water

MDPI

Review

Nitrate Water Contamination from Industrial Activities and Complete Denitrification as a Remediation Option

Karabelo M. Moloantoa [1,*], Zenzile P. Khetsha [2], Esta van Heerden [3], Julio C. Castillo [1] and Errol D. Cason [4,*]

[1] Department of Microbiology and Biochemistry, University of the Free State, Bloemfontein 9301, Free State, South Africa; castillohernandezj@ufs.ac.za
[2] Sustainable Crop Production Systems Group, Department of Agriculture, Central University of Technology, Bloemfontein 9301, Free State, South Africa; zkhetsha@cut.ac.za
[3] iWater Pty Ltd., Centre for Water sciences and Management, North West University, Potchefstroom 2520, North West, South Africa; esta@iwatersolutions.co.za
[4] Department of Animal and Wildlife and Grassland Sciences, University of the Free State, Bloemfontein 9301, Free State, South Africa
* Correspondence: kmmoloantoa@gmail.com (K.M.M.); casoned@ufs.ac.za (E.D.C.)

Abstract: Freshwater is a scarce resource that continues to be at high risk of pollution from anthropogenic activities, requiring remediation in such cases for its continuous use. The agricultural and mining industries extensively use water and nitrogen (N)-dependent products, mainly in fertilizers and explosives, respectively, with their excess accumulating in different water bodies. Although removal of NO_3 from water and soil through the application of chemical, physical, and biological methods has been studied globally, these methods seldom yield N_2 gas as a desired byproduct for nitrogen cycling. These methods predominantly cause secondary contamination with deposits of chemical waste such as slurry brine, nitrite (NO_2), ammonia (NH_3), and nitrous oxide (N_2O), which are also harmful and fastidious to remove. This review focuses on complete denitrification facilitated by bacteria as a remedial option aimed at producing nitrogen gas as a terminal byproduct. Synergistic interaction of different nitrogen metabolisms from different bacteria is highlighted, with detailed attention to the optimization of their enzymatic activities. A biotechnological approach to mitigating industrial NO_3 contamination using indigenous bacteria from wastewater is proposed, holding the prospect of optimizing to the point of complete denitrification. The approach was reviewed and found to be durable, sustainable, cost effective, and environmentally friendly, as opposed to current chemical and physical water remediation technologies.

Keywords: bioreactor; bioremediation; complete denitrification; contamination; nitrate; nitrogen cycling; wastewater

check for updates

Citation: Moloantoa, K.M.; Khetsha, Z.P.; van Heerden, E.; Castillo, J.C.; Cason, E.D. Nitrate Water Contamination from Industrial Activities and Complete Denitrification as a Remediation Option. *Water* **2022**, *14*, 799. https://doi.org/10.3390/w14050799

Academic Editor: Fabienne Battaglia-Brunet

Received: 18 January 2022
Accepted: 26 February 2022
Published: 3 March 2022

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).

1. Introduction

In the 21st century, water has become a scarce natural resource globally due to the increasing population, urbanization [1], and extensive agricultural practices [2]. These factors cause industrial water contamination, groundwater depletion [3,4] and emission of greenhouse gases that in turn cause global warming [5]. Human water demands continue to rise in arid environments, and groundwater supply is limited causing severe annual droughts [1,5]. Industries such as mining and agriculture account for over 70% of surface fresh water loss through contamination during mineral processing and excessive irrigation of crops, respectively [6,7]. Contamination of surface and groundwater sources by industrial waste exacerbates shortages of water, and the resulting severe health hazards threatening both aquatic and terrestrial life [1]. Domestic utilization and consumption of polluted water has been reported to account for over 90% of the transmission of infectious diseases, making water treatment to remove contaminants from the surface water prior to human use an imperative task [7,8]. Utilization of various chemicals during food preservation

and mineral processing contributes to water contamination, rendering the water toxic and unusable even for irrigation.

In this review, two of the major compounds of concern are ammonium (NH_4^+) and nitrate (NO_3^-), which are used in the manufacturing of the fertilizers, fossil fuels, and explosives widely used in the mines and other anthropogenic activities to blast rocks [9,10]. The intensive use of nitrogen-based products and their inappropriate disposal has contributed to NH_4^+ and NO_3^- contamination of both surface and groundwater sources. For instances, studies conducted in South Africa and the Kalahari of Botswana and Namibia revealed the presence of NO_3^- concentrations exceeding 700 mg/L in groundwater sources [11,12]. The deposits of NO_3^- concentrations were discovered to be from the use of cattle manure as fertilizer, overflow of septic tanks, and from mine tailings where ammonia-based explosives were used for detonation [11,13]. Nitrates are known to be a common contaminant in drinking water, affecting groundwater systems around sewage plants and companies producing fireworks, weapons and industrial explosives [14]. They are the second most common predominant anthropogenic surface and groundwater pollutants after pesticides, and are applied in the soil in a form of fertilizers during agricultural practices in similar fashion [15]. Over 50% of water pollution in streams, rivers and groundwater is due to agricultural practices via the application of pesticides and fertilizers that leach from their point of application into bodies of water [16]. Pesticides (i.e., herbicides, insecticides, fungicides, rodenticides, molluscicides, and nematicides) are applied globally during crop farming to protect and increase crop yields by killing biological entities that cause damage and disease to the plants [17].

