Majid Farhadi

Design and startup of a continuous-flow bioelectrochemical reactor for treating nitrate pollution from water

Pideva vooluga bioelektrokeemilise reaktori disainimine ja käivitamine nitraadireostuse puhastamiseks veest

Master`s Thesis

Environmental Governance and Adaptation to Climate Change

Supervisor: Mikk Espenberg, *PhD*

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Bioelektrokeemiliste süsteemid (BES) on osutunud paljulubavaks lahenduseks põhja- ja pinnaveekogude nitraadisaaste leevendamisel. Selle uurimistöö eesmärk oli hinnata NO3 eemaldamise tõhusust põhjaveest, kus elektronidoonorite tase on madal. Uuringu põhieesmärk, milleks oli NO3 likvideerimine, saavutati tõhusalt nii perioodilises kui ka pidevas režiimis; 94% nitraadist elimineeriti. Näidati negatiivset seost ammooniumi ja nitraadi kontsentratsiooni vahel reaktoris. Maksimaalset ammooniumikontsentratsiooni 2,8 mg/l täheldati nitraadieemalduse tippkiiruse ajal. Nitrifikatsiooniprotsessis muudetakse ammoonium nitraadiks, kuid denitrifikatsioon võib toimuda nitraadi assimileeriva ja dissimileeriva redutseerimise teel. Leiti, et denitrifikatsiooni domineerimine DNRA (dissimilatoorne nitraadi redutseerimine ammooniumiks) suhtes on positiivses seoses algse nitraadikontsentratsiooni vähenemisega. Anorgaanilise lämmastiku vähenemist ja nitritite kontsentratsiooni tõusu võivad negatiivselt mõjutada lahustunud hapniku tase ja hapniku olemasolu. Reaktori keskmine pH väärtus oli ligikaudu 8,2. Denitrifikatsiooniprotsessi tõhusust võivad takistada pH tasemed, mis langevad alla 6,0 või üle 8,0, kuna need võivad avaldada negatiivset mõju mikroobide aktiivsusele. Täheldati ka muutusi kogu orgaanilise süsiniku kontsentratsioonis seoses mikroobide biomassiga.

Märksõnad: bioelektrokeemilised süsteemid (BES), nitraat, läbivooluline süsteem, reovesi

Abstract of Master´s Thesis

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The present investigation centered on utilizing bioelectrochemical systems (BESs) to mitigate nitrate contamination in groundwater and surface water bodies. The objective of the current investigation was to evaluate the effectiveness of eliminating NO3 from groundwater that exhibits low levels of electron donors. The study's principal aim, which was the eradication of NO3, was effectively achieved in both batch and continuous modes; 94% of the nitrate was eliminated. The investigation revealed a negative relationship between the concentration of ammonium and nitrate in the reactor. The maximum ammonium concentration of 2.8 mg/l was observed during the peak nitrate removal rate. The nitrification process comprises a sequence of reactions that transform ammonium into nitrate. On the other hand, denitrification can take place via assimilatory and dissimilatory reduction of nitrate. The dominance of denitrification over DNRA (dissimilatory nitrate reduction to ammonium) was found to be positively related with a decrease in the initial nitrate concentration. The reduction of inorganic nitrogen and the elevation of nitrite concentration can be adversely affected by dissolved oxygen levels and the existence of oxygen. The mean pH value of the reactor was approximately 8.2. Denitrification process efficiency can be impeded by pH levels that fall below 6.0 or exceed 8.0, as they can have a negative impact on microbial activity. Alterations in the total organic carbon concentrations were also observed because of microbial biomass.

Keywords: Bioelectrochemical systems, nitrate, continuous feeding, microbial electrosynthesis (MES)

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In the realm of inspiration and guidance, a vision unfolds, revealing the presence of those who have played significant roles in your thesis journey.

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Contents

1 INTRODUCTION

Bioelectrochemical systems (BESs) employ bacteria, archaea, or fungi with complicated enzyme pathways for diverse energy production and treatment processes (Osman et al. 2011). BES is a promising technique for water and soil pollution removal. BES can remove organic pollutants like benzene, toluene, and other hydrocarbons (Shim et al. 2009), heavy metals like lead, cadmium, and arsenic (Sukrampal et al. 2020), chlorinated solvents, and nutrients like nitrogen and phosphorus from wastewater (Kelly and He 2014). Batch MFC reactors have been extensively researched for their capacity to extract nutrients from wastewater (Rabaey and Verstraete 2005). Over the past decade, more studies have examined MFC performance utilizing different designs, components, operational settings, and quality criteria.

The MFC technology's knowledge foundation was built on batch lab-scale research. As we approach full-scale usage, continuous-flow scalable MFC systems are being studied (Abdallah et al. 2019). Continuous flow systems have several advantages: (1) the medium's composition can be tuned for optimal production; (2) secondary metabolite synthesis, growth kinetics, and kinetic constants can be precisely regulated; (3) repeatable outcomes and reliable data allow for high productivity per unit volume; and (4) the continuous culture requires less effort (Rahimnejad et al. 2011). In areas with significant agricultural and animal activity and private and small communal supplies taken from shallow aquifers, nitrate concentrations exceed the permissible limit (Water quality and contamination by nutrients 2020). Many regions in Asia, Europe, Africa and some in America have groundwater with more than 50 mg-NO3/L nitrates (Abascal et al., 2022). Nitrate concentrations are limited to 44.3 mg-NO3/L by the US Environmental Protection Agency and 50 mg-NO3/L by the EU and WHO. However, smaller localities worldwide have greater nitrate concentrations. Nitrate has health repercussions in addition to environmental consequences. Nitrate can cause methemoglobinemia, cancer, hypertension, increased infant mortality, central nervous system birth abnormalities, diabetes, spontaneous abortions, respiratory tract infections, and immune system changes (Fewtrell 2004).

Microbial reactions in the nitrogen cycle make nitrogen available for living things. This cycle consists of denitrification, nitrification, dissimilatory nitrate reduction to ammonium (DNRA), and anaerobic ammonium oxidation (ANAMMOX). Denitrification turns nitrate and nitrite into gaseous nitrogen forms, nitrification links ammonia oxidation to fixed nitrogen loss, and DNRA reduces them to ammonium. ANAMMOX bacteria oxidize ammonium and produce dinitrogen gas to remove nitrogen from water and wastewater. The current study aimed to design, assemble and set up a continuous flow bioelectrochemical reactor to enhance the treatment of nitrate pollution and study the performance of these types of reactors.

2 THEORETICAL OVERVIEW

2.1 Bioelectrochemical systems

Over the past century, the concept of microorganisms being able to disintegrate organic compounds and release electrical energy, has greatly evolved (Pant et al. 2012). Bioelectrochemical technology has proven to be an effective new technology for addressing energy and environmental challenges. In addition to being studied for energy production and wastewater treatment, this technology is also being applied in various fields such as desalination, bioremediation of contaminated water and soil, recovery of nutrients, and synthesis of valuable chemicals (Ortiz-Martínez et al. 2015; San-Martín et al. 2018).

Bioelectrochemical systems (BESs) use microorganisms, including bacteria, archaea or fungi, that possess complex enzyme pathways for various reactions. BESs can use a comprehensive range of substrates and operate under different environmental conditions (Osman et al. 2011). There are different types of bioelectrochemical systems (BESs) based on microbial activity Figure 1. Microbial fuel cells (MFCs) convert chemical energy to electrical energy by the action of microorganisms; however, MFCs have also been used in wastewater treatment (Vishwanathan 2021). Microbial electrolysis cells (MECs) are a type of BES that can use microorganisms to produce hydrogen gas from organic matter, which can be used as a fuel source. In microbial electrolysis cells (MES), microorganisms use electrons to reduce carbon dioxide (CO2) to yield industrially relevant products. This is opposite to MFCs, in which microorganisms transfer electrons from the oxidation of compounds to an anode to generate an electric current (Schmoldt et al. 1975). Microbial desalination cells (MDCs) are another type of BES that can remove salt from brackish water by using microorganisms to drive an ion transport process.

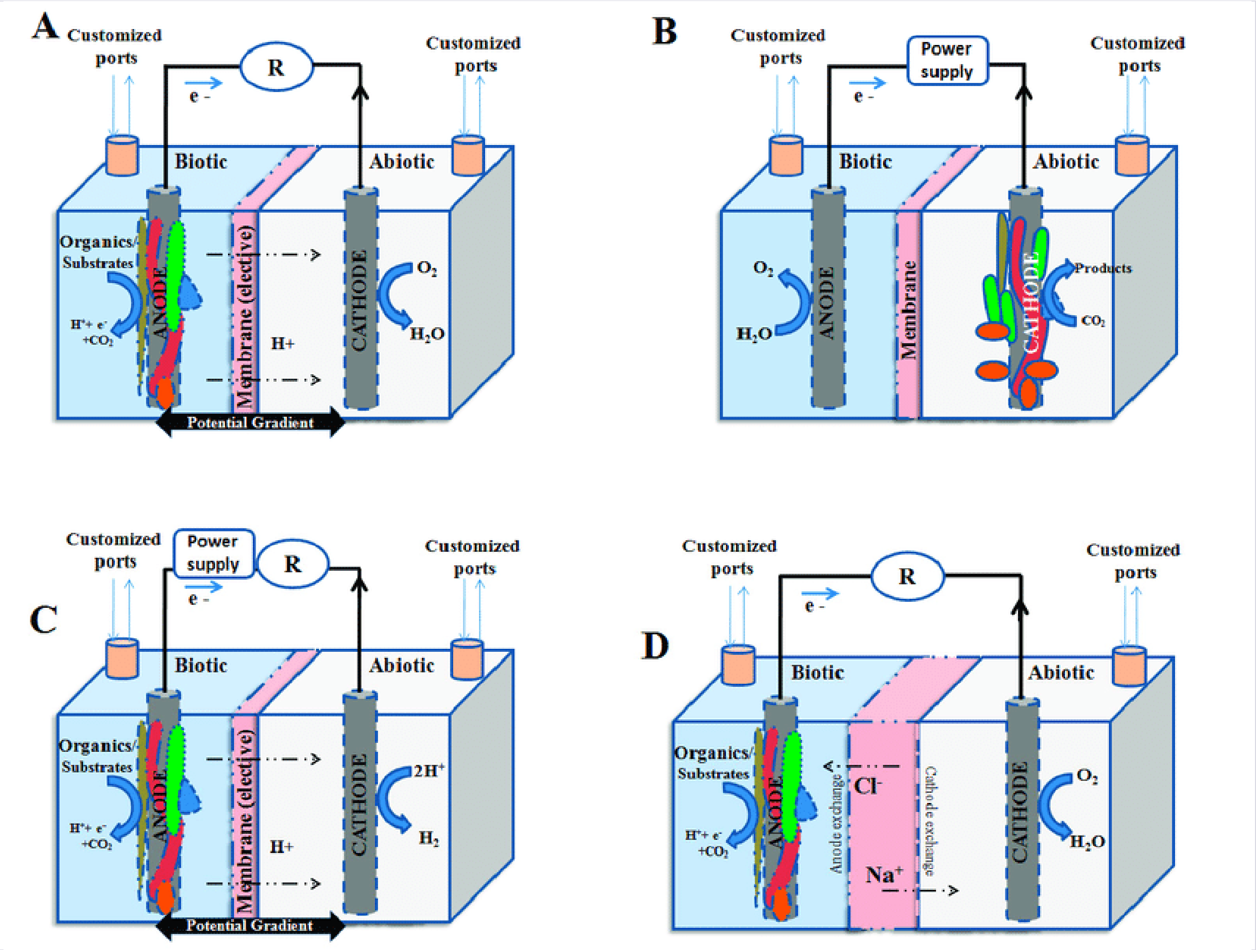

Figure 1. A) microbial fuel cells (MFC); (B) microbial electrolysis cell (MECs); (C) microbial electrosynthesis (MES); and (D) microbial desalination cells (MDC); (Schmoldt, Benthe, and Haberland 1975)

