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Special Edition

Kinetic of nitrogen consumption by Anammox process in membrane biofilm reactors operated in sequential batch

Cinética do consumo de nitrogênio por processo Anammox em reatores de biofilme aerado em membrana operados em batelada sequencial

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ABSTRACT

Biological nitrogen removal via Anammox is an advantageous technology in the nitrogen treatment effluents with a low Carbon/Nitrogen ratio, a process that makes this route interesting for the most different types of industries, agribusinesses, and urban effluent treatment plants. Achieving robust Anammox biomass for use in full-scale plants is still a challenge that motivates studies of biomass enrichment and the search for kinetic parameters of substrate consumption rate that help optimize the conduction of reactors. According to the previously mentioned, this work aimed to carry out the kinetic study of nitrogen consumption by the Anammox process in a membrane aerated biofilm reactors operated in sequential batches (MABR-BS). 6 MABR-BS reactors were used, each one of them inoculated with a specific Anammox sludge, obtained from the enrichment of anaerobic and aerobic sludges coming from 3 different sludge sources, namely, a municipal wastewater treatment plant, a landfill leachate treatment plant, and a swine slaughterhouse effluent treatment plant. For the kinetic study, 6 reactors were used, made in glass flasks with a total volume of 1L, with a useful volume of 500 mL, with the 300:200mL ratio between synthetic effluent (with 100mgN-NH_4^+.L^{-1}) and sludge from the sources: R1 - anaerobic sludge from a UASB reactor for urban sewage treatment; R2 - mixed sludge from a UASB reactor, consisting of waste sludge and supernatant scum; R3 - anaerobic sludge from landfill leachate treatment; R4 - mixed sludge consisting of aerobic and anaerobic sludge from landfill leachate treatment plant; R5 - anaerobic sludge from the swine slaughter effluent treatment plant and R6 - aerobic and anaerobic sludge from the swine slaughter effluent treatment plant. The experimental apparatus had 3 aerators coupled to 3 flowmeters with an air flow regulated at 1.0 L.min^{-1}; 30 cm of silicone membrane in a curved shape with one of the inlets connected to the aerator and flowmeter, the other outlet was immersed in a 75 cm water column, exerting negative pressure on the air inside the tubular silicone membrane, forcing the air to exit through the microporosity of the membrane. Aeration was intermittent, with an interval of 0.16 h between each minute of aeration, the reactors were shaken in a water bath at 30 rpm and temperature of 32°C. The kinetic test had a duration of 24 hours with sampling every 2.5 hours. The nitrogen removal efficiencies (%) determined in the kinetic test were 61.36 (R1); 61.01(R2); 59.03 (R3); 56.70 (R4); 62.77 (R5) and 64.40 (R6). Regarding pH, all reactors had an initial pH above 8.0 and a final pH close to neutral. The specific nitrogen removal rates (in mgN.gVSS^{-1}h^{-1}), were on average 29.43 (R1); 33.50 (R2); 33.62 (R3); 33.42 (R4); 28.90 (R5) and 30.34 (R6). The best performance in the kinetic assay was obtained in the R1 reactor, obtaining a specific activity of maximum nitrogen removal of 57.61 mgN.gVSS^{-1}h^{-1} and molar generation of residual nitrate with a stoichiometric coefficient of 0.018 mol.

Keywords: Nitrogen removal; Demonification kinetics; MABR-BS reactor

RESUMO

A remoção biológica de nitrogênio via Anammox se trata de uma tecnologia vantajosa no tratamento de efluentes nitrogenados com baixa relação Carbono/Nitrogênio, processo que torna essa via interessante para os mais diferentes tipos de indústrias, agroindústrias e estações de tratamentos de efluentes urbanos. Conseguir biomassa Anammox robusta para utilizar em plantas de escala real ainda é um desafio que motiva estudos de enriquecimento de biomassa e a busca por parâmetros cinéticos de velocidade de consumo de substrato que auxiliem na otimização da condução dos reatores. Diante do apresentado esse trabalho teve por objetivo realizar o estudo cinético do consumo de nitrogênio por processo Anammox em reatores de biofilme aerado em membrana operados em bateladas sequenciais (MABR-BS). 6 reatores MABR-BS foram utilizados, sendo cada um deles inoculado com um tipo de lodo Anammox, obtido do enriquecimento de lodos anaeróbios e aeróbios advindos de 3 diferentes fontes de lodo sendo elas, uma estação de tratamento de esgoto sanitário, uma estação de tratamento de lixiviado de aterro sanitário e uma estação de tratamento de efluente de abate de suínos. Para o estudo cinético foram utilizado 6 reatores confeccionados em frascos de vidro de volume total de 1L, com volume útil de 500 mL, sendo a relação 3:2 (v:v) entre efluente sintético (com 100mg$_{N\text{-}NH4+}$.L^{-1}) e lodo das fontes: R1 - lodo anaeróbio de reator UASB de tratamento de esgoto urbano; R2 - lodo misto de reator UASB, constituído por lodo de descarte e escuma sobrenadante; R3- lodo anaeróbio de tratamento de lixiviado de aterro sanitário; R4 - lodo misto constituído por lodo de lagoa aeróbia e anaeróbia da estação de tratamento de lixiviado de aterro sanitário; R5 - lodo anaeróbio da estação de tratamento de efluente de abate suíno e R6 - lodo aeróbio e anaeróbio da estação de tratamento de efluente de abate suíno. O aparato experimental contou com 3 aeradores acoplados a 3 fluxômetros com vazão de ar regulada em 1,0 L.min^{-1}; 30 cm de membrana de silicone em formato curvilíneo com uma das entradas conectadas ao aerador e fluxômetro, a outra saída foi imersa em coluna de água de 75 cm, exercendo pressão negativa sobre o ar no interior da membrana tubular de silicone, obrigando o ar a sair pela microporosidade da membrana. A aeração foi intermitente, com intervalo de 9,6 minutos entre cada minuto de aeração, os reatores foram agitados em equipamento banho maria ajustado em 30 rpm e temperatura de 32°C. O ensaio cinético durou 24h e contou com uma amostragem a cada 2,5h. As eficiências de remoção de nitrogênio (em %) determinada no ensaio cinético foram de 61,36 (R1); 61,01(R2); 59,03 (R3); 56,70 (R4); 62,77 (R5) e 64,40 (R6). Com relação ao pH todos os reatores apresentaram pH inicial acima de 8,0 e pH final próximo à neutralidade. As velocidades específicas de remoção de nitrogênio (em mgN.gSSV^{-1}h^{-1}), foram em média de 29,43 (R1); 33,50 (R2); 33,62 (R3); 33,42 (R4); 28,90 (R5) e 30,34 (R6). O melhor desempenho no ensaio cinético foi obtido no reator R1, obtendo atividade específica de remoção de nitrogênio máxima de 57,61 mgN.gSSV^{-1}h^{-1} e geração molar de nitrato residual com coeficiente estequiométrico de 0,018 mol.

