH_3). Nitrogen associated with organic molecules such as proteins, nucleic acids, or final metabolites such as urea and uric acid in municipal wastewater, is called organic nitrogen, and although it has no characteristic chemical effect, as organic matter, it is subject to consumption by microorganisms, which will eventually process and transform the nitrogen present into ammonia. The nitrogen present in the form of ammonia is called ammoniacal nitrogen. Its particular characteristic is that its behavior depends on pH; at acid pH, it remains dissolved in water as an ammonium ion, while at alkaline pH, it is transformed into ammonia gas which is susceptible to volatilization into the environment. Ammonia gas is irritating and lethal at high concentrations (Tchobanoglous *et al.,* 2003).

In an activated sludge system, the *nitrification* conversion of *ammonia* nitrogen to *nitrate* ions occurs under extensive aeration. Two genera of bacteria catalyze *Nitrification*: *Nitrosomonas*, which causes the transition of ammonia to nitrites, and *Nitrobacter* bacteria, which oxidize nitrites to nitrates. *Nitrites* have an acute toxicity that is even worse than ammonia, but they do not volatilize and are rapidly oxidized to nitrate. *Nitrates* are the most oxidized form of nitrogen and are much less toxic, unlike the previous arrangements. These nitrates can be reduced to nitrogen gas (N_2) in denitrification (WEF, 2008).

In the case of nitrification, nitrifying bacteria are characterized by their extraordinary sensitivity to parameters such as alkalinity, pH, dissolved oxygen (DO), temperature (T), sludge age (SRT), and toxic substances (Najman *et al.,* 2020). It is precisely here where through these parameters, mass balance can be used to quantify the degree of nitrification and denitrification at each stage of the activated sludge process. Based on the principle of mass conservation, a mass balance has the following general form:

Input + Reaction = Output + Accumulation

Mass balances can be based on a number of different process variables (e.g. Q, COD, N, P, TSS). An advanced way of using mass balances for fault identification is to set up parallel mass balances (mass balance of the same system utilizing different process variables) or overlapping mass balances (mass balances with different system boundaries with one common measuring point) (Rieger *et al.,* 2013).

In this context, this research work aimed to evaluate the degree of nitrification and denitrification in a biological treatment system of a wastewater treatment plant (WWTP) located in San Luis Potosi, Mexico. The evaluation was developed in the biological treatment system previously calibrated with the unified GMP protocol of the IWA Task Group (Rieger *et al.,* 2013) through mass balances and supported by simulation with GPS-X software. The GPS-X software is a tool that performs mathematical modeling, simulation, optimization, and evaluation of WWTP in a steady and dynamic state. It also optimizes advanced control systems and predicts effluent quality under variable conditions (Hydromantis, 2014). In sum, the specific contribution of this research work focused on the mass balance (flows and mass changes), which provided valuable information to analyze the transformation, nitrification and denitrification processes in the treatment system under study.
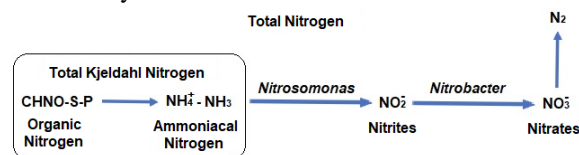

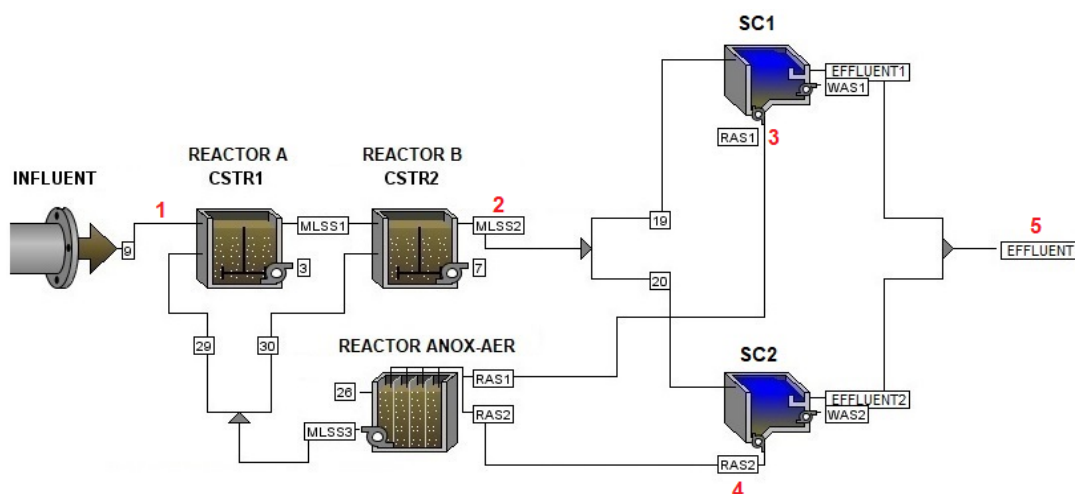Figure 1. Chemical composition of total nitrogen (N_{TOTAL}).

Figure 2. Delimitation of the activated sludge process and sampling points.

2   Materials and methods

2.1   Wastewater treatment plant (WWTP)

The WWTP under study was originally a contact-stabilization type activated sludge system, which later underwent some modifications to increase the amount of flow to be treated. Contact stabilization uses two separate aerated tanks for the treatment of the wastewater and stabilization of the activated sludge. In the stabilization tank, the bacteria digest (stabilize) the organic matter that they have assimilated in the contact tank. The process was developed for BOD removal, and the short contact time limits the amount of soluble BOD degraded and NH$_3$-N oxidation. The current plant comprises three stages: pretreatment, secondary, and tertiary treatment. Pretreatment consists of two grit channels, six bar screens, four mesh screens, a pumping sump, and a static screen. Secondary treatment consists of two reactors in series (A and B), an anoxic-aerobic reactor (R$_{ANOX-AER}$), and two secondary clarifiers (SC) with lamellae. Reactor "A" has a volume of 187.31 m^3, reactor "B" 231.75 m^3, and the R$_{ANOX-AER}$ 645.93 m3 (half of the volume is R$_{ANOX}$, and the other half corresponds to the R$_{AER}$). The two SC have a surface area of 90 m^2. The sludge return from the SCs goes to the R$_{ANOX-AER}$, then, from this reactor, the mixed liquor is sent to reactors A and B, 40% to reactor A and 60% to reactor B. The SCs purge sludge is treated by aerobic digestion and centrifuge for dewatering. Tertiary treatment consists of filters with layers of silica sand, regular sand, and gravel, each 30-40 cm thick. When the water from the filters has a color of less than 100 Pt-Co units, it goes directly to chlorination. On the other hand, if the color is more significant than 100 Pt-Co units, the water undergoes a coagulation-flocculation process and then disinfection with chlorine gas. In this study, the process delimitation for modeling and simulation focused on the activated sludge system (Figure 2).

2.2   Program of sampling and characterization of wastewater and sludge

Wastewater and sludge samples were taken at different points of the treatment process (Figure 2). Sampling was performed every two hours, 24 hours a day, for three days. For the characterization of the wastewater, the parameters of biochemical oxygen demand at 5 days and soluble (BOD$_5$), total suspended solids (TSS), volatile suspended solids (VSS), and alkalinity (as CaCO$_3$) were analyzed following the procedures of standard methods for the analysis of wastewaters (APHA, 2005). The BOD$_5$ was examined by the incubation method for five days, the TSS by the gravimetric method of drying at 103-105 °C, the VSS by the gravimetric method of incineration at 550 °C and the alkalinity by the titration method. The chemical demand oxygen total (COD$_{TOTAL}$) and chemical demand oxygen soluble (COD$_{SOLUBLE}$) was determined by the digestion method, the total ammoniacal nitrogen (N-NH$_3$) and soluble ammoniacal nitrogen (N-NH$_{3\ SOLUBLE}$) by the salicylate method, and the Total Kjeldahl Nitrogen (TKN) and Total Kjeldahl Nitrogen soluble (TKN$_{SOLUBLE}$) was obtained by the difference of total nitrogen (N$_{TOTAL}$), nitrites (NO$_2^-$) and nitrates (NO$_3^-$).

