Heterotrophic Denitrification of Wastewater by Using Melanoidin as Sole Carbon Source

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Removal of nitrate through heterotrophic denitrification is a highly effective method for treating water and wastewater containing these contaminants. Proper management of wastewater is critical to mitigating its harmful effects on both human health and the environment. This study investigates the biodegradation and removal of nitrate and nitrite by introducing melanoidin as a carbon source, focusing on the impact of a batch test strategy using heterotrophic denitrification. The process employed inoculum from a Master Culture Reactor (MCR) over 48 hours, involving four controls (C1, C2, C3, C4) and five test samples with varying dilutions of melanoidin—100 ppm, 250 ppm, 500 ppm, 700 ppm, and 1000 ppm. The results reveal a significant reduction in nitrate, nitrite, and Total Organic Carbon (TOC) levels, particularly in the 100 ppm, 250 ppm, and 500 ppm melanoidin dilutions, indicating successful toxicity removal. However, the 750 ppm and 1000 ppm dilutions did not show effective TOC removal through denitrification. The subsequent denitrification of samples T1, T2, and T3 demonstrated the potent synergy between treatment processes, with a C ratio of 2:1 and 3:1, leading to 98.14% nitrate removal within 48 hours. High-Performance Ion Chromatography (HPIC) analysis of the denitrified samples confirmed the complete elimination of toxicity. These findings emphasize the critical role of melanoidin as a carbon source in enhancing denitrification, enabling thorough nitrate degradation and detoxification. This study highlights the importance of adopting innovative treatment approaches to address the escalating challenges in wastewater management.

Keywords: Heterotrophic, Denitrification, Master Culture Reactor, Toxification 1.

Introduction:

Nitrogen pollution is a pervasive environmental issue with severe consequences for ecosystems and human health [1]. Biological nitrogen removal from wastewater is crucial, as excessive nitrogen levels can lead to detrimental effects, including eutrophication of surface waters and health conditions such as "blue baby syndrome" linked to high nitrate (NO_3^-) concentrations [2]. Therefore, effective nitrogen removal from wastewater is a vital goal in environmental management and engineering. Denitrification, a microbial process that converts nitrates to nitrogen gas, offers a promising solution by efficiently removing nitrogen from water [3].

Denitrification facilitates the conversion of nitrogen oxides (NO_3^-, NO_2^-) into reduced forms, primarily yielding gaseous by-products such as nitrous oxide (N_2O) and nitrogen gas (N_2). This biological process is driven by denitrifying microorganisms, or denitrifiers, that thrive under anaerobic conditions [4]. Heterotrophic denitrification, which involves the use of organic carbon sources, is considered the most cost-effective method for mitigating nitrate pollution [5], as most bacteria involved in anaerobic denitrification are heterotrophic [6].

To sustain bacterial growth and provide the energy needed for converting nitrate into gaseous nitrogen, an external carbon source must be supplied. However, the toxicity of certain carbon sources poses a significant challenge, making the use of less toxic alternatives more attractive. Various carbon sources, such as methanol, glycerol, and ethanol, have been explored. Zhang et al. (2009) reported successful outcomes using glucose and ethanol to initiate the growth phase of microorganisms. Nonetheless, the high costs associated with these sources limit their practicality for large-scale applications. This limitation has led to the exploration of melanoidin, a by-product of industrial processes like molasses fermentation and sugar refining, as a viable alternative.

Melanoidin, despite its complex composition and significant organic content, has emerged as a promising carbon source for wastewater treatment due to its dual role as both a recalcitrant pollutant and a resource for denitrifying bacteria. Utilizing melanoidin not only facilitates nitrogen removal but also addresses the challenge of managing this persistent pollutant [7].

This study investigates the use of melanoidin as a sole carbon source in heterotrophic denitrification for wastewater treatment. The research aims to evaluate the effectiveness of melanoidin in promoting denitrification, identify the specific microbial communities involved, and determine the operational factors that optimize the process. By enhancing our understanding of melanoidin's dual roles as a resource and a pollutant, this study contributes to the development of more sustainable wastewater treatment methods.