Palavras-chave: Remoção de nitrogênio; Cinética da desamonificação; Reator MABR-BS

1 INTRODUCTION

The nitrogen removal biological process through bacteria of the planctomycete phylum, known by the name Anammox (Anaerobic ammonium oxidation) has been attracting the attention of the scientific community for using a simplified way of nitrogen removal, in which ammoniacal nitrogen is converted to nitrogen gas using nitrite as an electron acceptor, generating a small amount of residual nitrate (MULDER, *et al.*, 1995; STROUS *et al.*, 1998). The stoichiometry of the general Anammox reaction is presented in equation 1.

$$NH_4{}^+ + 1{,}32\ NO_2{}^- + 0{,}066HCO_3{}^- + 0{,}13H^+ \rightarrow 1{,}02N_2 + 0{,}26\ NO_3{}^- + 0{,}066CH_2O_{0,5}N_{0,15} + 2{,}03H_2O \qquad (1)$$

Nitrogen removal through the Anammox process can be carried out in two ways: i) in a single-phase reactor where partial nitritation and the Anammox process (NP/A) occur in the same reactor and ii) in separate reactors, where partial nitritation takes place in an aerobic reactor and the Anammox process in another anaerobic reactor. Equation 2 represents the NP/A process in a single-phase reactor (SLIEKERS *et al.*, 2002).

$$NH_4{}^+ + 0{,}85O_2 \rightarrow 0{,}44N_2 + 0{,}11\ NO_3{}^- + 1{,}43\ H_2O + 1{,}14H^+ \qquad (2)$$

The Anammox process has a higher nitrogen removal rate than the conventional processes (nitrification + denitrification), especially in reactors operated with high Nitrogen loading rate (YAO, *et al.*, 2015). Chini *et al*. (2016) reported a specific rate of ammonium and nitrite consumption in an Anammox reactor of 50.08 and 58.18 mgN.mgSSV^{-1}.h^{-1}, respectively, whereas Cao (2018) obtained a specific nitrification rate of 14.90 mgN -NH$_4^+$.mgSAV^{-1}.h-1 and denitrification of 23.6 mgN-NO3-.mgSAV^{-1}.h^{-1}. In his study, Cao (2018) used biofilm reactors, which is why he expressed the content of solids in volatile adherents.

Other benefits are attributed to the use of the Anammox metabolic route, including Morales *et al*. (2015) highlight the low energy consumption required by aerators, a reduction of approximately 50%, the low sludge generation due to the cell replication slow rate, and the important reduction in the emission of nitrous oxide greenhouse gas, of the order of 76 %.

However, in the conduction of Anammox reactors, difficulty in obtaining a robust Anammox sludge for the start-up of treatment plants is reported, due to the slow replication of this group of bacteria (2.6 to 11 days) (ZHANG *et al*., 2017; ZHU *et al*., 2008). In the literature, this delay to confirm the onset of Anammox activity has been reported: Tang *et al*. (2011) 214 days, Gupta, *et al*. (2019) 119 days, Casagrande *et al*. (2011) 110 and 170 days for the two study reactors.

Membrane aerated biofilm reactors (MABR) have shown promise for the rapid start-up of the Anammox process. Augusto *et al*. (2018) presented start-up data in 21 days when operating an MABR reactor fed with synthetic effluent and continuous feed, keeping the process stable for 150 days. The membrane aerated biofilm reactor consists of one-phase reactors that promote partial nitritation and Anammox activity in the same reactor.

MABR systems use microporous membrane technology as a way of transport and dispersion of the DO, that is, they allow only enough DO to be dispersed in the reactors for partial nitritation, due to membrane (in silicone material or microporous carbon) due to its porosity, allows a low concentration of DO (~0.2 mg.L^{-1}) to disperse in the reactors, allowing the bacteria present in the sludge to form a biofilm adhered to the membrane, where partial nitritation occurs (AUGUSTO *et al*., 2018, LI *et al*., 2016; GONG *et al*., 2008). It is noteworthy that the literature data report the use of this reactor model for the Anammox process only with continuous feed, which is unexplored with sequential batch feed.

Kinetic studies of substrate consumption rate are important because they aim to demonstrate whether the nitrogen consumption temporal behavior can be optimized, or even attest to whether the time used for substrate degradation in reactors is being insufficient for microorganisms, also helping to generate mathematical models that collaborate in the bacteria behavior prediction (WANG, *et al*., 2021; MARTINS *et al*., 2018).

In this sense, this work aimed to carry out the nitrogen consumption kinetic study by the Anammox process in a membrane aerated biofilm reactors operated in sequential batches (MABR-BS). 6 MABR-BS reactors were used, each one of them inoculated with a specific sludge Anammox, obtained from the enrichment of anaerobic sludges coming from 3 different sludge sources, namely, urban sewage treatment plant, landfill leachate treatment plant sanitary, and swine slaughter effluent treatment plant.

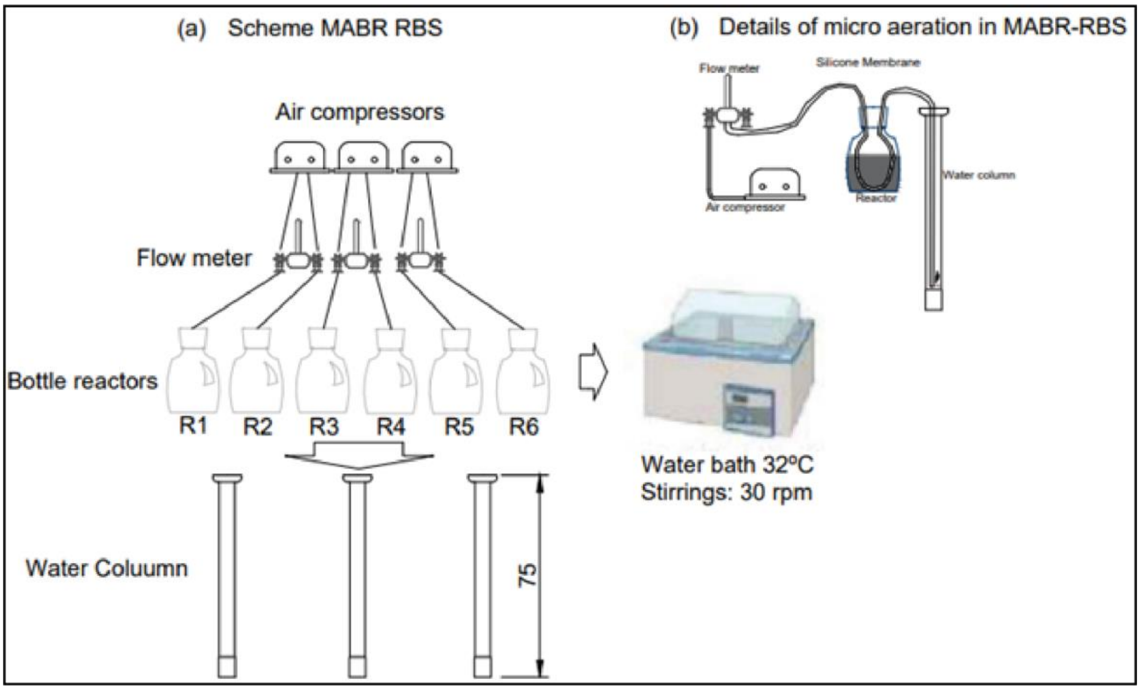
2 MATERIALS AND METHODS

To carry out the kinetic study of nitrogen consumption by the Anammox process, MABR reactors operated in sequential batches were used.