Nitrate contamination accounts for 4% loss of fresh water from sources such as aquifers in most developed regions of the world, such as United States of America (USA) and Europe [18]. Nitrate contamination has been on the rise globally, with water quality deteriorating compared to 1986, when over 20 mg/L of NO_3^- was detected in about 3% of surface water and 6% of groundwater in the USA. Such increasing and continuous losses of groundwater from NO_3^- contamination has led to the development and application of various physical, chemical, and biological NO_3^- remediation methods in attempt to mitigate contamination [19,20]. Most African countries, including South Africa, have been experiencing exponential increase of NO_3^- contamination in both surface and groundwater sources due to extensive agricultural [21] and mining practices [22], which has elicited major public concern and research into sustainable remediation strategies for NO_3^- contamination [12].

In oxygenated environments such as flowing rivers and the upper layers of the soil, biological nitrification is favored, converting most NH_4^+ to yield more NO_3^- compared to other nitrogen species. In contrast, in oxygen limited environments such as groundwater, denitrification is promoted, and nitrogen is predominantly present as NH_3 and NO [23]. Concentration of NO_2^- and NO_3^- above 10 mg/L in surface water are hazardous when consumed due to the toxicity of NO_3^-, which causes methemoglobinemia, colon cancer, and blue baby syndrome in infants [18,24]. Excess amounts of dissolved NO_3^- in water serve as nutrients to aquatic plants and algae as it is readily used as an N source for protein synthesis and rapid development of chlorophyll used for photosynthesis [25]. Due to this phenomenon, high NO_3^- concentration in water results in eutrophication, which is a process where rapid plant growth and development are promoted over the water surface. Eutrophication and algal blooms in rivers and streams are among the negative impacts NO_3 contamination has on the environment [26]. Nitrogen-based contaminants can occur from food production industries where nitrosyl compounds derived from nitrite are used in the preservation of meat, fish, and cheese. Chemical compounds such as Roussin's black salt $[Fe_4S_3\ (NO)_7]^-$ and saltpetre (KNO_3) are added to food products, where they serve as inhibitory agents against food spoilage bacteria such as *Clostridium botulinum* and *C.sporogenes* [27]. Nitrogen, as a main component of most commercial fertilizers and other commonly used products, contributes to surface water contamination, posing a health hazard and threatening biodiversity in the environment [7,28]. Nitrogen is the most abundant gaseous element in the atmosphere and is used extensively for

anthropogenic activities; its accumulation in the environment, mostly in soil and water, has become a concern and threat for human health, hence the need for its remediation. In this review, focus is afforded to details concerning the complete denitrification pathway using bacteria as a feasible bioremediation strategy to recycle oxidized NO_x back to its inert and environmentally-friendly gaseous state in the atmosphere.

2. Biogeochemical Nitrogen Cycle

Nitrogen gas makes up to over 78% of all gas in the atmosphere. Nitrogen gas is converted to multiple states in its biogeochemical cycle between the atmospheric, soil, and aquatic environments. The biogeochemical cycle of N is initiated by spontaneous actions such as volcanic eruptions, hydrothermal fields, lightning during rain, and the nutrient fluxes supplied by various biological processes in the biosphere [29]. Nitrogen and its cycling is essential for different forms of life, as it can react with other elements to change oxidation states from −3 to +5. These oxidation states are commonly represented by the following compounds: ammonia and ammonium (NH_3 and NH_4) with an oxidation state of −3, hydrazine (N_2H_4) −2, hydroxylamine (NH_2OH) −1, nitrogen gas (N_2) with a net oxidation state of 0, nitrous oxide (N_2O) +1, nitric oxide (NO) +2, nitrite (NO_2) +3, dinitrogen tetroxide (N_2O_4) +4 and finally nitrate (NO_3), with an oxidation state of +5; this variety grants nitrogen great biological significance in the context of microbial metabolism [30,31].

