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Environment

Efficiency comparison of heterotrophic denitrification in bioreactors treating agricultural drainage water with different organic matter sources

Comparação de eficiência da desnitrificação heterotrófica em biorreatores tratando água de drenagem agrícola com diferentes fontes de matéria orgânica

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ABSTRACT

Nitrate, when transferred in large amounts to surface waters, can cause serious problems for the environment and the health of humans and animals. The use of bioreactors with biodegradable organic supports emerges as an alternative for the treatment of nitrate-contaminated water. This study aimed to improve nitrate removal efficiency using different agricultural residues associated with Mini Biobobs® (a polyurethane foam biofilm carrier) at different hydraulic retention times (HRTs) and temperatures. Five reactors filled with different combinations of supports were evaluated: CS (corn stalk), CC (corn cobs), Mi (Mini Biobobs®), CS+Mi (corn stalk + Mini Biobobs®), and CC+Mi (corn cobs + Mini Biobobs®). The study was divided into two phases: Phase I, without temperature control and with HRTs of 24, 16, and 8 hours; and Phase II, with two temperatures (18 and 30 °C) and a fixed HRT of 8 hours. In both phases, the influent consisted of agricultural drainage water enriched with 20 mg L^{-1} of NO$_3^-$-N. In Phase I, all reactors, except Mi, produced effluents with a maximum NO$_3^-$-N concentration of 10 mg L^{-1}. In Phase II, the highest nitrate removal efficiency was achieved at 30 °C. The results indicate that corn residues are promising alternatives for removing nitrate from agricultural drainage water.

Keywords: Corn cobs; Corn stalk; Biological processes

RESUMO

Nitrato, quando transferido em grande quantidade para águas superficiais, pode causar sérios problemas ao meio ambiente e à saúde de humanos e animais. O uso de biorreatores com suporte orgânico biodegradável surge como uma alternativa para o tratamento dessas águas contaminadas com nitrato. O objetivo deste estudo foi aprimorar a eficiência de remoção de nitrato usando diferentes resíduos agrícolas associados ao Mini Biobobs® (suporte para crescimento de biomassa de espuma de poliuretano) em diferentes tempos de retenção hidráulica (HRT) e temperaturas. Cinco reatores preenchidos com diferentes combinações de suportes foram avaliados: CS (talo de milho), CC (espigas de milho), Mi (Mini Biobobs®), CS+Mi (talo de milho + Mini Biobobs®) e CC+Mi (espigas de milho + Mini Biobobs®). O estudo foi dividido em duas fases: Fase I, sem controle de temperatura e com Tempos de Retenção Hidráulica (TRH) de 24, 16 e 8 horas; Fase II, com duas temperaturas de 18 e 30ºC, e TRH fixo em 8 h. Em ambas as fases, o afluente era composto por água de drenagem agrícola enriquecida com 20 mg L^{-1} de N-NO_3^-. Na Fase I, todos os reatores, com exceção do reator Mi, produziram efluentes com concentração máxima de N-NO_3^- de 10 mg L^{-1}. Na Fase II, a melhor eficiência de remoção de nitrato foi obtida a 30°C. Os resultados indicam que os resíduos de milho são promissores e viáveis como alternativa para remover nitrato na água de drenagem agrícola.

Palavras-chave: Espigas de milho; Talo de milho; Processos biológicos

1 INTRODUCTION

Heterotrophic denitrification is a microbial process that removes nitrate under anoxic conditions with biochemical transformations that require an organic matter source. Organic carbon is used as an electron donor in the reduction reactions of nitrate and nitrite during nitrogen removal. During the treatment of nitrogenous residues on full scales, the organic carbon source represents a high cost for treatment systems, and the most used sources are methanol and ethanol (Burghate & Ingole, 2014).

Denitrifying bacteria uses nitrite and/or nitrate as final electron acceptors, transforming them into molecular nitrogen (N_2), with the intermediate products formation such as nitrite (NO_2^-), nitric oxide (NO), and nitrous oxide (N_2O). Enzymes associated with denitrification are synthesized when conditions become favorable. Several factors can affect the heterotrophic denitrification process, such as temperature, pH, quality and amount of carbon sources, HRT, toxic substances, and others (Elefsiniotis; Li 2006; Cheikh et al., 2013; Wang et al., 2013; Hou et al., 2019; Martin et al., 2019).

The use of bioreactors with support that also serve as a carbon source on the heterotrophic denitrification process can be a viable and promising alternative, since it meets the necessary conditions that the process requires, such as long durability and stable hydraulic characteristics in the treatment of agricultural drainage waters (Feyereisen et al. 2016).

The use of fertilizers in agricultural fields can result in drainage water rich in nitrates. Studies such as the present are justified for that reason (Yu et al., 2019; Jéglot et al., 2021; Krone et al., 2022).

Several bioreactors with organic carbon media supports are reported in the literature. Some authors report the denitrification activity in bioreactors with wood chips in several countries, including the United States, China, New Zealand, and countries in Europe (Fatehi-Pouladi et al., 2019; Moorman et al., 2010; Robertson, 2010; Yao et al., 2020). Its main disadvantage is the low carbon availability, due to the high material recalcitrance, which can limit the rate of denitrification, requiring studies to replace them with other materials that have greater availability of organic carbon in their composition (Hartz et al., 2017).