2.1 Experimental apparatus

Six glass vials with a volume of 1L, with 500 mL of useful volume and 500 mL of volume equivalent to the head-space were used as reaction environment; 3 aerators coupled to 3 flowmeters to control the air flow; 30 cm of silicone membrane in a curved shape with one of the inlets connected to the aerator and flowmeter, with an air flow regulated at 1.0 L.min^{-1}, the other outlet was immersed in a 75 cm water column, with the function to exert negative pressure on the air insufflated inside the tubular silicone membrane, forcing the air to exit through the porosity of the membrane. Aeration was intermittent, with an interval of 9.6 minutes between each minute of aeration, the reactors were kept in a water bath equipment with agitation at 30 rpm and temperature at 32°C (Figure 1).

Figure 1 – Details of the MABR experimental apparatus in Sequential Batch



Source: Authors, 2021

2.2 Inoculum

The inoculum used in the kinetic test demonstrated the Anammox activity from 20 days of operation of the MABR-BS reactors, but prior to the test, the reactors went through 261 days with synthetic feed to promote the enrichment of the Anammox biomass, with the objective of forming robust biomass. The raw sludge was collected in 3 effluent treatment stations and received the following nomenclature according to the place of sludge collection used in the inoculation: R1 - anaerobic sludge from a UASB domestic sewage treatment reactor; R2 - mixed sludge from a UASB reactor, consisting of waste sludge and supernatant foam; R3- anaerobic sludge from landfill leachate treatment; R4 - mixed sludge consisting of aerobic and anaerobic pond sludge from the landfill leachate treatment plant; R5 - anaerobic sludge from the swine slaughter effluent treatment plant and R6 - aerobic and anaerobic sludge from the swine slaughter effluent treatment plant.

The 6 reaction units were conducted with a 2:3 (v:v) ratio between anaerobic inoculum and synthetic effluent, which correspond to the R1, R3, and R5 reactors and in a 1:1:3 (v:v) ratio between the aerobic inoculum, anaerobic inoculum, and synthetic effluent, corresponding to reactors R2, R4, and R6.

2.3 Synthetic substrate

The synthetic substrate was adapted from Van de Graaf *et al*. (1996) following the formulation: NH_4Cl (100mg.L^{-1}); $NaHCO_3$ (1.5g.L^{-1}); KH_2PO_4 (0.0272g.L^{-1}); $MgSO_4 \cdot 7H_2O$ (0.3g.L^{-1}); $CaCl_2 \cdot 2H_2O$ (0.18g.L^{-1}); trace element solution I (for 1L of ultrapure water: EDTA, 5g; $FeSO_4 \cdot 7H_2O$, 9.15g) 1 mL of the solution per liter of synthetic effluent; Trace element solution II (for 1L of ultrapure water: EDTA, 15g; $ZnSO_4 \cdot 7H_2O$, 0.43g; $CoCl_2 \cdot 6H_2O$, 0.24 g; $MnCl_2 \cdot 4H_2O$, 0.99g; $CuSO_4 \cdot 5H_2O$, 0.25g ; $NaMoO_4 \cdot 2H_2O$, 0.22g; $NiCl_2 \cdot 6H_2O$, 0.19g; Na_2SeO_3 (anhydrous), 0.10g; H_3BO_4, 0.014g) 1mL of the solution per liter of synthetic effluent.

2.4 Kinetic Assay

The kinetic assay was carried out with a cycle time (TC) of 24h, corresponding to a load of 0.025 KgN.m^{-3}.d^{-1}. The parameters oxygen, temperature, and biomass agitation were controlled at ~0.5mg.O_2.L^{-1}; 32°C and ~30rpm, respectively. Samples were collected every 2.5h. Table 1 shows the initial conditions of the kinetic test for reactors R1 to R6.

It is noteworthy that all reactors received, in the enrichment step (261 days) prior to the kinetic assay, the same amount of sludge at the start (4.0 gL^{-1}) and fed with the same synthetic substrate, but the enrichment process from the Anammox biomass, promoted a natural selection of microorganisms causing at the time of the test different volatile suspended solids concentration between them. Although the test feed is synthetic, the initial assay data suffered interference from the previous batch.

Table 1 – Initial conditions of the kinetic assay performed in Anammox MABR-BS reactors

Parameters	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**
N-NH_4^+ (mg.L^{-1})	122.33	157.53	120.71	101.50	100.00	100.00
N-NO_2^- (mg.L^{-1})	0.56	12.07	0.82	0.51	0.21	0.21
N-NO_3^- (mg.L^{-1})	15.60	0.29	11.83	9.46	10.78	11.53
SSV (g.L^{-1})	2.64	5.93	0.41	2.55	3.82	1.71
pH	8.33	8.70	8.6	8.55	8.73	8.77

Source: Authors, 2021

2.5 Kinetic parameters

The parameters substrate consumption rate (r_S) and the specific substrate consumption rate (μ_S) were evaluated, which were determined from the exponential regression of substrate concentrations as a function of time, resulting from experimental kinetic assays, according to equations 3 and 4, respectively.

$$r_s = -\frac{dS}{dT} r_s = -\frac{dS}{dT} \tag{3}$$

r_S = substrate consumption rate (mgN. L^{-1}.h^{-1})
S-= substrate concentration (mgN.L^{-1})
T = time (h)

$$\mu_s = \frac{1}{X}(-\frac{dS}{dT}) \tag{4}$$

Where:
μs = specific substrate consumption rate (mgN.$gVSS^{-1}h^{-1}$)
X = biomass concentration express in Volatile Suspended Solid ($mgVSS^{-1}$.L^{-1})

2.6 Physicochemical analyses

The kinetic behavior of the reactors was followed in relation to the analyses: Ammoniacal Nitrogen (N-NH_4^+), Nitrate (N-NO_3^-), Nitrite (N-NO_2^-), pH and Inorganic Carbon. The analysis of Volatile Suspended Solids (SSV) was performed before the start of the kinetic test as an indirect measure of the biomass content. All analyzes were performed according to the APHA (2005), except for the Inorganic Carbon analysis which followed the IC method, using a TOC/TN/IC analyzer (TOC-LCPH/CPN, Shimadzu, Kyoto, Japan) following the manufacturer's recommendations.