As an inert gas in the atmosphere N_2 is non-reactive and non-flammable, which makes it easy to quickly harvest for applications in various industries, which then causes its cycle to be controlled by both natural and human activities, as shown in Figure 1. Conversion of this gas to liquid nitrogen has been carried out globally in industries and is used for cooling metallic industrial equipment used under high pressure and heat. The same cooling characteristics of nitrogen lead to the commercialization of its liquid form, where it is even transported in cryogenic liquid tankers due to increasing global demand [32]. Nitrogen is an essential element for all life forms, as it is an integral part of amino acids and proteins in all life forms, and part of the purines and pyrimidines that make up the DNA and RNA molecules in all biological entities. Biological and metabolic processes circulate different forms of N, from its least oxidized form, −3 (NH_3), to the most oxidized form, +5 (NO_3), which are mostly facilitated by enzymes present indifferent life forms. Bacteria account for most of the circulation processes, as they play major roles in decomposition breaking down organic matter and releasing different N species into the environment [33]. Breaking down of dead organic matter releases N, mostly in a form of NH_3, which is recycled into the soil to be reused by plants as fertilizer, while the remaining quantity is oxidized by bacteria to NO_3 [34]. Inorganic N species are present in the atmosphere and soil, and dissolved in various water bodies, forming part of the biogeochemical cycle. Their increasing quantity disturbs the spontaneous biogeochemical N-cycling, halting and modifying the natural ecosystem. N cycling has become more fast-paced with urbanization, as gasoline-powered vehicles and machinery that use fossil fuels that contributing to atmospheric gaseous N species (N_2O and N_2) in developed countries [35,36]. Out of all gaseous N species in the environment, various anthropogenic activities and the many metabolic processes of microorganisms together account for over 85% of the N_2O in the atmosphere, the accumulation of which results in detrimental conditions in the biosphere [37,38]. Nitrous oxide gas is a greenhouse gas, and N_2 is the most environmentally-friendly gas from the cycle to be released into the atmosphere, as it becomes an inert and non-reactive gas until fixed by rain and lighting to restart the cycle. Nitrogen is biologically cycled in five extensively-studied processes: nitrogen fixation, nitrification, assimilatory and dissimilatory nitrate reduction, denitrification, and anaerobic ammonium oxidation (ANNAMOX); these are facilitated by microorganisms and their different nitrogenous nutrients in the environment (Figure 1) [9,38]. Denitrifying bacteria, which can reduce N from its most oxidized form, NO_3 to N_2, are present in the environment and can facilitate the release of most nitrogenous gasses in the environment. Denitrifiers,

in synergy with other microorganisms facilitating nitrogen fixation, ammonification, and nitrification, are responsible for cycling N in both water and soil, making the control of the N cycle in the biosphere predominantly dependent on microorganisms [34].

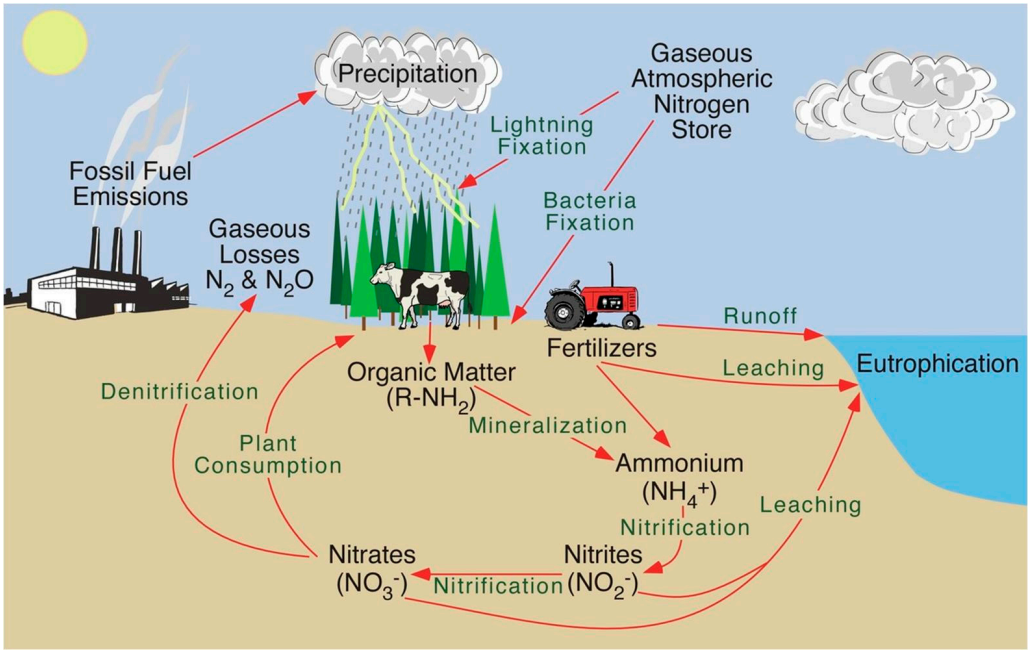

Figure 1. Influence of natural and human activities on the biogeochemical cycle of nitrogen (Used with permission from [38]).