The use of low-cost supports, such as agricultural residues that might otherwise pose environmental problems, has been studied. A variety of biodegradable organic supports have already been successfully tested, such as sawdust and wood chips, rice and walnut husks, cotton, barley, wheat, and corn straw, as well as corn cobs, across several countries such as the United States, China, New Zealand, and multiple European nations since 1995. These solid substrates provide an extended release of reducing power for denitrification and also serve as favorable supports for microbial biofilm development (Robertson; Cherry, 1995; Della Rocca et al., 2005; Moorman et al., 2010; Robertson, 2010; Wang et al., 2013; Nordström; Herbert, 2017; Satayeva et al., 2018; Fatehi-Pouladi et al., 2019; Yao et al., 2020; Hellman, 2021).

Agricultural residues contain cellulose in their composition, a renewable resource, and a basic component of all plant materials, which can replace other technologies that consume non-renewable chemicals and may pose toxicity risks (Wang et al., 2018; Zhang et al., 2021). This substitution also contributes to reducing the environmental impacts of residual materials left in agricultural fields, promoting sustainable development and aligning with public policies.

In this context, this work aimed to evaluate the nitrate removal from agricultural drainage water in bioreactors with (i) different organic matter sources as microbial support: corn stalk and corn cobs associated with commercial support medium (mini Biobobs®); (ii) different HRTs and (iii) different temperatures.

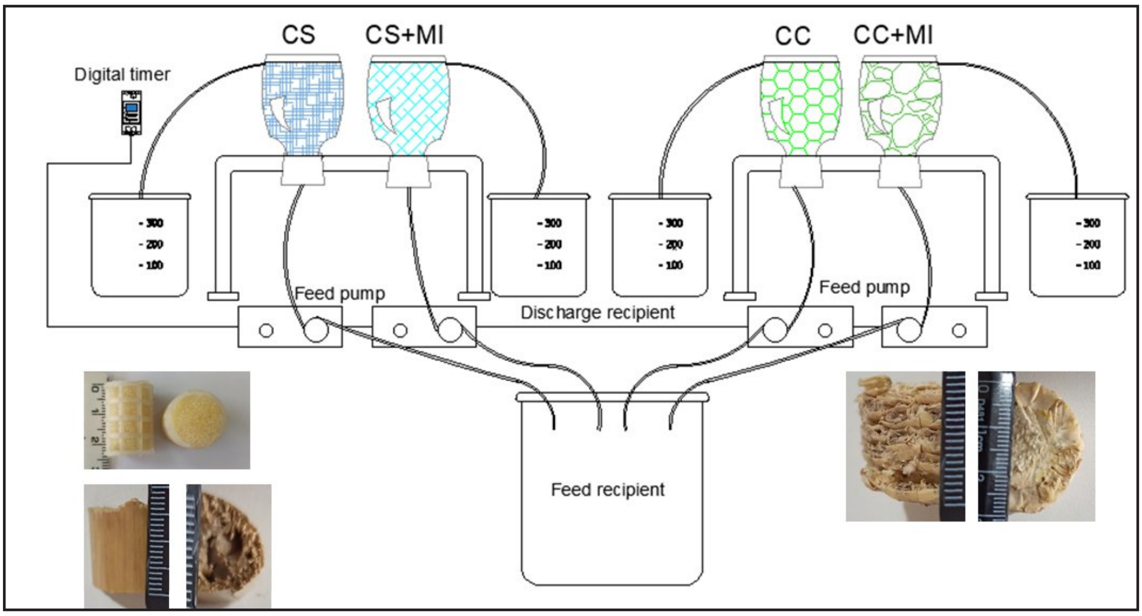
2 MATERIALS AND METHODS

2.1 Influent

The influent used was agricultural drainage water collected from a corn farm enriched with 121.42 mg L^{-1} of sodium nitrate ($NaNO_3$) which provided 20 mg L^{-1} of NO_3^--N.

2.2 Bioreactor design and operation

Figure 1 – Reactor scheme filled with corn by-products and Mini Biobobs®



Legend: CS: corn stalk; CS+Mi: corn stalk + Mini Biobobs®; CC+Mi: corn cobs + Mini Biobobs®; Mi: Mini Biobobs®; CC: corn cobs

Source: Authors (2024)

Five reactors were studied during 253 days. They consisted of cylindrical bottles made of polyethylene terephthalate (PET) with 16 cm high, internal diameter of 7.5 cm, and total volume of 0.5 L. The reactors were fed by the bottom using peristaltic pumps (INTLLAB brand, model DP-385) connected to a timer to permit intermittent feeding as shown in Table 2. The effluent outlet was at the top of the reactor (Figure 1). Three types of support material were used for the adhesion of microorganisms: Mini Biobobs®, corn stalks, and cobs. The Mini Biobobs® were manufactured and donated by the company Bioproj Tecnologia Ambiental. Corn stalks and cobs were obtained from external suppliers. Figure 1 illustrates the experimental apparatus.

The Mini Biobobs® are made up of an external high-density polyethylene (HDPE) frame measuring 1.5 cm in diameter, 2.0 cm in length, and an internal part of polyurethane foam (PU), with a 90% porosity and 0.196 g weight unit.

The corn stalks and cobs were cleaned before being brushed and rinsed with tap water. Before filling the bioreactors, the materials underwent size standardization, the stems were 1.0 cm in diameter and 1.5cm high, while the cobs were 3.0cm in diameter and 1.5 cm high.