Total nitrogen (N$_{TOTAL}$) and total soluble nitrogen (N$_{TOTAL\ SOLUBLE}$) were determined by the persulfate digestion method, total and soluble nitrites (N-NO$_2^-$, N-NO$_2^-$ $_{SOLUBLE}$, respectively) by the diazotization method, and total and soluble nitrates (N-NO$_3^-$, N-NO$_3^-$ $_{SOLUBLE}$, respectively) by the dimethylphenol method. The parameters referred to as soluble were analyzed by filtering the sample with a filter of
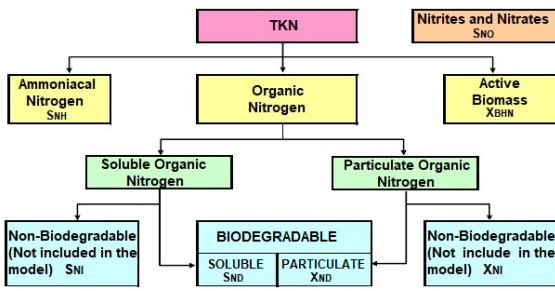
Figure 3. Fractionation of nitrogen according to ASM1 model.

0.45 μm. COD, N-NH$_3$, N$_{TOTAL}$, N-NO$_2^-$, and N-NO$_3^-$ were determined with a HANNA HI839800 digester and a HANNA HI83214 multiparameter spectrophotometer. Dissolved oxygen and temperature were measured in the field with a HACH HQ30d portable instrument, while pH was analyzed with a Thermo Scientific® Orion Star® A211 benchtop meter.

2.3 Nitrogen fractionation with Influent Advisor

Nitrogen fractionation according to the ASM1 model (Henze *et al.,* 2006), was determined using the Influent Advisor program, part of the GPS-X software. This fractionation is detailed in Figure 3. The TKN is fragmented into ammoniacal nitrogen (S$_{NH}$), organic nitrogen, and nitrogen contained in the biomass. Organic nitrogen is divided into soluble and particulate fractions with biodegradable and non-biodegradable parts. The biodegradable fractions of nitrogen S$_{ND}$ and X$_{ND}$ appear explicitly in the model. The nitrogen contained in the active biomass (heterotrophs and autotrophs) (X$_{BHN}$) is taken into account indirectly in the sense that decay produces biodegradable particulate organic nitrogen (X$_{ND}$) on the one hand and biodegradable soluble organic nitrogen (S$_{ND}$) on the other. The particulate inert organic nitrogen (X$_{NI}$) and the soluble inert organic nitrogen (S$_{NI}$) can be easily calculated, even if they are not shown in the model through the Influent Advisor. Assuming that the nitrogen contained in the active biomass in the influent is negligible, X$_{BHN}$ = 0, then the TKN$_{TOTAL}$ is defined by the following equation:

$$TKN_{TOTAL} = S_{NH} + S_{NI} + S_{ND} + X_{ND} + X_{NI} \quad (1)$$

2.4 Calibration of WWTP biological treatment system

The calibration of the model (Figure 2) was developed with the unified GMP protocol of the IWA Task Group (Rieger *et al.,* 2013). The methodology is shown in Figure 4.
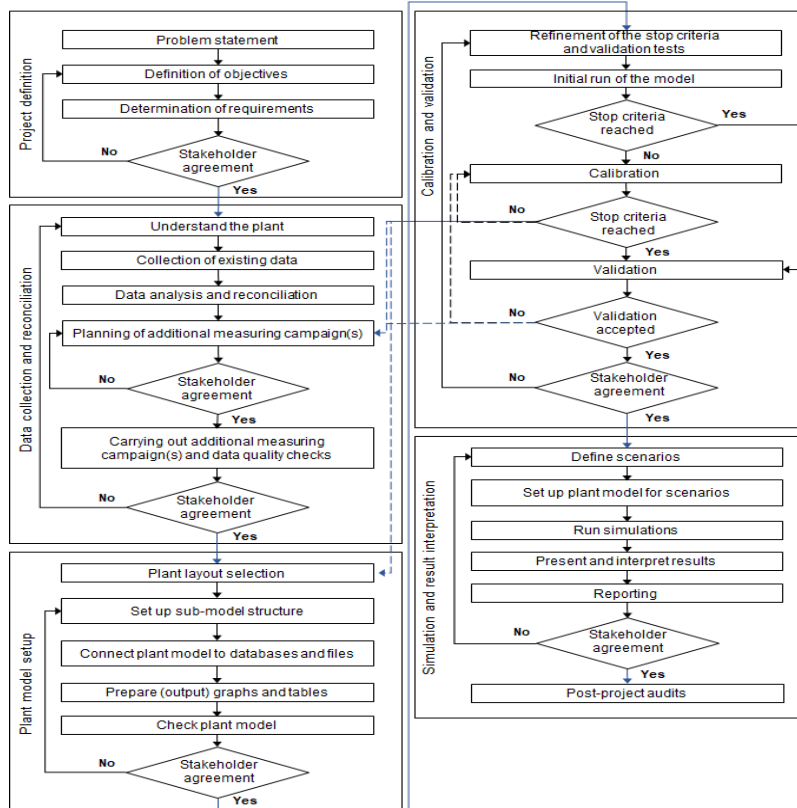

Figure 4. GMP unified protocol

Table 1. Average values of TSS and VSS in reactors and sludge recirculation (2020-2021).

	CSTR 1	CSTR 2	RAS1	RAS2	$R_{ANOX\text{-}AER}$
TSS (mg/L)	1306	1874	5350	5768	5870
VSS (mg/L)	1115	1506	4482	4856	4666

CSTR 1: Reactor A effluent; CSTR 2: Reactor B effluent; RAS1: Sludge recirculation of secondary clarifier 1, RAS2: Sludge recirculation of secondary clarifier 2; $R_{ANOX\text{-}AER}$: Reactor anoxic-aerobic effluent.

According to the GMP protocol, the purpose of model calibration and validation was to minimize the errors between measured data and model predictions. A calibrated model can give reliable results. To carry out the calibration and validation step (Figure 4), once the current model of the activated sludge system (Figure 2) was configured in the GPS-X software with the data obtained in the data collection and reconciliation (physical and operational parameters, influent characterization, internal flows, among others) and GPS-X software default parameters (kinetic and stoichiometric), the first simulation of the model was run. The results obtained in this simulation were compared with the data obtained in the sampling campaign, when there was no coincidence, an iterative adjustment was made in those parameters that can be modified until the simulation results coincided with the data measured in the intensive sampling campaign. The parameters that can be changed are:

- Internal flows: sludge recirculation (Q_{RAS}) and waste sludge (Q_{WAS}).

- Aeration: adjustment of dissolved oxygen in reactors.

- Kinetic and stoichiometric coefficients: organic fractions, nutrient fractions, heterotrophic and autotrophic active biomass.

To validate the model, the results obtained in the simulation can have a difference of ± 5 - 15% concerning the measured data (Rieger *et al.,* 2013).

2.5 Simulation scenarios using GPS-X for model calibration

For the calibration (actual operation) of the biological treatment system of the plant under study (Figure 2), the following data were initially introduced at the GPS-X simulator: influent characterization, nominal designs of the equipment, sludge characterization, and the XCOD/VSS ($COD_{PARTICULATE}$/VSS) ratio obtained in the reactors. The next phase was calibrating the mixed liquor suspended solids (MLSS; TSS in the mixed liquor) in the reactors. It was realized by adjusting XCOD/VSS to represent the actual operation of the treatment system (model calibration). XCOD/VSS represents the most important ratio in the calibration process, since it adjusts the solids in the reactors, considering the water quality results in the effluent of the secondary clarifiers (SC).

2.6 Nitrogen mass balance

Mass balances were performed in two ways:

1. Mass balance in each stage of the calibrated model, considering the main parameters involved in the nitrification and denitrification process.

2. Mass balance of total nitrogen of the calibrated model.

3 Results and discussion

3.1 Wastewater and sludge characterization

Table 1 shows the results of the sludge concentration determined at the WWTP under study during the years 2020 and 2021. The sludge was defined as total suspended solids (TSS) and volatile suspended solids (VSS).

Table 1 shows that the TSS in the complete mix reactors (CSTR1) and CSTR2) are within the operational range of 1500 - 4000 mg/L (Tchobanoglous *et al.,* 2003). However, this does not happen with the anoxic-aerobic reactor, which functions as a piston flow reactor ($R_{ANOX\text{-}AER}$) and for which the operational range is 1000 - 3000 mg/L (Tchobanoglous *et al.,* 2003). Table 2 shows the average wastewater characterization results determined at the sampling points (Figure 2) during the three days of data collection. The laboratory results were evaluated by verifying the methods and precision of the laboratory analyses so that values showing errors could be detected before they could be used in the calibration. The verification and empirical evaluations of certain relationships, such as BOD_5/COD, $COD_{TOTOTAL}$/BOD_5, $COD_{SOLUBLE}$/COD_{TOTAL}, TSS/COD_{TOTAL}, COD_{PART}/VSS VSS/TSS, N_{TOTAL}/COD_{TOTAL}, in the influent, and COD_{TOTAL}/VSS and VSS/TSS in the mixed liquor, facilitated the detection of out-of-range data.