2.7 Molecular Biology Analysis

After 261 days of enrichment of Anammox biomass, reactors with mixed biomass from the 3 initial biomass collection sites underwent PCR (Polymerase Chain Reaction) analysis in comparison with universal primer and showed the presence of Anammox bacteria in all studied sludges *Candidatus Anammoxoglobus Propionicus*.

2.8 Stoichiometric coefficients

In this study, the stoichiometric coefficients of partial nitritation and Anammox activity (NP/A) in a single-phase reactor were calculated according to equations 5 to 10, according to Third *et al*. (2001).

$$\mathrm{NO_2^-}\,\mathrm{Coefficient} = \frac{NO_2^- output - NO_2^- input}{NH_{3\,input} - NH_{3\,output}} \qquad (5)$$

$$NO_3^-\,\mathrm{Coefficient} = \frac{NO_3^- output - NO_3^- input}{NH_{3\,input} - NH_{3\,output}} \qquad (6)$$

$$H^+\mathrm{Coefficient} = \left(\frac{Alkalinity}{NH_{3\,input}}\right) \text{x} \left(\frac{14}{50}\right) \qquad (7)$$

$$N_2\ \mathrm{Coefficient} = \frac{1 - (NO_2^-\ coefficient + NO_3^-\ coefficient)}{2} \qquad (8)$$

$$H_2\text{O Coefficient} = \frac{(4 - Coeffcient\ H^+)}{2} \tag{9}$$

$$O_2\text{Coefficient} = \frac{(Coefficient\ NO_3^-\ x\ 3) + (Coefficient\ NO_2^-\ x\ 2) + (Coefficient\ H_2O)}{2} \tag{10}$$

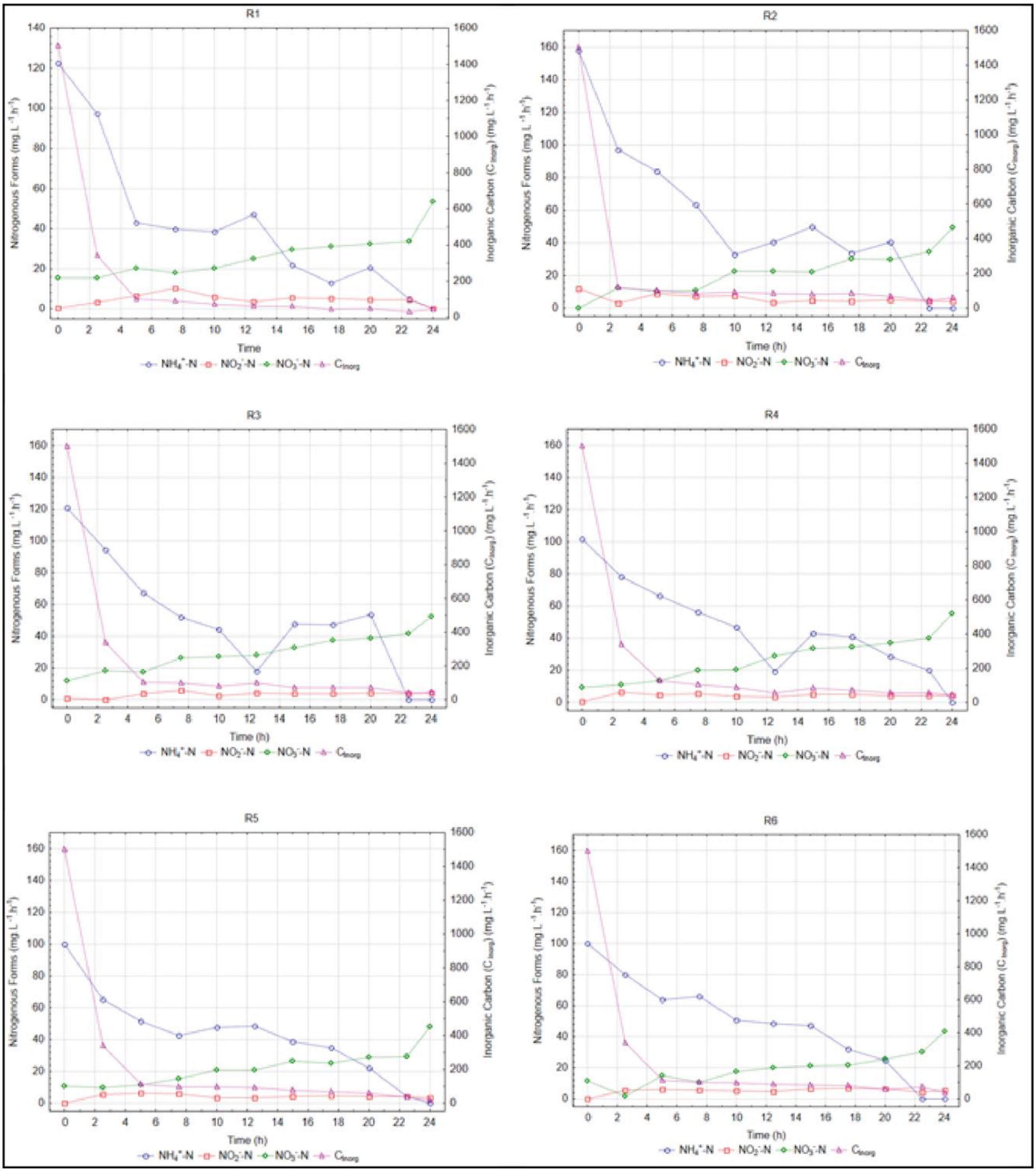
3 RESULTS AND DISCUSSION

Figure 2 shows the 6 reactors' temporal behavior in relation to the consumption of ammoniacal nitrogen, nitrite, nitrate, and inorganic carbon. Highlighting the positive result that in all reactors in the study, all the ammoniacal nitrogen present at the beginning of the kinetic assay was consumed.

In the 6 reactors, nitrite values were low, below 2.0 mg.L^{-1}, which is characteristic of reactors in which the Anammox process and Partial Nitritation occur simultaneously. In these reactors, as nitrite is formed, it is already consumed along with ammoniacal nitrogen in the Anammox process, in the ratio 1:1.32 (NH_4^+:NO_2^-) (equation 1). As the consumption of nitrite is higher, there is no noticeable reduction of the two compounds in the same proportion (STROUS, *et al.*, 1999).

About the nitrate behavior, all reactors showed similar behavior, with nitrate growth over time, this behavior is characteristic of active Anammox reactors that, according to equations 1 and 2, the process releases residual nitrate. Table 2 shows the residual nitrate and equivalent stoichiometric coefficient values for each reactor, as well as the removed ammoniacal nitrogen +nitrite concentration and the stoichiometric coefficients generated with the average data obtained in the kinetic test.

