|  |  |
| --- | --- |
| cetlogo ***CHEMICAL ENGINEERING TRANSACTIONS*** ***VOL. xxx, 2025*** | A publication ofaidiclogo_grande |
| The Italian Associationof Chemical EngineeringOnline at www.cetjournal.it |
| Guest Editors: David Bogle, Flavio Manenti, Piero SalatinoCopyright © 2025, AIDIC Servizi S.r.l.**ISBN** 979-12-81206-20-5; **ISSN** 2283-9216 |

Nitrate Removal in Wastewater Using Elephant and Vetiver Grasses

Magezi K. Mabaso\*, Evans M. Chirwa, Shepherd M. Tichapondwa

Water Utilisation and Environmental Engineering Division, Department of Chemical Engineering, University of Pretoria, Pretoria 0002, South Africa. Mabasom@dws.gov.za

Contamination of natural ecosystems by nutrients and organic pollutants poses a serious threat to the health and survival of humans, animals, plants, and microorganisms. Although various chemical and physical treatments have been used globally for centuries, these technologies often fall short due to high maintenance costs and limited effectiveness, especially in areas with excessive nitrate concentrations. Bioremediation offers an eco-friendly and efficient solution for restoring nitrate-contaminated waters through biological processes, particularly using grass species. Given their high effectiveness, low cost, and wide availability, perennial grasses, whether naturally occurring or genetically engineered, are gaining increasing attention for nutrient removal. This report provides a comprehensive review and critical analysis of nitrate removal in wastewater. Vetiver grass and elephant grass were used to compare their nitrate removal efficiency.

* 1. Introduction

Human activities in South Africa significantly accelerate the eutrophication of natural waters by releasing nutrients like phosphorus (P) and nitrogen (N). Freshwater contamination is a worldwide challenge that demands collective efforts, therefore, no one technology can deliver a complete solution to this problem (Razzak et al., 2022). Severe nitrate contamination, especially in water sources, can lead to serious health, environmental, and agricultural issues (Pietrelli et al., 2022). This is because nitrate is highly water soluble and can accumulate to harmful levels in groundwater, rivers, lakes, and drinking water supplies. Excessive nitrate in the receiving water contributes to excessive algal growth, which leads to oxygen depletion, resulting in fish kills (Mehariya et al., 2021)

Nitrate enters water bodies from point sources, such as wastewater treatment plants, and diffuse sources, like fertilizers and animal or bird excreta (Li et al., 2022). Controlling nutrient emissions from point sources is more manageable than from diffuse sources, so wastewater discharges are often subject to strict regulations (Faruque et al., 2023). Municipal wastewater treatment commonly uses various technologies, including trickling filters, activated sludge, and waste stabilization ponds (Sanchez-Zurano et al., 2020). The problem with these traditional processes primarily hinges on bacteria that emit carbon dioxide (CO2) into the environment (Ho and Goethals, 2020).

Nitrate removal in wastewater effluents is done through biological or chemical methods. Chemical removal is essential when using biological filters for wastewater treatment, while biological removal is more cost-effective and works well with various activated sludge systems. Each method has its advantages and disadvantages (Fazal et al., 2021).

The key nutrient elements essential for sustaining and reproducing life are oxygen (O), hydrogen (H), nitrogen (N), phosphorus (P), and carbon (C). Limiting the release of these nutrients helps control the growth of unwanted aquatic organisms (Hossain et al., 2022). Wastewater treatment removes organic and inorganic carbon through oxidation-reduction reactions. However, some soluble, non-biodegradable carbon remains and is discharged with the effluent. In contrast, the soluble, biodegradable carbon is rapidly broken down by microorganisms (Fallahi et al., 2020).

A portion of the non-biodegradable particulate matter is removed in the primary sedimentation tanks, while the rest is disposed of with the waste sludge. Meanwhile, the biodegradable particulate matter is slowly broken down by microorganisms (Rezvani et al., 2020).

In wastewater, nitrogen is converted from ammoniacal and organic forms to nitrate in an aerobic environment. Under anoxic conditions, this nitrate is reduced to nitrogen gas through denitrification. During this process, the most easily biodegradable carbonaceous material acts as the main food source for microorganisms (Shafiquzzaman et al., 2023).