According to the results in Table 2, the BOD_5/COD ratio for the reactor influent (point 1)

Table 2. Average values determined at the sampling points (Figure 2).

Parameter (mg/L)	Sampling points				
	Point 1	Point 2	Point 3	Point 4	Point 5
COD_{TOTAL}	409.0	1973.3	5,613.3	8,006.67	54.00
$COD_{SOLUBLE}$	217.0	38.7	41.0	44.33	24.67
BOD_5	195.0	753.0	2,733.0	2,733.00	8.30
$BOD_{5\ SOLUBLE}$	120.0	4.0	3.4	3.40	3.40
N_{TOTAL}	47.0	231.6	769.3	769.30	29.50
$N_{TOTAL\ SOLUBLE}$	23.0	21.6	22.7	22.75	22.75
TKN	47.0	213.0	750.7	750.70	10.90
$TKN_{SOLUBLE}$	22.9	9.5	9.5	9.50	9.50
$N-NH_3$	25.0	12.3	12.3	12.33	12.33
$N-NH_{3\ SOLUBLE}$	18.33	7.8	7.8	7.8	7.8
$N-NO_3^-$	0.0	18.45	18.36	18.36	18.36
$N-NO_3^-\ _{SOLUBLE}$	0.0	12.05	13.18	13.18	13.18
$N-NO_2^-$	0.0	0.15	0.24	0.24	0.24
$N-NO_2^-\ _{SOLUBLE}$	0.0	0.07	0.07	0.07	0.07
TSS	120.0	1,777	5,367	6,917	11.7
VSS	77.3	1,377	4,172	5,400	9.67
Alkalinity ($CaCO_3$)	298.7	207.7	255.3	263.00	234.33
DO	1.45	3.32	0.23	0.21	2.57
pH	7.38	7.31	7.21	7.15	7.64
Temperature (°C)	26.0	26.52	26.3	26.2	26.36

Table 3. Typical ratios for municipal WWTPs (Rieger et al., 2013) .

Ratios	Minimum	Maximum	Mean	Median	This study
Influent to reactors					
COD_{TOTAL}/BOD_5	0.5	3.0	1.874	1.9	2.10
$COD_{SOLUBLE}/COD_{TOTAL}$	0.15	0.75	0.449	0.495	0.53
TSS/COD_{TOTAL}	0.18	0.56	0.38	0.4	0.29
COD_{PART}/VSS	1.4	3.5	1.718	1.7	2.48
VSS/TSS	0.7	0.909	0.794	0.8	0.64
N_{TOTAL}/COD_{TOTAL}	0.05	0.36	0.134	0.12	0.11
Mixed liquor					
COD_{TOTAL}/VSS	1.266	1.6	1.434	1.42	1.43
VSS/TSS	0.65	0.9	0.739	0.75	0.77

was 0.48, which is within the established range of $0.45<BOD_5/COD<0.5$ for typical municipal waters (WERF, 2003), indicating that the wastewater arriving at the WWTP under study is largely of municipal or domestic origin. Concerning the ratio COD_{TOTAL}/VSS in the mixed liquor (point 2), where the result was 1.43, this also falls within the established range of $1.42<COD/VSS<1.48$ (WERF, 2003). Table 3 shows other relationships of interest obtained in several municipal WWTPs and is compared with the results of this study (Table 2).

The results of this study were within the range according of Rieger *et al.,* (2013) (Table 3), which reaffirms that the characteristics of the wastewater from the WWTP under investigation correspond to those of municipal or domestic water. Therefore, an activated sludge process can satisfactorily remove the organic load and nitrogen from wastewater (Henze *et al.,* 2006; Van Loosdrecht *et al.,* 2015).

Concerning COD_{TOTAL} and N_{TOTAL} removal (Table 2), 86.8% of COD_{TOTAL} and 37.2% of N_{TOTAL} are removed, indicating no problems in organic load removal. However, there are problems in nitrogen removal, which suggests that nitrification and denitrification rates are unacceptable.

3.2 Nitrogen fractionation with Influent Advisor

The results of the Total Nitrogen Kjeldahl (TKN) fractions according to Influent Advisor are $S_{TKN} = 22.9$ mg/L (filtered or soluble TKN) and $X_{TKN} = 24.1$ mg/L (particulate or non-soluble TKN). These results indicate a higher percentage of particulate TKN (51.3 %) which will be difficult to remove unless there is effective hydrolysis (Espinosa *et al.,* 2020), while soluble TKN with 48.7 % can be effectively biodegraded.

Table 4. Nitrogen fractionation (Influent advisor GPS-X).

Nitrogen fractions	Results (mg/L)	% TKN
S_{NH}	18.3	38.9
S_{ND}	4.58	9.8
X_{ND}	20.5	43.6
$S_{NI} + X_{NI}$	3.62	7.7

Table 5. Data initials entered in the GPS-X simulator.

Influent	Q_{INF} = 4320 m³/d
CSTR 1	V = 187.31 m³
	DO = 3.3 mg O_2/L
	Alkalinity = 298.7 mg $CaCO_3$/L
	XCOD/VSS = 1.48 g COD/g VSS (Default value in GPS-X™)
CSTR 2	V = 231.75 m³
	DO = 3.3 mg O_2/L
	Alkalinity = 207.7 mg $CaCO_3$/L
	XCOD/VSS = 1.48 g COD/g VSS (Default value in GPS-X™)
$R_{ANOX-AER}$	V = 645.93 m³
	DO_1 = 0.0 mg O_2/L and DO_2 = 3.3 mg O_2/L
	Alkalinity $_{INF-ANOX}$ = 263.0 mg $CaCO_3$/L
	XCOD/VSS = 1.48 g COD/g VSS (Default value in GPS-X™)
SC1 and SC2	DO = 2.57 mg O_2/L
	Alkalinity = 234.3 mg $CaCO_3$/L

Table 6. SC effluent results: measured and simulated data.

Parameter (mg/L)	Measured data*	% Removal	Simulated data	% Removal
BOD_5	8.3	95.7	6.6	96.6
COD	54.0	86.8	42.8	89.5
N_{TOTAL}	29.5	37.2	29.0	38.3
TSS	11.7	90.3	13.0	89.2

*Average of 3 days of sampling

According to Equation 1 of the TKN_{TOTAL} fractionation and the results obtained in Influent Advisor, the fractions shown in Table 4 are obtained.

The results shown in Table 4 indicate that biodegradable soluble organic nitrogen (S_{ND}) and biodegradable particulate organic nitrogen (X_{ND}), which together total 25.08 mg/L, correspond to 53.4% of the TKN, and ammonia nitrogen (S_{NH}) corresponds to 38.9%. The biodegradable fractions ($S_{ND} + X_{ND}$) and ammoniacal nitrogen (S_{NH}) can be adequately oxidized if there is a good nitrification process, achieving up to 92.3% of nitrogen oxidation. The fractionation of N was analyzed through TKN, equivalent to organic nitrogen plus ammoniacal nitrogen (Meijer *et al.,* 2002). For N fractionation, the following equations are used to help in the mass balance:

$$TKN_{INF\_TOTAL} = TKN_{INF\_SOLUBLE} + TKN_{INF\_PARTICULATE} \quad (2)$$

$$TKN_{INF\_SOLUBLE} = S_{NH} + S_{NI} + S_{ND} \quad (3)$$

$$TKN_{INF\_PARTICULATE} = X_{NI} + X_{ND} \quad (4)$$

According to equations 2, 3, and 4 and the data

obtained with *Influent Advisor*, we have data for TKN_{INF_TOTAL} (47 mg/L), $TKN_{INF\_SOLUBLE}$ (22.9 mg/L), $TKN_{INF\_PARTICULATE}$ (24.1 mg/L), S_{NH} (18.3 mg/L), S_{ND} (4.58 mg/L) and X_{ND} (20.5 mg/L), with which the fractions of S_{NI} (0.02 mg/L) and X_{NI} (3.6 mg/L) can be obtained.

3.3 Calibration of the WWTP biological treatment system

For the calibration, the data entered into the simulator initially are given in Table 5. The results obtained in the effluent of the secondary clarifiers (SC) by entering the data of Table 5 in the GPS-X simulator are shown in Table 6. Only the quality parameters that indicate removing organic load and nitrogen are considered.

The MLSS (TSS) concentration in the CSTR1, CSTR2, and $R_{ANOX-AER}$ reactors were 1517 mg/L, 2995 mg/L, and 10690 mg/L, respectively. These values, when compared to the current average operating data in the plant reactors (Table 1), represent a large difference: CSTR1 (1306 mg/L), CSTR2 (1874 mg/L), and $R_{ANOX-AER}$ (5870 mg/L), which has to do

Table 7. XCOD/VSS adjustment to calibrate MLSS of the biological system of the WWTP.