Figure 2 – Temporal behavior of nitrogen and inorganic carbon forms in MABR-BS R1 to R6 reactors



Source: Authors, 2021

About the nitrate generation, all reactors showed similar behavior, with nitrate growth over time, this behavior is characteristic of active Anammox reactors that, according to equations 1 and 2, the process releases residual nitrate. Table 2 shows the residual nitrate and equivalent stoichiometric coefficient values for each reactor, as well as the removed ammoniacal nitrogen + nitrite concentration and the stoichiometric coefficients generated with the average data obtained in the kinetic assay.

Table 2 – Reactors Behavior in the kinetic assay in relation to input and output parameters of greater importance for the Anammox process, as well as in relation to stoichiometric coefficients

Parameters	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**
Residual Nitrate ($mg.L^{-1}$)	37.84	49.22	40.69	46.04	37.31	32.01
$N\text{-}NH_4^+$ + $N\text{-}NO_2^-$ removal ($mg.L^{-1}$)	84.99	84.61	81.75	78.52	86.93	89.20
N removal efficiency (%)	61.36	61.01	59.03	56.70	62.77	64.40
O_2 Stoichiometric coefficient	1.00	1.40	1.50	1.69	1.58	1.51
NO_3^- Stoichiometric coefficient	0.018	0.31	0.33	0.45	0.37	0.32
N_2 Stoichiometric coefficient	0.44	0.48	0.43	0.42	0.46	0.43
H_2O Stoichiometric coefficient	1.94	1.96	1.95	1.94	1.98	1.96
H^+ Stoichiometric coefficient	0.10	0.07	0.09	0.11	0.03	0.07

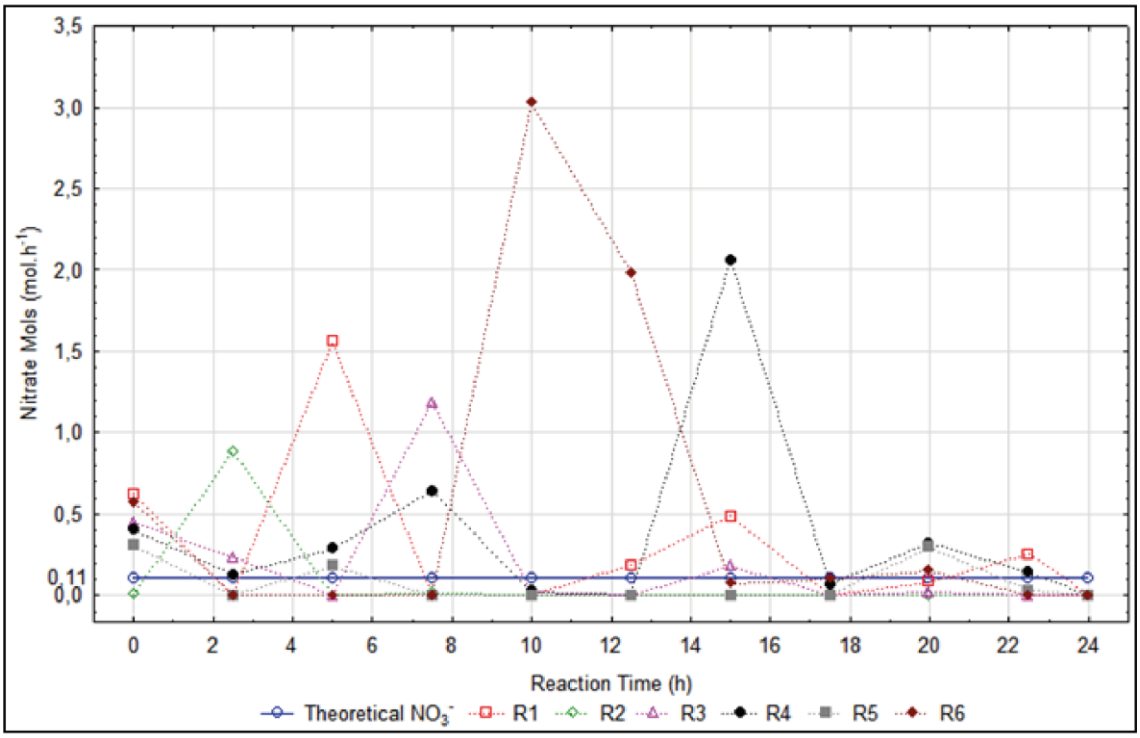
Source: Authors, 2021

According to Table 2, the reactor that showed most approximated the stoichiometric coefficient to the theoretical value for nitrate formation in equation 2 (0.11 mol) was the R1 reactor, with 0.018 mol of nitrate generated for each 1 and 1.32 mol of NH_4^+ and NO_2^- removed to gaseous form N_2. From an environmental point of view, the best result is related to the R1 reactor, as it has the lowest stoichiometric coefficient in nitrate production, thus, this reactor had the highest removal of ammoniacal nitrogen and nitrite with the lowest generation of nitrate, which in the environment becomes a toxic agent (CAO, 2018).

Martins *et al*. (2018) when performing a similar assay, reached a molar ratio between ammonium, nitrite, and nitrate of 1:1.24:0.34 for synthetic effluent with an input of 73/67 between $N\text{-}NH_4^+$ and $N\text{-}NO_2^-$, being the amount of nitrate found in that study like reactors R2, R3, R5, and R6. Yao *et al*. (2015) fed an Anammox reactor with synthetic effluent with 1:1.32 between N-NH_4^+ and N-NO_2^- and found 0.11 mol as a stoichiometric coefficient for nitrate, while the theoretical expected stoichiometry was 0.26, corroborating with a practical stoichiometric value of nitrate lower than the theoretical value, as found in reactor R1 in this research.