Table 1 – Support material and useful volume in each reactor

Reactor	Support material	Useful Volume (mL)	Support material amount in each reactor (units)
CS	Corn Stalk	180	40
CS+Mi	Corn Stalk + Mini Biobobs®	170	21 + 36
CC+Mi	Corn Cobs + Mini Biobobs®	160	13 + 33
Mi**	Mini Biobobs®	190	80
CC	Corn Cobs	270	16

Legend: CS: corn stalk; CS+Mi: corn stalk + Mini Biobobs®; CC+Mi: corn cobs + Mini Biobobs®; Mi: Mini Biobobs®; CC: corn cobs. **A previous test was made with one reactor filled only with Mini Biobobs® to prove that material could not offer organic carbon source to microorganisms present in the reactors
Source: Authors (2024)

To determine the useful volume, the procedure described by Robertson (2010) was carried out, where each reactor was filled with its respective supports, the inlet sealed, and the influent added until the volume was exceeded by the outlet. The reactors remained full and at rest for a period of 2 hours for the liquid to be adsorbed to the support material. After this period, the reactors were drained, and the volume was determined as useful volume (Table 1).

The reactors were covered with aluminum foil to block light and prevent algae growth. Afterwards, they were inoculated through batch feeding with the substrate itself until nitrate removal. This period lasted 15 days.

The reactor operation was divided into two phases, Phase I and Phase II, as shown in Table 2. In Phase I, the reactors were operated at room temperature and submitted to HRTs of 24, 16, and 8 hours. In Phase II, temperatures were fixed at 18 and 30ºC and HRT at 8h. The HRT in Phase II was chosen due to the efficiencies obtained in Phase I, where it was possible to reach the maximum limit of 10 mg L^{-1} NO_3^--N in all employed conditions.

Table 2 – Reactors operational parameters in Phases I and II

Phase	Reactors	HRT (h)	Temperature (ºC)	Time (d)	Pump feed control: pump on/ pump off (min/min)
I	CS, CS+ Mi, CC+ Mi, Mi	24	16.21 ± 3.12	29	1min/149min
	CS, CS+ Mi, CC+ Mi, CC, Mi	16	22.23 ± 3.22	91	1min/119min
	CS, CS+ Mi, CC+ Mi, CC	8	24.50 ± 1.91	50	2min/118min
II	CS, CS+ Mi, CC+ Mi, CC	8	30	14	2min/118min
	CS, CS+ Mi, CC+ Mi, CC	8	18	14	2min/118min

Legend: CS: corn stalk; CS+Mi: corn stalk + Mini Biobobs®; CC+Mi: corn cobs + Mini Biobobs®; Mi: Mini Biobobs®; CC: corn cobs
Source: Authors (2024)

2.3 Physicochemical analysis

The nitrate removal efficiency was evaluated by analyzing N-nitrate (NO_3^--N), N-nitrite (NO_2^--N), pH, and alkalinity content. The analyses were performed using the following standard methods: NO_3^--N by colorimetric method (APHA 4500-NO_3^-C), NO_2^--N by colorimetric method (APHA 4500-NO_2^-B), and pH by potentiometric method (APHA 4500-H^+B). All analyses were performed according to the APHA (2005), except for the alkalinity analysis which followed the method of DiLallo and Albertson (1961) modified by Ripley et al. (1986).

2.4 Support surfaces analysis

The surface analysis of the supports was performed at the beginning and end of the experiment. Corn stalk and cob samples were used. The analysis was performed in a Scanning Electron Microscope by field emission (FEG, Mira 3 Model, Tescan Brand).

2.5 Kinetic assay of denitrifying activity

The kinetic tests were carried out within the reactor themselves, operated in batch mode, at a temperature of 25 ºC and agitation of 120 rpm, and placed in an incubator with orbital agitation (Nova Técnica brand, model NT 714). The substrate was composed of agricultural drainage water enriched with 10 mg L^{-1} NO_3^--N. At each one-hour interval, an aliquot of 5 mL was withdrawn for nitrite and nitrate analyses. Reaction rates were determined by fitting substrate consumption curves to a linear model, plotting substrate concentration versus time. Thus, the velocities were determined after determining the equation of the straight line and defining the angular coefficient, carried out in the Microsoft Excel® software.