	CSTR 1	CSTR 2	$R_{ANOX-AER}$
XCOD/VSS adjusted	1.83	3.0	3.9
DO (mg/L)	3.3	3.3	0.0 – 3.3
SRT (d) simulated	1.7	1.7	1.7
SRT (Tchobanoglous *et al.,* 2003)	3 – 15	3 – 15	3 – 15
MLSS (mg/L) simulated	1271	1876	5918
MLSS (mg/L) laboratory data	1306	1874	5870
F/M (Kg BOD_5/Kg MLVSS- d) simulated	5.4	10.2	1.2
F/M (Tchobanoglous *et al.,* 2003)	0.2 - 0.6	0.2 - 0.6	0.2 - 0.4
Volumetric loading (kgBOD_5/m^3- d) simulated	4.5	10.0	3.3
Volumetric loading (Tchobanoglous *et al.,* 2003)	0.3 – 1.6	0.3 – 1.6	0.3 – 0.7

Table 8. Operational parameters of SC applying XCOD/VSS adjusted.

	SC1	SC2
Overflow rate (m^3/m^2- d) simulated	23.72	23.72
Overflow rate (Tchobanoglous *et al.,* 2003)	16 – 28	16 – 28
Solids loading (kg/m^2- d) simulated	61.23	61.23
Solids loading (Tchobanoglous *et al.,* 2003)	96 – 144	96 – 144

with the XCOD/VSS ($COD_{PARTICULATE}$/VSS) ratio, and, therefore, this parameter has to be adjusted since the default value was initially set in the GPS-X software.

The next phase was precisely to calibrate the MLSS of reactors adjusting XCOD/VSS to represent the actual operation of the biological system of the plant (model calibration). The results are shown in Table 7. By applying this adjustment, the SC effluent characteristics were BOD_5 = 8.2 mg/L, COD = 48.9 mg/L, N_{TOTAL} = 29.5 mg/L, and TSS = 12.2 mg/L, which are very similar to those obtained in Table 2 (point 5). Note the high concentration of N_{TOTAL} (29.5 mg/L) due to poor nitrification and denitrification.

When the XCOD/VSS adjustment is applied (Table 7), it is observed that the MLSS results from the simulation match the data measured in the intensive sampling campaign (Table 1). However, the solids retention time (SRT), F/M, and volumetric loading are outside the design and operating ranges handled in the literature (Tchobanoglous *et al.,* 2003). These out-of-range results imply sedimentation problems in the SC and, therefore, instability in the treatment system (WEF, 2008; D´Antoni *et al.,* 2017; Dionisi & Rasheed, 2018; Dlangamandla *et al.,* 2021; Sam *et al.,* 2022), as observed in the visits to the WWTP. As for nitrification in an activated sludge system, this is not possible unless the treatment system is provided with sufficient SRT for nitrifying bacteria to grow and oxidize ammonium; therefore, the system volume for nitrification is typically larger than that used for organic matter removal (WEF, 2008).

Table 8 shows the operating parameters of the secondary clarifiers (SC) when adjusting the XCOD/VSS ratio. The treatment system was calibrated with a Q_{RAS1} = Q_{RAS2} of 777.6 m^3/d and a Q_{WAS1} = Q_{WAS2} of 25 m^3/d, managed by the WWTP under study.

According to the Table 8, the overflow rate of secondary clarifiers (SC) is within the range, but not the solids loading, which is below the lower limit. Consequently, it causes sludge compaction problems at the bottom of the CS (Tchobanoglous *et al.,* 2003; WEF, 2008).

3.4 Mass balance by stages

To perform the mass balances, it was necessary to consider the flows in the calibrated model, which are shown in Figure 5.

In Figure 6, the stepwise mass balance of the calibrated model is shown, considering the main parameters that have to do with the nitrification and denitrification process. This balance is discussed below by parameters.

3.4.1 Alkalinity and pH

According to Figure 6, 1290.4 kg/d of alkalinity enters the influent, and 795.1 kg/d exits in the final effluent, therefore, 495.3 kg/d is consumed, and 795.1 kg/d remains unreacted, indicating sufficient alkalinity to support further nitrification. Many poor nitrification results can be explained by a deficit in the alkalinity of the water to be treated (Ronzano & Dapena, 2002). A low concentration of alkalinity (< 50 mg /L as $CaCO_3$) will lead to an unstable pH (insufficient buffering capacity), which could cause inhibition of nitrification and other problems in the treatment process (Hauduc *et al.,* 2013). Also, if all the alkalinity is consumed,
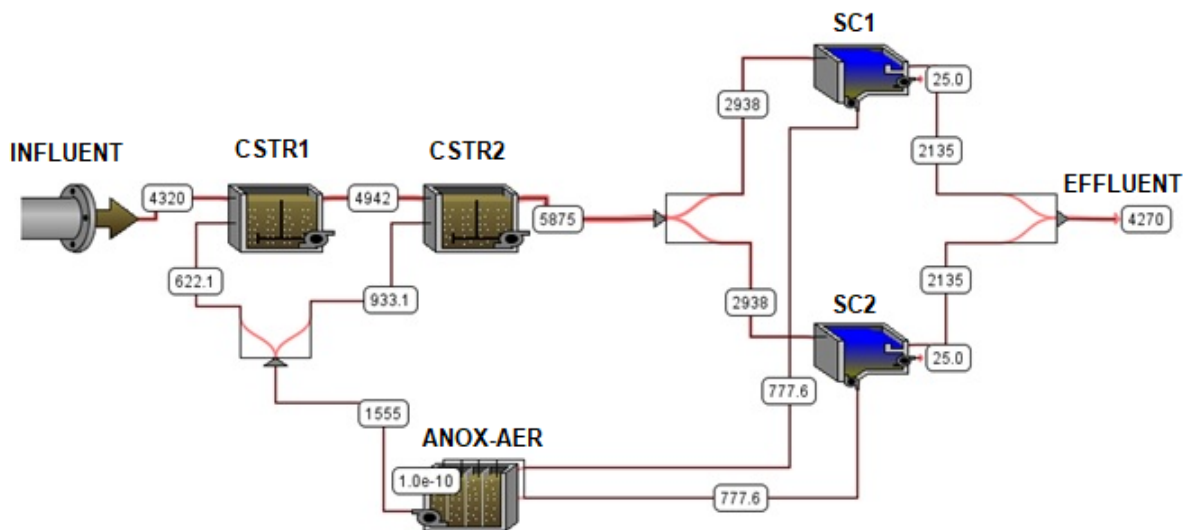
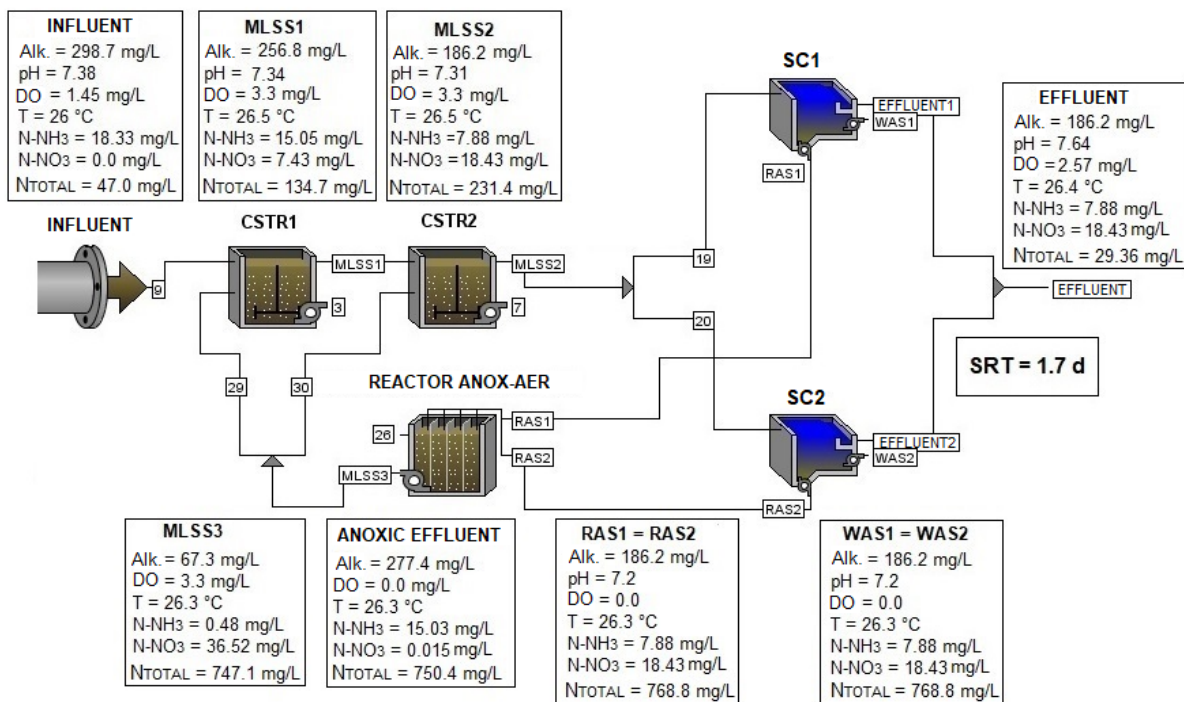Figure 5. Flows in the calibrated model (m³/d).

