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## BIOLOGICAL CONVERSION OF NITRATE IN PACKED BED ORGANIC MATERIAL REACTORS

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*Paulo Vitor de Figueiredo Bacovis*

Master's student in Sanitary and  
Environmental Engineering, UEPG, Brazil

*Gustavo Frederich Dechandt*

Technical Resident in Projects and Public  
Works, UEPG, Brazil

*Zacarias Alves Siqueira Neto*

Civil Engineer

*Guilherme Araújo Vuitik*

PhD in Hydraulic and Sanitation  
Engineering, UEPG, Brazil

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**Abstract:** With the growth of the timber and brewing industries, the production of waste such as malt bark and wood chips has increased, as has the generation of their respective effluents, which can be harmful because they are rich in nitrate, an essential nutrient for algae, and can therefore generate eutrophication. In order to remove nitrate, conventional reactors have two compartments, one for nitrification and the other for denitrification. With the aim of converting nitrate into molecular nitrogen and advancing research into reactors that promote nitrification and denitrification in a single reactor, this study was carried out to evaluate the possibility of using malt bark and wood chips as a carbon source and support medium in the process of biological conversion of nitrate into gaseous nitrogen. This research consisted of two phases, the first to fix the microorganisms in the bioreactor and the second to treat the effluent. In phase 1, samples were taken every 24 hours in order to visualize the inoculation through nitrate and chemical oxygen demand analysis, while phase 2 consisted of samples taken every 12 hours and comparisons of nitrate removal were made. After phase 2, the reactors and carbon sources were compared, after which nitrate removals of over 58% were achieved for the chip and over 65% for the malt husk. With these results, it is concluded that the sources are viable as a carbon source for the biological conversion of nitrate in NDS reactors.

**Keywords:** Effluent; Malt Bark; Wood Chip; Heterotrophic Bacteria; Nitrogen Removal.

## INTRODUCTION

Stagnating in 2019 and falling by 4.8% in 2020, Brazilian industry returned to growth in 2021, with growth of 4.9%, the country still lagging behind the world average, which after falling by 4.2% in the pandemic, recovered and closed 2021 with 9.4% growth (IEDI, 2021).

One of the main industries in Brazil is the brewing industry, which helped the country recover after the pandemic with growth of 7.6% in 2021 (ALVARENGA; SILVEIRA, 2022). In 2022, the country consumed almost 16 billion liters of beer, a record for the country (ALVARENGA; SILVEIRA, 2022). However, this industry has a problem in the production of waste, in the production of beer, it is estimated that for every 100 L of beer produced, 20 kg of malt husk are generated, this consists of approximately 85 % of the by-products generated, in addition to having no functionality for the industry, it can be a major polluting agent when disposed of incorrectly (MAIA, 2020).

Another prominent industry in the country is the timber industry, since Brazil has 12% of the world's total forest cover, approximately 495 million hectares. In 2022, the solid wood industry's production amounted to approximately R\$26.8 billion, in addition to a balance of U\$3.6 billion, or 5.8% of the country's total (ABIMCI, 2023). This industry generates a large volume of waste, up to 67% of the log volume. One way to make better use of it is to grind it into wood chips, which are rarely used (NASCIMENTO; DUTRA; NUMAZAWA, 2006).

In addition to the waste already mentioned, these and other industries, because they consume large quantities of water, end up generating even more effluents in their production (MATOS et. al. 2010). As industries have grown, so has the production of nitrogenous compounds, many of which are released in these effluents, such as nitrite, nitrate and ammonia (ZORZETTO, 2008).

When effluent rich in nitrogen compounds is disposed of incorrectly in a body of water, it can generate a process called eutrophication, which consists of excessive algae growth due to the high nutrient content, which hinders the development of aerobic beings due to the increase in chemical oxygen demand (COD) (BRANCO *et al.*, 2015). The high content of oxidized nitrogen in the form of

nitrate and nitrite makes the water unfit for consumption, as these can cause diseases such as methemoglobinemia, which can cause heart failure in people and animals. In order to limit concentrations of these and other nutrients, resolutions such as CONAMA 430/11 and CONAMA 357/05 have been introduced to improve public safety.