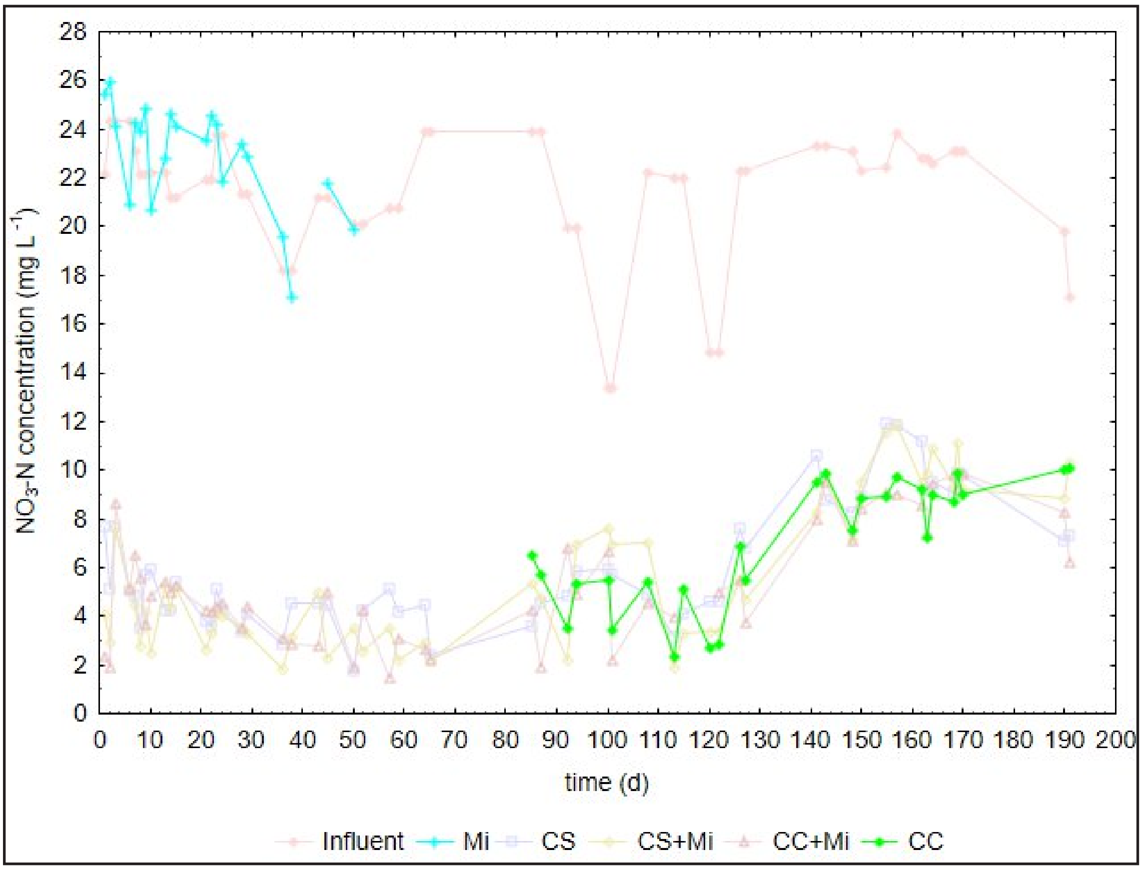
2.6 Statistical analysis

All results were expressed as mean followed by standard deviation. To verify the normality of the data, the Shapiro-Wilk test was used. For normally distributed data, Analysis of Variance (ANOVA) and Tukey's Test (p=0.05) were performed, for non-normally distributed data, a non-parametric test was used (Krusskall-Wallis test). All analyses were performed using the Statistica® software (v. 14.0).

3 RESULTS AND DISCUSSION

3.1 Removal of nitrate

Figure 2 – Influent and effluent nitrate concentration obtained in Phase I (Room temperature)



Legend: CS: Corn stalk; CS+Mi: Corn stalk + Mini Biobobs®; CC+Mi: Corn cobs + Mini Biobobs®; CC: Corn cobs; Mi: Mini Biobobs®
Source: Authors (2024)

The drainage water was characterized by a low concentration of organic matter. The organic matter concentration, referred to as COD reached levels of 0.0655 ± 0.0002 mg L^{-1}, with no detectable levels of NO_3^--N and NO_2^--N. Therefore, enrichment with $NaNO_3$ was necessary. Figure 2 shows the NO_3^--N concentrations within the reactors' influent and effluent during Phase I. It was verified that there was no removal of nitrate in the reactor filled only with Mini Biobobs®, Mi reactor. Knowing that the Mini Biobobs® are composed of polyurethane foam surrounded by a high-density polyethylene hollow structure and do not have carbon available in their composition, this result confirms the need for an external carbon source as the electron donor for denitrification. Thus, it was decided to stop the process and replace the Mini Biobobs® with corn cob, starting a new reactor “CC reactor”.

In the referred experimental alteration, the same amount of corn cobs present in the CC+Mi reactor was added to the new CC reactor, aiming to compare whether the foam could assist within the process or if only the corn cob would be sufficient. In Phase I, the effluents from the other reactors presented a NO_3^--N concentration below 10 mg L^{-1}, the concentration of 10 mg L^{-1} of NO_3^--N being the maximum limit accepted by current legislation (CONAMA 2005, 2008; Brasil, 2021) and recommended by WHO (2011).

Table 3 – NO_3^--N removal efficiency in the different reactors and phases studied

Phase I HRT (h)	**NO_3^--N Removal Efficiency (%)**				
	CS	**CS+Mi**	**CC**	**CC+Mi**	**Mi**
24	78.25 ± 5.40^{aA}	81.94 ± 5.27^{aA}	NA	79.29 ± 6.39^{aA}	0
16	76.44 ± 9.07^{aA}	79.00 ± 12.60^{aA}	75.90 ± 7.21^{aA}	80.64 ± 10.21^{aA}	2.00 ± 2.70^{Bb}
8	57.44 ± 5.23^{aB}	55.82 ± 7.18^{aB}	58.81 ± 6.73^{aB}	61.69 ± 3.78^{aB}	NA
Phase II T (oC)	**CS**	**CS+Mi**	**CC**	**CC+Mi**	**Mi**
30 °C	73.00 ± 3.87	73.00 ± 4.16	72.43 ± 6.11	73.86 ± 4.88	NA
18 °C	57.17 ± 3.54	56.83 ± 2.14	56.83 ± 2.93	62.50 ± 6.67	NA