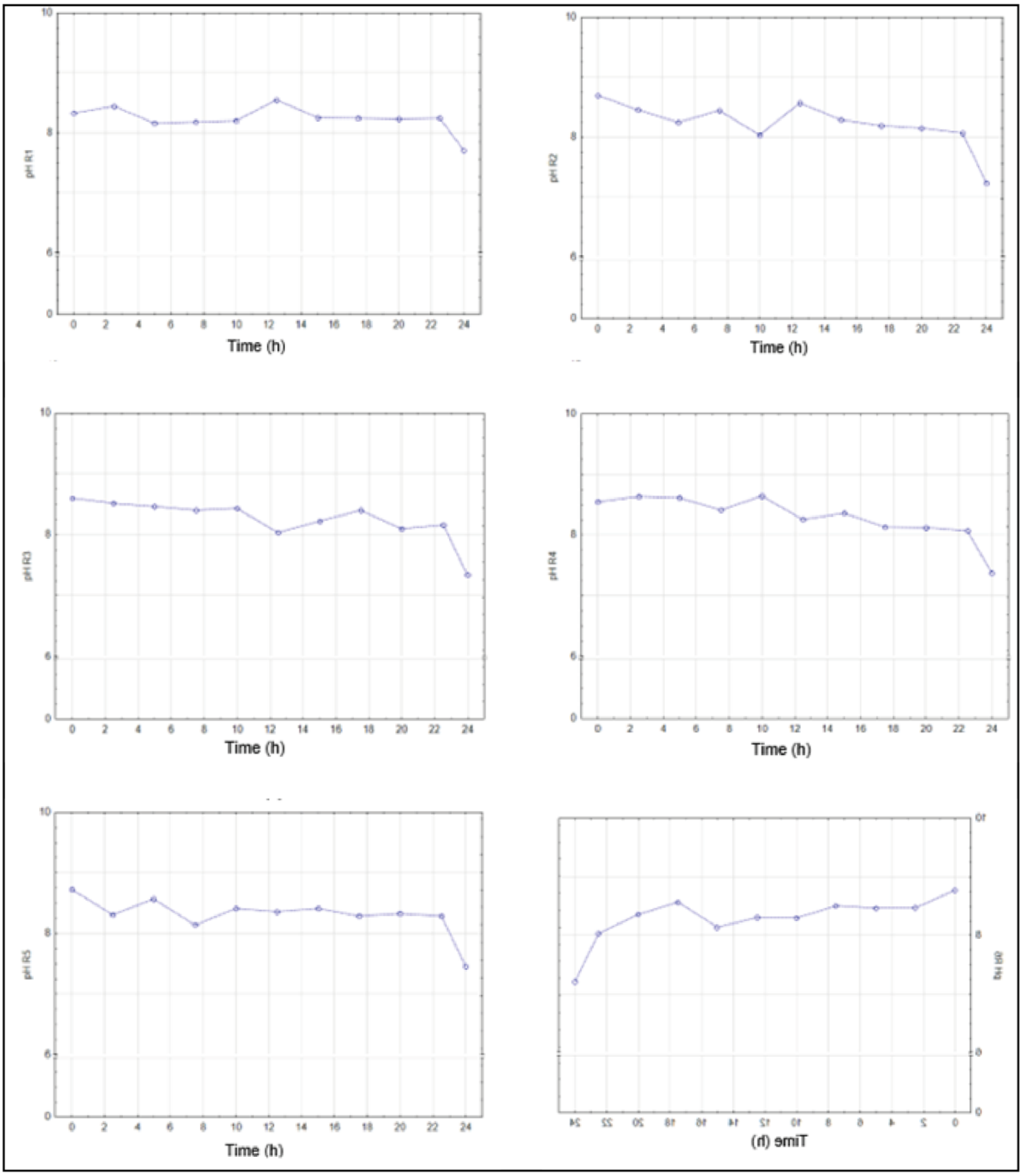
To demonstrate the molar nitrate formation behavior in relation to time, Figure 3 is shown. During the kinetics experimental time, all reactors showed concomitant activity of nitrate formation through Anammox microorganisms and nitrate-forming bacteria (BON) that act in the conventional N removal cycle, this inference is possible since the stoichiometric coefficient for nitrate in the Anammox reaction is 0.11 (Third *et al*., 2001) while there were peaks with a maximum value of 3.01 mol of nitrate in reactor 6, indicating mixed BON/Anammox activity.

Figure 3 – Temporal variation of molar nitrate formation, obtained by stoichiometric calculation, in MABR-BS R1 to R6 reactors



Source: Authors, 2021

Figure 4 – pH temporal variation in the kinetic assay conducted in MABR-BS R1 to R6 reactors



Source: Authors, 2021

Only reactor 5 showed, in Figure 3, NO_3^- generating activity predominant Anammox, since approximately 18h the stoichiometric nitrate coefficients formation were compatible with the theoretical reference value and from then on the amount of moles of NO_3^- increases, indicating that in this case, the cycle time could be adopted as 18h.

Figure 4 shows the pH values during the kinetic test in the 6 reactors. In all reactors, the initial pH was above 8.0, decaying to close to neutrality, due to the partial nitritation process that consists of the nitrogen transformation from the ammoniacal form to the nitrite form, with simultaneous consumption of alkalinity by the ammonia-oxidizing bacteria (BON) (7.14 mg of $CaCO_3$ per mg of $N\text{-}NH_4^+$) (METCALF & EDDY, 2003). Alkalinity consumption can also be seen in all graphs in Figure 2, through the consumption of carbon in inorganic form. The fastest alkalinity consumption was noted in reactor R2, in which in 2.5h the alkalizing level dropped from 1.5g to 0.12g. This higher consumption in R2 can be explained by the higher biomass concentration (5.93g.L^{-1} expressed in VSS).

Table 3 shows the average and maximum nitrogen removal rates and the specific average and maximum nitrogen removal rates, obtained in the kinetic assay in reactors R1 to R6.

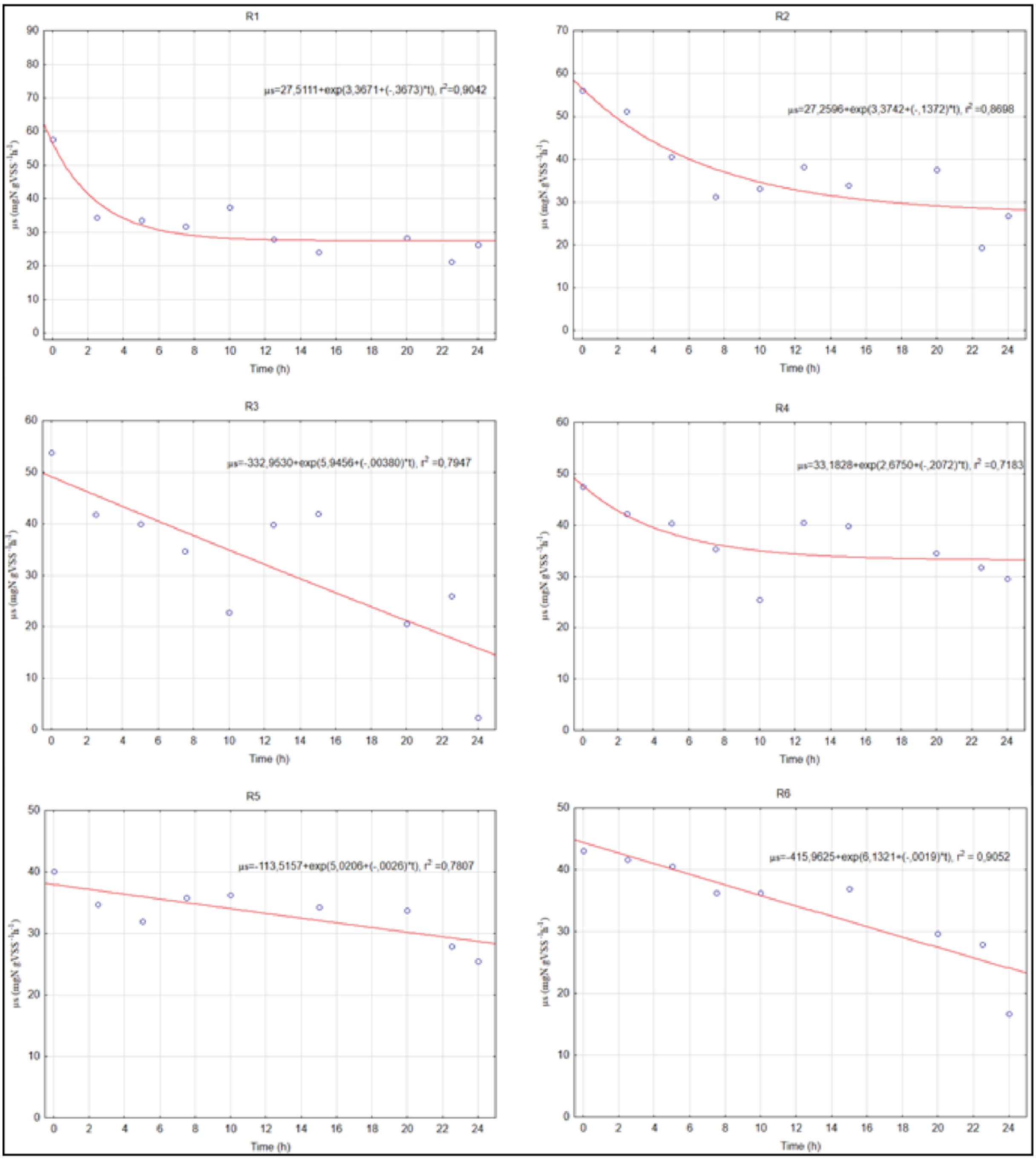
Table 3 – Nitrogen consumption velocities and specific nitrogen consumption velocities were expressed as mean values for each MABR-BS reactor in the study