In order to comply with resolutions such as these, in addition to improving existing technologies, methods have emerged to remove nitrogen from wastewater, such as the reactor that operates with simultaneous nitrification and denitrification (NDS), which has proven effective in reducing COD (SOUZA, 2019) and also organic matter in sanitary sewage (POLAK, 2018).

The NDS reactor is operated with heterotrophic bacteria, which need an external source of organic carbon for their metabolism (DOMINGUES et al, 2007). This reactor focuses on the denitrification and nitrification processes, which consist of transforming ammonia nitrogen into gaseous nitrogen (ZOPPAS; BERNARDES; MENEGUZZI, 2016).

Silva (2021) studied corn cobs as an electron donor and support medium, which proved to be effective in removing nitrate. Rice husk was investigated by Fowdar (2021) performing the same function in a wastewater treatment process and proved to be effective in secondary and tertiary treatment.

Another alternative carbon source studied was polycaprolactan (PCL), which is a biodegradable polymer and was also used as a support medium in an NDS reactor to promote nitrogen removal.

In addition to these, Ling (2021) studied six other carbon sources acting as a support medium: Rice straw, wheat straw, corn stalks and corncobs, soybean stalks and hulls, all of which achieved similar and positive results in the removal of ammoniacal nitrogen.

## OBJECTIVES

### GENERAL OBJECTIVE

To evaluate the possibility of using malt bark and wood chips as a carbon source and support medium in the process of biological conversion of nitrate into gaseous nitrogen.

### SPECIFIC OBJECTIVES

To evaluate the start-up process of batch-type biological reactors with beds packed with malt bark and wood chips, in the biological conversion of nitrate into molecular nitrogen;

To study the efficiency of batch-type biological reactors with beds packed with malt bark and wood chips in the biological conversion of nitrate into molecular nitrogen;

## METHODOLOGY

This study investigated the viability of malt bark and wood chips as a support medium and carbon source in a batch and fixed-bed simultaneous nitrification and denitrification reactor operating with hydraulic detention times of 24 and 12 hours. The methodology consisted of operating three reactors with different media, mini Biobob<sup>®</sup>, malt bark and wood chips, comparing the nitrate removals of each. The following flowchart represents the stages of the research.



## REACTOR

Three bench-scale bioreactors operated in batches were used for the experiment. These consisted of a cylindrical acrylic container with a volume of approximately 574 ml, a height of 13 cm and a diameter of 7.5 cm.

These reactors used two different pumps, one brand Tecnoyon and model LDP-104-6 operating at a flow rate of 160 mL/min, which recirculated the wastewater inside the reactors with malt bark and wood chips. The reactor operating with the mini Biobob® used an OFA environmental pump with an average flow rate of 83 mL/min. The Biobob® in the reactor only acted as a support medium, unlike the reactors with organic matter, which also acted as a carbon source.

## EFFLUENT

The effluent used was agricultural drainage water from a farm in the Castrolanda region of Castro, Paraná (Figures 1 and 2). After it was obtained, it was taken to the laboratory and characterized using tests to determine the following parameters: Solids, nitrate, nitrite, pH, alkalinity and COD, as well as temperature which was measured on site.



Figure 2 - Agricultural drainage water

Source: Authors, 2023

For its recirculation, the effluent was enriched with sodium nitrate in a range of 30 to 50 mg/L in order to better visualize nitrate removal in the malt husk and wood chip reactors.

## INOCULATION (PHASE 1)

Inoculation consists of the stage in which the microorganisms responsible for the process are fixed in the reactor, coming entirely from the collected drainage water. For this process to take place, the effluent is recirculated in the reactors and the concentration of nitrate and COD is periodically assessed. This recirculation took place nine times with a TDH of 24 hours.

