Steady state modeling of autotrophic membrane bioreactor – a new approach to quantify biomass

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Abstract

Purpose – The main purpose of this study resides essentially in the development of a new tool to quantify the biomass in the bioreactor operating under steady state conditions.

Design/methodology/approach – Modeling is the most relevant tool for understanding the functioning of some complex processes such as biological wastewater treatment. A steady state model equation of activated sludge model 1 (ASM1) was developed, especially for autotrophic biomass (XBA) and for oxygen uptake rate (OUR). Furthermore, a respirometric measurement, under steady state and endogenous conditions, was used as a new tool for quantifying the viable biomass concentration in the bioreactor.

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Findings – The developed steady state equations simplified the sensitivity analysis and allowed the autotrophic biomass (XBA) quantification. Indeed, the XBA concentration was approximately 212 mg COD/L and 454 mgCOD/L for SRT, equal to 20 and 40 d, respectively. Under the steady state condition, monitoring of endogenous OUR permitted biomass quantification in the bioreactor. Comparing XBA obtained by the steady state equation and respirometric tool indicated a percentage deviation of about 3 to 13%. Modeling bioreactor using GPS-X showed an excellent agreement between simulation and experimental measurements concerning the XBA evolution.

Originality/value – These results confirmed the importance of respirometric measurements as a simple and available tool for quantifying biomass.

Keywords Bioreactor, Steady state modeling, Activated sludge model 1 (ASM1), State variable, Respirometric tool

Paper type Research paper

1. Introduction

In biological purification of urban wastewater, it is challenging to identify all the basic processes necessary to describe the biological system's functioning. In addition, the properties of the reaction medium, composed of a purifying culture, are constantly evolving due to variations in influent flow, composition and concentration (Giwa and Hasan, 2015; Potrykus *et al.*, 2020). However, it is necessary to have a sufficient number of parameters needed to well conducted the design of different components of wastewater treatment system as well as to have control tools that allow optimal exploitation.

For a long time, the design equations were based on material balance on the reactor operating in stationary condition and associated with a kinetic approach based on irreversible first-order reactions (Resat *et al.*, 2009). Moreover, determining parameters for the kinetic coefficient was often macroscopic quantities (Mardani *et al.*, 2011), such as the organic loading rate, which represents the inlet flow of pollution, the hydraulic retention time (HRT) which corresponds to the theoretical time that spent the effluent in the bioreactor and the sludge retention time (SRT), which represents the time spent by sludge in the treatment unit.

However, these simplified approaches often lead to oversizing the units. They are unable to provide tools for controlling and understanding intrinsic phenomena. Also, they need to predict the system response in dynamic conditions (Khan, Hasnain, Fareed, & Ben Aim, 2019). Representing viable biological population in the bioreactor through the volatile suspended solid (VSSs) parameter does not distinguish between either viable bacterial populations or organic compound fractions (Camejo, Barat, Murgui, Seco, & Ferrer, 2018; Regmi *et al.*, 2022).

Researchers (Gujer and Henze, 1991) tried to solve in part to this problem by defining the state variables, the results of an elementary fraction of compounds present in wastewater. This new decomposition made it possible to (i) classify pollutants according to their nature (i.e. organic or mineral, particulate or soluble) and biodegradability (easily, slowly and non-biodegradable) and (ii) separate purifying populations following their character, heterotrophic or autotrophic and field of activity (Elnaker *et al.*, 2018). Furthermore, introducing digital tools and software allowed the development of these models. Such new concepts have lifted a technological barrier that had been challenging to overcome (Cadet, 2014) and made it possible to develop numerous tools promoting the comprehension of elementary processes (e.g. degradation of organic matter and transformation of nitrogen compounds) (González-Cabaleiro, Curtis, & Ofiţeru, 2019; Vielela *et al.*, 2022) and define online process control tools (Jeon *et al.*, 2019). The most widely accepted in wastewater treatment technology was the activated sludge model 1 (ASM1) (Van Loosdrecht, Lopez-Vazquez, Meijer, Hooijmans, & Brdjanovic, 2015), which was developed to describe ammonium and organic carbon removal.

Currently, many new analytical methods allow for characterization substrates and biomass in polluted and treated water, especially the chemical oxygen demand (COD) fractionation (Ravndal *et al.*, 2018). The technologies used for identifying and quantifying bacteria, such as the polymerase chain reaction and scanning electron microscopy

(Zhang *et al.*, 2017; Islam *et al.*, 2017), were very sophisticated and were not available to some researchers. Moreover, modeling tools still need to be expanded to identify active bacterial populations and measure their own reactions (Monti and Hall, 2008). Therefore, this work focuses on the development of a new tool for quantifying the active biomass and characterizing the specific activity of autotrophic populations in a wastewater treatment reactor. It was built around two tasks. The first is the linearization of basic equations of the ASM1 for the biological operation in a steady state condition. A sensitivity analysis will be follow this development. The second task is dedicated to active biomass concentration quantification using a respirometric measurements. This value will be compared to those obtained from the steady state equation and GPS-X simulation. This approach will make it possible to define new criteria for characterizing the nitrifying population.

Nomenclature

X_{BA}	Autotrophic biomass (mgCOD/L)	Y_A	Autotrophic Yield g(cellCOD formed).g(N oxidized)$^{-1}$
X_{BH}	Heterotrophic biomass (mgCOD/L)	K_{NH}	Ammonia half-saturation coefficient for autotrophs (mgN/L)
S_{NH}	Soluble ammonia nitrogen substrate (mgN/L)	Ks	for heterotrophic biomass ((mgCOD/L)
S_{ND}	Soluble biodegradable organic nitrogen (mgN/L)	K_h	Maximum specific hydrolysis (d^{-1})
Ss	Biodegradable soluble organic substrate (mgCOD/L)	K_x	Hsc for hydrolysis of slowly biodegradable (g(slowly biodegr.COD).g(cellCOD)/d)$^{-1}$)
S_{NO}	Nitrate nitrogen (mgN/L)		
So	Oxygen concentration ($gO_2.m^3$)		
X_S	Biodegradable organic particulate fraction (mgCOD/L)	K_a	Ammonification rate (m^3.(gCOD.day)$^{-1}$)
X_{ND}	Biodegradable nitrogen particulate fraction (mgN/L)	b_H	Heterotrophic decay rate (d^{-1})
X_P	Non-biodegradable particulate fraction (mgCOD/L)	b_A	Autotrophic decay rate (d^{-1})
f_p	Fraction of particular inert from biomass lysis (dimensionless)	μ_{Amax}	Autotrophic maximum growth rate (d^{-1})
i_{xb}	Nitrogen content in the active biomass (gN.gCOD^{-1})	μ_{Hmax}	Heterotrophic maximum growth rate (d^{-1})
i_{xp}	Nitrogen (N) content of products of biomass decay (gN.gCOD^{-1})	μ_{BHend}	Heterotrophic growth rate in endogenous condition (d^{-1})
Y_H	Heterotrophic Yield g(cellCOD formed).g(COD oxidized)$^{-1}$	Q	Feed flow ($m^3.d^{-1}$)
		Qw	Withdrawal flow ($m^3.d^{-1}$)
		V	Volume of bioreactor (m^3)
		SRT	Sludge retention time (d)
		HRT	Hydraulic retention time (d)

2. Materials and methods

2.1 Experimental set up

The experimental setup consisted of a 30-L of aerobic bioreactor equipped with a continuous pH controller and a 0.8 L submerged hollow fiber membrane module (0.05 µm pore size and 0.2 m^2 of surface area) (Figure 1). Due to the high mixing rate, the reactor and the membrane module were considered perfectly mixed. The concentrated synthetic feed solution, the diluting water and the permeate were injected or extracted by peristaltic pumps. Aeration was continuously provided through membrane diffusers at the bottom of the reactor and just below the fibers in the membrane module enabling to operate without dissolved oxygen (DO) limitation.