Legend: CS: Corn stalk; CS+Mi: Corn stalk + Mini Biobobs®; CC+Mi: Corn cobs + Mini Biobobs®; CC: Corn cobs; Mi: Mini Biobobs®. NA: Not Analyzed. Means followed by the same lowercase letter do not differ statistically in the same row ($p \leq 0.05$). Means followed by the same capital letter do not differ statistically from each other in the same column ($p \leq 0.05$)
Source: Authors (2024)

The mean denitrification efficiency obtained in the different reactors during Phases I and II is presented in Table 3. In Phase I, the highest removal efficiencies (75.90 – 81.94%) were obtained in the 24 and 16 h HRTs, with no statistically significant difference between the results of the reactors filled with carbon sources. The only reactor that did not show nitrate removal was the one filled only with Mini Biobobs®, Mi reactor. This data confirms that nitrate removal was carried out by heterotrophic pathways and therefore a carbon source is required.

When the HRT was reduced to 8h, the efficiencies decreased in all reactors, obtaining values in the range of 55.82 to 61.69%, with no statistically significant difference between them except for the reactor containing only Mini Biobobs®. Zhang et al. (2019) explain that a shorter HRT in the denitrification process with good removal efficiency is related to the biofilm firmly adhered to the pores on the irregular and rough support surface. Hoover et al. (2016), Martin et al. (2019), and Kouanda and Hua (2021) verified the impact of HRT on nitrate removal in their studies, and the authors observed that as HRT increased, the NO_3^--N efficiency removal also increased, in agreement with results obtained in the present study.

The denitrification rate also varies according to the carbon source, due to differences in material composition. Carbon release from agricultural residues occurs in two stages: initially, there is a rapid release of low-molecular-weight, water-soluble organic compounds due to the swelling of the residues; subsequently, insoluble compounds such as cellulose and lignin are slowly released through bacterial hydrolysis, a process also influenced by the HRT (Cao et al., 2016).

In Phase II (Table 3), with temperature control set at 18ºC and 30ºC, it was verified that the temperature affects denitrification efficiency, where the highest efficiencies (72.43-73.86%) occur at a temperature of 30ºC. It is verified that the temperature range where the greatest removals occurred corresponds to the optimal temperature range of 25-35ºC (Liao et al., 2018).

Temperature is an important control factor for the development and activity of the organisms involved within the process. Groh et al. (2019) found that the denitrification rate is positively affected by temperature. Temperatures from 4 to 23ºC were used during the experiment with three reactors with nitrate and/or carbon limitations, where the authors generally pointed out that lower temperatures resulted in a lower denitrification rate for all reactors, and when the temperatures increased, the denitrification efficiencies also increased. The highest rates occurred at the highest temperature (23°C) which is justified because it is a process mostly carried out by mesophile microorganisms. In addition, the closer to the optimum temperature, the greater the efficiency of microbial metabolism; it is also believed that these temperatures increase oxygen consumption, creating more anaerobic points for denitrification to occur (Madigan et al., 2016).

Table 4 presents the pH values obtained in Phase I. The pH of the influent ranged from 5.57 to 6.78, considered slightly acidic. Denitrifying microorganisms act effectively within a pH range of 6.0 to 8.5, with microbial activity being optimal near neutrality and inhibited when initial pH values fall outside this range (Elefsiniotis & Li 2006; Cheikh et al., 2013; Wang et al., 2013; Hou et al., 2019).

A significant increase in pH was observed in the majority of the reactor effluents. The only reactor who did not present a notable difference with the influent was the one filled with Mini Biobobs®, since the denitrification process did not occur, as previously discussed.

He et al. (2016) estimated the initial pH effects on denitrification capacity in the pH range of 6.0-9.0, using pre-treated corn husk hydrolyzate as a carbon source for nitrate removal. In all tests, the pHs increased during the removal process, and the results demonstrated that hydrolyzate is a viable source in a wide pH range, with denitrification efficiency of 88.45% at initial pH 6.0 and 96.91% at pH 8,5, which was considered ideal during the experiment.

Table 4 – pH values of the influent and effluent from the reactors at the three Hydraulic Retention Times (HRTs) studied

HRT	**Reactor**					
(h)	**Influent**	**CS**	**CS + Mi**	**CC + Mi**	**Mi**	**CC**
24	5.57 ± 0.21^{a}	6.89 ± 0.21^{bA}	6.90 ± 0.30^{bA}	6.71 ± 0.35^{bA}	5.87 ± 0.40^{aA}	NA
16	6.78 ± 0.32^{a}	7.53 ± 0.56^{bB}	7.56 ± 0.51^{bB}	7.34 ± 0.47^{abB}	6.37 ± 0.49^{abA}	7.62 ± 0.28^{bA}
8	6.67 ± 0.41^{a}	7.46 ± 0.47^{bB}	7.14 ± 0.64^{abAB}	7.14 ± 0.51^{abAB}	NA	7.10 ± 0.59^{abB}

Legend: CS: Corn stalk; CS+Mi: Corn stalk + Mini Biobobs®; CC: Corn corbs; CC+Mi E: Corn corbs + Mini Biobobs®; Mi: Mini Biobobs®. NA: Not Analyzed. Means followed by the same lowercase letter do not differ statistically in the same row ($p \leq 0.05$). Means followed by the same capital letter do not differ statistically from each other in the same column ($p \leq 0.05$)