As the aim of this stage is to fix the heterotrophic bacteria, which are responsible for removing the nitrogen present in the drainage water in the reactor, this fixation is observed when the chemical oxygen demand (COD) tends to zero associated with the drop in nitrate concentration, as the former indicates the amount of dissolved oxygen and the latter indicates that the bacteria present there have transformed the nitrate into molecular nitrogen.



Figure 1 - Representation of the municipality of Castro in the state of Paraná

Source: Authors, 2023

## REACTOR OPERATION (PHASE 2)

Once the inoculation phase was complete, the reactor began operating. The effluent was enriched with sodium nitrate and recirculated in the reactor. A sample of the effluent was taken every 12 hours to determine the amount of nitrate removed, after which the effluent was discarded and the reactors refilled.

## EXPERIMENTAL PLANNING

The input variable in the experiment was the TDH and the output variable was the nitrate conversion efficiency. Analysis of variance (ANOVA) was carried out to determine whether there was a statistically significant difference between the data in order to determine whether wood chips and corn cobs were efficient in the biological conversion of nitrate into molecular nitrogen.

## RESULTS

### CHARACTERIZATION OF WASTEWATER

The first result found was about the wastewater, since laboratory tests were carried out to really understand the characteristics of the effluent used in the study. The parameters analyzed were: Alkalinity, conductivity, COD, nitrate, nitrite, pH, solids and temperature.

Parameter	Value
COD (mg/L)	0
Temperature (°C)	13
Nitrite (mg/L)	0,003
Nitrate (mg/L)	2,36
Total solids (mg/L)	53,33
Fixed solids (mg/L)	20
Volatile solids (mg/L)	33,33
Alkalinity (ppm)	9,194
pH	6,04

Table 1 - Wastewater parameters

Source: Authors, 2023

The chemical oxygen demand showed a zero result in the analysis. This value represents the absence of organic matter from carbon, which is essential for the heterotrophic conversion of nitrate and molecular nitrogen (PHILIPS, 2008). On the other hand, the concentration of volatile solids (33.33 mg/L) indicates the possible presence of nitrogenous organic matter in the crop wastewater (SOUSA, 1999).

The temperature of 13 °C is within the range of 8 to 28 °C indicated by Zoppas (2016) as a range in which there is an increase in bacterial activity. In addition, the hydrogen potential value (6.04) is close to neutrality (ph = 7.00), and because of this value, it was not necessary to add an alkalizing agent in addition to the alkalinity present (9.194 ppm) (SOUTO, 2009).

With regard to nitrogen compounds, enrichment was necessary throughout the experiment in order to obtain a better visualization of the experiment, this was due to the low concentration of nitrate in the effluent (2.36 mg/L), while nitrate showed a low concentration (0.003 mg/L), which indicated that nitrification of the wastewater had already occurred (VON SPERLING, 1996).

### PHASE I

After collecting the drainage water, the first phase began, which consisted of supplying the raw effluent enriched with nitrate in order to obtain a value between 8 and 28 mg/L. Tests were carried out daily respecting the TDH of 24 h per collection, there was no change of effluent during the process. The values found can be seen in figure 3.

At this stage, it can be seen from the values in the graph that the inoculation process did not take place in the reactor filled with malt husk, since the nitrate concentration did not reduce considerably over time. Consequently, it can be concluded that malt husk was not suitable as a support medium and carbon source for the biological nitrogen conversion process.