Figure 6. Mass balance by stages of the calibrated model.

the pH may drop so that the flocs of the mixed liquor in the reactors may disintegrate, leading to problems in secondary sedimentation (Tchobanoglous *et al.,* 2003; WEF, 2008). Considering from influent to effluent (Figure 6), 6.3 g of alkalinity (as $CaCO_3$) is consumed for each gram of ammonia (N-NH₃) converted to nitrate (N-NO₃⁻) during the nitrification process; the US Water Environment Federation has as a theoretical value, 7.14 g of alkalinity consumed for each gram of ammonia oxidized to nitrate ion (WEF, 2008), while

Ronzano & Dapena, (2002) mention as a practical value 6.5 g of alkalinity (as $CaCO_3$) consumed for each gram of N-NO₃⁻ produced.

On the other hand, denitrification in the anoxic reactor (Figure 6) recovers 141.8 kg/d of alkalinity and reduces 28.6 kg of nitrate (N-NO₃⁻), equivalent to 4.95 g of alkalinity produced for each gram of nitrate reduced. The literature mentions a theoretical value of 3.57 g of alkalinity (as $CaCO_3$) formed for each gram of nitrate (N-NO₃⁻) consumed or denitrified

(Tchobanoglous *et al.,* 2003; WEF, 2008). Ronzano & Dapena, (2002) mention 3.5 g of alkalinity produced for each gram of reduced nitrate as a practical value.

However, in the nitrification reaction, the concentration of bicarbonates (HCO_3) is reduced, and carbonic acid (H_2CO_3) is produced, as shown in the following reaction (Randall & Buth, 1984).

$$NH_4^+ + 1.83O_2 + 1.98HCO_3^- \longrightarrow 0.021C_5H_7O_2N + 0.98NO_2^- + 1.041H_2O + 1.88H_2CO_3$$
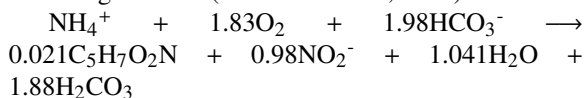
With the above reaction, the pH tends to decrease, which is observed in the effluent of the CSTR1 and CSTR2 reactors (Figure 6). It has been observed that the maximum nitrification rate in activated sludge occurs at pH values between 7.2 and 9.0. In contrast, at values below 6.5, the nitrification rate is significantly reduced (WEF, 2008; Zornoza *et al.,* 2014). Other authors mention that pH substantially influences the degree of growth of *Nitrobacter* bacteria and mainly on *Nitrosomonas* so that the maximum nitrification rate occurs at pH between 7.5 and 8.5 (Tchobanoglous *et al.,* 2003; Najman *et al.,* 2020; Espinosa *et al.,* 2020; EPA, 2009). Mozumder & Hossain, (2020) mention that the optimum pH for ammonia nitrogen removal is in the range of 7 to 8, and nitrification and denitrification are inhibited at pH higher than 8 and lower than 5, respectively, in different biological nutrient removal (BNR) processes.

On the other hand, the optimum pH for aerobic denitrification ranges between 7.0 and 8.0 (Ji *et al.,* 2015), while in anoxic denitrification, the optimum pH is close to that of nitrification, i.e., between 7.5 and 9.0 (Ronzano & Dapena, 2002).

Kokina *et al.,* (2022) mention that acidic pH affected the conversion of ammonia to nitrite in an activated sludge system. It was attributed to the limitation of the inorganic carbon source (due to the pH change itself). They also observed that rapid pH changes from optimum to acidic had a more significant impact than rapid pH changes from optimum to alkaline.

3.4.2 Dissolved oxygen and temperature

The influence of the DO concentration in the mixed liquor of aerobic reactors has to do with the rate of oxygen diffusion through the cytoplasmic membrane of *Nitrosomonas* bacteria. A DO concentration of 2 mg/L can be considered a minimum acceptable value at any point in the reactor, however, 4 mg/L does not improve the growth rate of *Nitrosomonas* by more than 10%. Poor oxygenation (below 2 mg/L DO) will result in partial nitrification (Ronzano & Dapena, 2002; Mozumder & Hossain, 2020).

The autotrophic bacteria in charge of nitrification are strictly aerobic, and it is considered that for DO concentrations between 3 and 4 mg/L, the maximum growth rate of nitrifying microorganisms begins to decrease, with a significant reduction below 2 mg/L. At concentrations below 0.5 mg/L, the effect of the decrease in nitrification is greater for *Nitrobacter* than for *Nitrosomonas.* In this regard, since the autotrophic bacteria Nitrosomonas and *Nitrobacter* are strictly aerobic, their growth is arrested in the anoxic reactor, i.e., nitrifying catabolism is delayed by oxygen limitation at concentrations that do not affect many heterotrophs. In a successful nitrification process, heterotrophic bacteria compete with nitrifying bacteria for both DO and space (EPA, 2009).

In the treatment system under study, the aerobic reactors operate with a DO concentration of 3.3 mg/L, which implies a lot of air supply in the reactors. However, this does not indicate higher nitrification but higher energy expenditure. This was observed by lowering the DO concentration to 2 mg/L in a simulation scenario, where the results were similar to those observed in Figure 6. The airflow for the calibrated model (Figure 6) with 3.3 mg/L of DO in the CSTR1, CSTR2, and $R_{AEROBIO}$ reactors were 527.3, 870.6 and 1696.0 m^3/h respectively, and by lowering the DO concentration to 2 mg/L, the air flows were 373.9, 643.4, and 1279.0 m^3/h in the CSTR1, CSTR2, and $R_{AEROBIO}$ respectively, having a decrease in air consumption of 25.8% in total. Undoubtedly, DO is a critical parameter governing nitrification efficiency (Gupta *et al.,* 2021). However, the lower the DO concentration, the lower the N-NH_3 oxidation efficiency, and the higher the DO concentration, the more N-NO_3^- is produced.

In bioprocesses, controlling the DO in a liquid medium is essential for cell growth. Oxygen transfer is usually considered a limiting factor in aerobic processes, due to the low solubility of oxygen in the liquid medium (Melgarejo *et al.*, 2022). The lack of aeration significantly affects the biological processes involved in an activated sludge process, being the heterotrophic activity the metabolic process most affected (Romualdo *et al.,* 2022).

In the case of denitrification, it is known that there is aerobic denitrification (in the presence of oxygen) and anoxic denitrification, which is more sensitive to the presence of oxygen and inhibits nitrate reduction (Gupta *et al.,* 2021). The oxygen threshold that inhibits aerobic denitrification ranges from 0.08 to 7.7 mg/L (Ji *et al.,* 2015), while DO concentrations greater than 0.2 mg/L inhibit anoxic denitrification (Tchobanoglous *et al.,* 2003; EPA, 2009). It should be noted that the anoxic-aerobic reactor of the treatment system under study does not have a separation barrier, so the oxygen injected into the aerobic zone likely diffuses into the anoxic zone. According to the balance of matter in the anoxic reactor (Figure 6), there is a large number of reduced nitrates (28.6 kg/d) reaching concentrations in the effluent of this reactor of 0.015 mg/L (0.0233 kg/d), which indicates that

denitrification is not inhibited.

Temperature is another parameter that significantly affects the nitrification, denitrification, and metabolic activities of microorganisms. Several studies have reported good simultaneous nitrification and denitrification in aerobic reactors with temperatures between 20 and 30 °C (Gupta *et al.,* 2021). Wang *et al.,* (2010) examined nitrogen removal in a CAST (cyclic activated sludge technology) type activated sludge system at 27 and 30 °C temperatures and observed N-NH$_3$ and N$_{TOTAL}$ removal efficiency of 51% and 42%, respectively. While at temperatures between 10 - 15 °C, N-NH$_3$ removal and N$_{TOTAL}$ were inefficient due to poor biomass activity. In this study, according to the calibrated model (Figure 6), the N-NH$_3$ removal efficiency was 57% and N$_{TOTAL}$ 38% at a temperature of 26.5 °C in the CSTRs, in which there may be simultaneous nitrification and denitrification.