2.2 Biological conditions

Two successive experiments were carried out under the operational requirements, as shown in Table 1. At the beginning of the first run, the reactor was filled with sludge inoculums from a domestic wastewater plant operated with low organic loading rate (<0.1 kg COD/kgVSS/d). The reactor was then fed with a synthetic solution containing ammonium chloride (NH_4Cl) with additional phosphorus salts as diammonium phosphate $(NH_4)_2HPO_4$. Sodium carbonate (Na_2CO_3) was added to ensure the necessary alkalinity for the nitrification reaction. No organic carbon was in the reactor, as the feeding solution's COD/N ratio was always zero. Other elements (Mg^{2+}, K^+, etc.) were supplied by tap water used as diluent.

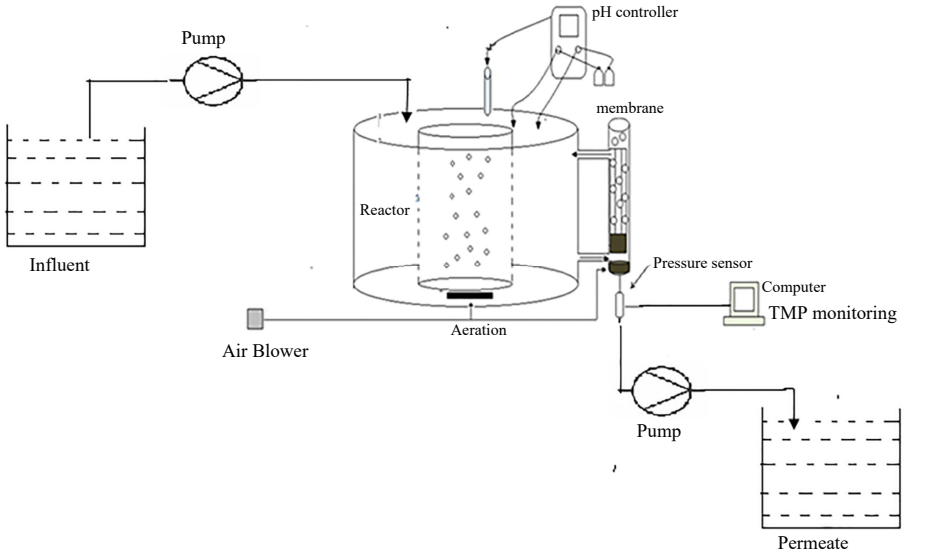

Figure 1.
Schematic representation of the experimental unit

Source(s): Figure by authors

run	I	II	
SRT (d)	20	no sludge withdrawal	40
Membrane flux (L/m^2/h)	10	17	17
HRT (d)	0.625	0.334	0.334
NLR (kgN/m^3/d)	0.22	0.374	0.374

Table 1.
Operational conditions

Source(s): Table by authors

During the first period, a nitrogen load rate (NLR) of 0.22 kgN/m^3/d and a sludge age of 20 days were imposed. For the second run, the NLR decrease, from 0.44 to 0.374 kgN/m^3/d, and the SRT was set at 40 days. At the beginning of the second run, sludge extraction was temporarily halted to achieve the expected concentration values of total suspended solids rapidely. The bioreactor was operating for 125 successive days. The monitoring during run I and II was done for 46 and 79 days, respectively.

During these runs, the pH was adjusted in the range of 7.5 ± 0.5 by the ez-control system which an automatic pH controller using a conventional proportional integral derivative (PID) control. The executive element was a peristaltic pump dosing the acid solution when the pH is increasing and alkaline solution when the pH is decreasing.

2.3 Analytical methods

Total suspended solids (TSS) and volatile suspended solids (VSS) were analyzed according to the Standard Methods (APHA, 2005). Ammonia, nitrate, and nitrite in the influent and effluent were measured using a colorimetric method (HACH DR/2500). The analysis of extracellular polymeric substances (EPS), which are polymer materials secreted by cells, was accomplished through the determination of protein and carbohydrate content according to Frolund, Griebe, and Nielsen (1995) and Dubois *et al.* (1956), respectively.

2.4 Respirometric analysis

A respirometric measurements was set up to study the kinetics of biological reactions by monitoring the evolution of DO concentration in the reactor throughout time. There are several methods for measuring the respirometry needs of a bacterial population (Gasmi, Heran, Hannachi, & Grasmick, 2015). In this work, we relied on one method that was carried out in a closed batch reactor, because it has the advantage to overcome the oxygen transfer phenomena from air to the environment.

The following protocol was adopted to perform these measurements: A volume of 250 ml of sludge from the continuous reactor is taken and placed in another batch and stirred reactor. The pH and temperature were controlled to be not a limiting factor to the biological reaction. The requirement for oxygen is evaluated by measuring the instantaneous concentration of DO in the medium using an oximeter (Oxi 330i). The rate of DO consumption over time is known as Oxygen Uptake Rate (OUR (mgO_2/L/d)). The experimental device used is presented in Figure 2.

The respirometric tests were carried out in endogenous respiration. The sludge in bioreactor was aerated without supplying of substrate for 24 hours; thus, it can then be assumed that the biodegradable substrates were consumed during this time. This duration is sufficient to achieve a constant total endogenous OUR noted OUR_{endt}. A sample from bioreactor was then transferred to the batch reactor to monitor the DO over time. Moreover, two specific inhibitors were added to the sludge sample placed in the batch reactor to quantify the relative activity of the different populations present in the bacterial culture. The first is the allythiourea solution (ATU) (20 $mmol.L^{-1}$) that is known as an inhibitor of autotrophic microorganisms and, more particularly, of the Nitrosomonas bacteria (Gorska, Gernaey, Demvunck, Vanrolleghem, & Verstraete, 1995). The second inhibitor is the sodium azide (24μM) or of the sodium chlorate ClO_{3^-} (2.3 mol/L), known as The Nitrobacter inhibitor (Chandran and Smets, 2000).

The nitrification reaction is the net result of two distinct processes (Heil, Vereecken, & Brüggemann, 2016).