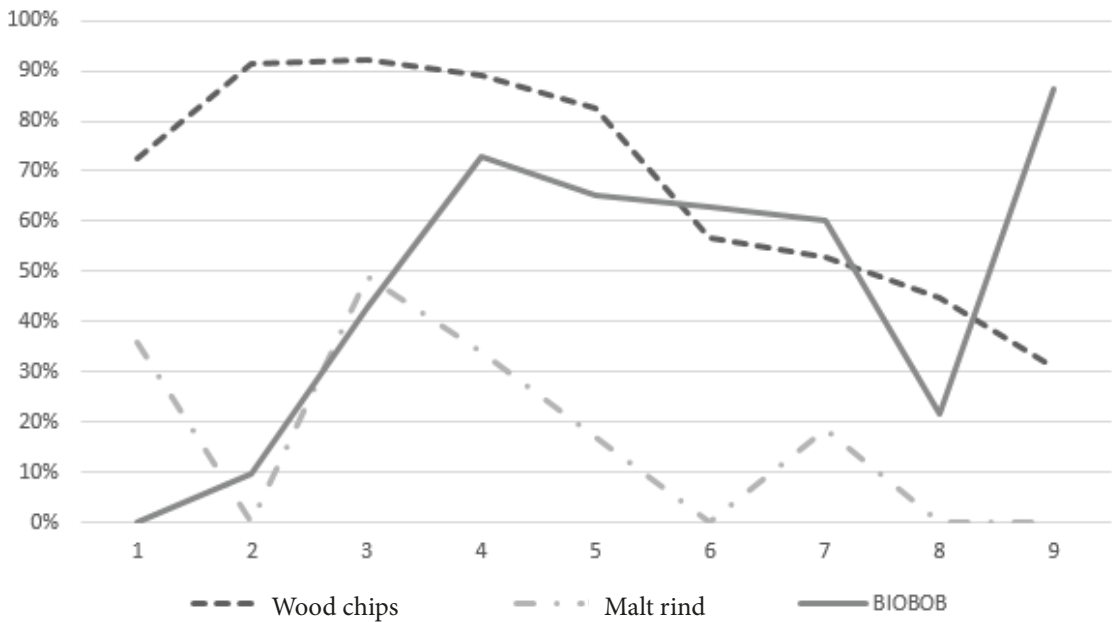


Figure 3 - Nitrate removal comparison - Phase 1

Source: Authors, 2023

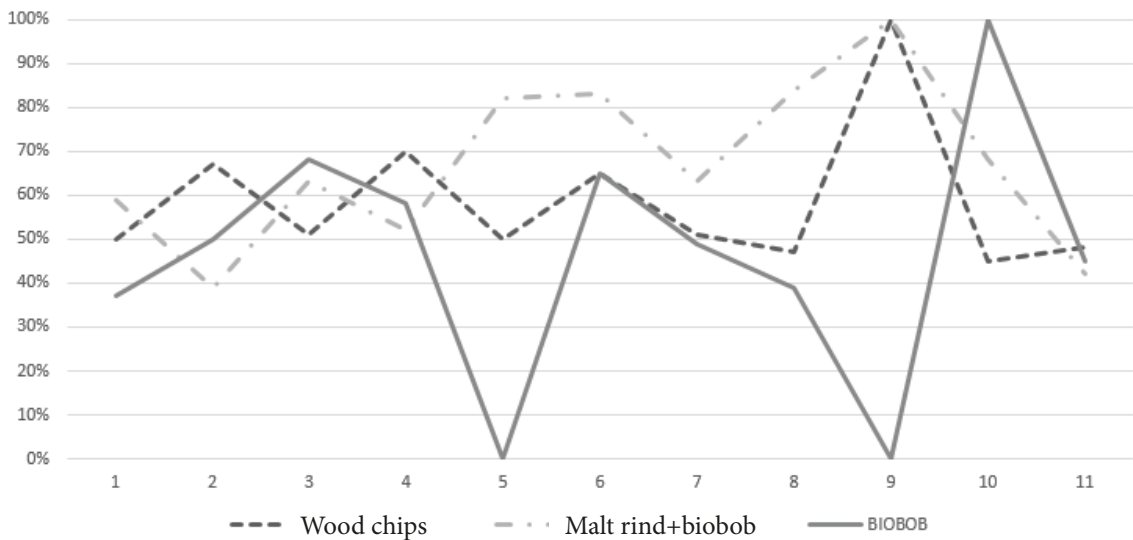


Figure 4 - Nitrate removal comparison - Phase 2

Source: Authors, 2023

The reactor operated with wood chips showed a high conversion efficiency in the first five tests, but this efficiency decreased over the course of the process. This increase may be due to the hydrolysis of nitrogen compounds present in the chips as a result of their prolonged immersion in aqueous media.