When microorganisms enter an anoxic or aerobic environment, they utilize the SCFA for growth and replenish their poly-P stores by absorbing orthophosphate from the wastewater, resulting in greater phosphorus uptake compared to systems without anaerobic zones. The stored phosphorus is then removed from the wastewater along with the sludge that is discarded from the system (Wang et al., 2020)

In wastewater treatment, phosphorus can also be removed through chemical methods, using metallic salts such as aluminum or iron, or by adding lime (Zhou et al., 2023). The drawback of using metallic salts is that they reduce the pH and alkalinity of the water while increasing the concentration of dissolved salts in the effluent. In contrast, the disadvantage of using lime is that the required dosage varies depending on the pH and alkalinity of the wastewater (Zubair et al., 2020).

**2. Materials and Methods**

2.1 Nitrate Solution

Sodium nitrite (NaNO2) was utilized to prepare nitrate ions (NO₃⁻) solution. 13.799 g of nitrite salt was dissolved in 500 mL of distilled water in a glass beaker. The solution was stirred until NaNO2 had completely dissolved. This was then transferred into the 3L bucket, where 1.5L of distilled water was added up to the 2L mark. Hydrochloric acid was used for pH adjustment. Tap water was used to replenish the buckets, while distilled water was employed for water sample analysis.

3. Elephant grass and Vetiver grass

Elephant grass was obtained from SMR Africa (Pty) Ltd in Bronkhorstspruit, South Africa, while Vetiver grass slips were obtained from Free Choice Progressive learning (Pty) Ltd, Vetiver Grass South Africa in White River. The two grasses, which were initially grown in soil, were introduced to the water medium with careful handling to prevent root damage. They were cleaned and set aside in water for two weeks to acclimate. Chemical elements such as nitrogen, phosphorus, and potassium, which are used as nutrients, were introduced to support root and shoot growth to a length of 35 cm. After acclimatization, the shoot length was trimmed to 30 cm.

Each of the two grasses was then placed in 2 L of nitrate ions (NO₃⁻) solutions in three transparent 3 L buckets to begin the nitrate ions (NO₃⁻) removal efficiency experiment. Water level reductions over time were attributed to evaporation and plant uptake. To ensure that concentration changes were not caused by evaporation, water was replenished in each bucket before samples were taken, assuming the evaporated water did not have nitrate ions (NO₃⁻) solution.

Analysis for the water sample was done every two days, whereas grass samples were gathered at a 10-day interval. The shoots and roots for each grass were carefully separated, thoroughly washed with tap water to remove any unwanted particles and then rinsed again with distilled water.  The washed shoots and roots for the 2 grasses were thoroughly dried at 60°C until all moisture was removed. The grinder and bowl were used to grind the dried shoots and roots.

 10 mL of nitric acid and 2 drops of hydrogen peroxide were used to treat 0.1 g of each grass sample for 2 days. Nitrate (NO3) and phosphate (PO4) analysis were performed using a Spectrophotometer after the leachate was filtered through 0.45 μm syringe filters and diluted with 10 mL of distilled water.

The first phase of the study aimed to determine the impact of initial nitrate ions (NO₃⁻) concentrations on nitrate ions (NO₃⁻) uptake, nitrate ions (NO₃⁻) accumulation, and their effects on elephant grass and vetiver grass growth. To achieve this, six 3L buckets containing 2-liter nitrate ions (NO₃⁻) solutions were prepared at initial concentrations of 5 ppm, 10 ppm, and 30 ppm with two slips of elephant grass and vetiver grass, which were placed in each bucket. The experiment lasted for three months and was conducted in triplicate to minimize the impact of grass harvesting on nitrate ions (NO₃⁻) uptake rates.

The second phase focused on assessing the impacts of nitrate ions (NO₃⁻) uptake on the grass density of elephant and vetiver grasses. Experiments were performed using 5 ppm, 10 ppm, and 30 ppm concentrations of nitrate ions (NO₃⁻) solutions in 3-liter buckets, with slip densities categorized into three groups: low (10 slips), medium (15 slips), and high (20 slips) of each grass. nitrate ions (NO₃⁻) uptake was analyzed by monitoring concentration changes in the buckets and nitrate ions (NO₃⁻) accumulation in different grass parts.