- Oxidation of ammonium (NH_4^+) to nitrite (NO_2^-) by nitrosomonas bacteria:

$$2NH_4^+ + 3O_2 \rightarrow 2NO_2^- + 2H_2O + 4H^+ \quad (1)$$

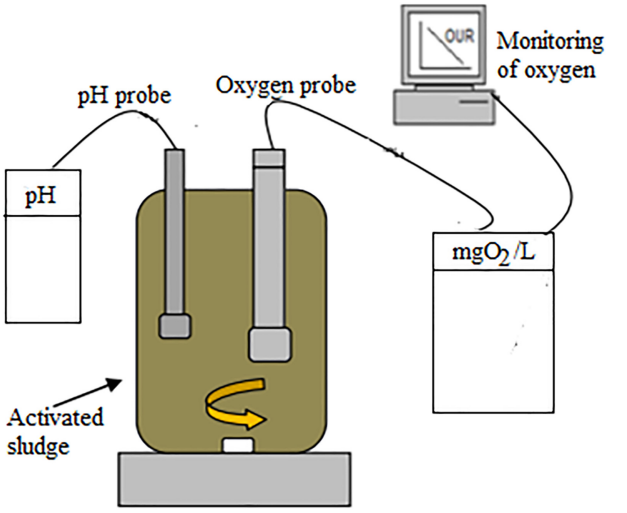

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Figure 2.
Experimental device for monitoring bacterial activity

- Oxidation of nitrite (NO_2^-) to nitrate (NO_3^-) by the Nitrobacter bacteria:

$$2NO_2^- + O_2 \rightarrow NO_3^- \quad (2)$$

Thus, after auditing two inhibitors, the active species in the medium is only the heterotrophic bacteria responsible for organic substrate oxidation.

Figure 3 represents an example of a curve obtained after successive additions of both inhibitors. In fact, after reaching the endogenous respiration, a sample was taken from the membrane bioreactor for DO monitoring as it aformentioned. The first slope of the line segment AB represents the total oxygen uptake rate (OUR_{endt}). After the injection of ATU, the Nitrosomonas bacteria will be inhibited. The second slop of segment BC represents the oxygen uptake rate resulted only from the endogenous respiration of Nitrobacter and heterotrophic bacteria. The injection of sodium chlorate inhibits the Nitrobacter bacteria, therefore, the slope of segment CD represents the endogenous oxygen uptake rate only of

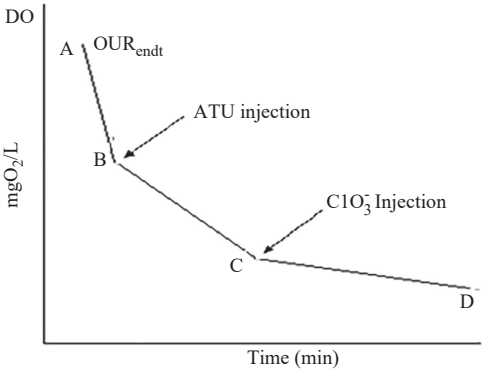

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Figure 3.
Curve obtained after injections of inhibitors into bioreactor

heterotrophic bacteria. The differences in slope obtained allow going back to the specific oxygen requirements of the different species of the sample.

The slope of the linear portion of the DO profile with time is the OUR.

3. Results and discussion

3.1 Performance of membrane bioreactor (MBR)

Table 2 summarizes the effluent qualities under steady-state condition. During the first run the TSS and VSS concentrations decrease compared to the effluent, this was likely due to (i) the continuous sludge withdrawn (SRT = 20 days) and (ii) the decrease of heterotrophic bacteria related to the lack of organic substrate in the influent. However, during the second run the TSS and VSS concentration increase due to the increase of SRT (40 days) and stop sludge withdrawal at the beginning of the second run. The average effluent TSS and VSS concentrations were: 485 ± 51 and 351 ± 38 mg/L for run I, 842 ± 65 and 748 ± 46 mg/L for run II.

The monitoring of nitrogen species throughout the study, shows that MBR was able to achieve satisfactory nitrogen removal, nitrate were the major nitrogen species in the effluent of the MBR suggesting complete nitrification in the treatment process. The removal efficiencies were more than 94% and 96 % for run I and II respectively. It should mention that, the monitoring of DO concentration exhibit that there is no oxygen limitation for nitrification reaction with a value of 6 mg/L.

3.2 Effect of SRT on fouling membrane

The major constraint for MBR application was membrane fouling since it cause an increase of operational cost (Rahman *et al.*, 2023). Tracking transmembrane pressure (TMP) or permeate flux variations over time are the two conventional methods for membrane-fouling monitoring. In fact, the flow of wastewater through a porous membrane was described by the Darcy's law given by Equation (3):

$$J = \frac{TMP}{\mu . R_t} \tag{3}$$

where:

J is the permeate flux ($L \cdot m^{-2} \cdot h^{-1}$).

TMP: The transmembrane pressure (Pa).

μ: The viscosity (Pa·s).

R_t: The total resistant which the sum of The fouling resistance and membrane resistance.

Variable	Influent	Effluent	
		Run I	Run II
NH_4^+(mgN/L)	125	6.2 ± 0.8	3.7 ± 0.8
NO_{3^-} (mgN/L)	–	118 ± 5	122 ± 3
NO_{2^-}(mgN/L)	–	0.3 ± 0.1	0.1 ± 0.05
TSS (mg/L)	1125 ± 85	458 ± 51	842 ± 65
VSS (mg/L)	710 ± 58	351 ± 38	748 ± 46

Source(s): Table by authors

Table 2. Effluent and influent qualities of the MBR under steady-state condition

The method of R_t determination has been widely investigated in previous works (Gasmi, Heran, Hannnachi, & Grasmick, 2012, 2013). The monitoring of TMP with time allowing the determination of fouling rate that is defined by the evolution of resistance or TMP with time. Table 3 presents the fouling rate obtained in our study as well as for other works.

The SRT has an important effect on the sludge properties, including the TSS concentration, the presence of extracellular polymeric substances (EPS) and soluble microbial products (SMP) resulted from bacteria activities and known among the responsible of fouling membrane. As it showed in Table 3, the membrane fouling rate in this study was highest at SRT equal to 20 days operation compared to SRT equal to 40 days. These results were consistent with some other studies that suggested MBRs operated under a prolonged SRT tend to have a lower fouling potential (Ouyang & Liu, 2009; Deb *et al.*, 2022). In this study, It seems that the increase of TSS during the second run hasn't affected the fouling propensity. Thus, higher air flow intensity through membrane (200NL/h) was sufficient to prevent sludge deposition on surface membrane. However, it was found during this study that the difference of EPS concentration inside the bioreactor (EPSs) and in the permeate (EPSp), (EPSs-EPSp) was found equal to 5-20 mg/L for run I and 2-12mg/L for run II. Therefore, the membrane has a significant role in the quality of permeate regarding to the soluble fractions and these materials contribute to fouling mechanism. Ahmed *et al.* (2007)

Table 3.
Fouling rate results from previous literature studies in comparison with the present study