2.1.1 Working principles of bioelectrochemical systems

MFCs consist of an anode chamber and a cathode chamber separated by a Proton Exchange Membrane (PEM) Figure 1. The anode, commonly constructed of carbon or graphite and coated with a conductive substance to facilitate electron transmission (Rabaey and Verstraete 2005), is important in biofilm generation and electron transfer, which influences power output. The generated current depends on electroactive metabolic reactions of the biofilm that develops on the anode. Microbes attached to the anode form an electroactive microbial film, which acts as an electrocatalytic unit and assists substrate oxidation. The produced electrons are transferred to the cathode through an external circuit. MFC can be configured as a two-chambered or single-chambered system, with the latter being preferred due to its simplicity. MFCs have potential applications in treating various wastewater and monitoring water quality as a biosensor(Banu et al. 2019; Gul and Ahmad 2019). MES cells consist of two electrodes, a cathode and an anode, separated by a cation-exchange membrane (CEM). These cells facilitate a reductive reaction, which involves the conversion of CO_2 into organic acids, and an oxidative reaction which involves the oxidation of water or organic compounds. Microorganisms present in the MES cells help convert CO_2 into organic acids. These microorganisms take up electrons either directly from the electrode or indirectly through intermediates such as hydrogen, which is produced through (bio)electrochemical means. (Dessì et al. 2021). MECs are a new bioelectrochemical technology that converts various organic sources into pure hydrogen gas. The production of hydrogen in MECs depends on the substrate used and the ability to convert waste into by-products such as methane, hydrogen peroxide, and hydrogen gas. The technology relies on exoelectrogenic bacteria that consume organic waste and discharge electrons in the anode, while electrotrophs in the cathode receive and transfer electrons to generate methane gas. The hydrogen and/or methane gas produced can be used in internal combustion engines to produce electricity, making MECs suitable for power generation in waste biorefineries. However, the design of the MEC reactor is critical for efficient hydrogen generation (Rivera, Schröder, and Patil 2019).

2.1.2 Removing pollutants with bioelectrochemical systems

BES is an emerging technology that has demonstrated its potential for removing various pollutants from water and soil. BES has shown promising results in the removal of different types of contaminants, including organic pollutants such as benzene, toluene, and other hydrocarbons (Shim et al. 2009), heavy metals like lead, cadmium, and arsenic (Sukrampal, Kumar, and Patil 2020), excess nutrients like nitrogen and phosphorus from wastewater, and chlorinated solvents like 1,4-Dioxane (Kelly and He 2014). The removal of nitrate in BES has also gained great attention recently (Gadegaonkar et al. 2023). A recent review highlighted the effectiveness of BES technology in removing a wide range of contaminants and discussed the potential challenges and opportunities for scaling up the technology for real-world applications (Li, Li, and Zhou 2021).

Malik et al. (2023) discussed several studies that have demonstrated the effectiveness of MFCs in removing organic pollutants from wastewater. One promising application of BES technology is sediment microbial fuel cell (SMFC) to bioremediate polycyclic aromatic hydrocarbons (PAHs) in water originating from soil (Sherafatmand and Ng 2015). SMFC demonstrated its bioremediation capabilities by achieving 76.9%, 52.5%, and 36.8% removal of naphthalene, acenaphthene, and phenanthrene, respectively, in the anaerobic environment. These results demonstrated the ability of SMFCs to stimulate microorganisms for the bioremediation of complex and recalcitrant PAHs (Sherafatmand and Ng 2015).

MFCs, have been investigated as a potential method for cleaning water of various pollutants(Lv et al. 2022). In a study from 2020, Beretta and colleagues examined the effectiveness of bioreduction, bioelectrochemical reduction, and solely electrochemical reduction in the process of eliminating chromium from water. In comparison to the other methods, the research indicated that bioelectrochemical reduction had both a greater overall efficiency and a faster elimination rate. In their study on the use of MFCs for the reduction of perchlorate, they discovered that a denitrifying biocathode was applied. As a result, they were able to accomplish a maximum perchlorate removal of 24 mg/L-d and a cathodic conversion efficiency of 84%(Beretta et al. 2020). The researchers Rabaey et al. (2006) explored the use of MFCs to convert dissolved sulfide into elemental sulfur. They demonstrated that the conversion of sulfide to sulfur is potential-dependent and that MFCs can remove up to 514 mg of sulfide L-1 from the net anodic compartment (NAC) every day. MFCs can remove up to 98% of the sulfide and 46% of the acetate when used in conjunction with an anaerobic up flow anaerobic sludge blanket reactor. In addition, they can remove sulfate in an effective manner through the process of sulfide reduction. According to the findings of these research, MFCs have the ability to rid water of a variety of pollutants, enhance the quality of digester effluents, and recover energy that would otherwise be lost during the process of methane digestion (Rabaey and Verstraete 2005; Banu et al. 2019).

The potential of MFCs in the treatment of pharmaceutical wastewater has been demonstrated by their capacity to expedite the degradation of pharmaceutical pollutants through the decomposition of organic molecules into CO_2, H_2O, and energy (Cecconet et al. 2017). Research has indicated that the implementation of MFCs can result in notable reductions in reaction time and acceleration of the degradation of pharmaceutical pollutants. Specifically, degradation rates of 98% for penicillin within 24 hours and 79.1% for tetracycline within 7 days have been achieved, as reported by Wen et al. (2011) and Wang et al. (2017). Furthermore, it has been demonstrated by Xie et al. (2021) that MFC coupled constructed wetland systems are efficacious in eliminating carbamazepine, which is a priority pollutant among pharmaceuticals and personal care products(Wen et al. 2011; Wang et al. 2017; Xie et al. 2021).

In their study, Greenman et al. (2021) conducted a comprehensive analysis of the efficacy of microbial fuel cells (MFCs) in removing nutrients from wastewater. The authors identified several crucial factors that impact MFC performance, including the microbial community, electrode composition, operational parameters, and nutrient characteristics and concentrations(Greenman et al. 2021). The selection of electrode material can influence the microbial population in MFCs, while the operational parameters, including pH, temperature, and flow rate, can significantly impact the performance of MFCs. Optimizing MFC designs and operations enhance the capacity of MFCs to eliminate nutrients from wastewater (Paucar and Sato 2021).

2.1.3 Batch reactors

Batch reactors are a form of MFC that deliver a specified quantity of substrate (such as wastewater) to a reactor and allow the microbial population to break down over a certain time. This type of MFC generates energy by breaking down the substrate. During this process, electrons are transported from the oxidation of the substrate caused by microorganisms to the anode, which produces energy (Logan 2010). Batch MFC reactors have been extensively researched for their capacity to extract nutrients from wastewater (Rabaey and Verstraete 2005). Nutrient removal is accomplished by the microbial decomposition of organic materials, which is utilized as a carbon source by the anode chamber microbial community (Rabaey and Verstraete 2005). The investigation carried out by Zeng et al. (2020) focused on a three-phase single-batch chamber MFC (TP-MFC) by introducing a phase containing immobilized cells into a typical bipolar single-chamber MFC (common MFC). The TP-MFC had a total nitrogen removal rate of 63.4% after 72 hours, which was 38.4% greater than the rate of the regular MFC (Zeng et al. 2020). In 2022, Lust et al. (2022) examined the single-chamber microbial electrosynthesis reactor for nitrate reduction from waters with a low-electron donors' concentration, they reached the nitrate removal rate of 3.8 ± 1.2 mgN-NO_3/(L×day) (Lust et al. 2022). Guo et al. (2015) achieved 99% NH_4 and 94% TN removal from mixed species biocathode MFCs batch reactor equipped with Mustard tuber wastewater (MTWW) catholyte. Concerning nitrogen removal, aerobic and anaerobic microenvironments could be formed within the cathodic biofilms, and both heterotrophic denitrification and bioelectrochemical denitrification were involved (Guo, Fu, and Zhang 2015).

2.1.4 Continuous-flow reactors

During the last decade, an increasing number of published research have investigated the performance of MFCs using a variety of designs, components, operational parameters, and quality measures. Each design and operational factor had a distinct impact on MFC performance regarding power production and treatment efficiency. Nonetheless, most prior and continuing research investigations were conducted in batch lab scale setups, which was critical in establishing the MFC technology's knowledge base. Further study on continuous-flow scalable MFC setups has been undertaken as we move closer to full-scale use (Abdallah et al. 2019). Continuous flow systems have various advantages:

1) the medium's composition may be tuned for optimal production

2) secondary metabolite synthesis can be precisely regulated, as can growth kinetics and kinetic constants

3) the continuous culture produces repeatable outcomes and reliable data, allowing for high productivity per unit volume

4) the continuous culture requires less effort (Rahimnejad et al. 2011).

Although some researchers have employed the fed-batch system for long-term operation in the production of bioelectricity, continuous mode is more appropriate for future practical applications than batch and fed-batch systems. The most of the research on continuous MFC systems has focused on determining their suitability for wastewater treatment (Rahimnejad et al. 2011).

The feasibility of a continuous MFC with anoxic/oxic (A/O) modification was investigated by You et al. (2010), and some factors such as hydraulic retention time (HRT), salinity, and pH were investigated, as well as archived COD removal up to 80% and biological nitrification (You et al. 2010). In a separate investigation, Puig et al. tested the treatment and power production capabilities of MFCs for landfill leachate under conditions with high nitrogen concentration and conductivity

(6033 mg N/L), respectively (73,588 S/cm). Leachate from an urban dump was treated with an air-cathode MFC over an extended length of 155 days. During generating electricity (344 mW/m^3), up to 8.5 kg COD m^3/d of biodegradable organic waste was removed (Puig et al. 2011). Gao et al. achieved the anode-acquired good removal efficiency of 98.6% for COD and 52.1% for N in a system that combined an anaerobic fluidized bed with a membrane filtration column to treat high-salinity wastewater (Gao et al. 2019).