3. Sources and Effects of Nitrate Contamination in Water

Different industrial activities contribute to NO_3^- contamination in the environment, as shown in Table 1, with surface and groundwater bodies being the most affected [39]. Sources of NO_3^- in the environment can be natural; however, anthropogenic activities contribute most of the NO_3^- that accumulates in different localized water bodies, resulting in devastating environmental and health impacts [40]. With rapid urbanization and industrialization globally, N in the form of NO_3^- occurs in the environment in varying concentrations, depending on its sources. Nitrate concentrations as high as 3000 mg/L are discharged from industrial sites with other chemical waste and leach into nearby rivers and streams, threatening both aquatic and terrestrial life [41,42].

Table 1. Sources of anthropogenic nitrate contamination.

Source	Location	Contaminated Water Body	NO_3^- Concentration mg/L	Reference
Explosives factory	China	Wastewater catchment	3600	[43]
Explosives factory	Poland	Wastewater catchment	3000	[42]
Farm	Pakistan	Groundwater	1610	[44]
Farm	Namibia	Groundwater	1000	[13]
Sewage	Tanzania	Groundwater	929	[45]
Farm	South Africa	Surface water	193	[46]
Sewage	Iran	Aquifer	166	[47]
Mine	China	Groundwater	109	[22]
Mine	South Africa	River	50	[41]

Various established industries utilize N-derived chemicals daily, making it inevitable that NO_3^- contamination will occur in the environment. Primarily, contamination is due to agricultural practices, where N based fertilizers, pesticides, and herbicides are used in crop farming practices [48]. Due to its toxicity, NO_3^- is used in negligible quantities as an

N supplement in feedstock added to replace urea and serve as a nutritional additive for ruminants, with care taken not to cause poisoning to the livestock [49].

According to the Environmental Protection Agency (EPA) of the USA and the Department of South African Water Affairs and Forestry, up to 10 mg/L of NO_3^- is an acceptable maximum limit of NO_3^- in drinking water. However, this amount is usually exceeded in domestic water used in most rural areas in South Africa, especially around farming and mining sites [18]. South Africa, as an extensive mining country, has alarmingly high NO_3^- water contamination, resulting in up to 200 and 760 mg/L of NO_3^- in ground and surface water systems, respectively [11,46]. In Figure 2, it is noticeable that the distribution of NO_3^- pollution exceeding the maximum accepted limits of 10 mg/L up to over 200 mg/L is spread throughout the country for groundwater sources, which is very alarming given the health hazard posed [46]. Water contaminated solely with NO_3^- is tasteless, odorless, and colorless, which makes it seem less of a threat because it cannot be detected visually or through taste and smell [50]. Studies conducted by [51] proved that up to 25% of the South African population uses surface and groundwater without any treatment, which poses a risk of consumption of NO_3^-, which is not easily noticeable when dissolved. Drinking water with over 10 mg/L leads the digestive system, through the activity of microbiota, to convert most of the NO_3^- into NO_2^-. The NO_2^- in the blood stream reduces the oxygen transport capacity of the blood, resulting in a condition called methemoglobinemia (Blue Baby Syndrome) in infants [18]. The NO_2^- in the bloodstream oxidizes the iron centre of hemoglobin, producing methemoglobin, which is unable to bind and transport oxygen in the blood stream [9]. The condition is lethal in infants and in adults with impaired immune systems, as well as in certain animal pecies, where it mostly causes colon cancer [13,52,53]. Other health effects that NO_2^- can have on humans include hyperthyroidism (goitre) and bladder, breast, and colorectal cancers [18]. In pregnant women, congenital methemoglobinemia can cause spontaneous abortions and malformations in the unborn child [52]. Constant consumption of NO_3^- contaminated water by humans has resulted in fatalities around the world and while contamination is inevitable, its accumulation and spread presents an exception that requires treatment [18].