References	Operating conditions details	Fouling rate dR/dt ($\times 10^{12}$ $m^{-1}.d^{-1}$)
Ouyang and Liu (2009)	HRT = 12 h	
	OLR = 0.79 kgCOD/m^3/d	
	SRT = 10d	0.53
	SRT = 40 d	0.38
	No sludge withdrawal	0.24
Van den Broeck *et al.*, 2012	HRT = 15 h	0.24
	ORL = 0.39-0.65kgDCO/m^3/d	0.07
		0.0042
	SRT = 10 d	0.24
	SRT = 30 d	0.07
	SRT = 50 d	0.0042
Han *et al.* (2005)	HRT = 12 h	
	SRT = 50 d	0.6
	SRT = 70 d	1
	SRT = 100 d	1.3
Huang *et al.* (2011)	HRT = 12 h	
	OLR = 1.1 kgDCO/m^3/d	
	SRT = 30 d	0.14
	SRT = 60 d	0.52
	(No sludge withdrawal)	0.68
Deb *et al.* (2022)	HRT = 5h	
	SRT = 10 d, OLR = 0.22 kgDCO/m^3/d	0.22
	NLR = 0.022 kgN/m^3/d	
	SRT = 25 d, OLR = 0.19 kgDCO/m^3/d	0.056
	NLR = 0.022 kgN/m^3/d	
	SRT = 40 d, OLR = 0.22 kgDCO/m^3/d	0.19
	NLR = 0.021 kgN/m^3/d	
Our study	HRT = 8-15 h	
	NLR = 0.22-0.374 kgN/m^3/d	
	SRT = 20 d	0.26
	SRT = 40 d	0.16
	(No sludge withdrawal)	

Source(s): Table by authors

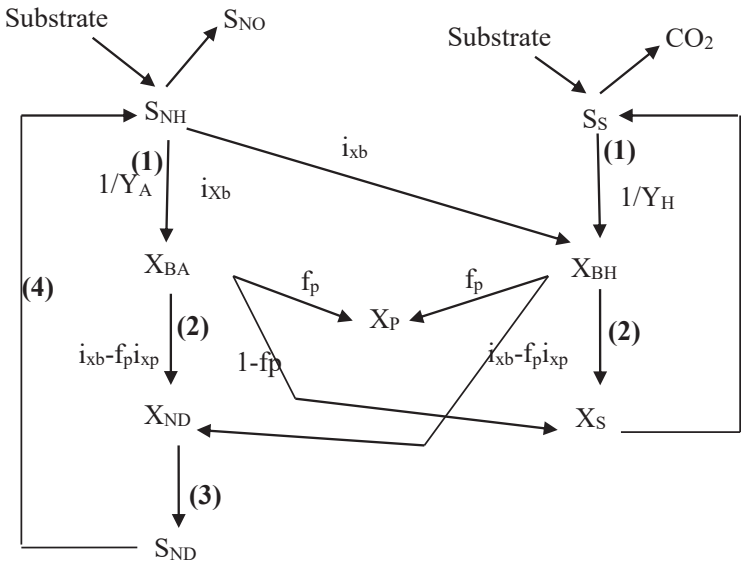
found that the bound of EPS per biomass unit increase as the SRT decreased. Nevertheless, some other researchers observed that at a long SRT, the SMP and EPS concentrations were higher (Faridizad *et al.*, 2022). Huang *et al.* (2011) found that in short SRT, the microorganisms metabolized more actively, however less SMP were produced, which restricted biofilm growth and membrane fouling.

3.3 Steady state equation developing

3.3.1 Activated sludge model 1 model (ASM1) description. The components of relevance in the ASM1 model are biomass, substrate and dissolved oxygen. These components are known as the state variables. Two fundamental processes occur which are biomass growth and decay. The oxygen utilization and substrate removal (organic and nitrogen substances) also occur, and they are coupled to biomass process through the system stoichiometry. According to ASM1 model soluble components are given the symbol S and the insoluble components X. Figure 4 shows the different interactions between the state variables and processes according to ASM1 and illustrates the transformation of soluble ammonia nitrogen (S_{NH}) and biodegradable soluble organic (Ss) substrates initially present in the feed flow.

The autotrophic bacteria (X_{BA}) allow the oxidation of S_{NH} to nitrate (S_{NO}) and, the heterotrophic population (X_{BH}) oxidize the organic substrate (Ss) from the cell lysis into carbon dioxide ($CO_{2\ (g)}$). As a result, the substrate S_{NH} undergoes oxidation of $1/Y_A$, and the consumption of i_{XB} fraction of nitrogen was needed for cell maintenance. Similarly, the oxidation of S_S promotes a $1/Y_H$ of cell synthesis and the consumption of i_{XB} fraction of nitrogen needed for cell maintenance.

When only the nitrogen substrate was fed to the reactor, X_{BH} died over time. This death causes the production of particular metabolites in the reactor that are differentiated by their (i)



(1)growth; (2) Decay; (3) Hydrolysis; (4) Ammonification

Source(s): Figure by authors

Figure 4.
Concept of death regeneration according to ASM1 model

biodegradable fraction (i.e. the organic particulate fraction (Xs) and the nitrogen particulate fraction (X_{ND}) and (ii) non-biodegradable particulate fraction (Xp). The particulate biodegradable fractions X_S and X_{ND} will undergo a hydrolysis process generating (Ss) and (S_{ND}), respectively.

S_S is then easily assimilated by the heterotrophic populations; and S_{ND} undergoes ammonification to reform the ammonia S_{NH}, which can be used as a substrate by nitrifying populations.

Thus, nitrogen compound follows these main transformations:

- An important part of nitrogen substrate is oxidized to nitrate by nitrification reaction. The oxidation reaction releases energy that supports the growth of autotrophic populations. The dynamic growth of these bacteria results in an actual growth rate $r_{X_{BA}}$ expressed through an homographic relation of Monod (Monod, 1949). The production rate of nitrates $r_{S_{NO}}$ is then assumed to be proportional to the autotrophic growth rate:

$$r_{S_{NO}} = (1/Y_A)\, r_{X_{BA}} \tag{4}$$

- The fraction of nitrogen S_{NH} instantly used to generate new cells is assumed to be proportional to the growth rate of the concerned population; for the autotrophic part alone, it is given by the product ($i_{XB}.r_{X_{BA}}$).
- The mortality of bacteria leads to the production of co-products: a fraction f_p of inert compounds (X_p) and ($i_{XB}-f_p i_{xp}$)fraction of (X_{ND}) rapidly hydrolyzed to organic nitrogen S_{ND}, which will be transformed to S_{NH} after ammonification.

The processes, kinetics and state variables involved in the nitrogen cycle were presented according to the matrix (Table 2) (Gujer and Henze, 1991). The rate equations of each process are recorded in the rightmost column. Four processes are listed in the leftmost column. The kinetic and stoichiometric parameters are given inside Table 4.

The matrix presentation of each component helps in the developement of mass balance equations.

3.3.2 Mass balance. The Relationships developed in this study correspond to the case of open perfectly stirred reactor operating under steady state conditions (Figure 5).

S_{NHe}, S_{NDe} and X_{NDe} represent the inlet nitrogen concentration, and S_{NH} and S_{NO} are the nitrogen concentration in outlet flow, respectively.