Kinetic parameters	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**
r_s average (mgN. $L^{-1}.h^{-1}$)	29.73	33.64	35.62	33.74	29.11	30.82
r_s maximum (mgN. $L^{-1}.h^{-1}$)	58.08	56.19	56.32	47.85	40.35	43.70
μs average (mgN.$gSSV^{-1}h^{-1}$)	29.43	33.50	33.62	33.42	28.90	30.34
μs maximum (mgN.$gSSV^{-1}h^{-1}$)	57.71	56.02	53.88	47.46	40.09	43.12

Source: Authors, 2021

Chini *et al*. (2016) when operating an Anammox EGSB reactor (Expanded Granular Sludge Bed) fed with synthetic effluent, reached an average rate of the N removal reaction (r_S) of 19.53 and 28.66 mg.$L^{-1}.h^{-1}$, for the ammonium and nitrite forms, respectively. In the present study, the total r_S kinetic parameter for sum ($N\text{-}NH_4^+ + N\text{-}NO_2^-$) was maintained in all reactors above 29 mgN.$L^{-1}.h^{-1}$ and reached in reactors R1, R2, and R3 maximum values of (r_S) higher than the sum of the velocities of those authors (19.53 + 28.66 = 49.19 mgN.L^{-1}.h^{-1}), indicating the compatibility of the sludges in the present study with the high-performance Anammox sludge used by Chini *et al*. (2016).

Figure 5 – Specific nitrogen consumption rate and exponential mathematical models better adjusted to the biological nitrogen removal behavior achieved in MABR-BS reactors



Source: Authors, 2021

The kinetic data obtained by Equation 2 (μ_s) were fitted to the exponential mathematical model, and the equations that describe the behavior of the specific general consumption rate of nitrogenous forms (N-NH4+ + N-NO2-) are shown in Figure 5.

Table 4 – The specific rate of Nitrogen Consumption by Anammox microorganisms reported in the literature

Parameters	**Valor de** μs	**Reactor model**	**Fed conditions**	**Authors**
μ_s (mgN-NH_4^+.gVSS^{-1}.h^{-1}.;mgN-NO_2^-.gVSS^{-1}.h^{-1})	Assay 1: 20.11 and 30.03; Assay 2: 19.38 and 14.22 for N-NH_4^+ and N-NO_2^-, respectively	Anammox sludge activity tests conducted in batch	Synthetic feed, rich in ammonium and nitrite.	Chini *et al.*, (2016)
μ_s (mgN.gVSS^{-1}.d^{-1})	0.4 as maximum value	RBS reactor inoculated with Anammox enriched Sludge from municipal WWTP, with 3 months of enrichment.	Synthetic feed, with 273 mg.L^{-1} of $NaNO_2$ and 210 mg.L^{-1} of $(NH_4)_2SO_4$ and micronutrient solution.	Noophan *et al.*, (2015)
μs (mgN-NH_4^+.gSSV^{-1}.h^{-1}.;mgN-NO_2^-.gSSV^{-1}.h^{-1})	342.01 and 337.80 to N-NH_4^+ and N-NO_2^-, respectively	SBR, testing different power ratios H_2N_2:NH_4^+:NO_2^-.	Synthetic fed with a maximum ratio of 100:65 between NH_4^+:NO_2^-.	Yao *et al.*, (2015)
μs (mgN.gSSV^{-1}.d^{-1})	850 for the 1st day of the experiment; after 100 days of operation 528.	SBR with temperature control at 15°C and 30°C.	Synthetic fed 1:1 ratio between NH_4^+:NO_2^-.	Wang *et al.*, (2021)
μs (mgN.gSSV^{-1}.h^{-1})	67,1; 64,2; 72,5; 61,0	SBR with different agitation ranges.	Synthetic fed with NH_4^+:NO_2^- 31,6/32,8 73/67 92,2/67,3	Martins *et al.*, (2018)

Source: Authors, 2021

The kinetic assays that presented the highest correlation coefficients (r^2) between the practical values and the mathematical model used in the adjustment were the assays performed in reactors R1 and R6 with r^2 = 0.9042 and r^2 = 0.9052, respectively. The lowest correlation was found in reactor R4 with r^2 = 0.7183. Yao *et al*. (2015) also used an exponential model to adjust the data reaching r^2 = 0.98 and r^2 = 0.97 for the consumption of ammonia and nitrite, respectively.

To corroborate the results of the present work with other research, Table 4 presents similar works on Anammox nitrogen removal kinetics assays.

Most research presented in Table 3, showed specific nitrogen removal rate compatible with the average and maximum values of μs found in the present study reactors. Between all research, the results presented by Martins *et al*. (2018), had had the input conditions closer to the present study, and the reaction rate was very similar corroborating more precisely with the present result.

An important observation to be made when studying the kinetics of the Anammox process is related to the assays performed by Wang *et al*. (2021), which reached an initial rate of 850 $mgN.gVSS^{-1}.d^{-1}$ and maintained even lower a high N removal rate with 528 $mgN.gVSS^{-1}.d^{-1}$, demonstrating the high performance of Anammox reactors operated with high applied nitrogen loading rates.