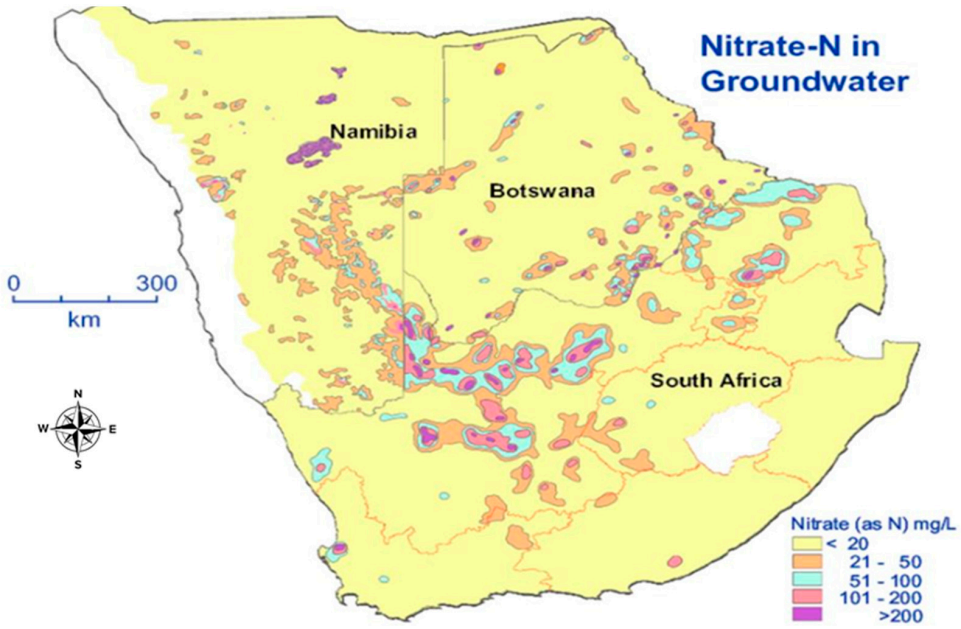

Figure 2. Map of Southern Africa showing sampling points where more than 10 mg/L of nitrates were detected in groundwater (Used with permission from [46]).

In addition, excess NO_3^- in water affects the environment by disturbing the ecosystem through eutrophication [54]. Plants tend to grow in areas with excess $NO_3^-$and the common

primary source is fertilizers washed from nearby farms into streams, rivers, and other uncultivated land [54,55]. Eutrophication on land leads to afforestation beyond natural forest development. This promotes growth of parasitic plants growing closer to farms, sequestering natural nutrients from the soils which are needed by beneficial plants [54]. Aquatic life, such as fish are affected as well, as the purity of water decreases, disturbing the biodiversity in the water through nutrient depletion. Sunlight limitation becomes a growth-limiting factor to aquatic photosynthetic plants, as algal blooms and aquatic weeds cover the surface of stagnant water bodies and those with slow currents [40]. In southern Africa, mining and crop farming are predominantly practiced at both domestic and industrial scales, further exacerbating NO_3^- contamination in the water.

3.1. Nitrate Contamination from Inorganic Fertilizers

It has been estimated that by 2050 the global population will have increased to over nine billion people, while water and food shortage will gradually become a threat to humanity [56]. Shortages of food lead agricultural industries to increase crop production through intensive planting, which carries with it excessive need of water for irrigation, utilizing mostly fresh available surface water [56,57]. In addition, need for fast-growing crops with full nutritional content has led to advances in the science of crop breeding and genetic improvements of plants, including application of inorganic nutrients in the form of fertilizers to meet the required nutritional standards and food demands [2,57].

Application of different organic and inorganic fertilizers can increase crop yields by over 50%, depending on the nutritional need of the particular plant being grown [58]. Inorganic fertilizers (NPK) with high N content were reported to increase maize (*Zea mays* L.) crop yields by 76.2% in the southern part of Africa, which makes them recommendable for application in other crop faming [59]. In 2017, it was estimated that 181.9 million metric tons (Mt) of fertilizer was applied in crop farming globally, and maize was recognized as the greatest consumer of fertilizer in agriculture [60]. Maize, as a basic source of food in Southern Africa, accounts for over 40% of the total fertilizer used in the country annually [60], which is estimated to be 184,500 Mt. The high demand for N-based fertilizer of maize followed by sugar cane and fruit production, accounting for 18% and 20%, respectively, of the total annual fertilizer usage in the country, which is close to 92,000 Mt each. With intensive crop farming practices, the need to protect and nurture crops through further application of mostly N-based pesticides applied to control biological pests and weeds in planted crops grows [13,61].

Organic fertilizers sourced from discomposing organic material and byproducts such as cattle manure are more readily absorbed by plants; however, their supply is limited for large-scale farming, hence utilization of commercial synthetic inorganic fertilizers [60,62,63]. The elements in the inorganic fertilizers: N, P, and K, are regarded as the 'big three' primary nutrients in commercially-produced fertilizers each of these three elements play fundamental roles in plant nutrition [57,64]. Among them, N is considered the most important, as plants absorb it more readily than the others due its importance for plant growth and protein synthesis in growing crops. In global fertilizer demands studies, it was estimated that N accounts for 102.5 Mt of the nutritional requirements in commercial fertilizer, higher than P and K, which account for 45.9 and 33.5 Mt, respectively [61,65]. The quantities of N ($NH_3/NH_4(NO_3)$), P (P_2O_5), and K (K_2O) used in commercial fertilizers define the grade of the fertilizer, which is determined by the nutritional needs of the crops and nutrient deficiencies in the soil determining the required balance of the nutrient supplements in the fertilizers [9,65,66]. Nitrogen is usually added in larger amounts to commonly-used fertilizers in the form of inorganic N compounds such as NH_4^+, NO_2^-, and NO_3^- [61,63]. However, up to 50% of N and 90% of P in inorganic fertilizer are not assimilated by plants and run off from the crop fields during irrigation and rain to be deposited in the nearby water bodies [67,68]. Commercial fertilizers and pesticides for crop farming contribute to most NO_3^- traced in contaminated drinking water sourced from surface and groundwater systems, which causes environmental hazards that threaten

both terrestrial and aquatic life [59,69]. The Nitrogen quantity in fertilizer is estimated to increase by 172% by the year 2050, which poses the risk of further elevating NO_3^- concentrations in the environment [64]. Continuous application of inorganic fertilizers remains on the rise, as crop farming is inevitable due to food demands which consequently result in water contamination [70]. Nitrogen removal strategies are being tested globally in the quest to recycle N from water back into the atmosphere, and this review further explores one promising option.