The basic equation of mass balance within any defined system boundary is:

$$\text{Input} - \text{Output} + \text{Reaction} = \text{Accumulation}.$$

Table 4. Different state variables for nitrogen in ASM1

State variables Processes	X_{BA}	X_p	S_{NO}	S_{NH}	S_{ND}	X_{ND}	S_o	Rate [$ML^{-3}T^{-1}$]
Aerobic growth of autotrophs	1		$1/Y_A$	$-(i_{XB} + 1/Y_A)$			$-(4.57 - Y_A)/Y_A$	$\mu Amax \frac{SNH}{SNH+KNH} XBA$
Decay of autotrophs	−1	fp				$(i_{XB} - \text{fp}.\, i_{XP})$		$b_A X_{BA}$
Ammonifi-cation of soluble organic nitrogen				1	−1			$kaS_{ND}X_{BH}$
Hydrolysis of organic nitrogen					1	−1		$k_h \frac{X_s/X_{BH}}{K_X+(X_s/X_{BH})} X_{BH} X_{ND}/X_{Xs}$

Source(s): Table courtesy of Gujer and Henze (1991)

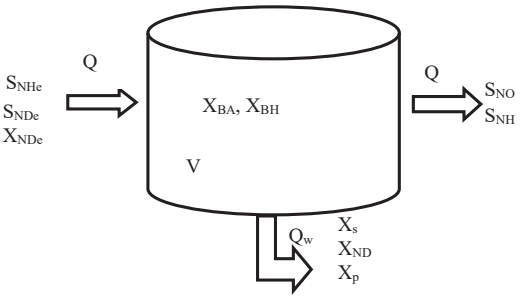


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Figure 5.
Open reactor and flow material associated

The system reaction term is obtained by summing the product of the stoichiometric coefficient and the process rate expression for the considered component.

a. Expression of X_{BA} and nitrogen compounds

In a perfectly stirred reactor operating in steady-state, the mass balance for the nitrogen substrate was written for autotrophic activity, according to Equation (5):

$$\frac{(S_{NHe} + S_{NDe} + X_{NDe})}{HRT} + (i_{XB} - f_P i_{XP}) b_A X_{BA} = \mu_{Amax}\left(i_{XB} + \frac{1}{Y_A}\right)\left(\frac{S_{NH}}{S_{NH}+K_{NH}}\right)X_{XB} \quad (5)$$

The first term (that is $(S_{NHe} + S_{NDe} + X_{NDe})/HRT$) is the nitrogen loading rate. The second term $(i_{XB}-f_p i_{xp}).b_A X_{BA}$ represents the flow of nitrogen provided by cell lysis. Finally, The term in the right side of Equation (4) reflects the loss of nitrogen through (i) the production of new cells autotrophic (i_{XB}. $r_{X_{BA}}$) and (ii) the oxidation of nitrogen into nitrate.

The growth rate of biomass is expressed as follows:

$$r_{X_{BA}} = \left(\mu_{Amax}\frac{S_{NH}}{K_{NH} + S_{NH}}\right)X_{BA} \quad (6)$$

Taking into account the death of biomass, the apparent rate of growth appears as follows:

$$r_{X_{BA}apparent} = \left(\mu_{Amax}\frac{S_{NH}}{K_{NH} + S_{NH}} - b_A\right)X_{BA} \quad (7)$$

The X_{BA} microorganisms concentration becomes constant in the bioreactor when the apparent flow product is equal to the flow withdrawn:

$$Vr_{X_{BAapparent}} = Q_w X_{BA} \quad (8)$$

Taking into account Equation (8), Equation (7) can be written as:

$$\mu_{Amax}\frac{S_{NH}}{K_{NH} + S_{NH}} = \frac{1}{SRT} + b_A \quad (9)$$

Combining Equations (5) and (9) gives the concentration of the active biomass concentration in a steady state condition in the bioreactor (Equation (10)):

$$X_{BA} = \frac{\frac{1}{HRT}(S_{NHe} + S_{NDe} + X_{NDe})}{\left(\left(\frac{1}{Y_A} + i_{XB}\right)\left(b_A + \frac{1}{SRT}\right) - (i_{XB} - f_P i_{XP}) b_A\right)} \quad (10)$$

When the values of b and Y_A are known, and i_{XB}, fp and i_{XP} referred to from default values in ASM1. The X_{BA} concentration can be easily calculated in a steady state condition through Equation (10) under imposed values of HRT and SRT. Also using Equation (10) could be helpful to calculate the concentration of the outlet water in steady state expressed by Equation(11):

$$S_{NO} = \frac{(1 + b_A SRT)}{Y_A SRT}.HRT.X_{BA} \tag{11}$$

The experimental measurement of nitrate concentration (S_{NO}) in the treated water (assuming no denitrification under the operating condition) is also a tool to determine the concentration of X_{BA} in steady state.

b. Equation of the required oxygen (oxygen uptake rate, OUR)

The required oxygen is related to the rate of oxygen consumption by the bacteria in endogenous condition. Thus, in the absence of an available exogenous substrate, the death-regeneration concept allows the maintenance of bacterial activity on the oxidation products of lysis. The required oxygen for the oxidation of the substrate from the bacterial lysis in endogenous condition represents the OUR_{endt}. Regarding Table 2 and Figure 4, the oxygen requirement for autotrophic species in the endogenous condition noted OUR_{endaut} is given by Equation (12):

$$OUR_{endaut} = (4,57 - Y_A)[(i_{XB} - f_P i_{XP})(b_A X_{BA} + b_H X_{BH}) - i_{XB}.\mu_{BHend}.X_{BH}] \tag{12}$$

The endogenous oxygen needs, corresponding to autotrophic and heterotrophic bacteria death that generates a fraction (i_{XB}-f_p.i_{XP}) of particulate organic nitrogen (XND), after hydrolysis and ammonification reveal a substrate SNH to be oxidized. In addition, cell lysis of both populations leads to producing a particular organic substrate (Xs), which will generate a soluble organic substrate Ss assimilated by heterotrophic cultures. Thus, even under endogenous condition, bacterial growth occurs depending on this substrate and will need to assimilate a portion of SNH from bacterial lysis. Equation (13) shows that the heterotrophic cell growth is a function of the *S*s released.

$$r_{X_{BH}} = \mu_{BHend} X_{BH} \tag{13}$$

where: μ_{BHend} is the heterotrophic growth rate in endogenous condition (d^{-1}).