  

  **Elephant Grass Vetiver Grass**

3.1 Nitrate (NO₃⁻) Measurement

Nitrate ions (NO₃⁻) concentrations were determined using a visible-range spectrophotometer at a wavelength of 540 nm. This measurement followed the reduction of NO₃⁻ to NO₂⁻ by using cadmium as a reducing agent. The measurement followed the reaction of NO₂⁻ with sulfanilamide and N-(1-naphthyl) ethylenediamine dihydrochloride in an acidic medium solution, which produces a pink azo dye. Trace amounts of total NO₃⁻ in plant samples were analyzed using a Colorimetric (Griess) method.

3.2 Removal Efficiency

Removal performance refers to the degree of nutrients removed from the solution. where C0 is the initial concentration of solution and Ct is the final concentration of solution at time t. It was calculated as follows:

$$ x= \frac{C\_{0}- C\_{t}}{C\_{0}} ×100 (1) $$

3.3 Bioaccumulation Factor

The Bioaccumulation factor indicates the plant’s ability to accumulate the metal relative to the metal concentration in the external solution. The BAF is an essential parameter for assessing a plant's ability to accumulate (NO₃⁻) from its surrounding environment. It is calculated using the subsequent equation:

$$x= \frac{C\_{p}}{C\_{w}} (2)$$

where Cp​ = Concentration of NO₃⁻ in the 2 grases (mg/kg or μg/g) while Cw is the initial concentration of NO₃⁻ in the wastewater (mg/L or μg/mL)

* 1. Results and Discussion
		1. Effects of varying NO₃⁻ concentrations

The rhizofiltration efficiency of lemon grass, elephant grass, and vetiver grass at varying NO₃⁻ concentrations of 5ppm, 10ppm, and 30ppm was assessed to determine the impact of concentration levels on NO₃⁻ uptake and plant growth

*Table 1: Initial* NO₃⁻ *concentration of 5ppm with elephant and vetiver grass over 10 days*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Time (d)**  | **Elephant Grass (ppm)**  | **Vetiver Grass (ppm)**  |  |  |
| **Day 1** | **5.00**  | **5.00**  |  |  |
| **Day 2** | **4.16**  | **4.05**  |  |  |
| **Day 3** | **3.71**  | **3.59**  |  |  |
| **Day 4** | **3.23**  | **3.15**  |  |  |
| **Day 5** | **2.74**  | **2.67**  |  |  |
| **Day 6** | **2.33**  | **2.21**  |  |  |
| **Day 7** | **1.82**  | **1.75**  |  |  |
| **Day 8** | **1.41**  | **1.29**  |  |  |
| **Day 9** | **1.14**  | **1.03**  |  |  |
| **Day 10** | **0.94**  | **0.57**  |  |  |

*Figure 1. Shows elephant grass and vetiver grass’s NO3- removal efficiency.*

*Table 2:* NO₃⁻ *concentration of 10ppm with elephant and vetiver grass for 10 days*

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Time (d)**  | **Elephant Grass (ppm)**  | **Vetiver Grass (ppm)**  |  |  |  |
| **Day1** | **10.00**  | **10.00**  |  |  |  |
| **Day 2** | **9.31**  | **9.19**  |  |  |  |
| **Day 3** | **8.85** | **8.72**  |  |  |  |
| **Day 4** | **8.39**  | **8.25**  |  |  |  |
| **Day 5** | **7.93**  | **7.79**  |  |  |  |
| **Day 6** | **7.47**  | **7.33**  |  |  |  |
| **Day 7** | **7.01**  | **6.87**  |  |  |  |
| **Day 8** | **6.55**  | **6.41**  |  |  |  |
| **Day 9** | **6.09**  | **5.95**  |  |  |  |
| **Day 10** | **5.69**  | **5.49**  |  |  |  |