It has been reported that the optimum temperature for nitrifying bacteria is between 20 to 30 °C, and then low temperatures can lead to DO supersaturation and hypoxic denitrification, further affecting total nitrogen removal (Li *et al.,* 2023). Mozumder & Hossain, (2020) mention that the optimum temperature for nitrification is between 28 and 38 °C in different BNR processes.

On the other hand, the optimum temperature range for aerobic denitrification is 25 to 37 °C and is considerably reduced at temperatures below 10 °C (Ji *et al.,* 2015). In the case of anoxic denitrification, this can occur between 5 and 30 °C (Ronzano & Dapena, 2002). Carrera *et al.,* (2004) mention that the optimum temperature for anoxic denitrification is 25 °C.

Therefore, and according to these first four parameters analyzed (alkalinity, pH, DO, and T), it is deduced that the conditions exist to achieve a certain degree of nitrification and denitrification of the treatment system under study, since these parameters are within the operational range obtained by several researchers.

3.4.3   SRT (solids retention time or sludge age)

Although the parameters analyzed (alkalinity, pH, DO, and T) are within the operational ranges for nitrification to occur in the treatment system under study, there is another crucial factor in achieving complete nitrification, the SRT. In addition, it should be noted that wastewater cannot be denitrified unless it has first been nitrified.

Nitrification is only possible by providing the treatment system with sufficient SRT for nitrifying bacteria to grow and oxidize ammonium. The SRT required for nitrification in an activated sludge process is a function of the maximum growth rate of nitrifying microorganisms, which is related to alkalinity, pH, DO, and T (Zhou *et al.,* 2023; WEF, 2008; EPA, 2009). Merton, (2004) mentions that maintaining an SRT of

15 days or more at a defined temperature ensures good nitrification in an activated sludge system. Also, the concentration of ammonia (N-NH$_3$) to be nitrified must be considered, since wastewater with high concentrations of ammonia requires a very long SRT, Elawwad, (2018) recommends an SRT of 40 - 50 days for N-NH$_3$ concentrations of 611 mg/L.

Many WWTPs operate with long SRTs to enhance nitrification and reduce excessive sludge production. However, the SRT affects not only the nutrient removal performance and settling characteristics of the sludge but also the production of secondary pollutants such as nitrous oxide (N$_2$O) during biological nutrient removal (BNR). In an activated sludge system, the main BNR process, bacteria must be flocculated for efficient gravity sedimentation, for this, the biomass requires a minimum SRT of 3 to 4 days, too large SRTs lead to poorly settleable flocs and the growth of filamentous bacteria (Hreiz *et al.,* 2015). In this regard, other authors have restricted the SRT in activated sludge between 4 to 27 d, 5 to 35 d, and 4 to 30 d (Hreiz *et al.,* 2015). Li & Wu, (2014) determined that the optimum SRT for nitrogen removal in a BNR process was 20 d.

As shown in Figure 6, the SRT at which the WWTP under study operates is 1.7 d, which is very low compared to those referred to in the literature, but partial nitrification occurs. The minimum SRT should be 3 d (Tchobanoglous *et al.,* 2003).

3.4.4   Ammonia (N-NH$_3$) and nitrates (N-NO$_3^-$)

Table 9 and Table 10 present the balances of matter of oxidized N-NH$_3$ and N-NO$_3$ produced during the nitrification process in the aerobic reactors, according to Figure 6.

As shown in Table 9, oxidation efficiency in each of the aerobic reactors of the treatment system under study increases from CSTR1 to R$_{AEROBIO}$, which is related to the reactor volume, the treated flow, and the rate of N-NH$_3$ to be oxidized. The highest oxidation efficiency occurs in the aerobic reactor (96.79%), which has a larger volume, a lower treatment flow, and a lower rate of N-NH$_3$ to be oxidized. This N-NH$_3$ oxidation process is reflected in the production of N-NO$_3^-$ during nitrification (Table 10); the highest number of nitrates produced is in the R$_{AEROBIO}$, which presented the highest oxidation efficiency.

According to the results obtained in Table 10 concerning the N-NO$_3$ produced, the nitrification rates of CSTR1, CSTR2, and R$_{AEROBIO}$ (R$_{AER}$) were 3.683, 7.502, and 8.907 mgN/L-h, respectively. In the case of denitrification in the anoxic reactor (R$_{ANOX}$), 28.66 kg/d of N-NO$_3^-$ enter and 0.0233 kg/d leave, so 28.6 kg/d of nitrates are removed, equivalent to a denitrification rate of 88.67 mgN/L-d with respect to nitrates reduced. However, this good rate of denitrification, is unfavorable because more N-

Table 9. Mass balance of N-NH$_3$ oxidized during nitrification.

Reactor	V$_{REACTOR}$ (m^3)	Q (m^3/d)	N-NH$_{3\ INF}$ (kg/d)	N-NH$_{3\ EFF}$ (kg/d)	N-NH$_{3\ OXIDIZED}$ (kg/d)	Oxidation Efficiency (%)
CSTR1	187.31	4942	79.5	74.4	5.1	6.42
CSTR2	231.75	5875	74.85	46.3	28.55	61.86
R$_{AEROBIC}$	322.96	1555	23.4	0.75	22.65	96.79

Table 10. Mass balance of N-NO$_3^-$ produced during nitrification.

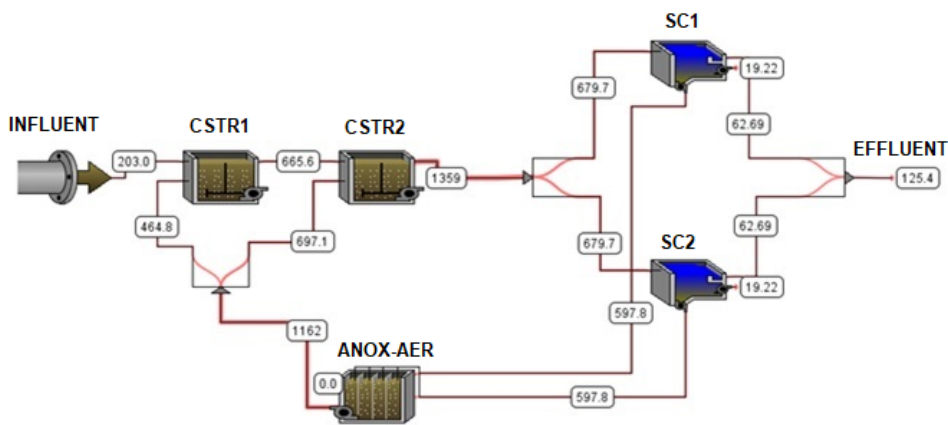| Reactor	V$_{REACTOR}$ (m^3)	Q (m^3/d)	N-NO$_3^-{}_{INF}$ (kg/d)	N-NO$_3^-{}_{EFF}$ (kg/d)	N-NO$_3^-{}_{PRODUCED}$ (kg/d)
CSTR1	187.31	4942	22.7	36.7	14.0
CSTR2	231.75	5875	70.8	108.3	37.5
R$_{AEROBIC}$	322.96	1555	0.02	56.8	56.78

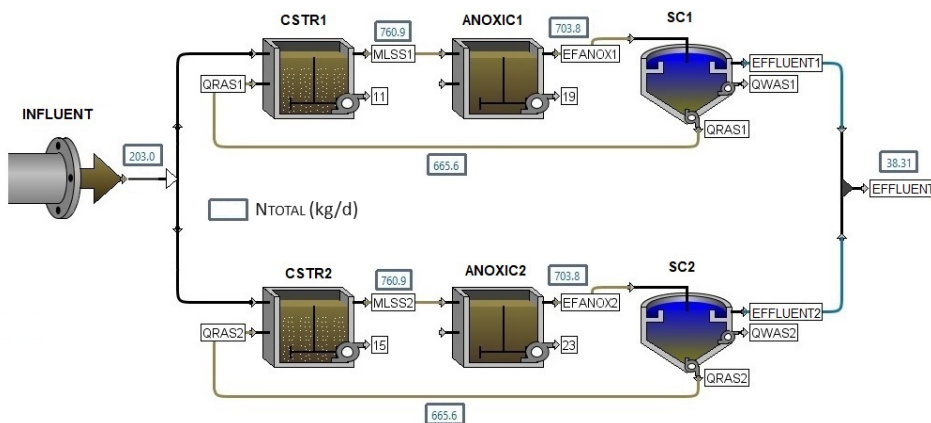Figure 7. Mass balance of N$_{TOTAL}$ of the calibrated model (kg/d)

Figure 8. CSTR - R$_{ANOXIC}$ - SC model and mass balance of N$_{TOTAL}$ (kg/d).