The nitrogen needs for such cell growth is (i_{XB}. $r_{X_{BH}}$). Thus, the amount of nitrogen released by lysis and could be oxidized. This quantity must be reduced to estimate the oxygen requirements in endogenous conditions for autotrophic bacteria as given in Equation (11). Hence, the two bacterial populations (autotrophic and heterotrophic ones) could coexist in the bioreactor, even under COD/N ratio equal to 0. The equations (14) and (15) show the heterotrophic population's oxygen needs in endogenous condition and the X_{BH} equation, according to Héran, Wisniewski, Orantes, and Grasmick (2007).

$$X_{BH} = \frac{Y_H(1 - f_p) b_A X_{BA}}{\frac{1}{SRT} + b_H(1 - Y_H(1 - f_p))} \tag{14}$$

$$OUR_{endhet} = \begin{array}{l}(1 - f_p)(1 - Y_H) b_H X_{BH} + \\ (1 - fp)(1 - Y_H) b_A X_{BA}\end{array} \tag{15}$$

The total endogenous uptake rate OURendt was the sum of OURendaut and OURendhet:

$$OURendt = OUR_{endaut} + OUR_{endhet} \tag{16}$$

c. Production of biomass and co-products

(i) Concentration of Xp

The production rate of inert matter r_{Xp} resulted from bacterial lysis could be given by Equation (17):

$$r_{Xp} = f_p(b_A X_{BA} + b_H X_{BH}) \tag{17}$$

Including the fact that the system operates in a steady state condition, the flow of inert products must be compensated by the flow withdrawn (Q_w.Xp/V). The mass balance leads to the expression of the Xp concentration as follows:

$$X_P = f_P(b_A X_{BA} + b_H X_{BH})\,SRT \tag{18}$$

(ii) Concentration of particulate biodegradable nitrogen matter from bacterial lysis(X_{ND})

The production rate of X_{ND} ($r_{X_{ND}}$), after bacterial lysis was given by Equation (19):

$$r_{X_{ND}} = (i_{XB} - f_p i_{XP})(b_A X_{BA} + b_H X_{BH}) \tag{19}$$

The hydrolysis rate of X_{ND} ($r'_{X_{ND}}$) is supposed to be written in the following form:

$$r'_{X_{ND}} = k_h \frac{(X_{ND}/X_{BH})}{K_x + (X_S/X_{BH})} X_{BH} \tag{20}$$

The steady state is reached when the production flow X_{ND} is equal to the sum of hydrolysis and extraction flows:

$$r_{X_{ND}} V = r'_{X_{ND}} V + Q_W X_{ND} \tag{21}$$

Therefore, the X_{ND} expression was given by Equation (22):

$$X_{ND} = \frac{(i_{XB} - f_p i_{XP})(b_A X_{BA} + b_h X_{BH})}{\frac{1}{SRT} + \frac{k_h}{K_X + \frac{X_S}{X_{BH}}}} \tag{22}$$

The growth rate μ_{Amax} does not appear in the steady state equations. However, researchers (Choubert *et al.*, 2008) highlighted strong links between μ_{Amax} and b_A. Also, K_{NH} does not appear in the equations defined in steady-state conditions; however, its influence is still related to the concentration of S_{NH} in the bioreactor through the switching function S_{NH}/($K_{NH} + S_{NH}$).

3.3.3 Advantage of steady state equation: sensitivity analysis. 3.3.3.1 Sensitivity analysis method. In biological wastewater treatment, sensitivity analysis is essential to consider when assessing the influence of input parameters (e.g. kinetic, stoechiometric parameters, and operating conditions) on the output response, especially the state variables. One of the most straightforward ways to perform a sensitivity analysis is to vary each model input parameter one at a time (OAT) while other input parameters remain constant (Saltelli *et al.*, 2019; Upadhyaya, Singh, Chaurasia, Baghel, Kumar, & Dohare, 2018). However, this method generates a large number of simulations to perform with significant computing time for integrating transient responses. Hence, The developments of steady equations promote to identify the main parameters influencing the state variables, making sensitivity analysis easier to conduct. In this study, the local sensitivity analysis (LSA) method is used since the analytic expression of the output variable was known (Lin *et al.*, 2021). The LSA can be seen as a particular case of the OAT approach. Five state variables related to the nitrogen

transformation (XBA, SNO, OURendt, Xp and XND) were considered on the sensitivity analysis. The sensitivity of these five state variables has been studied through the influence of fourteen parameters, which are divided into four categories: operating parameters (HRT and SRT), kinetic parameters (bA,bH,kh and kx), stoichiometric parameters (YA,fp,ixb and ixp) and state variables (XBA, XBH, Xs and SNHe).

The sensitivity of a state variable F to a parameter θ can be expressed as Equation (23):

$$S_\theta = dF/d\theta \tag{23}$$

To compare the sensitivity of different parameters, the normalized sensitivity index (SI) is calculated using Equation (24):

$$SI = \frac{\theta}{F}\frac{dF}{d\theta} \tag{24}$$

The sensitivity index can be classified to five levels listed in Table 5 for evaluating relative sensitivity of the parameters (Castillo, Hadi, Conejo, & Canteli, 2004).

3.3.3.2 Sensitivity analysis results. The sensitivity analysis considers five model outputs: X_{BA}, X_p, S_{NO}, X_{ND} and OUR_{endt}. The SI evaluated for the five outputs are given in Table 6.

As shown in Table 6, the X_{BA} is very sensitive to the yield (Y_A) and ordinarily sensitive to the HRT and b_A. Lahdhiri *et al.* (2020) studied the sensitivity analysis of organic compounds to the operating condition. The results showed that X_{BH} was influenced by HRT and SRT especially for the value above 30 days. However, a slight influence of parameters (f_P, i_{XB}, i_{XP}) and type of substrate in the inlet of biological system S_{NHe} have been observed on X_{BA}. The lysis

Table 5. Sensitivity index levels

Level	Value	Sensitivity
I	[0.00,0.05)	Not sensitive
II (*)	[0.05,0.2)	Slight sensitive
III (**)	[0.2,1.00)	Normal sensitive
IV(***)	[1,00,∞)	Very sensitive

Source(s): Table by authors

Table 6. Sensitivity analysis results

Parameters		State variables X_{BA}	Xp	S_{NO}	X_{ND}	OUR_{endt}
Operating parameters	HRT	**		**		
	SRT	*	**	**	*	*
Stoichiometric	Y_A	***		**		
	f_p	*	*		*	*
	i_{XB}	*			***	***
	i_{XP}	*			*	*
Kinetic	b_A	**	**		***	***
	b_H		**		***	***
	k_h				***	***
	K_x				**	**
State variables	X_{BA}		***		***	***
	X_{BH}		***		***	***
	X_s				**	**
	S_{NHe}	*				

Source(s): Table by authors

products (X_p and X_{ND}) are strongly influenced by the biomass concentration in the reactor and death rates respectively (b_A and b_H) than other parameters. Indeed, the increase of death rate results in more X_P and X_{ND} production from biomass lysis. The autotrophic and heterotrophic coefficient decay and bacteria concentration in the MBR were the most sensitive parameters for the total oxygen demand in endogenous conditions (OUR_{endt}) confirming the interest of respirometric measurement for the estimation of active biomass, X_{BA} and X_{BH}. Therefore, the obtained results of sensitivity analysis suggesting the adjustment of parameter with high sensitivity influence (e.g. Y_A, b_A,. . .). However, For the parameters with low sensitivity, the typical default values of the ASM1 model can be used directly.

3.4 X_{BA} and X_{BH} evaluation by respirometric measurements

The respirometric measurements in endogenous condition was used to calculate the biomass concentration (X_{BH} and X_{BA}). The obtained values were compared with those obtained from the equations proposed. Moreover, the quantification was done by integrating the heterotrophic activity developed on biodegradable products resulting from the lysis of autotrophic bacteria.

The steady state was reached in runs I and II. The respirometric measurements are made in endogenous condition without inhibitor (OUR_{endt}) and after injection of two inhibitors (OUR_{endhet}).