In relation to the reactor fed with Biobob®, it showed considerably high values in nitrate removal (above 40%, a result that was not expected, since the wastewater had low carbonaceous organic matter, necessary in the denitrification process, so the reactor with Biobob® should not remove nitrate, but the continuous increase in removal efficiency indicates adaptation of the biomass.

## PHASE II

In the second phase, the tests were carried out every 12 hours, with the effluent being changed between each test, i.e. the reactor was emptied at the end of each test and fed with enriched effluent for the next cycle. In addition, malt husk proved to be unviable if used as a support medium and carbon source, so this phase tested whether it has the capacity to optimize nitrate removal, as a carbon source, if the reactor has another support medium, in this case Biobob®.

The graph shows the nitrate removal efficiency of the three reactors. The reactor filled with malt husk and Biobob® showed an average efficiency of  $66\% \pm 22\%$ , while the reactor operating with wood chips showed an average conversion of  $58 \pm 16\%$  and the one filled only with Biobob®  $46 \pm 29\%$ .

Denitrification was observed in the reactor used only with Biobob®, which was not expected, but it is believed that autotrophic denitrification occurred, i.e. without an organic carbon source. This is due to an alternative route for ammonia oxidation which occurs simultaneously with nitrite reduction in an anaerobic environment, such as the ANAMMOX process (ALVES, 2018).

The woodchip reactor was found to comply with the Conama 430/2011 resolutions, according to which the maximum concentration of nitrate allowed in effluent discharges is 10 mg/L, with the exception of test 7. It showed an efficiency of more than 58 % and can therefore be used for the biological conversion of nitrogen

Based on the efficiency shown, the malt husk reactor can be used to treat effluents, as we can see considerable efficiency (above 65 %) for a carbon source that does not cost the industry anything.

## CONCLUSION

Malt husk did not prove to be viable for use as a support medium and carbon source in the biological conversion of nitrate, as it did not prove useful for aggregating the bacteria themselves, since the concentration did not drop from 7.33 mg/L, reaching an average of only 11.82 mg/L, showing that inoculation did not occur, but acting only as a carbon source it proved to be viable for the industry.

During the start-up process (inoculation) of the reactor with wood chips, it proved capable of removing nitrate. Despite this, its average efficiency decreased over the course of the inoculation, which may be due to the hydrolysis of nitrogenous compounds present in the wood chips.

During the second phase, the efficiency of the biological conversion of nitrate into molecular nitrogen in the reactor with wood chips ( $58.5 \pm 16\%$ ) was again higher than in the reactor with the Biobob® support medium ( $46.5 \pm 29\%$ ). This difference, however, is not considered significant, since both reactors showed large deviations from the standards of their average removal efficiencies. Furthermore, these removals possibly originated from different metabolic routes

It is concluded that the use of wood chips as a carbon source and support medium in the process of biological conversion of nitrate into molecular nitrogen is feasible, since it was able to comply with the regulations regarding nitrate concentrations in effluents. Based on the nitrate removal efficiency in a reactor using malt bark and Biobob® ( $66 \pm 22.81\%$ ), with a TDH of 12 h, it can be concluded that malt bark optimizes the biological conversion of nitrogen when used only as a source of organic matter in a reactor with Biobob® as a support medium, with possible autotrophic denitrification routes, since the reactor with Biobob® showed an efficiency of only  $20 \pm 80.03\%$ .

The analysis of variance (ANOVA) test was then carried out to determine whether there was a statistically significant difference between the data, for which a significance level of 5% was set, after which it was concluded

that the reactors filled with malt husk and the one filled with malt husk and Biobob® showed no statistically significant difference between them and the Biobob® reactor ( $F=1.896$   $p$ -value=0.168).

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