2.2 Excessive nitrogen in the water

Nitrogen, a component of plant and animal proteins, is essential to life. It is essential to life, but too much can be toxic (Shaaban et al. 2022). Nitrogen pollution has disrupted the nutritional cycle between living things, soil, water, and air. Disruptions caused major issues. Humans greatly affect biogeochemical cycles. From 1986 to 2010, reactive nitrogen released into the environment increased from 15 megatons (Mt) to 185 Mt, whereas nitrogen fertilizers used in agriculture increased from 12 Mt in 1961 to 110 Mt in 2014. Nitrogen is soluble in water and can enter drinking water and groundwater (Rožić et al. 2000; Yu et al. 2019).

Agricultural activities are the primary cause of water quality deterioration for rivers and lakes and the third for estuaries (Mateo-Sagasta et al. 2017). Furthermore, nutrient contamination in agriculture is recognized as the second most significant contributor to river and lake pollution and the first in significance for estuaries. Nutrient pollution in water is typically caused by both point and nonpoint sources. The largest agricultural nonpoint source of nutrients is commercial fertilizer (Puckett, L. J 1995). Nonpoint source pollution in agriculture is mainly produced by nitrogen and phosphorus fertilizers, herbicides, and other organic or inorganic nutrients released along surface runoff or agricultural effluents. Asymmetric information, high uncertainty, unpredictability, moral hazard, and adverse selection are characteristics of agricultural nonpoint source pollution (Ambulkar 2017).

Global manure production from concentrated livestock feeding facilities has skyrocketed recently. Manure mismanagement frequently results in the direct release of liquid manure to surface waters via runoff, causing eutrophication, characterized by excessive concentrations of N and P, causing an ecological imbalance in the water system. Depending on the weather, such drainage might occur quickly after application. Animal manure applied wisely and sustainably to land will provide nutrients to crops while reducing the demand for mineral fertilizers (Chien et al. 2011). Unfortunately, many nations have been sluggish in implementing sustainable and ecologically sound manure management (production, storage, and application) methods because manure has been viewed as a waste rather than a resource. By best practices in livestock and manure management, there is every chance to decrease pollution hazards (N and P input to surface and ground waterways) and improve soil quality (Sakadevan and Nguyen 2017). Storm runoff collects sediments, pathogens, and pollutants when impermeable surfaces replace plants and soil. Increased impermeable area and surface runoff damage urban water quality. Stream quality declines with 10% impermeable surface cover (Schueler et al. 2009). Stormwater flows N and P, which can cause eutrophication, harmful algal blooms, and fish death. Urban stormwater runoff's nutrient sources and metabolic transformation pathways may help managers minimize environmental nutrients. Natural, anthropogenic, and biogenic sources supply urban N and P. Understanding watershed N and P sources and transport mechanisms that remove nutrients can assist downstream aquatic ecosystems regulate N and P (Yang and Lusk 2018).

WWTP effluent nitrogen content also impacts receiving streams. WWTPs require a discharge permit, but nutrients can still pollute (Carey and Migliaccio 2009). Many aquatic systems have very low ambient nutrient concentrations; therefore, even small nutrient load adjustments can alter community structure (Rabalais 2002). WWTP effluent affected nutrient loading more than nonpoint sources, according to research. As best management practices (BMPs) minimize nonpoint source pollution, WWTP effluent may dominate aquatic system nutrient inputs (Carey and Migliaccio 2009).

2.2.1 Groundwater

Depending on the hydro-geological circumstances, the pace of nitrogen percolation into the deeper layers of the soil is often relatively slow, and the presence of high nitrogen concentrations results from the pollution that occurred on the surface up to forty years earlier. The nitrate concentration threshold stated by the United States environmental protection agency is 44.3 mg-NO_3/L, and by the European Union (EU) and World Health Organization (WHO) regulations, the upper limit for nitrates is 50 mg-NO_3/L. Nitrate concentrations that are more than allowed are typically found in private and small communal supplies drawn from shallow aquifers, as well as in regions with heavy agricultural and animal activity (Water quality and pollution by nutrients 2020). In general, continent-wise, 20 regions in Asia (e.g., China, India, Palestine, Saudi Arabia, Pakistan), 9 in Europe (e.g., Italy, Malta, Spain, Lithuania, Belgium), 30 in Africa (e.g., Democratic Republic of the Congo, Zimbabwe, Mozambique), and 1 in America (Fortaleza, Brazil) have observed to have more than 50 mg-NO_3/L nitrates in groundwater (Abascal et al., 2022). However, different smaller places with higher nitrate concentrations are all over the world.

Groundwater resources abound in Estonia, and most Estonians can access safe drinking water. Despite this, more than 200,000 individuals in Estonia may have their well water quality degraded owing to nitrate or pesticide contamination caused by agriculture, mineral resource exploitation, or untreated wastewater from residences (Helerin Kõrvemaa 2018). In the Pandivere and Adavere-Põltsamaa Nitrate-Vulnerable Zone, the share of arable land and livestock is significantly above the Estonian average, and the groundwater is only partially or slightly protected, has higher nitrate concentrations in Silurian-Ordovician groundwater bodies in East and West Estonia. Excessive water abstraction may also have an impact on groundwater quality. The National Audit Office found that nitrate contamination of groundwater has grown, and fertilizer use is thought to be the primary source of nitrates. Groundwater quality has not improved in Ida-Viru County, which has been extensively impacted by oil shale extraction, and extraction permits have not implemented procedures to avoid future groundwater harm (Freshwater - State and impacts (Estonia) 2020). Apart from nitrate, nitrogen is applied in ammonium (NH_4^+) and amide (NH_2^-) forms, which create nitrate in the soil system via mineralization, which occurs rather quickly in tropical and subtropical soils (Kader et al. 2013). Nitrate is prone to leaking to the subsurface layers and eventually groundwater due to its high solubility in water and limited retention by soil particles if not taken up by plants or denitrified to N_2O and N_2. Soil characteristics and the amount of water in the soil system determine the leaching rate. Shallow groundwater tables, excessive use of fertilizers, organic manure, and irrigation, and plentiful rainfall can all increase nitrate arrival in groundwater (Pereira et al. 2014). Other prominent sources of nitrate in groundwater are livestock feeding, barnyards, septic tanks, and animal and human pollution. Municipal and industrial wastes may contribute large quantities of nitrate to groundwater in and around regions of high urbanization and industrialization. When nitrate-rich groundwater is pumped out and consumed, it causes various health problems (Deepanjan Majumdar Navindu Gupta 2000).

Nitrate has health repercussions in addition to environmental consequences. Nitrate has been linked to methemoglobinemia and other health consequences: cancer (through bacterial formation of N-nitroso compounds), hypertension, increased infant mortality, central nervous system birth abnormalities, diabetes, spontaneous abortions, respiratory tract infections, and immune system alterations (Fewtrell 2004). When nitrate is consumed, it is absorbed into the bloodstream via the stomach and upper intestine. The majority is eliminated in the urine, although some can be converted to nitrite, particularly in the intestine. Nitrite oxidizes the iron in hemoglobin to generate methemoglobin (metHb). If more than 10% of the hemoglobin is converted to metHb, the blood's oxygen-carrying capacity is reduced to the point when symptoms of anoxia arise. Increased metHb levels have been linked to brain damage and mortality (Keeney and Follett 2015).

2.2.2 Surface water

Anthropogenic nitrogen enters water from various industrial, municipal, residential, and agricultural sources. Another large anthropogenic nitrogen source that is released into surface water is nitrogen deposition from fossil fuel burning in the atmosphere. Chemical inputs to aquatic bodies are also categorised as coming from point sources like municipal wastewater treatment plants and nonpoint sources like agricultural operations and air deposition. Human nitrogen fixation now exceeds natural nitrogen fixation on an annual basis. Human activities increase the nitrogen influx into water over time. Nitrogen flow into water bodies is projected to continue to rise worldwide. According to some reports, 90% of the dissolved inorganic nitrogen load in the world's rivers will be anthropogenic by 2050 (Hu et al. 2021). Excessive nitrogen addition to aquatic systems may cause eutrophication by promoting excessive plant growth rates. Water eutrophication is the process by which an excess of nitrogen, phosphorus, and some other inorganic nutrients enter relatively isolated and slow-moving aquatic ecosystems, such as a lake, pond, river, or wetland, stimulating the proliferation of algae and other plankton in the water, resulting in lower dissolved oxygen (DO), increased chlorophyll-a (Chlo) content, and the degradation of water quality. This can lead to the demise of fish and other aquatic organisms. Under anoxic circumstances, the breakdown of algae releases more nutrients, such as nitrogen and phosphorus, back into the water for the next generation of algae to use (Yao et al. 2018). The Fifth Global Environment Outlook (GEO-5) reveals that more than 40% of the world's water sources suffer from varying degrees of eutrophication. The most prevalent impact of nutrient enrichment in aquatic environments is the proliferation of algae and aquatic plants. Nonetheless, the impacts of nutrient enrichment are more severe and intricate(Mohammad Nazari-Sharabian et al. 2018). Many studies have demonstrated that eutrophication is one of the most significant contributors to the spread of hazardous algal blooms (HABs), particularly cyanobacterial blooms. Certain cyanobacterial species create poisons, deplete oxygen, change food webs, and degrade water quality by forming huge surface growths. The repercussions of cyanobacterial blooms may represent a significant danger to the availability of potable and agricultural water (Zhang et al. 2021).

2.2.3 Removal of excess nitrogen

The biological nitrogen cycle is a complex process involving living organisms' assimilation and dissimilating of nitrogen compounds. Nitrogen is essential for life and is required to synthesize amino acids and nucleotides. The nitrogen cycle is driven by microbial transformations Figure 2, such as nitrogen fixation, nitrification, denitrification, and dissimilatory nitrate reduction to ammonium (DNRA), as well as geological processes (Thamdrup 2012). Denitrification is the microbiological process that converts nitrate and nitrite to gaseous nitrogen forms, primarily nitrous oxide (N_2O) and nitrogen (N_2). A wide variety of microorganisms may denitrify. Denitrification occurs because of changes in oxygen (O_2) content in their immediate surroundings. Denitrifiers will only convert from aerobic to anaerobic respiration when O_2 is scarce, employing nitrite (NO_2) as an electron acceptor (Skiba 2008). Nitrification is also the nitrogen cycle stage that connects ammonia oxidation (generated by organic matter breakdown) to the loss of fixed nitrogen in dinitrogen gas. It is carried out by various microorganisms, including ammonia-oxidizing bacteria, ammonia-oxidizing archaea, and nitrite-oxidizing bacteria. All these bacteria are aerobes that are mostly autotrophic. Their specific metabolism gives them a distinct niche but also causes sluggish and wasteful development. ANAMMOX organisms are another type of microorganism that performs direct anaerobic oxidation of ammonia to nitrogen gas (Ward 2008). DNRA stands for dissimilatory nitrate reduction to ammonium. The DNRA procedure DNRA is a two-step process that shares the first stage of nitrate reduction to nitrite with denitrification but then conducts direct nitrite to ammonium reduction in the second phase. $NO_3^- \rightarrow NO_2^- \rightarrow NH_4^+$ DNRA can be linked to the oxidation of organic carbon molecules as well as inorganic electron donors like sulphide or hydrogen (Herrmann and Taubert 2022). Moreover, ANAMMOX (Anaerobic Ammonium Oxidation) is an important microbial process in the nitrogen cycle, notably in removing excess nitrogen from aquatic and wastewater settings (Long et al. 2013). ANAMMOX bacteria can oxidize ammonium (NH_4^+) in anaerobic circumstances by using nitrite (NO_2^-) as an electron acceptor and producing dinitrogen gas (N_2). This process is conducted by a distinct group of bacteria from the *Planctomycetes* phylum that contains the ANAMMOX some, a subcellular compartment containing the enzymes essential for the ANAMMOX reaction (Abbas et al. 2020).