Source: Authors (2024)

Table 5: Measured and theoretical alkalinities (mg of $CaCO_3$ L^{-1}) of the effluent from different reactors at three Hydraulic Retention Times (HRTs)

Reactor	HRT (h)					
	24		16		8	
	Alkalinity (mg de $CaCO_3$ L^{-1})					
	Measured	**Theoretical**	**Measured**	**Theoretical**	**Measured**	**Theoretical**
Influent	4.17	-	10.52	-	10.80	-
CS	72.82 ± 38.22	59.17	64.74 ± 8.62	44.83	44.63 ± 6.57	34.93
CS+Mi	93.94 ± 47.92	62.20	65.50 ± 11.60	45.44	45.38 ± 7.38	33.95
CC	NA	-	64.88 ± 7.91	44.56	52.98 ± 8.13	36.64
CC+Mi	73.97 ± 31.29	59.63	59.33 ± 9.69	40.43	54.80 ± 6.47	38.15
Mi	1.38 ± 3.96	-20.28	0.48 ± 3.16	5.63	NA	-

Legend: CS: Corn stalk; CS+Mi: Corn stalk + Mini Biobobs®; CC: Corn corbs; CC+Mi E: Corn corbs + Mini Biobobs®; Mi: Mini Biobobs®. NA: Not Analyzed
Source: Authors (2024)

Table 5 presents the alkalinity values obtained in Phase I. The theoretical alkalinity production is 3.57 mg expressed in $CaCO_3$ per mg NO_3^--N reduced (EPA, 1993), which makes alkalinity another opportunity to show the occurrence of heterotrophic denitrification. Within the reactor containing only Mini Biobob®, alkalinity production was not observed, since the values showed no significant difference compared to the influent, indicating that no nitrate removal occurred in this reactor. In the other reactors, alkalinity production was observed, the highest alkalinity value (93.94 ± 47.92 mg of $CaCO_3$ L^{-1}) produced was in the 24 h HRT in the CS + Mi reactor, with a nitrate removal efficiency of 81.94%. In the 8 h HRT, where the lowest nitrate removal efficiencies occurred, from 55.82 to 61.69%, the lowest alkalinities were generated, from 44.63 to 54.80 mg of $CaCO_3$ L^{-1}.

Table 6 presents the alkalinities produced during Phase II. The highest alkalinity productions from 49.16 to 66.96 mg of $CaCO_3$ L^{-1} occurred at a temperature of 30ºC, also corresponding to the highest nitrate removal efficiencies, from 72.43 to 73.86% (Table 3). The increase in pH and alkalinity observed in the reactor effluent in this study is considered an indication of denitrification, as explained by the EPA (1993), which associates this behavior with bacterial respiratory metabolism involving nitrate reduction and H^+ consumption during denitrification.

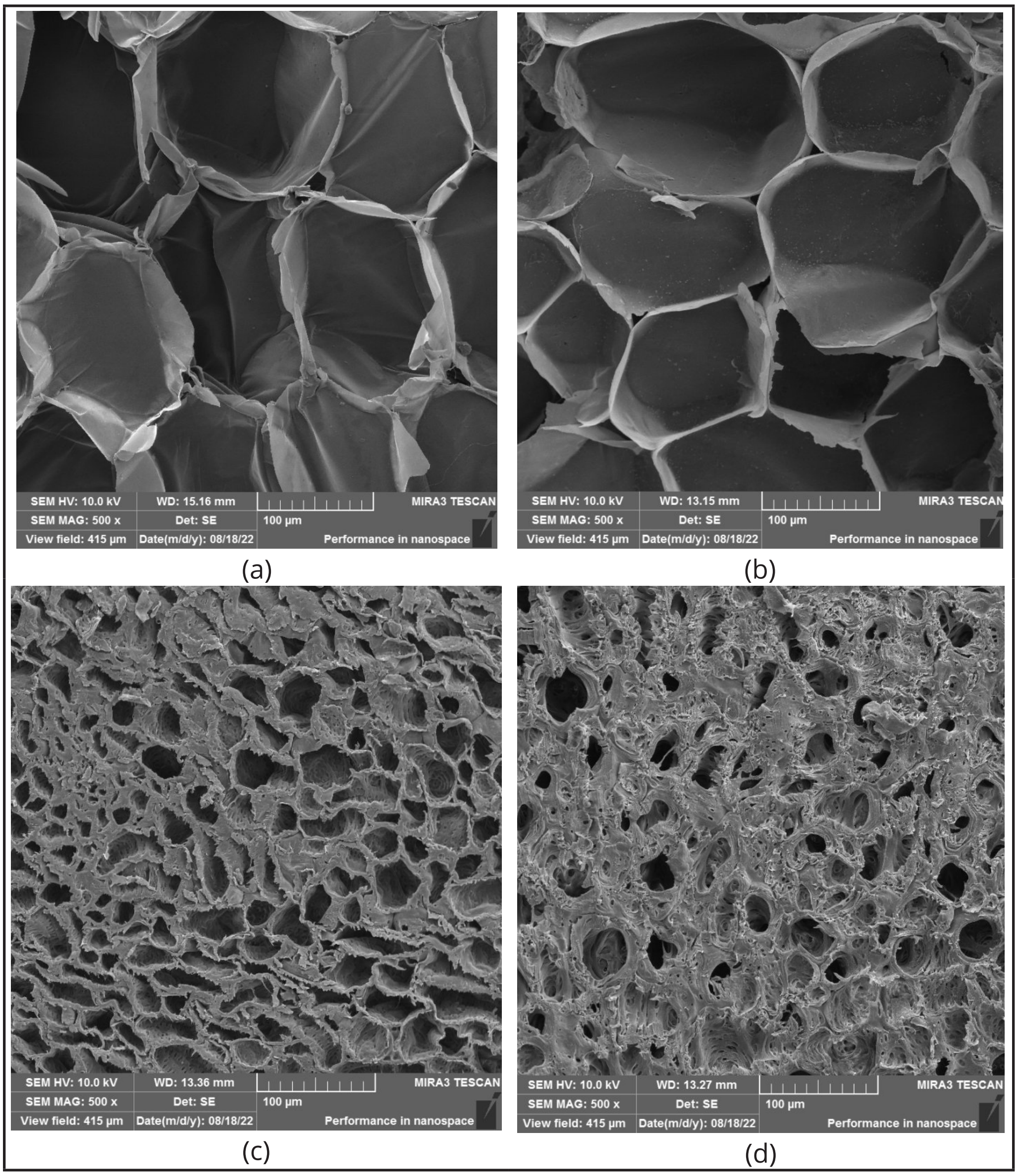
Table 6 – Alkalinity (mg de CaCO3 L-1) and pH obtained in Experimental Phase II