Objectives:

- To analyse the feasibility of using melanoidin as the sole carbon source for heterotrophic denitrification.
- Design and optimize bioreactor systems to effectively remove melanoidin and nitrates concurrently from wastewater.
- To determine the reduction of Nitrate and Nitrite.

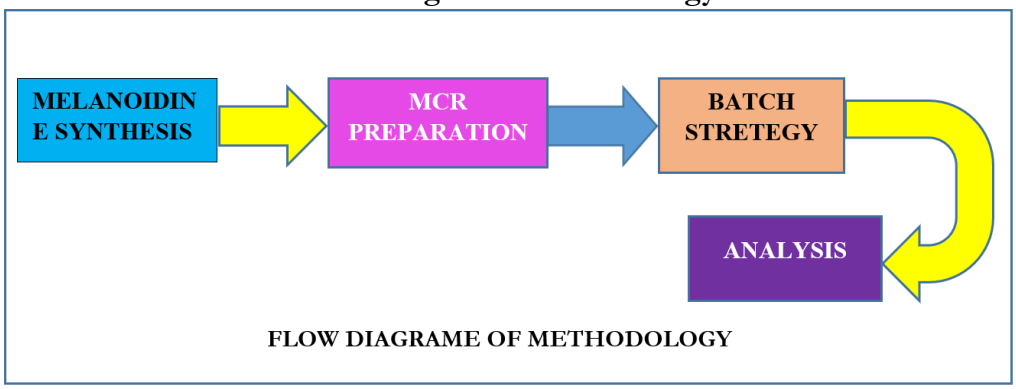
Novelty Statement:

This study presents a novel approach to wastewater purification by utilizing melanoidin as a carbon source. As an industrial by-product, melanoidin poses challenges for degradation, making its use in heterotrophic denitrification complex. However, melanoidin offers a dual benefit: it not only addresses the challenge of disposal but also enhances nitrogen removal efficiency. The research provides critical insights into the microbial communities and metabolic processes involved, enabling the optimization of key operational parameters for effective denitrification. This innovative approach supports sustainability by reducing both operational

costs and environmental impact, with promising potential for broader applications in environmental biotechnology. By integrating melanoidin into wastewater treatment processes, this study contributes to significant advancements in developing more sustainable and efficient practices in the field.

Materials and Methods:

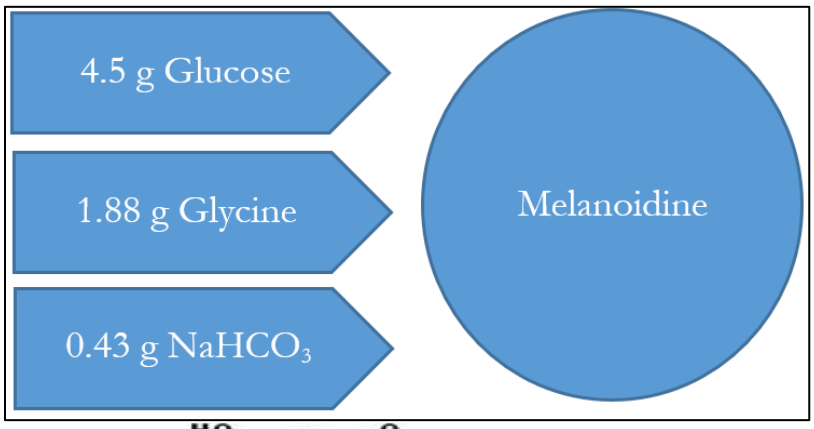
Flow Diagram of Methodology

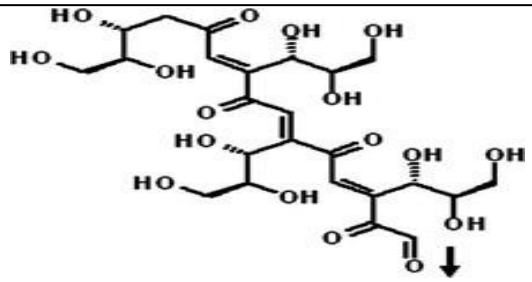


FLOW DIAGRAME OF METHODOLOGY

Melanoidin Synthesis:

To synthesize melanoidin, 4.5 grams of glucose, 1.88 grams of glycine, and 0.43 grams of sodium bicarbonate (NaHCO3) were dissolved in 100 mL of distilled water. The resulting solution was then heated to 70°C and maintained at this temperature for 7 hours to facilitate the reaction.