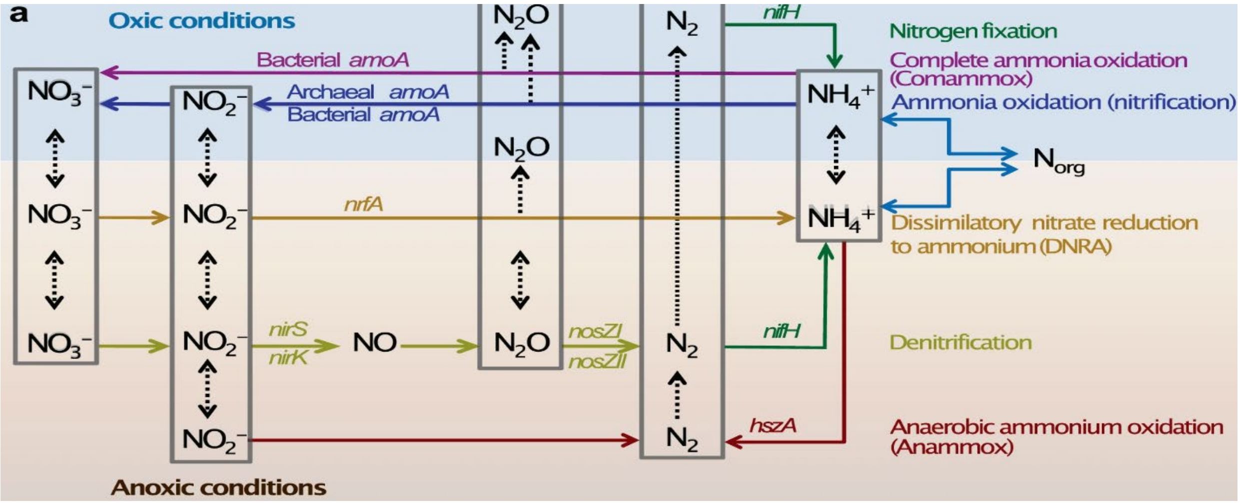

Figure 2. Schematic view of nitrogen cycle and the key genes involved (Bahram et al. 2022).

3 MATERIALS AND METHODS

3.1 Reactor design

For the purpose of continually cleaning wastewater, a reactor made of Plexiglas (HDPE (High-density Polyethylene)) with dimensions 700 mm by 596 mm by 195 mm was created. A see-through chamber is located at the very top of the reactor, where the holders are kept. When designing the reactor, both the continuous flow and the daily output needed to supply a family of four with the approximately 20 L of water they use each day were taken into consideration. To achieve this goal, the parameters of the number of electrons and the pattern of water flow in the reactor were chosen in such a manner as to optimize the removal of nutrients, and the output was designed to have the features and volume that were wanted. To install the electrodes in the pilot, holders were first mounted on top of the reactor. These holders were then used to position the electrodes in such a way that they would fill the whole center space of the reactor. These holders have had 10 sets of electrodes, each with its own anodes and cathodes attached to them. Figure 3 illustrates how the anode and cathode should be installed, as well as how they should be designed. They are composed of 3 mm thick graphite felt (Graphite felt PGF 3*1.200 m, CGT Carbon GmbG, Asbach, Germany), and after being cut into 10 pieces measuring 38*42 cm, they have three rows of titanium wires (Grade-1 Titanium Round Wire 0.2 mm × 50 m, Metal Clays 4 You, Mayfield, United Kingdom) run through them to increase the strength of the titanium wires made of epoxy (Premium Epoxy Fix&Coat 507, Penosil, Estonia) and carbon particles (Cabot VULCAN®XC-72R, BC Berlin Catalysts GmbH, Germany) in a ratio of 3 carbon particles to 1 epoxy particle. After that, a three-layer plastic mesh with the size was used to separate the anode and cathode from each other. This was done to achieve this goal by first covering the area where the wire passes through with the desired compound, then insulating the titanium wires with electrical insulation (Heat Shrink PO 1.5-0.5mm 3:1 WHT, Distrelec, Switzerland) that was 25 cm long. Anode from the cathode was employed as proton exchange membrane(PEM). To do this, three layers of polymer were stacked atop one another, and the anodes were entirely stitched with plastic thread Figure 4.

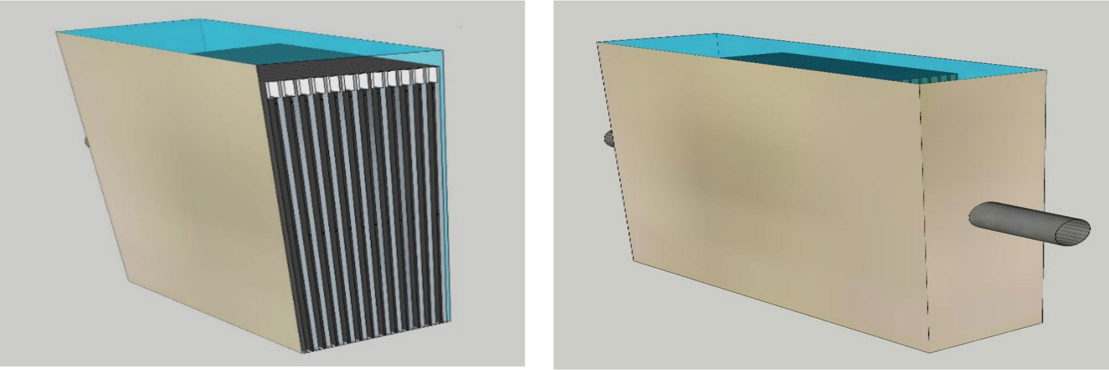

Figure 3. The 3D schematic of the reactor with a cross section to illustrate the electrodes installation.

Figure 4. Assembling the reactor, anodes and cathodes, wiring before and after installation.

Next step, anodes were mounted on the holders. After that, the same material was used to create the cathode, which was then chopped to dimensions of 120 mm by 38 mm and had six layers of titanium wire put through it, much like the anode. Insulating and connecting the titanium wires was accomplished using the same technique. It can be seen in Figure 4 that the two-way cathode was mounted on the holders and that it encircled it. While the titanium wires were being installed, they were done so in such a way that the output wires of the anode were directed to one side of the reactor, while the output wires of the cathode were directed to the opposite side of the reactor. Finally, the anode wires were connected from the reactor outlet, and the cathode wires were connected from the reactor inlet. This was followed by the connection of a potentiostat (Interface 5000, Gamry Instruments, USA), which measures the voltage and current generated by the MEC to monitor the MEC's performance over time Figure 5.

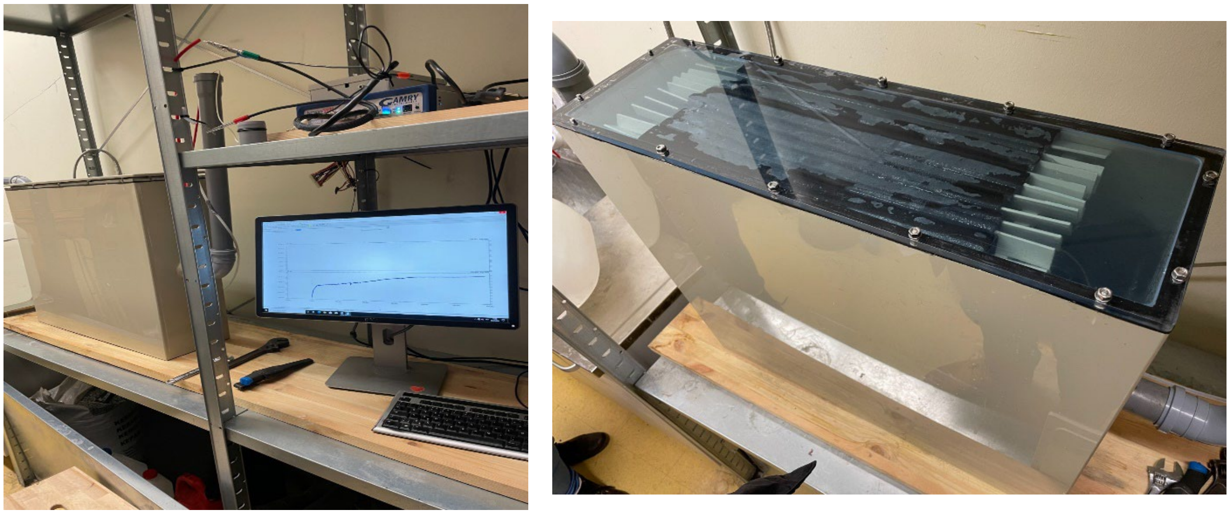

Figure 5. The final assembled reactor and running potentiostat in laboratory.

3.2 Operating conditions

After completing the installation and design of the reactor, synthetic wastewater was added to the reactor with a concentration of 100 mg/L of nitrate. For this purpose, potassium nitrate was added to the tap water to reach 100 mg/L of nitrate. Water from previous experiments (Lust et al. 2022) was used as an inoculum, which was based on a culture of T. denitrificans. At first, the reactor was run in a batch mode from the 23rd of January until the 3rd of May, and <0.5 L of synthetic wastewater (concentration of 100 mg/L of nitrate) was added each week for taking the samples. In addition, 15 L and 5 L of synthetic wastewater (concentration of 100 mg/L of nitrate) were added on the 31st of March and 28th of April. Since the 3rd of May, the reactor has been running in a continuous mode, and every day 1 L of synthetic wastewater (concentration of 100 mg/L of nitrate) was added. From the 9th of May, the continuous mode continued, but 2 L of synthetic wastewater (concentration of 100 mg/L of nitrate) was added every day. For this thesis, sampling was carried out until the 18th of May.