3.2. Nitrate Contamination from Mining Activities

The mining industry has become a pillar of the economy in the current generation. This industry depends largely on the technology, power, and economic drive from trading base metals and energy fuels such as oil, coal, gas, and uranium, which are mostly sourced from underground [71]. Mining practice started in the mid-1800s with copper, followed by diamonds, which were discovered in 1868, then gold in 1886, of which up until today, remains the most abundant in most African countries [72]. For the extraction of minerals from underground, ammonium nitrate-based (NH_4NO_3) explosives are used to detonate and crush rocks to expose minerals. This practice has been applied globally from the 1900s and continues to this day, resulting in negative environmental effects such as soil, air, and water pollution [71]. In 2005, it was recorded that South Africa had recorded 1113 mines both active an banoned, which produced 55 different valuable minerals that are used locally and exported to most countries in the world [72]. By the end of 2019, it was reported that there were 526 active mines producing 22 minerals in South Africa standing in the top five global leading producers of Pt, Au, coal, and diamonds [73]. Due to such extensive mining, groundwater easily becomes contaminated by various chemicals, including heavy metals such as Cu, U, Fe, and Zn that leach from subsurface rocks. Residual NH_4 that becomes oxidized to NO_3 during detonation of explosives, forms part of these contaminants. Chemicals such as nitric and sulphuric acids are used in the processing of the raw mined minerals and tailings to extract the remaining undetected minerals, which contributes to pollution of both surface and groundwater systems around the mining sites.

A study conducted by [41] revealed the impact of NO_3^- contamination of over 50 mg/L from one mine that was detected in water supplied to a village situated close to a mine in the Limpopo province of South Africa. Groundwater sampled around the Selian mining area in China revealed a maximum NO_3^- concentration of 109 mg/L, which is alarmingly higher than the global acceptable 10 mg/L limit [22]. Most South African mines use ammonium-based explosives, although less data is available on water quality contaminated with NO_3^- from explosives used during mining. On average, mining requires around 0.4 to 1 kg of explosives to crush one ton of rock prior to mineral extraction [74,75]. Mining explosives are made up of chemical mixtures that produce pressure (over 2000 bar), heat (over 3000 K), and gasses that generate waves to crack and break rocks at different speeds depending on the type of rocks and minerals being extracted [75,76].

Detonation of mining explosives uses mixtures such as ammonium nitrate - fuel oils (AN-FO), Amonex-1, and Majdanit 10, which produce high quantities of carbon monoxide and nitrogen oxides (mainly NO_2) as their byproducts, amongst other chemicals and gases [76,77]. AN-FO are low-cost N-based explosives commonly used in both pit and open cast mines; they contain 94.5% ammonium nitrate (NH_4NO_3) and 5.5% fuel oil, which are delivered separately and only mixed at the mining site prior to denotation, hence a great contributor to N contamination [78]. Their resistance to water affords most of the residual NH_4NO_3 a chance to buildup in the mine tailings, eventually dissolving in water over time and causing high NO_3^- concentrations in mine wastewaters [79]. Emulsified explosives are the second most commonly used type of explosives in mining. As with AN-FO, they have a high NO_3^- content, containing 70–90% NO_3^-, over 10% water, and 4% fuel oil, as well as other bulking and packaging additives [80]. The emulsified explosives differ, with phases ranging from liquid to almost solid, allowing them to be delivered to the site ready to be applied to rocks for detonation. There are emulsified water-resistant explosives known to

produce less harmful gasses with low residual NO_3^- content that dissolve in water after detonation; however, these are used for small-scale projects such as pillar erections sites during road constructions [81]. Dynamite is one of the safest types of explosives, and is used widely as it is easy to handle and has few activation needs. Dynamite consists mainly of nitroglycerin (which makes it an N-based explosive) packaged in a suitable material such as plastic cartridges or tubes prior to detonation [82]. Ammonium nitrate explosives are widely used in most industries, including mining and large construction sites in urban areas where at times replacing them with AN-FO in sites where there are nearby water bodies such as rivers and streams becomes vital.