Structure of melanoidin (Singh and Santal, 2013) [7]

MCR Preparation:

The master culture reactor (MCR) was operated under heterotrophic conditions, with melanoidin used as an external carbon source. To enhance the process, 10 mL of activated sludge from a municipal wastewater treatment plant was introduced into the reactor. Additionally, 100 mg/L of NO3-N was supplied to support the denitrification process. The MCR was successfully operated using a fed-batch approach, where 40% of the reactor's liquid volume was removed and replaced with a fresh medium containing NO3-N every four days. The reactor was sealed

with a plastic cover and a butyl rubber stopper to maintain anoxic conditions, which were further ensured by purging the system with N2 gas for ten minutes. The reactor was then incubated at 30±1°C with continuous agitation at 150 rpm.

Batch Test Strategy:

A 20 mL serum vial was used as the vessel for the mixed culture batch mode experiment. The trial involved the use of distilled water, inoculum, and treated samples. Each vial was prepared with 8.1 mL of medium, 0.4 mL of NO3, and 5 mL of inoculum at a pH of 6. Various melanoidin dilutions—100 ppm, 250 ppm, 500 ppm, 750 ppm, and 1000 ppm—were tested. The total liquid volume in each vial was 18 mL, leaving a 2 mL headspace. Additionally, four control trials were conducted using test vials that were shaken at 150 rpm, incubated at 30°C, and purged with N2 gas for five minutes to create anoxic conditions.

Analysis:

The pH was measured using a "Hanna HI8424" pH meter, while Total Organic Carbon (TOC) analysis was performed with a TOC analyzer. Nitrate and nitrite concentrations were determined using the anion column of a Shimadzu LC20Adsp High-Performance Ion Chromatography (HPIC) system.

Results and Discussion:

Following the reactor's start-up, denitrification conditions evolved in response to variations in the carbon-to-nitrogen (C) ratio, the type of carbon source used, and the dilution levels of melanoidin. For the batch tests, inoculum was sourced from a Master Culture Reactor (MCR) that had been incubated for 28 days, during which denitrification occurred through the removal of NO3-N.

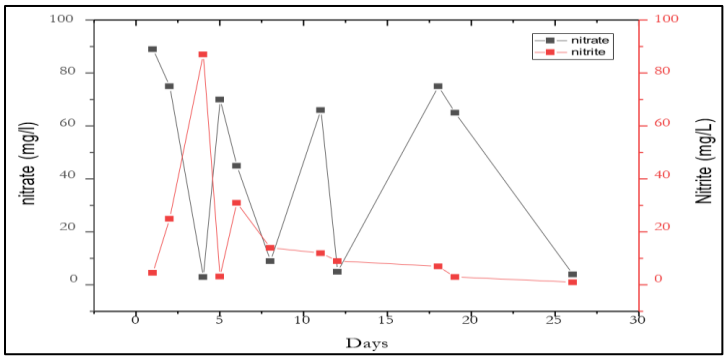


Figure 1: MCR Profile

The master culture reactor successfully eliminated nitrate within 26 to 30 days. Initially, nitrite levels increased over the first 4 to 5 days but gradually decreased until they were completely eliminated by the end of the period. To enhance the efficiency of this process, external carbon sources, specifically melanoidin, were introduced during the same timeframe.