The concentrations of autotrophic and heterotrophic biomasses can be calculated using the measured values of the OUR_{endt}, OUR_{endhet} and OUR_{endaut} given by Equations (12), (15) and (16).

An approximation of μ_{BHend} is made based on Equation (25). Finally, the value of soluble biodegradable substrate in the endogenous state is calculated according to Equation (26) (Héran, Wisniewski, Orantes, & Grasmick, 2007).

$$\mu_{BHend} = \mu_{Hmax}\frac{S_s}{K_s + S_s} \tag{25}$$

$$S_s = \frac{K_s(1 + SRTb_H)}{\mu_{Hmax}SRT - (1 + SRTb_H)} \tag{26}$$

Since the bioreactor was operated under two SRTs, Ss and μ_{BHend} have two different values. The obtained values were 0.66 and 0.64 d^{-1} for SRT equal to 20 and 40 days, respectively. An average of 0.65d^{-1} value was adopted for μ_{BHend}. The monitoring was done in the run I and II after reaching the steady state condition during days: 20, 40, 95, 115, and 120. The measurement's results were summarized in Table 7.

Table 8 gives the kinetic and stoichiometric parameters used to calculate X_{BA} and X_{BH} with a comparison to the default ASM1 values. One stoichimetric parameter (e.g Y_A) and three kinetic parameters (e.g. b_A, μ_{Amax}, K_{NH}) were adjusted from the labscale tests (Gasmi, Heran & Hannachi, (013), so that the predictions of the model accurately agreed with the actual performance of MBR. Once a steady state was reached, the maximum growth rate of

Time (d)	20	40	95	115	120
OUR_{endt} ($mgO_2/L/d$)	31.89	36.12	77.18	76.78	75.91
OUR_{endhet}($mgO_2/L/d$)	23.12	24.32	56.27	55.12	55.41
OUR_{endaut}($mgO_2/L/d$)	8.77	11.8	20.91	21.66	20.5

Source(s): Table by authors

Table 7. Respirometric test results

Parameter	Values	Typical values
Y_A (mgCOD.mgN^{-1})	0.25	0.24
Y_h (mgCOD.mgCOD^{-1})	0.67	0.67
b_A (d^{-1})	0.14	0.2
b_H (d^{-1})	0.46	0.62
μ_{Hmax} (d^{-1})	6	6
μ_{Amax} (d^{-1})	0.33	0.8
Ks (mgCOD.L^{-1})	17	20
K_{NH} (mgN.L^{-1})	1.6	1
K_h (d^{-1})	3	3
f_p	0.08	0.08
i_{xb} (gN.gCOD^{-1})	0.086	0.086
i_{xp} (gN.gCOD^{-1})	0.06	0.06

Source(s): Table by authors

Table 8. Kinetic and stoichiometric parameters obtained

nitrifiers obtained in this study is compared to literature value as obtained by Choubert, Racault, Grasmick, Beck, and Heduit (2005). These authors analyzed and simulated the performance of activated sludge bioreactor for the treatment of nitrogen pollution. Since, μ_{Am} and b_A are very correlated, their simultaneous identification need a stabilized active biomass concentration (i.e. steady state condition). Therefore, there is one unique couple (μ_{Am}, b_A) that can predict the nitrogen elimination performance in MBR, b_A was set at 0.14 d^{-1}. The values of the autotrophic yield (Y_A) are close to the default values. Regarding to the half-saturation coefficient for ammonia nitrogen (K_{NH}), the value obtained in this study (1.6 mgN.L−1), Leyva-Díaz, González, Muñío, and Poyatos (2015) have been obtained a close value in the treatment of nitrogen compounds by moving bed biofilm reactor-membrane bioreactor (MBBR-MBR). Moreover, Mannina *et al.* (2018), found that the factor mostly influencing the total nitrogen removal is the bacteria affinity factor for O_2, confirming the interest of respirometric measurement for biomass quantification.

Table 9 recapitulates the X_{BA} and X_{BH} values obtained after respirometric measurements and steady state equations and the deviation percentage.

day	Active biomass (mgCOD/L)	Using respirometric measurements	Using steady state equations	% of deviation
20	X_{BA}	167.25	216	22.5
(SRT = 20d, HRT = 0.625d and NLR = 0.22(kgN/m^3/d)	X_{BH}	74.32	79.02	6
40	X_{BA}	212.68	216	3.37
(SRT = 20 d,HRT = 0.625d and NLR = 0.22(kgN/m^3/d)	X_{BH}	67.52	79.02	15
95	X_{BA}	401	454	11.67
(SRT = 40 d, HRT = 0.334d and NLR = 0.374(kgN/m^3/d)	X_{BH}	165.48	185.74	10.9
115	X_{BA}	409.47	454	9.8
(SRT = 40 d, HRT = 0.334d and NLR = 0.374(kgN/m^3/d)	X_{BH}	176.77	185.74	5
120	X_{BA}	393.44	454	13.33
(SRT = 40 d, HRT = 0.334d and NLR = 0.374(kgN/m^3/d)	X_{BH}	178.26	185.74	4

Source(s): Table by authors

Table 9. Obtained X_{BH}, X_{BA} values and the percentage of deviation

Under steady condition, measuring OUR under endogeneous conditions allowed the evaluation of autotrophic and heterotrophic biomasses through Equations (11), (14) and (15). At day 40 (SRT = 20 d), the steady state was established and the percentage deviation of active biomass concentration between the equations developed in steady state and those obtained by respirometric analysis were 3.37% and 15 % for X_{BA} and X_{BH}, respectively. Whereas for SRT equal to 40 d, the deviation percentage was 13 and 4 % for X_{BA} and X_{BH}, respectively. The results demonstrated the effectiveness of respirometric measurements in access to the active biomass in the bioreactor.

Nevertheless, the highest percentage of deviation could be explained by the specific limitations of the respirometric method, which influenced the result's precision. The sensor's measurement accuracy and response time are severely constrained by the aging of the probe membrane, resulting in low accuracy and poor stability (Nei & Lillenberg, 2009). Bubbles on the sensor's surface can also generate a signal disturbance and cause imprecision in the concentration measurement.

Although inlet flow is devoid of organic substrate, the heterotrophic biomass exists in the bioreactor, confirming that the heterotrophic bacteria are developed on biodegradable products resulting from the lysis of bacterial autotrophs.

3.5 Simulation of active biomass evolution

To better understand the evolution of biomass concentration in the membrane bioreactor (MBR) over time, the bioreactor has been modeled using GPS-X software. Indeed, GPS-X is a dynamic wastewater treatment plant simulator, which allows the simulation of a variety of different biological wastewater treatment systems like activated sludge systems with reactors functioning under different situations (aerobic, anoxic, anaerobic), including sludge return and internal recirculation streams, batch reactors, and MBR.

Simulation results (Figure 6) show the evolution of the concentration of the different bacterial species over time for two SRT values (20 and 40 days). The simulation was conducted using kinetic and stoichiometric parameters mentioned in Table 7.