NO$_3^-$ is produced in the R$_{AER}$ (56.8 kg/d) even more than what entered the R$_{ANOX}$ (28.66 kg/d); this makes denitrification in the R$_{ANOX}$ counterproductive in this system (Figure 6).

From the results discussed above, it is clear that the ability to achieve complete nitrification of the wastewater depends on the nitrification rate inside the aeration tanks. The nitrification rate is controlled by maintaining the system with an SRT that provides the desired degree of nitrification (WEF, 2008). On the other hand, to achieve a high denitrification rate in an anoxic reactor, it is necessary to have a good design of this reactor, such that dissolved oxygen is limited as much as possible.

Table 11. Equipment sizing, Q_{RAS} and Q_{WAS}.

Volume of CSTR1 = Volume of CSTR2 (m³)	600
Volume of anoxic reactor 1 = Volume of anoxic reactor 2 (m³)	1000
Area of SC1 = Area of SC2 (m²)	95
Q_{RAS1} = Q_{RAS2} (m³/d)	1400
Q_{WAS1} = Q_{WAS2} (m³/d)	40

Table 12. Operational results of the CSTR-R_{ANOXIC}-SC model.

	CSTR1 = CSTR2	$R_{ANOXIC1}$ = $R_{ANOXIC2}$
XCOD/VSS adjusted	1.48	1.48
DO (mg/L)	2.0	0.0
SRT (d) simulated	14.86	14.86
SRT (Tchobanoglous *et al.,* 2003)	3 – 15	3 – 15
MLSS (mg/L) simulated	2774	2743
MLSS (Tchobanoglous *et al.,* 2003)	1500–4000	1500–4000
F/M (Kg BOD₅/Kg MLVSS- d) simulated	0.37	1.3
F/M (Tchobanoglous *et al.,* 2003)	0.2 - 0.6	-
Volumetric loading (kgBOD₅/m³- d) simulated	0.7	2.4
Volumetric loading (Tchobanoglous *et al.,* 2003)	0.3 – 1.6	-
	SC1	**SC2**
Overflow rate (m³/m²- d) simulated	22.32	22.32
Overflow rate (Tchobanoglous *et al.,* 2003)	16 – 28	16 – 28
Solids loading (kg/m²- d) simulated	102.8	102.8
Solids loading (Tchobanoglous *et al.,* 2003)	96 – 144	96 – 144
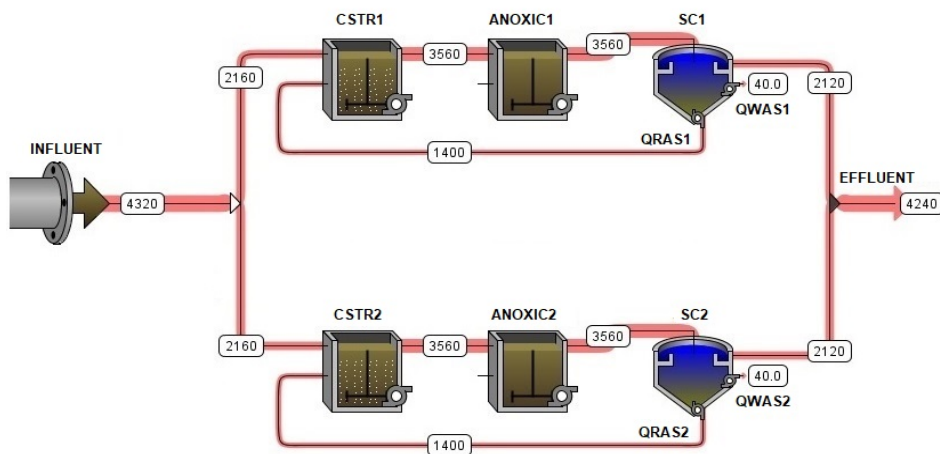
Figure 9. Flows in the CSTR - R_{ANOX} - SC model (m³/d).

The R_{ANOX} design should have a hydrodynamic configuration closer to plug-flow than complete mixing, and minimize surface oxygen exchange by carefully choosing the agitation system (Raboni *et al.,* 2020).

3.5 Mass balance of total nitrogen of the calibrated model

Figure 7 shows the mass balance of the N_{TOTAL} of the calibrated model (Figure 6). From the results shown in Figure 7, it can be determined that the percentage of removal of N_{TOTAL} is 38%. In this regard, a new model was developed to increase the rate removal of N_{TOTAL} and thus comply with the maximum permissible limits for wastewater discharge to a water body according to NOM-001-SEMARNAT-2021 (DOF, 2022). Additionally, in this new model, it was sought that the range of the most decisive operational parameters in terms of nitrification and denitrification, should be within the limits set in the literature. Figure 8 shows the new model that was simulated with GPS-X software.

According to Figure 8, the equipment sizing in the best simulation scenario is shown in Table 11. Table 12 shows the operational results.

According to the results obtained in Table 12, the treatment system would have an efficient operation without problems related to F/M, volumetric loading, and solids retention time (SRT). In addition, there would be no operational problems in the secondary clarifier (SC) due to the effects of overflow rate and solids loading. On the other hand, the effluent quality recorded for this model (Figure 8) was 3.9 mg/L of BOD_5, 38.6 mg/L of COD, 12.3 mg/L of TSS and 9.0 mg/L of N_{TOTAL}, which are within the maximum permissible limits according to NOM-001-SEMARNAT-2021 (DOF, 2022). By lowering the concentration of N_{TOTAL} in the effluent to 9 mg/L, 81% removal of this parameter would be achieved, as observed in the N_{TOTAL} mass balance (Figure 8), determined from the treatment system flows (Figure 9).

It should be noted that, in this new biological treatment model, the volume of aerobic reactors (greater aeration), anoxic reactors, and the SRT had to be increased to achieve a reasonable degree of nitrification and denitrification. However, in the case of the two aerobic reactors (CSTR1 and CSTR2), a total of 1902 m^3/h of air would be consumed, which, compared to the calibrated model (Figure 6) that consumes a total of 3093.9 m^3/h of air, would result in 38.5% less air to be used. Nitrification occurs in an activated sludge system when extensive aeration occurs (WEF, 2008). Nitrification is only possible with sufficient SRT for nitrifying bacteria to grow and oxidize ammonia nitrogen. Hence the system volume for nitrification is larger than that used for organic matter removal (Zhou *et al.,* 2023; WEF, 2008; EPA, 2009; Merton, 2004; Elawwad, 2018).

Conclusion

The parameters of alkalinity, pH, DO, T, and SRT were useful indicators to observe the nitrification and denitrification process in an activated sludge system, and these can be analyzed appropriately through a mass balance.

In the system under study, the degree of nitrification and denitrification needs to be improved. To achieve complete nitrification of the wastewater, a reasonable nitrification rate inside the aeration tanks and an adequate denitrification rate in the anoxic reactor are required. By creating a new treatment system model, it was possible to increase the removal efficiency of N_{TOTAL} from 38% to 81%, for which it was necessary to rearrange the processes for nitrification and denitrification, in addition to increasing the volume in aerobic reactors, anoxic reactors, and the SRT.

Mass balance and GPS-X software, in conjunction, are handy tools for studying the operation of a WWTP and for detecting problems that may occur.

References

APHA (2005). Standard methods for the examination of water and wastewater. American Public Health Association/American Water Works Association/Water Environment Federation, Washington DC.

Carrera, J., Vicent, T. & Lafuente, J. (2004). Effect of influent COD/N ratio on biological nitrogen removal (BNR) from high-strength ammonium industrial wastewater. *Process Biochemistry 39(12),* 2035-2041. http://doi.org/10.1016/j.procbio.2003.10.005

D´Antoni, B., Iracá F. & Romero, M. (2017). Brief review on filamentous foaming and bulking in activated sludge treatments: Causes and mitigation actions. *PantaRei Water Solutions,* 1-10. https://doi.org/10.13140/RG.2.2.29506.58560

Dionisi D. & Rasheed, A. (2018). Maximization of the organic load rate and minimization of oxygen consumption in aerobic biological wastewater treatment processes by manipulation of the hydraulic and solids residence time. *Journal of Water Process Engineering 22,* 138-146. https://doi.org/10.1016/j.jwpe.2018.02.002

Dlangamandla, C., Basitere, M., Ifeoluwa, B., Silas, B. & Karabo, S. (2021). Biofoam formation and defoamation in global wastewater treatment systems. *Water Practice &. Technology 16 (1),* 1-18. https://doi.org/10.2166/wpt.2020.113

DOF (2022). Norma oficial mexicana NOM-001-SEMARNAT-2021. Diario Oficial de la Federación, México.