3.3 Water sampling and analyses

In this experiment, the potential was controlled by a potentiostat (Interface 5000, Gamry

Instruments, USA) that can apply and measure currents from 5 A to ~0.05 µA. At the beginning of the experiment, the reactors were filled with synthetic wastewater. The potential was posed at −756 mV. The nitrate concentration was measured by Pocket Water Quality Meters (LAQUAtwin NO_3-11C/NO_3-11S/NO_3-11, Horiba, Japan). The current density was observed. Once a week, dissolved oxygen (DO), temperature and pressure were measured by Optical Dissolved Oxygen Probe (GDX-ODO, Vernier, USA), and pH was measured by pH electrode (CPO-401 with IJ44A, Elmetron, Poland) and cyclic voltammetry (CV) measurements were performed to control the reactor performance. Once a week, water samples also were taken to the Estonian Environmental Research Centre (EKUK) lab to determine concentrations of ammonium, nitrite, nitrate and total organic carbon (TOC) (Table 1).

Table 1. The methods for determining the concentrations of chemical parameters.

Parameter	**Standard method**	**Unit**
Ammonium (NH_4^-)	EVS-EN ISO 11732	mg/l
Nitrite (NO_2^-)	EVS-EN ISO 13395	mg/l
Nitrate (NO_3^-)	EVS-EN ISO 10304-1	mg/l
Total organic carbon (TOC)	EVS-EN 1484	mgC/l

4 RESULTS

4.1 System startup and addition of inoculum

The reactor was run in batch mode for a long time to get it going (development of the microbial community, reduction of the nitrate, etc.) before making it as a continuous-flow reactor. The reactor was exposed to a potential of −756 mV based on previous studies (Lust et al. 2022). The removal of NO_3 was not observed prior to inoculation (Figure 6), but after adding the inoculum, all parameters started to change.

4.2 Nitrate (NO_3) concentration

Following the inoculation process, a notable shift in the trend of NO_3 concentration was observed. Specifically, between the 1st of March (the day of inoculation) and the 8th of March, the concentration of NO_3 decreased from 90 to 30 mg/l. Similarly, on the 1st of April, the concentration of NO_3 decreased to 6 mg/l. However, it is worth noting that the removal rate was not consistent throughout the observation period. Specifically, between the 8th to 24th of March, the removal rate decreased. During the third measured period, which spanned from the 24th of March to the 1st of April, the concentration of NO_3 dropped from 11 to 6 mg/l. Throughout this time frame, NO_3 was introduced into the reactor on multiple occasions. The initial addition occurred subsequent to the introduction of the inoculum, specifically on the 31st day of March, whereby 15 L of synthetic polluted water were introduced. Subsequently, another addition (5 L) was made on the 28th of April. Following a comparable pattern, the initial two weeks of May saw the daily addition of 1 L and subsequently 2 L to the reactor to examine the ongoing removal process. The concentration of NO_3 exhibited an initial increase following the introduction of polluted water, but subsequently decreased. For instance, on May 24th, the concentration was recorded at 24 mg/l, but by May 28th, it had dropped to 18 mg/l. This trend persisted despite continuous feeding and the addition of NO_3 to the reactor, with the NO_3 concentration never exceeding 12 mg/l. These findings are illustrated in Figure 6. This study presents an analysis of the NO_3 concentration rate and trend during various measurement intervals before and after inoculation. For example, on the 31st of March, NO_3-polluted water was introduced to the reactor, resulting in observable fluctuations in NO_3

concentration and trend.

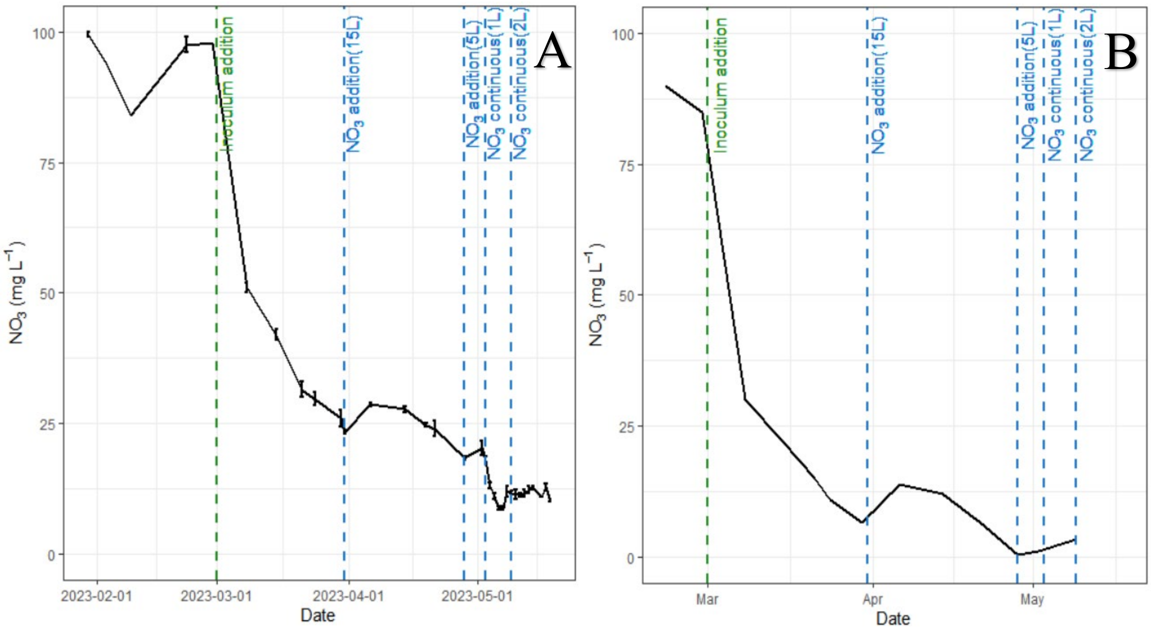

Figure 6. The trend of NO_3 removal: the measured concentrations with nitrate meter (A) and EKUK laboratory (B).

4.3 Ammonium (NH_4) concentration

The concentration of NH_4 exhibited an inverse relationship with NO_3, whereby it increased from an initial value of 0.2 mg/l to a final value of 0.64 mg/l during the first week of March following inoculation. Subsequently, there was a slight decline in NH_4 concentration, reaching a value of 0.59 mg/l by the 15 of March. Similarly, there was a sharp increase in the NH_4 concentration to 2.5 mg/l on the 31st of March, with a maximum concentration of 2.8 mg/l being recorded. The diagram depicted in Figure 7 displays the temporal variation of NH_4 concentration after inoculation and the introduction of nitrate into the reactor. In the continuous feeding of reactor the NH_4 concentration reached to 0.2 mg/l in the last measurement in May and experienced a decrease. The NO_3 concentration can be contrasted during the aforementioned interval in Figure 6.

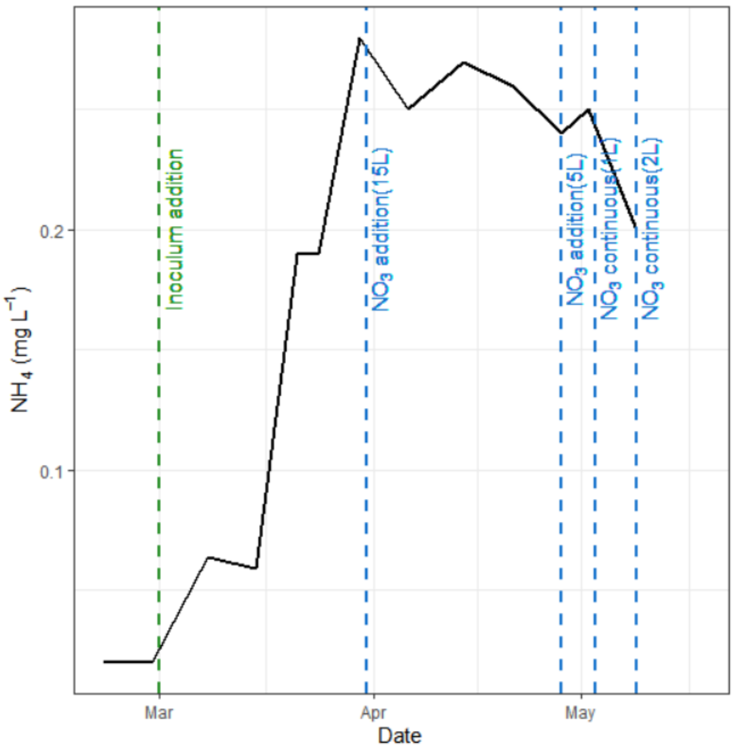

Figure 7. The NH_4 concentration trend measured by EKUK laboratory.

4.4 Nitrite (NO_2) concentration

Figure 8 illustrates the NO_2 amount in the reactor. The concentration of NO_2 reached a level of 0.25 mg/L within 8 days after inoculation, and subsequently attained a level of 0.52 mg/L on the 31st of March. During this period, the concentration of NO_2 exhibited fluctuations, with a decrease to 0.57 mg/L on the 15th of March, then the concentration decreased to 0.4 mg/l on the 21st of March and got the highest level in the middle of April. In continuous feeding of the reactor, the amount of NO_2 was stable.

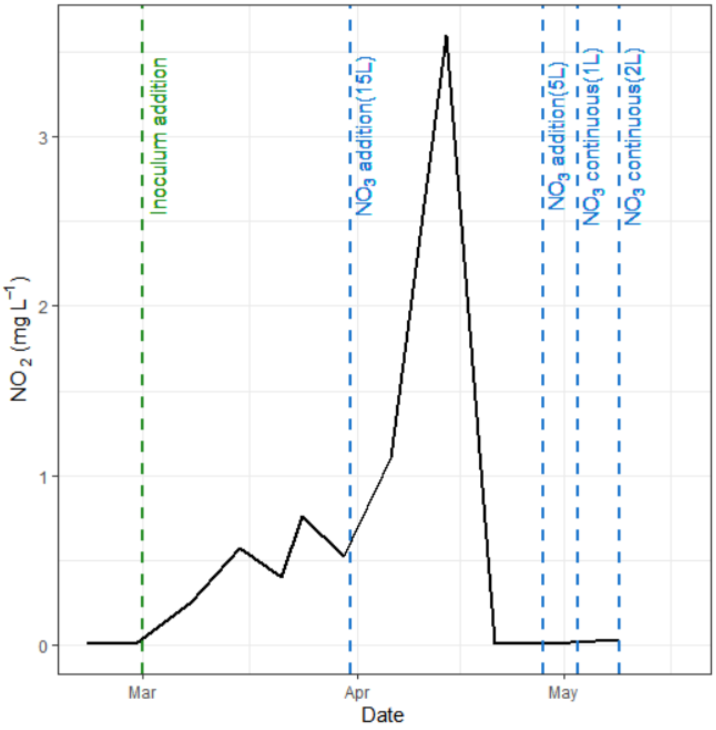

Figure 8. the NO_2 concentration trend measured by EKUK laboratory

4.5 Total organic carbon (TOC) concentration and pH

The monitoring of total organic carbon (TOC) was conducted to analyze the microbial community in the reactor and assess its growth or reduction. As depicted in Figure 9, following the inoculum phase, there was an increase in the number to 26 mgC/l during the first week. This increase was accompanied by slight fluctuations and was followed by a subsequent decrease to 24 mgC/l on the 24th of March. Subsequently, there was a notable increase in the TOC levels, observed on the 1st of April, followed by a further increase to 30 mgC/l on the 6th of April. The amount of TOC increased to maximum point, on the 21st May, where the amount of TOC was 38 mgC/l after feeding with 15 L synthetic water. During the continuous feeding, the TOC decreased 27 mgC/l.

pH was a measured parameter in each sampling event (Figure 9). The pH values ranged from 7.3 on the initial day of reactor operation on February 21st to a maximum of 8.4 on March 31st. During this period, the pH exhibited fluctuations. Following the first feeding event on April 1st, the pH remained stable at 8.3. However, with continuous feeding, the pH decreased to 7.9.