The NH_4NO_3 content of different explosives overtime gets converted to other N species such as NO and NO_2^- as the compounds react with water and oxygen. Spontaneous reaction of oxygen with reduced nitrogen compounds happens rapidly in aqueous solutions, forming nitrate (NO_3^-) [83]. Equations (1)–(3) highlight the dissolution reactions of NH_4NO_3 in the environment, where the NH_4NO_3 dissociates into NO and NO_2^-, then NO_3^-:

$$5NH_4NO_3 + CH_2 \rightarrow 4N_2 + 2NO + 11H_2O + CO_2 \quad (1)$$

$$2NO + O_2 \rightarrow 2NO_2 \quad (2)$$

$$2NO_2 + O_2 \rightarrow 2NO_3 \quad (3)$$

Nitrate contamination in mining wastewater is mostly associated with the explosives used in open pit mines, which require more explosives to extract minerals such as coal, diamonds, and copper [77,84]. The pH of mine wastewater is determined mainly by the way in which the geochemistry of the host rocks types alters the chemistry of the wastewater, which further affects the microbial communities present in the environment [85,86]. Wastewaters with an acidic pH close to neutral (between 5 and 7) and mostly alkaline pH (between 7 and 9) have been reported to contain elevated concentrations (400 to 500 mg/L) of NH_3^- and NO_3^- and lower concentrations of dissolved heavy metals. In contrast, highly acidic mine drainages with pH below 5 have been found to contain more dissolved metals and less NO_3^- concentration [87,88]. During mining, tailings that are transported to the surface from the pits are piled next to the mine, where the residual explosives are washed off and dissolve in rain water, which eventually flows into nearby streams and rivers, resulting in elevated NO_3^- concentrations [9,19,22]. Mine wastewater remediation strategies tend to focus on metal and SO_4^{2-} remediation due to their known toxicity and lethality, while NO_3^- remediation garners less focus. With over 300 million tons of commercial explosives used in South Africa, a remediation strategy for NO_3^- removal is required for restoration of the polluted water, soil, and air contaminated by mining explosives.

4. Methods Applied for Mitigating Nitrate Contamination in Wastewater

Due to the environmental hazards NO_3^- contamination has in water, a remediation strategy which is environmentally friendly, sustainable, affordable, and effective is needed in extensive mining and crop farming areas [89]. There are a variety of mitigation and remediation strategies for removal of high NO_3^- concentrations in both water and soil, which can either be biological [90] or chemical [91]. Nitrate is removable in low amounts is possible by conventional physical water remediation techniques such as filtration, sedimentation, flocculation, and coagulation; however, due to its water solubility and various oxidation states, these methods are not always feasible [19,92]. Biological systems are applied globally to remove NO_3^- in industrial wastewaters, although mostly without the production of N_2 as the terminal product of the system [42]. Thus, additional steps to reduce NO_3^- to N_2 gas are needed, which can be achieved through additional chemical, physical, and biological processes. Biological approaches such as phytoremediation of NO_3^- by plants in wetlands, and microbial bioremediation, where denitrifying bacteria in continuous flow reactors are applied, have yielded promising results as the most effective and efficient approach to the removal of NO_3^- from water [93]. However, little information is available regarding bacterial biofilm reactors for NO_3^- removal from industrial wastewaters and

its conversion to inert N_2 gas [94,95]. Due to various sources of NO_3^- in the environment, isotopic compositions of nitrates ($^{15}N/^{14}N$ and $^{18}O/^{16}O$) are used to determine the source of contamination and aid the selection of a suitable remediation technique, as these data can indicate the origin of N in water [96]. Discussed below are different chemical, physical, and biological remediation techniques applied in NO_3^- removal.

4.1. Chemical Nitrate Remediation

Chemical remediation is based on the reaction of the reactive chemical with the contaminant, converting it to a lesser toxic form, mostly into precipitates when the contaminant is a metal [97]. There are various inorganic N removal methods applied in different treatments based on the contaminant, including conventional chemical denitrification, zero-valent metal nano-particles, and H_2-driven catalytic denitrification [98]. A study conducted by [91,93] reported the use of Al powder in the reduction of NO_3^- to NH_3, NO_2^-, and eventually N_2 as a conventional chemical denitrification approach based on the level of the alkaline pH of the water. Lithium niobate ($LiNbO_3$) was shown to remove over 98% of dissolved NO_3^- ions under neutral pH conditions [99]. Iron (Fe) in its different oxidation states has been applied in different studies to reduce NO_3^-, mainly to NH_3 as the terminal product, with large deposits of iron sludge [93,99]. Due to constantly increasing NO_3^- concentrations in surface and ground water systems, Al and Fe reactions become easily saturated and require catalysts such as Cu, Rh, and Pd to remove NO_3^-, which further introduces solid metal waste as byproducts in the water bodies [100]. Spontaneous reduction of NO_3^- to NO_2^- and NH_4 is possible, especially when NO_3^- is present in high concentrations. Due to this redox factor, catalytic reduction of NO_3 by different metals such as Cu, Pd, Pt, Rh, and TiO_2 has been explored as an option to reduce NO_3^- [101]. The catalytic method has been advanced to the point of using two different metals in the process to optimize NO_3^- reduction, with a catalytic metal (Cu, Pd, Pt, Ti) applied on a supporting metal such as Titanium (TiO_2) that aids and promotes the catalytic reduction, however, this seldom affords the reduction of NO_3^- to N_2 [101,102].