Elawwad, A. (2018). Optimized biological nitrogen removal of high-strength ammonium wastewater by activated sludge modeling. *Journal of Water Reuse and Desalination 8(3),* 393-403. https://doi.org/10.2166/wrd.2017.200

EPA (2009). Nutrient control design manual. EPA/600/R-09/012. Environmental Protection Agency, USA.

Espinosa, M. A., Delgado, R. & Hidalgo, A. (2020). Evaluación de un proceso anóxico-aerobio-reactor biológico de membrana con alto

contenido de nitrógeno. *Revista Internacional de Contaminación Ambiental 36(2),* 303-320. https://doi.org/10.20937/RICA.53111

Espósito, M., Blanco, M., Sequeira, M., Paoloni, J., Fernandez, S., Amiotti, N. & Díaz, S. (2016). Contaminación natural (As, F) y eutrofización (N, P) en la cuenca del arroyo el divisorio, Argentina. *International Journal of Experimental Botany 85,* 51-62.

Guevara, M. & Ramírez, L. (2015). *Eichhornia crassipes*, su invasividad y potencial fitorremediador. *La Granja: Revista de Ciencias de la Vida 22(2),* 5-11. DOI:10.17163/lgr.n22.2015.01

Gupta, R., Poddar, B., Nakhate, S., Chavan, A., Singh, A., Purohit, H. & Khardenavis, A. (2021). Role of heterotrophic nitrifiers and aerobic denitrifiers in simultaneous nitrification and denitrification process: a nonconventional nitrogen removal pathway in wastewater treatment. *Letters in Applied Microbiology* 74(2), 159-184. https://doi.org/10.1111/lam.13553

Hauduc, H., Rieger, L., Oehmen, A., van Loosdrecht, M., Comeau, Y., He´duit, A., Vanrolleghem, P.A. & Gillot, S. (2013). Critical review of activated sludge modeling: State of process knowledge, modeling concepts and limitations. *Biotechnology and Bioengineering 110(1),* 24-46. https://doi.org/10.1002/bit.24624

Henze, M., Gujer, W., Mino, T. & van Loosdrecht, M. (2006). Activated Sludge Models ASM1, ASM2, ASM2d and ASM3. IWA Publishing, London UK.

Hreiz, R., Latifi, M. & Roche, N. (2015). Optimal design and operation of activated sludge processes: State-of-the-art. *Chemical Engineering Journal 281,* 900-920. http://dx.doi.org/10.1016/j.cej.2015.06.125

Hydromantis (2014). Software GPS-X. Environmental Software Solutions, INC.

Ji, B., Yang, K., Zhu, L., Jiang, Y., Wang, H., Zhou, J. & Zhang, H. (2015). Aerobic denitrification: A review of important advances of the last 30 years. *Biotechnology and Bioprocess Engineering 20*, 643-651. https://doi.org/10.1007/s12257-015-0009-0

Kokina, K., Mezule, L., Gruskevica, K., Neilands, R., Golovko, K. & Juhna, T. (2022). Impact of rapid pH changes on activated sludge process. *Applied Sciences 12(11),* 5754. https://doi.org/10.3390/app12115754

Li, B. & Wu, G. (2014). Effects of sludge retention times on nutrient removal and nitrous oxide emission in biological nutrient removal processes. *International Journal of Environmental Research and Public Health 11(4),* 3553-3569. Available on line: https://doi.org/10.3390/ijerph110403553

Li, X., Yang, Y., Liu, G., Sun, D. & Ma, X. (2023). Enhanced nitrogen removal at low temperature with mixed anoxic/oxic process. *Water Science and Technology 16(1),* 67-75. https://doi.org/10.1016/j.wse.2022.08.005

Meijer, S., van der Spoel, H., Susanti, S., Heijnen, J. & van Loosdrecht, M. (2002). Error diagnostics and data reconciliation for activated sludge modelling using mass balances. *Water Science & Technology 45(6),* 145-156. https://doi.org/10.2166/wst.2002.0102

Melgarejo, R., Rosales, D., Polo, M., Fernández, G., Morales, M., Pérez, S., Arce, M & Palmerín, D. (2022). Mathematical model to estimate volumetric oxygen transfer coefficient in bioreactors using conformable calculus. *Revista Mexicana de Ingeniería Química 21(2),* 1-18. https://doi.org/10.24275/rmiq/Bio2701

Merton, A. (2004). What SRT and MCRT mean to treatment plant design and operation. *Water Environment & Technology 16(6),* 65-66. https://www.jstor.org/stable/43888802

Mozumder, M. & Hossain, M. (2020). Interaction between biological nitrogen removal processes and operating parameters: A review. *Journal of Scientific Research 12(4),* 757-774. http://dx.doi.org/10.3329/jsr.v12i4.46092

Najman, K., Panglish, S. & Katayama, V. (2020). Effect of different parameters of the biological processes on the specific growth rate of nitrifying bacteria by means of mathematical models. *Journal of Duhok University 23(2),* 372-386. https://doi.org/10.26682/csjuod.2020.23.2.30

Raboni, M., Viotti, P., Rada, E., Conti, F. & Boni, M. (2020). The sensitivity of a specific denitrification rate under the dissolved oxygen pressure. *International Journal of Environmental Research and Public Health 17(24),* 9366. https://doi.org/10.3390/ijerph17249366

Randall C.W. & Buth D. (1984). Nitrite build-up in activated sludge resulting from temperature

effects. *Journal Water Pollution Control Federation 56(9),* 1039-1044. https://www.jstor.org/stable/25042426

Rieger, L., Gillot, S., Langergraber, G., Ohtsuki, T., Shaw, A., Takács, I. & Winkler, S. (2013). Guidelines for using activated sludge models. IWA Publishing, London-New York. https://doi.org/10.2166/9781780401164

Romualdo, I., Hernández, A., González, G. & Beristain, R. (2022). Metabolic and kinetic changes of activated sludge because of failures in the aeration system in a WWTP. *Revista Mexicana de Ingeniería Química 21(3),* 1-7. https://doi.org/10.24275/rmiq/IA2914

Ronzano E. & Dapena J. L. (2002). Tratamiento Biológico de las Aguas Residuales. Díaz de Santos, S.A., Madrid España.

Sam, T., Le Roes-Hill, M., Hoosain, N. & Welz, P. (2022). Strategies for controlling filamentous bulking in activated sludge wastewater treatment plants: The old and the new. *Water 14(20),* 1-21. https://doi.org/10.3390/w14203223

Tchobanoglous, G., F., Burton F. & H. Stensel, H. (2003). Wastewater engineering, treatment and reuse. McGraw Hill, New York.

Van Loosdrecht M., López C., Meijer S., Hooijmans C. & Brdjanovic D. (2015). Twenty-five years of ASM1: past, present and future of wastewater treatment modelling. *Journal of Hydroinformatic 17 (5),* 697-718. https://doi.org/10.2166/hydro.2015.006

Wang, Y., Zhang, Z., Yan, M., Gao, N., Yang, J. & Ren, M. H. (2010). Impact of operating conditions on nitrogen removal using cyclic activated sludge technology (CAST). *Journal Environment Science Health*, *part a*, *45(3),* 370–376. https://doi.org/10.1080/10934520903467964

WEF (2008). Operation of municipal wastewater treatment plants. Water Environment Federation, USA.

WERF (2003). Methods for wastewater characterization in activated sludge modelling. Water Environment Research Federation, Alexandria, Virginia.

Zhang, J., Gilbert, D., Gooday, A., Levin, L., Naqvis, S., Middelburg, J., Scranton, M., Ekaus, W., Dewite, B., Oguz, T., Monteiro, P., Urban, E., Rabalais, N., Ittektots, V.,Kemp, W., Ulloa, O., Elmgren, R., Escobar, E., & Van der Plas. A. (2010). Natural and human-induced hypoxia and consequences for coastal areas: synthesis and future development. *Biogeosciences* 7, 1443-1467. https://doi.org/10.5194/bg-7-1443-2010

Zhou, Y., Zhu, Y., Zhu, J., Li, Ch. & Chen, G. (2023). A comprehensive review on wastewater nitrogen removal and its recovery processes. *International Journal of Environmental Research and Public Health 20(4),* 3429. https://doi.org/10.3390/ijerph20043429

Zornoza, A., Avendaño, L., Aguado, D., Borrás, L. & Alonso, J. L. (2014). Análisis de las correlaciones entre la abundancia de bacterias nitrificantes, parámetros operacionales y físico-químicos relacionados con el proceso biológico de nitrificación en fangos activos. *Tecno Agua 5,* 1-12.