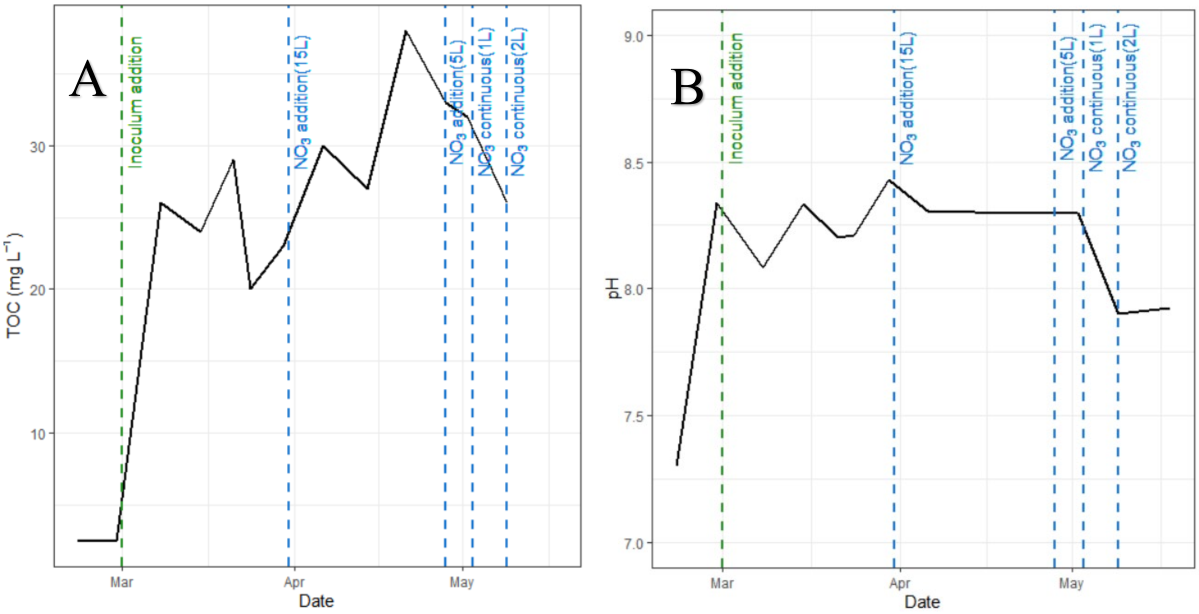

Figure 9. Total organic carbon (TOC) concentration (A) measured by EKUK laboratory and pH trend (B).

4.6 Dissolved oxygen (DO), temperature and pressure

Throughout the experimentation process, parameters such as dissolved oxygen (DO), temperature, and pressure were monitored to see the reactor's performance and evaluate the impact of each of these factors on the elimination of NO_3 (Figure 10). The significance of dissolved oxygen (DO) was crucial, considering the reactor's objective to enhance denitrification. The dissolved oxygen (DO) concentration initially increased on March 15th. Following some fluctuations, it stabilized at a nearly constant level thereafter. On the 31st of March, the temperature served as an indicator of the microbial activity within the reactor, as evidenced by its increase. However, the temperature was pretty constant as all the changes were inside ca 1 °C.

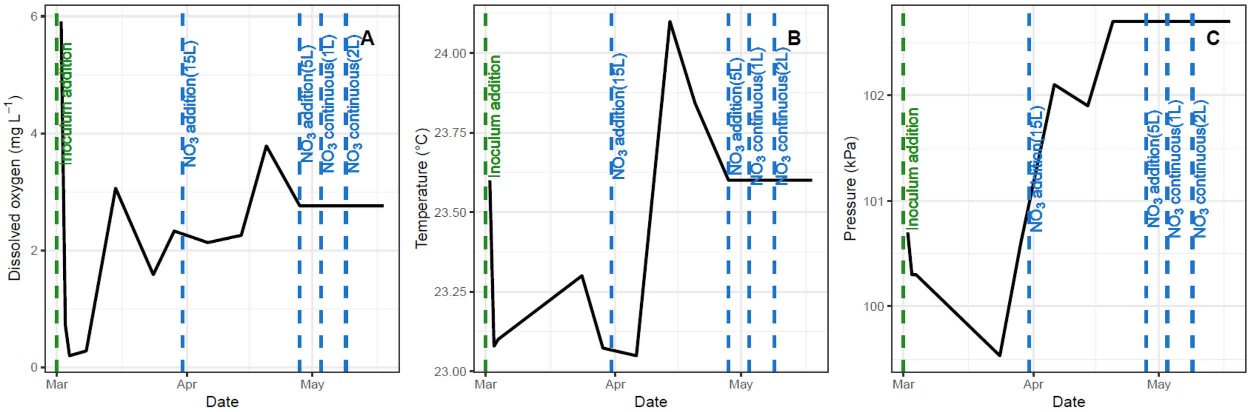

Figure 10. Dissolved oxygen (A), temperature (B) and pressure (C) trend in reactor.

4.7 Chronoamperometry

The experiments involved the measurement and regulation of current density, with the potential being established at a value of −756 mV. The observation of maximum NH_4 levels (as depicted in Figure 7) and minimum NO_3 levels (as depicted in Figure 6) was prior to the introduction of synthetic water. The current density exhibited a fluctuation in a similar pattern, with a subsequent decrease observed on May 21st when the reactor was supplied with contaminated water, and this trend persisted with continuous feeding. The sharp decreases in current density imply the decline in NO_3 concentration (Figure 11).

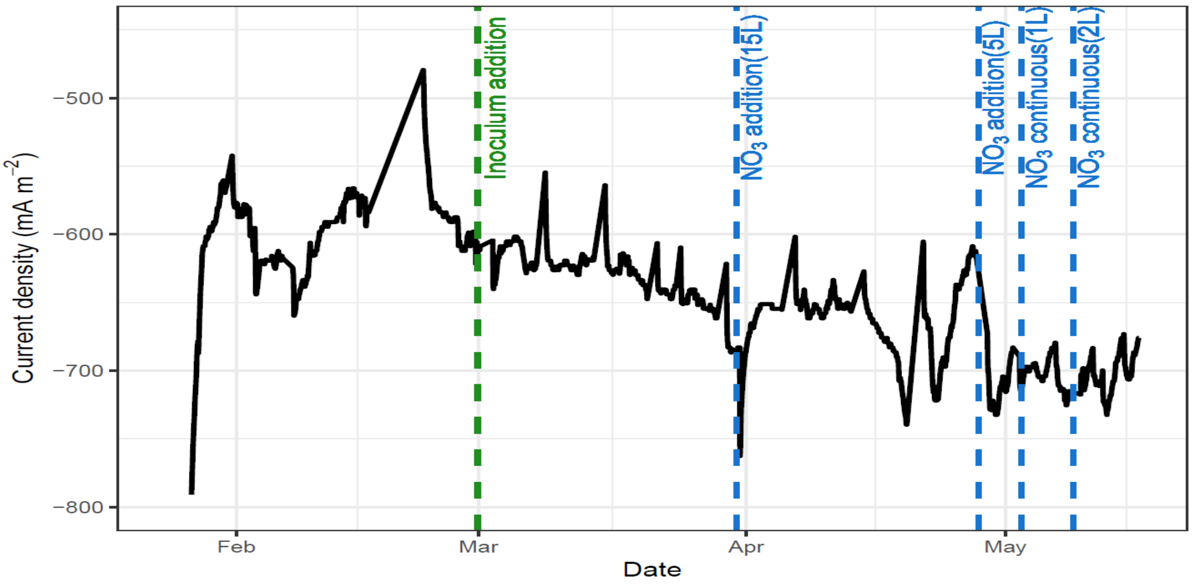

Figure 11. Chronoamperometry measured the current density of the system to control the performance and NO_3 removal.

4.8 Cyclic voltammetry (CV)

Cyclic voltammetry (CV) measurements were made during the experiment to characterize the changes that were taking place in the reactor. The selection of the potential sweep range and methodology was based on prior research findings (Lust et al. 2022). The measurements in the below figures were all obtained at various periods, which are depicted in Figure 11, Figure 12 and Figure 13 with various colors. The measurements were conducted in three different sweep rates when the rates changed from 0.5 to 1 rate 1mVs Figure 11, 0.5 to 1.5 rate 5 mVs Figure 12 and 0.5 to1 mVs rate 5 mVs Figure 13; the dates of these measurements are also presented. The changes in the cycle shape show that NO_3 removal or nitrification occurs, the graphs showed possibly on the dates 20th, 23rd and 28th of March the removal when some deformation in the shapes happened, moreover during the continuous feeding, the curves in the 2nd and 9th of May experienced some deformation in the graphs, the data on Figure 6 also showed possibly the removal of NO_3 in mentioned dates.