Figure 6 shows that the reactor's autotrophic (X_{BA}) and heterotrophic bacteria (X_{BH}) concentrations increase over time until reaching steady-state conditions. The concentration X_{BA} was much higher than the X_{BH} due to the imposed operating condition COD/N equal to 0 and it confirmed the idea that the heterotrophic biomass growth deponds on the substrate from the autotrophic biomass. Same pattern of biomass evolution as the TSS concentration.

A good agreement between experimental and simulated results was observed. For an SRT equal to 20 days, the average concentrations of X_{BA} and X_{BH} obtained from the respirometric measurements were about 212 (considering the value obtained on day 40, in which the steady-state condition was more established) and 65 mgCOD/L, respectively. These concentrations are close to those obtained by simulation (220 and 73 mgCOD/L for X_{BA} and X_{BH}, respectively). The same trend was noticed between experimental and simulation on steady-state bacteria concentrations at SRT 40 (e.g. for autotrophic bacteria, the obtained values were 393 (day 120) and 390 mgCOD/L for experimental measurements and simulation, respectively). The increase of SRT from 20 to 40 days, leads to increase the TSS, VSS concentrations as it mentioned in Table 2, as a result increasing of bacterial concentrations. These results revealed that the ASM1 model had been successfully established to simulate the biological process of the membrane bioreactor. Table 10 gives some results of MBR modeling by other researchers. Baek *et al.* (2009) reported in their research that the simulated results of X_{BH} evolution in MBR treating dilute municipal wastewater increase by SRT increasing and has the same pattern as the TSS evolution.

The simulation's results confirmed the importance of respirometric tools for biomass quantification. In addition, the autotrophic bacteria quantity represent approximately

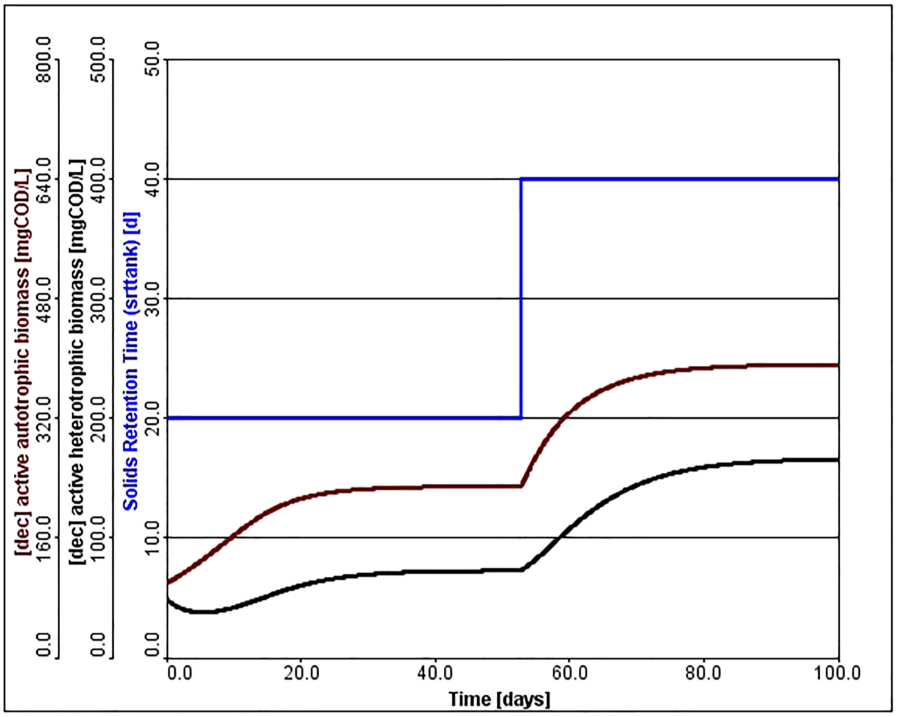

Figure 6.
Evolution of X_{BA} and X_{BH} concentrations over time under SRT 20 and 40 days

Source(s): Figure by authors

$60 \pm 6\%$ and $52 \pm 3\%$ of the total volatile solids for run I and II, respectively. In fact, this result highlights the importance of autotrophic biomass quantification, as the measurements of apparent removal rates of ammonium (i.e. expressed through the VSS concentration(kgN/kgVSS/h)) seem irrelevant to characterize their specific activity.

4. Conclusion

This work aimed at developing of a new tool to quantify the viable biomass in the bioreactor operating under steady state conditions. This technique was based on respirometric measurements by monitoring the oxygen uptake rate under endogenous (OURend) conditions coupled with the development of steady state equations based on material balances at the bioreactor integrating the rate described by activated sludge model 1 (ASM1). These equations describing the performance of the bioreactor and highlight the parameters that significantly affect the state variable. Thus, they explain any sudden change in the evolution of this variable under actual operating conditions. The respirometric measurements, specifically in the endogenous phase lead to differentiate autotrophic (X_{BA}) and heterotrophic (X_{BH}) biomass and quantify their concentration. importance of respirometric tools as a simple and available technique for biomass quantification. Then, the results were compared to those calculated with a steady state equation. The discrepancy varies from 4 to 22%. Finally, the membrane bioreactor (MBR) was simulated using GPS-X. The findings showed a very good agreement between simulation and experimental measurement, confirming the importance of respirometric tools as a simple and available technique for biomass quantification.

References	Operating conditions details	Observations
Baek *et al.* (2009)	MBR, dilute municipal wastewater, COD/N = 2.4 9 runs, HRT = 0.5 and 1 day SRT = 165,197,107,74,52,29,85,103,108 ASM1 modeling, AQUASIM 2.0	- The most sensitive parameters were b_H, Y_H to TSS evolution - The model predicted well the performance of MBR - X_{BH} varied by the changes in the operational conditions - X_{BA} was relatively stable regardless of HRT
Mannina, Cosenza, Viviani and Ekama (2018)	MBR, COD/N = 10 ASM2d modeling, two nitrification step	- For the TSS, the most important model factors are (Y_H) and the decay rate (b_H) - Ammonia oxidizing bacteria mostly influenced by half saturation coefficients related to O_2
Spérandio and Espinosa (2008)	MBR, COD/N = 6.8 ± 0.1 SRT = 10, 37, 53, 110 ASM1 and ASM3 modeling, GPS-X	- ASM1 provided good prediction when the SRT 10, 37 and 50 days but values were overestimated at high SRT equal to 110 days - Increase of active autotrophic biomass concentration (*XBA*) with SRT increasing - A larger quantity of active autotrophic biomass (*XBA*) is predicted with ASM1 compared to ASM3
Kapumbe, Min, Zhang, Kisoholo, and Yongfenf (2019)	MBR, COD/N = 25 and 33 HRT = 5h, no sludge was discharged ASM3	- The effluent nitrogen was sensitive to yield coefficient - NH_3-N effluent simulation was sensitive to maximum growth rate, K_{NH}, Y_h, the maximum specific growth rate - ASM3 simulation values and the measured values were in good agreement for TN effluent - ASM3 simulation of NH3-N effluent and N-NH3 measured value has an average relative error 24.44%

Source(s): Table by authors

Table 10. Modeling results from previous literature studies in comparison with the present study

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