*Figure 2. Shows elephant grass and vetiver grass’s NO3- removal efficiency.*

*Table 3:* NO₃⁻ *concentration of 30ppm with elephant and vetiver grass for 10 days*

|  |  |  |  |
| --- | --- | --- | --- |
|  **Time (d)**  | **Elephant Grass (ppm)**  | **Vetiver Grass (ppm)**  |  |
|  **Day 1** | **30.00**  | **30.00**  |  |
|  **Day 2** | **29.32**  | **29.23**  |  |
|  **Day 3** | **28.76**  | **28.64**  |  |
|  **Day 4** | **28.21**  | **28.11**  |  |
|  **Day 5** | **27.64**  | **27.55**  |  |
|  **Day 6** | **27.08**  | **26.99**  |  |
|  **Day 7** | **26.52**  | **26.43**  |  |
|  **Day 8** | **25.96**  | **25.87**  |  |
|  **Day 9** | **25.41**  | **25.33**  |  |
|  **Day 10** | **24.82**  | **24.70**  |  |

*Figure 3. Shows elephant grass and vetiver grass’s* *NO3- removal efficiency.*

Figures 1-3 illustrate NO₃⁻ removal at varying concentrations over a 10-day period. At an initial concentration of 5 ppm, NO₃⁻ level decreased to 0.74 ppm for elephant grass and 0.38 ppm for vetiver grass, corresponding to removal efficiencies of 85.2% and 92.4%, respectively. At the second concentration of 10 ppm, NO₃⁻ level dropped to 5.17 ppm for elephant grass and 5.03 ppm for vetiver grass, reflecting removal efficiencies of 42.3% and 49.7%, respectively. For the highest concentration of 30 ppm, NO₃⁻ levels decreased slightly to 24.28 ppm for elephant grass and 24.16 ppm for vetiver grass, indicating removal efficiencies of 19.06%, 5.6%, and 19.46%, respectively.

* 1. Conclusion

This research supports Sustainable Development Goals (SDG) 6 and 14. SDG 6 targets to ensure the supply and sustainable control of water and sanitation for all, while SDG 14 pursues to preserve and sustainably use the oceans, seas, and marine resources for sustainable development. Elephant grass and vetiver grass exposed to a lower NO₃⁻ concentration of 5 ppm exhibited increased NO₃⁻ uptake. This enhanced absorption is likely due to the greater mobility of NO₃⁻ solution to the root cell walls via a diffusion-driven and passive process. However, at higher NO₃⁻ of 10 ppm and 30 ppm, both grasses showed a decline in NO₃⁻ uptake. This reduction is attributed to the toxic effects of NO₃⁻ at higher concentrations because it binds micronutrients, making them unavailable, resulting in chlorosis, which causes early plant desiccation, ultimately halting. Among the two species, vetiver grass demonstrated the highest removal efficiency, achieving rates of 92.4%, 49.7%, and 19.46% at NO₃⁻ concentrations of 5 ppm, 10 ppm, and 30 ppm, respectively. Elephant grass followed with removal efficiencies of 85.2%, 42.3%, and 19.06%, for the same concentration levels. Further research is recommended on a pilot scale, involving similar experiments on soil not previously exposed to any of these grasses, to better evaluate their treatment performance in more natural conditions.

* 1. Acknowledgments

This study was partially funded by the National Research Foundation (NRF) under Grant No. SRUG2204072544 and by Rand Water Company through Grant No. RW01413/18, awarded to Prof. E.M.N. Chirwa in the Department of Chemical Engineering at the University of Pretoria. Additional gratitude is extended to the Department of Water and Sanitation for providing scholarship support to the PhD student, Kenneth Mabaso. Sincere gratitude is extended to SMR Africa (Pty) Ltd for their valued contribution to this research through the supply of elephant grass.

* 1. References

Fallahi, A., Hajinajaf, N., Tavakoli, O., Sarrafzadeh, M.H., 2020. Cultivation of mixed microalgae using municipal wastewater: Biomass productivity, nutrient removal, and biochemical content. Iran. J. Biotechnol. 18, 88–97. <https://doi.org/10.30498/> IJB.2020.2586.

Faruque, M.O., Hossain, M.M., Farooq, W., Razzak, S.A., 2023. Phototrophic bioremediation of municipal tertiary wastewater coupling with lipid biosynthesis using scenedesmus dimorphus: effect of nitrogen to phosphorous ratio with/without CO2 supplementation. Sustainability 15, 1409. <https://doi.org/10.3390/> su15021409.