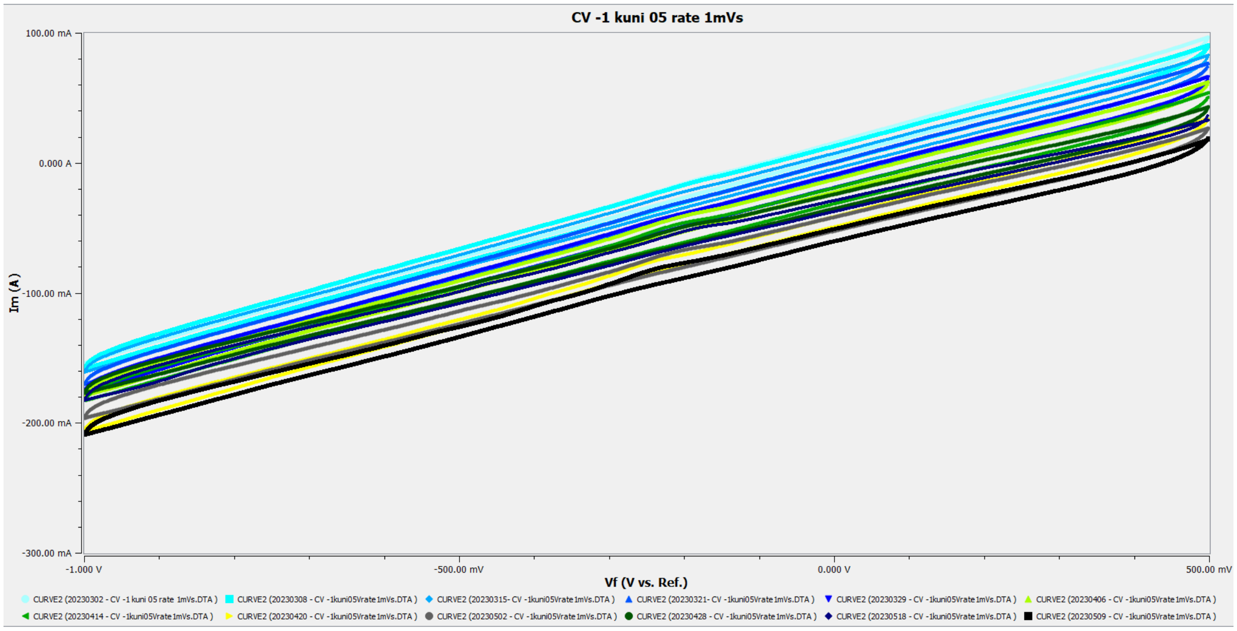

Figure 12. Cyclic voltammetry graph sweep rates from 0.5 to 1 rate 1mVs.

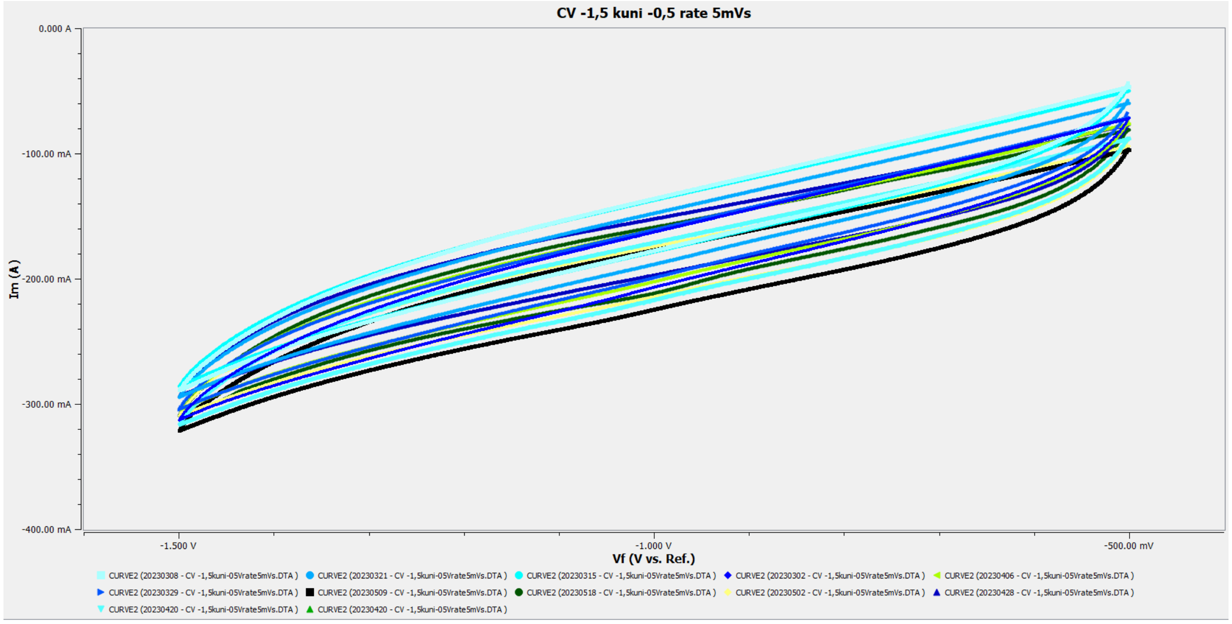

Figure 13. Cyclic voltammetry graph sweep rates from 0.5 to 1.5 rate 5mVs.

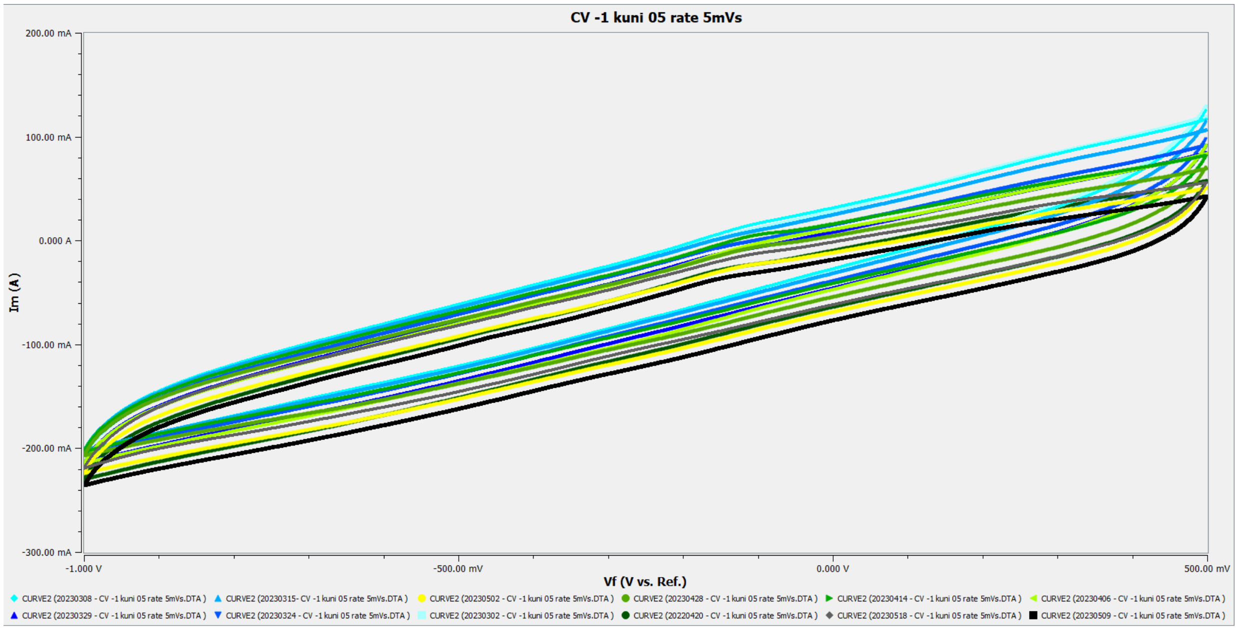

Figure 14. Cyclic voltammetry graph sweep rates from 0.5 to 1 rate 5mVs.

5 DISCUSSION

Prior to the introduction of the inoculum, nitrate removal did not occur. The present study examines the removal of NO_3 in a reactor utilizing groundwater with low levels of carbon compounds. It appears that the microbial community was insufficient in its ability to remediate NO_3 in the synthetic groundwater prior to the introduction of inoculum. There is a potential correlation between the rise in TOC concentration and the proliferation of microorganisms (Lust et al. 2022). Following the introduction of inoculum, the TOC concentration exhibited a notable increase from 2 to 26 mgC/l. Subsequently, due to the presence of microbial activities, a shift in the outcome occurred, leading to the removal of NO_3. Specifically, after a period of 8 days, approximately 66% of NO_3 was eliminated, and this percentage increased to 92% after one month. Nitrate assimilation in cytoplasm (*Clostridium perfringens*), dissimilation in periplasm (*Ralstonia eutropha*), and respiration in inner membrane (*Simplicispira psychrophila*) were the three pathways responsible for denitrification owing to different types of microbes. These reactions produced the final products NH_4, NO_2, and N_2, respectively (Zhu et al. 2017). Studies on fermentation balance have been conducted on *Clostridium perfringens* under varying conditions of nitrate presence in the growth medium (Hasan and Hall 1975). The presence of nitrate enables these bacteria to function as an electron acceptor, thereby leading to enhanced growth yields as compared to the conditions where nitrate is not present. The observed rise in the proportion of metabolite molecules that can engage in substrate-level phosphorylation reactions is attributed to the presence of an inorganic acceptor. The process of nitrate reduction in these bacteria can be considered as a rudimentary type of anaerobic respiration, as it is evidently linked to their energy metabolism and does not serve an assimilative purpose (Hasan and Hall 1975).

The ammonium concentration in the reactor had an inverse trend with nitrate concentration, the maximum amount of 2.8 mg/l occurred on the 31st of March when the NO_3 removal was at the highest rate. The most oxidized compounds for nitrogen are from ammonium to nitrate, in that order. The process of converting nitrate to ammonium is known to require a significant amount of energy for microorganisms (Prosser 2005). The process of reducing nitrate to ammonium and oxidizing ammonium to nitrate requires the involvement of numerous reactions and enzymes. The process of nitrification entails a series of three distinct steps that facilitate the conversion of ammonium to nitrate. Conversely, denitrification can be achieved through two distinct pathways, namely assimilatory and dissimilatory reduction of nitrate. The process of assimilatory denitrification involves the conversion of nitrate to ammonium in a two-step mechanism, with the ultimate goal of creating biomass. Subsequently, the ammonium undergoes a conversion process leading to the production of glutamine, which is utilized in the process of synthesizing proteins. The enzymes implicated in the distinct pathways are glutamine synthetase and glutamate synthase, as well as glutamate dehydrogenase (Cabello et al. 2004; Harris et al. 2021).

Furthermore, The initial concentration of 100 mg/l of NO_3 was chosen for this study, this amount was taken based on previous studies (Lust et al. 2022; Yang and Lusk 2018). The purpose was to remove NO_3 to less than 50 mg/l, which is the European Union standard. The initial concentration of nitrate is a crucial factor in determining the proportion of nitrate reduction achieved through DNRA and denitrification. As the concentration of nitrate initially decreases, the prevalence of DNRA over denitrification is observed through an increase in the number of electrons at the working electrode. The proposed pathway for electrode oxidation and subsequent production of ammonia through dissimilatory reduction of nitrate/nitrite accounts for a maximum of 34% of the overall reduction of nitrate/nitrite (Su et al. 2012). Elevated levels of nitrate have the potential to enhance the ion exchange processes, thereby leading to increased rates of nitrate elimination. The observed correlation between the rate of nitrate removal and the initial concentration of nitrate indicates that the nitrate removal process in the current bioelectrochemical system exhibits characteristics consistent with a first-order reaction (Tong and He 2013). The initial NO3 concentration was determined based on previous articles (Cecconet et al. 2018; Molognoni et al. 2017; Tong and He 2013). Moreover, the cathodic nitrate overloading resulted in a decrease in the current production rate, as opposed to an increase. The present study investigates the inhibitory impact of nitrite ($NO_2^{\,-}$), an intermediate denitrifying product, on denitrification and current generation processes of denitrifying microorganisms. The spontaneous formation of free nitrous acid (FNA), which is the protonated variant of nitrite ion HNO_2, is linked to the formation of nitrite in the aqueous chemical system (Al-Mamun and Baawain 2015; Jiang et al. 2011).