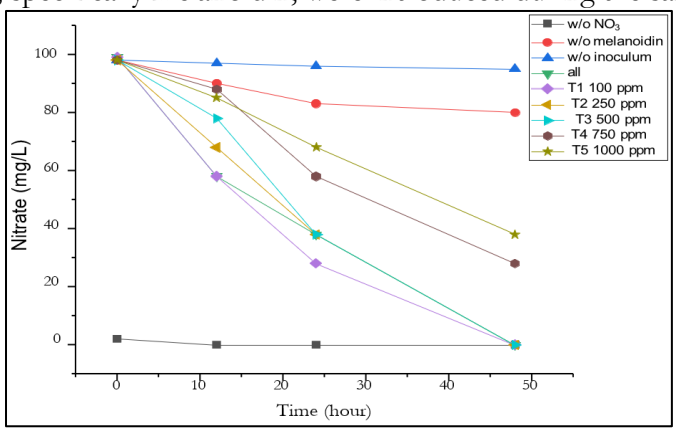


Figure 2: Nitrate reduction in denitrification

Effects of Denitrification on Nitrate:

Recent research findings indicate that nitrate elimination was successfully achieved within 48 hours during a batch test experiment. The experiment included four control runs: C1 (without melanoidin), C2 (without NO3), C3 (without inoculum), and C4 (with all components present). All diluted test samples were included in the experiment, and the controls (C1 to C4) were used to evaluate the influence of various factors on the denitrification process.

Effects of Denitrification on Nitrite:

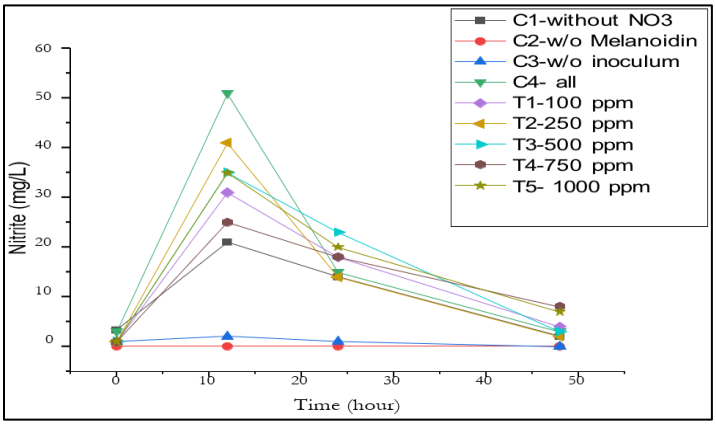


Figure 3: Nitrite reduction in denitrification

The graph illustrates the elimination of nitrite, which initially accumulated within the first 10 to 15 minutes. However, the nitrite levels steadily decreased over time until they were completely eradicated. In contrast, no changes were observed in the C2 and C3 controls, indicating that denitrification did not occur in these conditions.

Effects of pH on Denitrification:

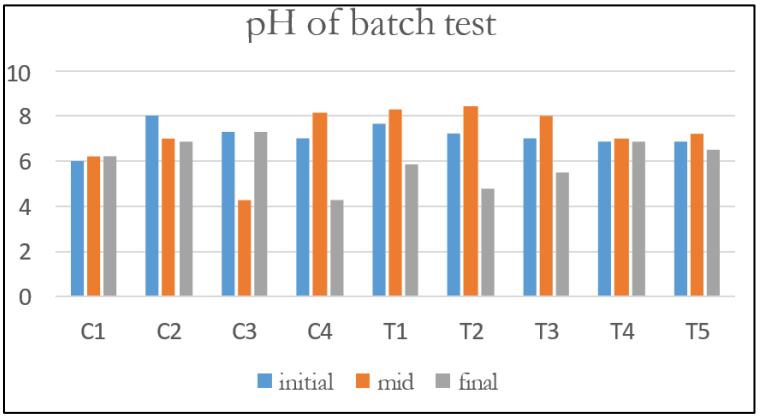


Figure 4: pH of batch test

The graph illustrates the pH changes during the batch test. A decrease in pH in the C4 sample indicates that complete denitrification occurred. Samples T1, T2, and T3 show pH variations associated with denitrification, while T4 and T5 exhibit only minimal changes.

Effects of Denitrification on TOC:

This graph demonstrates the reduction in Total Organic Carbon (TOC). Significant TOC reduction was achieved in samples C4, T1, T2, and T3. In contrast, no changes in TOC were observed in controls C1, C2, and C3, while T4 and T5 showed a slight decrease. This process effectively reduced nitrate and nitrite concentrations, thereby mitigating the risk of nitrate-nitrite toxicity. The specified reduction was successfully achieved within 48 hours.