Fazal, T., Rehman, M.S.U., Javed, F., Akhtar, M., Mushtaq, A., Hafeez, A., Alaud Din, A., Iqbal, J., Rashid, N., Rehman, F., 2021. Integrating bioremediation of textile wastewater with biodiesel production using microalgae (Chlorella vulgaris). Chemosphere 281. <https://doi.org/10.1016/j.chemosphere.2021.130758>.

Ho, L., Goethals, P.L.M., 2020. Municipal wastewater treatment with pond technology: historical review and future outlook. Ecol. Eng. 148. <https://doi.org/10.1016/j>. ecoleng.2020.105791

Hossain, S.M.Z., Sultana, N., Jassim, M.S., Coskuner, G., Hazin, L.M., Razzak, S.A., Hossain, M.M., 2022. Soft-computing modeling and multiresponse optimization for nutrient removal process from municipal wastewater using microalgae. J. Water Process Eng. 45. <https://doi.org/10.1016/j.jwpe.2021.102490>.

Li, X., Zhang, C., Qu, W., Xie, P., Xie, Y., Chang, J.S., Ho, S.H., 2022. Role of nitrogen transport for efficient energy conversion potential in low carbon and high nitrogen/ phosphorus wastewater by microalgal-bacterial system. Bioresour. Technol. 351. https://doi.org/10.1016/j.biortech.2022.127019.

Mehariya, S., Goswami, R.K., Verma, P., Lavecchia, R., Zuorro, A., 2021. Integrated approach for wastewater treatment and biofuel production in microalgae biorefineries. Energies 14. <https://doi.org/10.3390/en14082282>.

Pietrelli L., Ferro S., Reverberi A., Vocciante M., 2022, Sustainable Removal of Nitrates from Wastewater Using Membrane Bioreactors, Chemical Engineering Transactions, 91, 217-222.

Razzak, S.A., Faruque, M.O., Alsheikh, Z., Alsheikhmohamad, L., Alkuroud, D., Alfayez, A., Hossain, S.M.Z., Hossain, M.M., 2022. A comprehensive review on conventional and biological-driven heavy metals removal from industrial wastewater. Environ. Adv. 7. https://doi.org/10.1016/j.envadv.2022.100168.

Rezvani, F., Sarrafzadeh, M.H., Oh, H.M., 2020. Hydrogen producer microalgae in interaction with hydrogen consumer denitrifiers as a novel strategy for nitrate removal from groundwater and biomass production. Algal Res. 45. https://doi.org/10.1016/j.algal.2019.101747.

Sanchez-Zurano, A., G´omez-Serrano, C., Aci´en-Fern´andez, F.G., Fern´andez-Sevilla, J.M., Molina-Grima, E., 2020. A novel photo-respirometry method to characterize consortia in microalgae-related wastewater treatment processes. Algal Res. 47. <https://doi.org/10.1016/j.algal.2020.101858>.

Shafiquzzaman, M., Hasan, M.M., Haider, H., Ahmed, A.T., Razzak, S.A., 2023. Comparative evaluation of low-cost ceramic membrane and polymeric micro membrane in algal membrane photobioreactor for wastewater treatment. J. Environ. Manag. 345, 118894. https://doi.org/10.1016/j.jenvman.2023.118894.

Wang, H., He, X., Nakhla, G., Zhu, J., Su, Y.K., 2020. Performance and bacterial community structure of a novel inverse fluidized bed bioreactor (IFBBR) treating synthetic municipal wastewater. Sci. Total Environ. 718, 137288. <https://doi.org/10.1016/j.scitotenv.2020.137288>.

Zhou, Y., Zhu, Y., Zhu, J., Li, C., Chen, G., 2023. A comprehensive review on wastewater nitrogen removal and its recovery processes. Int. J. Environ. Res. Public Health 20. https://doi.org/10.3390/ijerph20043429.

Zubair, M., Wang, S., Zhang, P., Ye, J., Liang, J., Nabi, M., Zhou, Z., Tao, X., Chen, N.,Sun, K., Xiao, J., Cai, Y., 2020. Biological nutrient removal and recovery from solid and liquid livestock manure: recent advance and perspective. Bioresour. Technol. 301, 122823. <https://doi.org/10.1016/j.biortech.2020.122823>.