Temperature	Reactors				
	Influent	CS	CS+Mi	CC+Mi	CC
30 °C	9.66 ± 3.40	66.96 ± 14.32	49.16 ± 14.58	62.53 ± 9.55	50.75 ± 12.19
18 °C	13.26 ± 5.82	45.76 ± 14.04	39.23 ± 14.13	54.45± 10.59	38.11 ± 9.56

Legend: CS: Corn stalk; CS+Mi: Corn stalk + Mini Biobobs®; CC: Corn corbs; CC+Mi E: Corn corbs + Mini Biobobs®; Mi: Mini Biobobs®
Source: Authors (2024)

3.2 Analysis of the surface of the supports

Figure 3 – Scanning Electron Microscopy (SEM) of the investigated agricultural residues (500x)



(a) (b) (c) (d)

Legend: a: CS surface before denitrification; b: CS surface after denitrification; c: CC surface before denitrification; d: CC surface after denitrification
Source: Authors (2024)

The morphologies of corn cob and corn stalk are presented in Figure 3. Zhang et al. (2019), Wang et al. (2013), and Ling et al. (2021) associated higher denitrification rates with the structure and surface area of the material used.

Figures 3a and 3c show, respectively, the corn stalk and the corn cob structure before the denitrification process. The corn stalk had larger pores and smaller amounts when compared to the corn cob surface. The supports showed a rough surface morphology, with clear pores distributed over the entire surface, which is consistent with the morphology observed by Wang et al. (2013) and Ling et al. (2021). In addition, the authors concluded that reactors filled with cobs provided intermediate spaces and this acted in favor of the denitrifying microorganism's colonization.

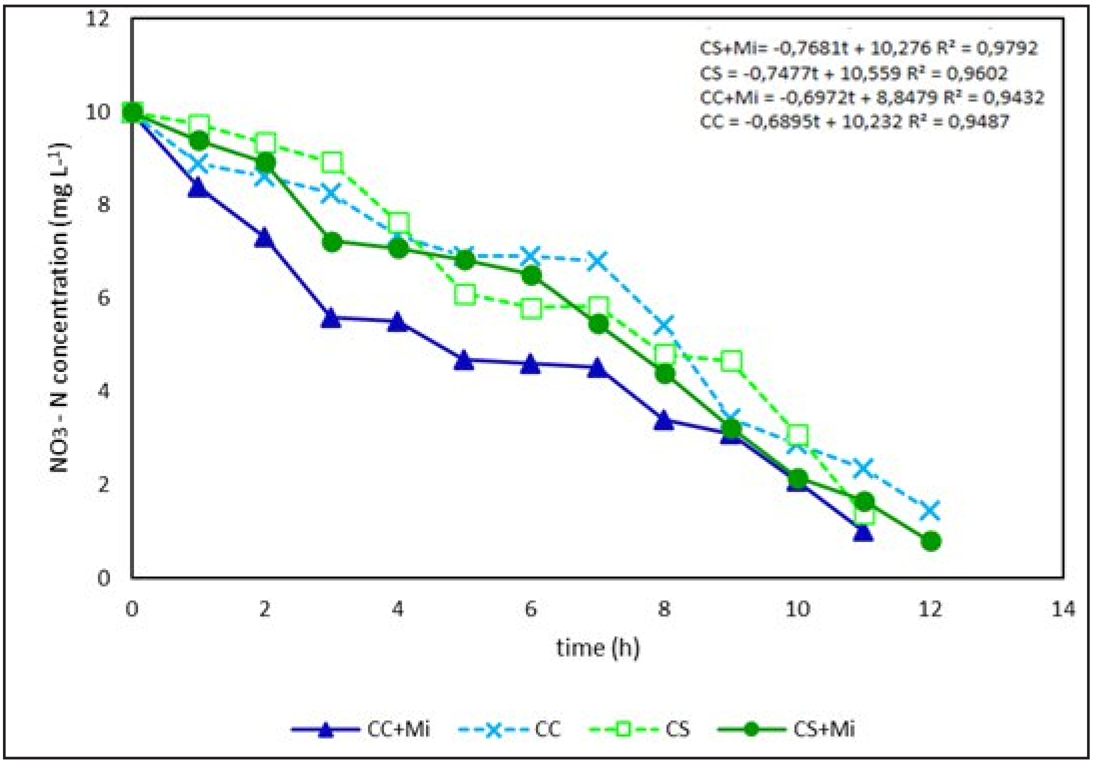
After the denitrification process, the surface and structure of the supports underwent modifications. The corn stalk pore wall (Figure 3b) showed a smoother laminar structure when compared to the structure before denitrification. The corn cob structure (Figure 3d) was replaced by a rough surface and grouped structure; modifications also observed by Yu et al. (2019).