Discussions:

The study focuses on the removal of total organic carbon (TOC), nitrate, and nitrite from wastewater through heterotrophic denitrification, using melanoidin as a carbon source. The findings provide significant insights into the mechanisms and effectiveness of denitrification

in the presence of melanoidin [8], a complex and resistant organic molecule. Various dilutions were utilized to identify the optimal operating conditions for the system. Specifically, carbon-to-nitrogen (C) ratios of 2:1 and 3:1 were employed, as the capacity for denitrification depends on the concentration of biodegradable substrates, particularly the C ratio [9, 10]. The pH decrease observed in sample C4 indicates that denitrification was fully achieved, while samples T1, T2, and T3 displayed pH variations related to denitrification. In contrast, T4 and T5 showed minimal changes. The study found that denitrification was complete at a pH below 4, and neutral pH levels enhanced nitrate removal [11]. Nitrite accumulation initially increased over 4 to 5 days but gradually decreased until complete elimination. The introduction of melanoidin as a carbon source significantly improved nitrate removal [12]. In the control samples, nitrate removal was sluggish, except in C4 and the diluted samples T4 and T5 with a specific C ratio [12]. Throughout the denitrification process, TOC concentrations progressively decreased. Within 48 hours, all carbon sources were depleted, correlating with fluctuations in NO3-N concentration. The effluent TOC concentration in the T4 and T5 groups was higher than in the T1, T2, and T3 groups, likely due to reduced carbon source addition [13]. This outcome not only reflects the breakdown of organic matter but also signifies the removal of precursors for nitrate and nitrite formation [14].

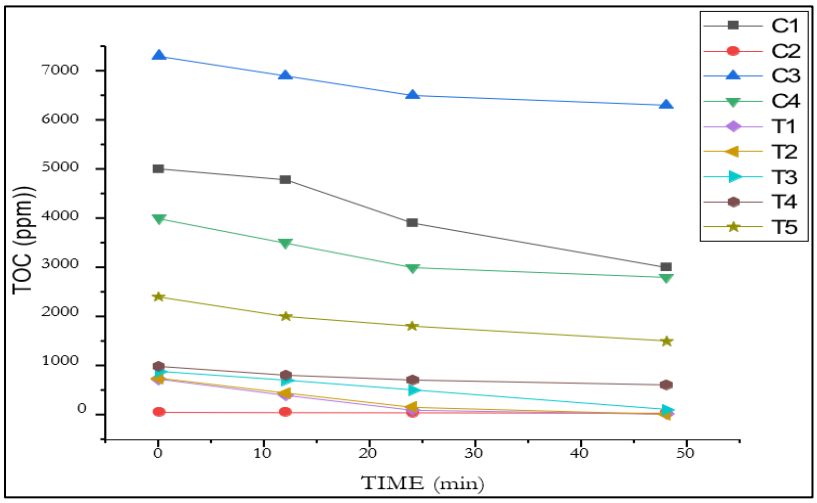


Figure 5: TOC removal

Conclusion:

Biological treatment of wastewater through heterotrophic denitrification using melanoidin as the sole carbon source demonstrated that optimal denitrification was achieved at concentrations of 250 ppm and 500 ppm. The batch test results reveal a concentration-dependent impact of melanoidin on denitrification. Lower concentrations (100 ppm, 250 ppm, and 500 ppm) exhibited a significant increase in denitrification efficiency (T1, T2, and T3), indicating that these levels fall within an optimal range for melanoidin application. Conversely, higher concentrations (750 ppm and 1000 ppm) led to only slight decreases in denitrification efficiency, as well as in TOC and nitrate removal (T4 and T5), highlighting the importance of optimizing melanoidin concentration. The findings suggest that the optimal concentration range for effective denitrification lies between 100 ppm, 250 ppm, and 500 ppm. Concentrations outside this range may have adverse effects on other parameters. Using a C ratio of 2:1 and 3:1, it was possible to remove 98.14% of nitrate within 48 hours. Further investigation is needed to elucidate the underlying mechanisms and refine concentration guidelines for the use of melanoidin in denitrification processes.

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