The utilization of O_2 as an electron acceptor in water has been found to have a negative impact on the removal of inorganic nitrogen (Gómez 2002). During the experiment the DO was monitored, after a fluctuation at first days the amount became almost stable around 2mg/l which is in the range of denitrification. The detrimental impact is amplified as dissolved oxygen levels escalate and is contingent upon the type of electron donor utilized during the procedure. Autotrophic denitrification can occur within the range of dissolved oxygen levels that are less than 4.5 mg/l (Rahmani et al. 1995). In addition to the adverse impact of dissolved oxygen DO on the elimination of inorganic nitrogen, the existence of oxygen O_2 also results in an elevation of nitrite concentration in the processed water (Gómez 2002).

In addition to the fact that the current density did not alter, the results demonstrated that the application of electrical manipulation did not appear to have a major impact (Lust et al. 2022), in comparison to the findings of previous investigations, these ones had quite different outcomes. The use of graphite anode was successful in removing 28.4% of the total nitrogen when the current was held constant at 12 mA (Liang et al. 2022; Lust et al. 2022), an examination of a reactor revealed a NO_3 removal rate of up to 96%. The removal was observed through both cyclic voltammetry and chronoamperometry techniques, which demonstrated more changes in the curves and removal of NO_3 over time.

The electric manipulation exerted a significant impact on the pH (Lust et al. 2022). The reactor exhibited an average pH value of approximately 8.2. The consumption of alkalinity during the nitrification process may lead to a considerable reduction in pH levels in wastewater, which could impede denitrification. According to literature reports, the denitrification process may experience hindrance when the pH levels fall below 6.0 or exceed 8.0 (Saeed and Sun 2012). The significance of pH lies in its impact on bacterial activity, which in turn affects the efficiency of nitrogen removal (Rezaee et al. 2015).

6 CONCLUSIONS

The present study aimed to examine the efficacy of NO_3 removal from groundwater characterized by low electron donor content. The primary objective of the study, namely the elimination of NO_3, was successfully accomplished. The reactor exhibited favorable outcomes in the context of sustained feeding, which constituted an additional objective of the investigation. Up to 94% of the nitrogen was removed. The study observed an inverse trend between ammonium concentration and nitrate concentration in the reactor, with the highest ammonium concentration of 2.8 mg/l occurring when nitrate removal was at its highest rate. Converting nitrate to ammonium requires a significant amount of energy for microorganisms. Nitrification involves a series of steps converting ammonium to nitrate, while denitrification can occur through assimilatory and dissimilatory reduction of nitrate. The initial nitrate concentration of 100 mg/l was chosen based on previous studies and the goal was to reduce it to less than 50 mg/l, which is the European standard. The prevalence of denitrification over DNRA (dissimilatory nitrate reduction to ammonium) increased as the initial nitrate concentration decreased. The current production rate decreased with cathodic nitrate overloading. Dissolved oxygen levels and the presence of oxygen can negatively impact inorganic nitrogen removal and increase nitrite concentration. Electrical manipulation did not have a significant impact on the current density in this study. The pH of the reactor averaged around 8.2, and pH levels below 6.0 or above 8.0 can hinder denitrification process efficiency due to their effect on microbial activity.

7 SUMMARY

7.1 Summary in English

Bioelectrochemical systems (BESs) represent a technology with great potential, wherein microorganisms are employed and electricity is used to treat wastewater and produce energy. Various categories of bioelectrochemical systems (BESs) exist, such as microbial fuel cells (MFCs), microbial electrolysis cells (MECs), microbial electrosynthesis (MES), and microbial desalination cells (MDCs). Bioelectrochemical systems (BESs) exhibit promising prospects in various domains such as energy generation, wastewater management, and desalination of water, among others. Notwithstanding, there exist certain obstacles that must be overcome to enhance the technology's suitability for practical implementation, including but not limited to expenses, scalability, stability and activity of microbial communities, and efficacy in removing pollutants.

The presence of an excessive amount of nitrogen in aquatic ecosystems can result in adverse impacts on both the environment and human health. The degradation of water quality in rivers and lakes is predominantly attributed to agricultural practices. Agricultural nonpoint source pollution is primarily generated by the discharge of nitrogen and phosphorus fertilizers, herbicides, and other organic or inorganic nutrients through surface runoff or agricultural effluents. Elevated levels of nitrogen in groundwater may arise even due to surface contamination that transpired up to four decades ago. The contamination of groundwater can be attributed to various anthropogenic activities such as agricultural practices, mineral resource extraction, and the discharge of untreated domestic wastewater.

The reactor designed, equipped with a chamber designed to accommodate holders located at the uppermost part of the apparatus. The optimization of the number of electrodes and the pattern of water flow in the reactor was conducted to effectively eliminate nutrients. Additionally, the reactor was specifically engineered to generate a daily output of approximately 20 liters of water, which is deemed sufficient for a household of four individuals. Ten distinct sets of electrodes, comprising both anodes and cathodes, were affixed to holders. Carbon felt and titanium wires were utilized to construct the anodes and cathodes, respectively. A three-layer mesh was employed to isolate the titanium wires. The reactor's performance was monitored over time by connecting a potentiostat to it. The reactor was supplemented with synthetic wastewater containing a nitrate concentration of 100 mg/l, followed by the introduction of inoculum mainly based on *T. denitrificans* culture for inoculation purposes. The concentration of nitrate was assessed through the utilization of Pocket Water Quality Meters, while the measurements of dissolved oxygen, temperature, and pressure were obtained through the use of an Optical Dissolved Oxygen Probe, usually on a weekly basis.

The presented research investigated the outcomes of an experimental investigation aimed at assessing the impact of electrical manipulation on the elimination of nitrate from polluted water. The study was carried out within a BES that was equipped with a solitary chamber. The research revealed that the elimination of nitrate was not detected prior to inoculation. Subsequent to the process of inoculation, a significant reduction in the concentration of nitrate was discerned. Specifically, the concentration exhibited a decline from 90 to 30 mg/L during the period spanning from the first to the eighth day of March. The research additionally observed a rise in the concentration of ammonium and variations in the concentration of nitrite, suggesting the occurrence of denitrification. Upon inoculation, the levels of total organic carbon (TOC) within the reactor exhibited an increase. To ensure optimal performance of the reactor, a range of parameters including dissolved oxygen, temperature, and pressure were closely monitored.

7.2 Summary in Estonian

Bioelektrokeemilised süsteemid (BES) kujutavad endast suure potentsiaaliga tehnoloogiat, milles kasutatakse reovee puhastamiseks ja energia tootmiseks mikroorganisme ja elektrit. On olemas mitmesugused bioelektrokeemiliste süsteeme (BES), nagu näiteks mikroobsed kütuseelemendid (MFC), mikroobsed elektrolüüsielemendid (MEC), mikroobsed elektrosünteesid (MES) ja mikroobsed magestuselemendid (MDC). Bioelektrokeemilistel süsteemidel (BES) on paljutõotavad väljavaated erinevates valdkondades (nt energia tootmine, reoveekäitlus ja vee magestamine). Selle tehnoloogia on praktiline rakendamine on siiski olnud väljakutse erinevatel tehnilistel ja sisulistel põhjustel nagu näiteks kulud, mastaapsus, mikroobikoosluste stabiilsus ja aktiivsus ning tõhusus saasteainete eemaldamisel.

Liigne lämmastikusisaldus veeökosüsteemides avaldab kahjulikku mõju nii keskkonnale kui ka inimeste tervisele. Jõgede ja järvede veekvaliteedi halvenemise põhjuseks on valdavalt põllumajandustavad. Põllumajandusest tekib reostus peamiselt lämmastik- ja fosforväetiste, herbitsiidide ja muude orgaaniliste või anorgaaniliste toitainete eraldumisest pindmiste või põllumajanduslike heitvete kaudu. Kõrgenenud lämmastikusisaldus põhjavees võib tekkida isegi kuni neli aastakümmet tagasi toimunud pinnareostuse tõttu. Põhjavee saastumist võib seostada mitmesuguste inimtegevustega, nagu põllumajandustavad, maavarade kaevandamine ja puhastamata olmereovee ärajuhtimine.

Selle töö reaktor on konstrueeritud ühekambrilisena, mille keskosas asuvad hoidikud elektroodide jaoks. Toitainete tõhusaks eemaldamiseks viidi läbi elektroodide arvu ja veevoolu mustri optimeerimine reaktoris. Lisaks on reaktor konstrueeritud nii, et see suudaks puhastada päevas kuni 20 liitrit vett, mida peetakse piisavaks neljaliikmelise leibkonna joogivee vajaduse katmise jaoks. Hoidikutele kinnitati kümme elektroodide komplekti, mis koosnesid nii anoodidest kui katoodidest. Anoodide ja katoodide ehitamiseks kasutati vastavalt süsinikvilti ja titaantraate. Katoodi ja anoodi isoleerimiseks kasutati kolmekihilist plastikust võrku. Reaktorit tööd kontrolliti potentsiostaadiga. Reaktorisse lisati sünteetilist reovett, mis sisaldas nitraadikontsentratsiooni 100 mg/l. Inokuleerimiseks kasutati peamiselt T. denitrificans kultuuril põhinevat inokulumi varasemast BES-i katsest. Nitraadi kontsentratsiooni hinnati nii kohapeal kui ka laborianalüüside alusel. Lahustunud hapniku, temperatuuri ja rõhu väärtused saadi hapnikumõõturi abil iganädalaselt või mõnikord sagedamini.

Eksperimentaaluuringus hinnati elektrilise manipuleerimise mõju nitraatide eemaldamisele saastunud veest. Uuring näitas, et enne inokulumi lisamist ei tuvastatud nitraadi eemaldamist. Pärast inokuleerimisprotsessi täheldati nitraadikontsentratsiooni olulist vähenemist. Kontsentratsioon vähenes 90-lt 30 mg/l-le perioodil, mis kestis kaheksa päeva. Lisaks täheldati uuringus ammooniumi kontsentratsiooni tõusu ja nitriti kontsentratsiooni muutusi. Inokuleerimisel suurenes orgaanilise süsiniku (TOC) tase reaktoris. Reaktori optimaalse jõudluse tagamiseks seirati mitmesuguseid parameetreid, sealhulgas lahustunud hapnikku, temperatuuri ja rõhku.

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