Yang et al. (2015), Lai and Xiugui (2021), and Ling et al. (2021) explain that the modifications are correlated to the chemical compositions of the supports, since the cellular structures formed by lignin from agricultural residues take time to biodegrade due to their stable structure, whereas cellulose and hemicellulose are degraded faster and are more easily used by denitrifying microorganisms, reducing their percentages during the process. The corn stalk has 36.9% cellulose, 20.4% hemicellulose, and 17.4% lignin in its composition (Zhang et al., 2021), whereas the corn cob has 31.7% cellulose in its composition, 34.7% hemicellulose and 20.3% lignin (Wang et al., 2018), which explains the changes undergone during the experiment.

Wang et al. (2013), Lai and Xiugui (2021), Jéglot et al. (2021), and Ling et al. (2021) commented that extracellular enzymes secreted by microorganisms can hydrolyze cellulose and hemicellulose in substrates of small and soluble molecules, where the organic carbon released by microbial degradation can be used as co-metabolites and electron donors to promote denitrification. Therefore, it is presumed that efficient nitrate removal is the result of multiple mechanisms acting together.

3.3 Kinetic analysis of nitrate removal

Figure 4 – Nitrate concentrations obtained during kinetic tests performed in different reactors, along with the zero-order kinetic equations describing the process



Legend: CS: Corn stalk; CS+Mi: Corn stalk + Mini Biobobs®; CC: Corn corbs; CC+Mi E: Corn corbs + Mini Biobobs®
Source: Authors (2024)

Figure 4 shows the nitrate concentrations graph obtained during the kinetic tests in the 4 reactors. Robertson (2010) pointed out that a linear fit is considered appropriate for a zero-order kinetic model, where the nitrate removal rate remains constant regardless of the nitrate concentration. On the other hand, a non-linear fit is appropriate for a first-order kinetic model, where the nitrate removal rate varies in proportion to the nitrate concentration, i.e., when nitrate is the limiting substrate. Schipper et al. (2010) explain in a simplified manner that if the nitrate removal rate is related to the nitrate concentration, then first-order reaction kinetics apply. However, if the nitrate removal rate is independent of the nitrate concentration and controlled by an independent parameter instead, such as the availability of labile organic carbon, then zero-order kinetics would be applied.

Based on this information, the present study kinetic analyses were adapted to zero-order kinetics (Figure 4), as it appears that there was a correlation between the denitrification process and zero-order kinetics ($R^2 > 0.9$) in all the assays (GAO et al., 2022). The zero-order behavior was also observed in the tests by Robertson (2010) and Hou et al. (2019), demonstrating that the nitrate concentration present within the reactor did not limit its removal. This suggests that, at the concentrations studied, the nitrate substrate was not toxic to the heterotrophic microorganisms present in the reactors. However, the CC+Mi and CS reactors showed higher substrate degradation rates, resulting in shorter times for complete NO_3^--N removal.

4 CONCLUSION

The denitrification of agricultural drainage water using two agricultural by-products, corn stalk, and corn cob, proved to be feasible in all studied conditions, as they made it possible to remove nitrate efficiently, in compliance with current legislation. However, the reactor filled only with Biobob® did not show nitrate removal, confirming the need for an external electron donor to support the heterotrophic denitrification process. The highest nitrate removal rates were obtained in the HRTs of 24 and 16h, with no statistical difference between the 4 reactors filled with corn stalk and cob in different proportions.

After reducing the HRT to 8 h, there was a decrease in the efficiency of nitrate removal, but the concentrations were below the limit established in the legislation nonetheless. In addition, the denitrification rate decreases depending on the type of carbon source, as carbon release occurs gradually in two stages, meaning that sufficient time is required for the process to take place — which also helps explain the reduction in efficiency when the HRT is shortened.

With the temperature control, it was confirmed that the removal efficiency is influenced by this parameter, where the highest efficiencies, from 72.43 to 73.86%, were obtained at a temperature of 30ºC since it is a process performed by mesophilic bacteria.

The zero-order kinetic study indicated that the nitrate concentration was not a limiting factor for this nutrient removal through heterotrophic denitrification, however, the CC+Mi and CS reactors showed a higher velocity of substrate degradation. Analyzing the environmental aspect, the use of two agricultural by-products in the nitrate removal process, acting as a support medium and source of organic carbon, proved to be relevant due to the large generation and the possibility of bringing added value to these generally discarded materials. Considering the availability of larger treatment areas under field conditions, operating with higher HRTs is recommended when using lignocellulosic by-products. This approach ensures more stable and complete denitrification by leveraging their gradual carbon release.

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