In accordance with the previous comment, reactors R1, R2 and R4, observing Figure 5, showed signs of entering a low linear rate in the range of 8 to 16h, indicating that to take advantage of the exponential phase of nitrogen consumption, the time of detention could be reduced allowing for the amplification of the nitrogen load rate to be applied.

4 FINAL CONSIDERATIONS

Kinetic assays showed that there is an exponential progression in nitrogen consumption as a time function, where the highlight was the R1 reactor, which presented μ_{smax} of 57.71 $mgN.gVSS^{-1}.h^{-1}$. Reactors R1, R2 and R4 presented a more visual exponential phase that varied between 8 and 16h, indicating that the hydraulic detention time has the potential to be reduced, optimizing the Anammox nitrogen removal process.

The reactor with the best correlation of the fit of the data to the model and with the lowest residual nitrate production index was the R1 reactor, which was inoculated with anaerobic sludge from a domestic sewage wastewater treatment plant. Related to the nitrate production in the other reactors, all presented similar behavior with molar production of nitrate 3 times greater than the theoretical stoichiometry. The R4 reactor showed the highest nitrate production (R4 = 0.44 mol of $N\text{-}NO_3^-$, theoretical = 0.11 mol of $N\text{-}NO_3^-$).

It is important to highlight that although Anammox biomass was developed in all reactors, the biomass source with the best performance was the sludge collected in the domestic sewage wastewater treatment plant. This result is positive because this sludge is an easily accessible source of biomass and has great microbiological diversity, which can be easily exploited in the development of robust biomass for application in full-scale Anammox reactors.

The MABR-BS model proved to be satisfactory in terms of nitrogen removal rate, corroborating the results of the literature and showing indications that the reactors in particular R1, R2 and R4 have the potential to react positively to the increase in load in future research.

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REFERENCES

AMERICAN PUBLIC HEALTH ASSOCIATION – APHA. **Standard methods for the examination of water and wastewater**. 21. ed. Washington, DC: APHA, 2005.

AUGUSTO, M.R.; CAMILOTI, P.R.; SOUZA, T.S.O. Fast start-up of the single-stage nitrogen removal using anammox and partial nitritation (SNAP) from conventional activated sludge in a membrane-aerated biofilm reactor. **Bioresource Technology**, v. 266, p.151–157, 2018.

CAO, S.M.S. **Nitrificação e desnitrificação em situações críticas de substrato**. (Thesis) – Rio de Janeiro: UFRJ/COPPE, 2018.

CHINI, A.; KUNZ, A.; VIANCELLI, A.; SCUSSIATO, L.A.; DIAS, J.R.; JACINTO, I.C. Recirculation and aeration effects on deammonification activity. **Water, air. Soil Pollut.** v. 227, p. 67, 2016.

GONG, Z.; YANG, F.; LIU, S.; BAO, H.; HU, S.; FURUKAWA, K. Feasibility of a membrane-aerated biofilm reactor to achieve single-stage autotrophic nitrogen removal based on Anammox. **Chemosphere**, v. 69, n. 5, p. 776–784, 2007.

GUPTA, H.; ARORA, S.; VERMA S.; DAVEREY, A. Evaluation of corncob as a bio-carrier for the enrichment of anammox bacteria using activated sludge as seed. **Biocatalysis And Agricultural Biotechnology**, v. 19, p. 101140-101144, 2019.

LI, X.; SUN, Y.; WANG, Z.; HE, Z. Theoretical understanding of the optimum conditions for a mainstream granular nitritation-anammox reactor coupled with anaerobic pretreatment. **Science Of The Total Environment, v.** 669, p. 683-691, 2019.

MARTINS, T. H.; SOUZA, T. S. O.; VARESCHE, M. B. A. The Influence of Stirring Speed, Temperature and Initial Nitrogen Concentration on Specific Anammox Activity. **Brazilian Archives of Biology and Technology**, v. 60, p. 1–11, 2018.

METCALF & EDDY. **Wastewater engineering: treatment, disposal and reuse**. 3. ed. New York: Mc Graw Hill, 2003.

MORALES, N.; VAL DEL RÍO, Á.; VÁZQUEZ-PADÍN, J.R.; MÉNDEZ, R.; MOSQUERA-CORRAL, A.; CAMPOS, J.L. Integration of the Anammox process to the rejection water and main stream lines of WWTPs. **Chemosphere,** v. 140, p. 99–105, 2015.

MULDER, A.; VAN DE GRAAF, A. A.; ROBERSON, L. A.; KUENEM, J. G.. Anaerobic ammonium oxidation discovered in a denitrifying fluidized reactor. **FEMS Microbiol. Ecol.** v. 16, p. 177-184, 1995.

NOOPHAN, P.; BOONYAWAT, T.; SAENGSUWAN, S.; LARPKIATTAWORN, S.. Mixing Intensity Effects of Attached Growth on Enriched Anammox Cultures. **Environment Asia**, v. 7, n.1, p. 104–111, 2015.

SLIEKERS, A.O.; DERWORT, N.; GOMEZ CAMPOS, J.L.; STOUS, M.; KUENEN, J.G.; JETTEN, M.S.M. Completely autotrophic nitrogen removal over nitrite in one single reactor. **Water Research**, v. 36, n.10, p. 2475–2482, 2002.

STROUS, M., HEIJNEN, J.J., KUENEN, G.J., JETTEN, M.M.S. The sequencing batch reactor as a powerful tool for the study of slowly growing anaerobic ammonium-oxidizing microorganisms. **Appl. Microbiol. Biotechnol,** v. 50, p. 589–596, 1998.

Third, K.A.; SLIEKERS, A.O.; KUENEN, J.G., JETTER, M.S.M. The CANON system (Completely Autotrophic Nitrogen-removal Over Nitrite) under ammonium limitation: interaction and competition between three groups of bacteria. **Systematic and applied microbiology**, v. 24, n. 4, p. 588–596, 2001.

WANG, S.; ISHII, K.; YU, H.; SHI, X.; SMETS, B. F.; PALOMO, A.; ZUO, J. Stable nitrogen removal by anammox process after rapid temperature drops: Insights from metagenomics and metaproteomics. **Bioresource Technology,** v. 320, p. 124231, 2021.

YAO, Z.; LU, P.; ZHANG, D.; WAN, X., LI Y.; PENG, S. Stoichiometry and kinetics of the anaerobic ammonium oxidation (Anammox) with trace hydrazine addition. **Bioresource Technology**, v. 198, p. 70–76, 2015.

ZHANG, L., NARITA, Y., GAO, L., ALI, M., OSHIKI, M., OKABE, S. Maximum specific growth rate of Anammox bacteria revisited. **Water Reasearch**, *116*, 296–303, 2017.

ZHU G.; PENG Y.; LI B.; GUO J.; YANG Q.; WANG S. **Biological Removal of Nitrogen from Wastewater: Reviews of Environmental Contamination and Toxicology**. Springer, New York, NY, 2008.

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