For treatment of large volumes of industrial wastewater, this method is very expensive to maintain and apply as a continuous remediation system [103]. Most chemical remediation approaches produce byproducts that require special disposal methods due to their potential to cause secondary contamination. Solid state byproducts of the chemical remediation process require a joined polishing step to remove them from remediated water in order to prevent re-dissolution back in to the water, causing recontamination. Certain byproducts can be more harmful to the environment than the primary contaminant, even after the system has stopped. To prevent secondary contamination, careful chemical application and thorough research regarding the chemical agents used for remediation is needed [104]. The above discussed chemical remediation methods are based on NO_3^- reduction to lesser oxidized nitrogen species (NH_3 and NO_2^-), and as a result, physical and electrochemical methods are needed to accomplish the reduction of all oxidized N species to inert N gas [98]. Such remediation strategies are laborious and costly, with multiple steps, making the method inapplicable for treating large volumes of industrial wastewater contaminated with NO_3^-.

4.2. Physical Nitrate Remediation

Common physical NO_3^- remediation strategies include reverse osmosis (RO), ion exchange (IX), membrane filtration, adsorption, and electro-dialysis, which due to limitations are often coupled with the addition of various chemicals to aid the water treatment process [98,100]. Physical NO_3^- remediation from water includes the elimination and removal of NO_3^-, generally with a membrane that filters, repels, and in other applications binds to the NO_3^- ions [105]. Several of these membranes can be chemically charged with compounds that react to the NO_3^-, converting its state to a lesser oxidized form, hence referred to as physico-chemical remediation [106].

Nano-filtration through cation charged membrane filters is one of the techniques used to physically remove high concentrations of NO_3^-. The method is 60–80% effective, and in remediation systems where complete reduction of NO_3^- is required a supplemented polishing or secondary remediation method must be applied [107]. Sulphates attach to the nano-filters when present, as they are common co-contaminants in most mine wastewaters, resulting in less removal of NO_3^- [107,108]. Application of adsorbent material which is negatively charged serves as a porous membrane barrier that repels NO_3^- ions in the contaminated water from passing through the material. This was proven to have an NO_3^- remedial effect of up to 88.8% [109,110]. The physicochemical stability and surface properties of clay have led to its application in direct removal of NO_3^- through adsorption without any chemical or bacterial addition [100,111].

Ion exchange (IX) has been widely applied in most drinking water plants with low NO_3^- and has proven effective, reliable, and sustainable. The IX method uses resins that are chemically charged with a strong base anion, allowing exchange of chloride or carbonates that exchange with NO_3^- ions on the resin [93]. In industrial wastewater remediation, application of IX removes SO_4^- prior to NO_3^-, which is a disadvantage when having both contaminants are present in high concentrations [106]. The method requires extensive maintenance, including washing and recharging the resins, and creates brine dumps of NO_3^- that are time-consuming to dispose, hence becoming an environmental burden and causing secondary contamination [103].

Reverse osmosis (RO) is a high pressure-based approach where water contaminated with NO_3^- is passed through semi-permeable filters, membranes, and brine seals, trapping the NO_3^- ions [112]. The method is efficient for small-scale remediation sites and has the capability to remove multiple contaminants without any chemical additives. Reverse osmosis is dependent on high pressure pumps that require a constant electrical power supply [113,114]. It is not ideal for industrial wastewater that could have various suspended solids, extreme low or high pH, and metals which can easily clog the system, necessitating regular membrane and filter changes [1]. In such instances, pretreatment of the water is needed prior to application of RO, commonly through chemical neutralization and filtration to remove solids, which is a setback for system efficiency [112]. The greatest limitation of physical NO_3^- removal approaches is their low efficiency in removal of high NO_3^- concentrations from industrial wastewater, which commonly includes multiple contaminants. These methods require high maintenance costs and mostly result in accumulation of NO_3^- waste brine and different ions that become challenging to dispose and cause secondary contamination [54,101]. The limitations of the chemical, physical, and physicochemical NO_3^- removal methods discussed above demonstrate the importance of an efficient, effective, reliable, and cost-effective remediation approach toNO_3^- water contamination. To circumvent the above-mentioned challenges, the biological NO_3^- remediation approach discussed below could serve as a green and environmentally-friendly method that could mitigate the contamination and restore most industrial wastewaters.

4.3. Bioremediation as an Option for Mitigating Nitrate Contamination

The use of living organisms for the remediation of contaminated water (bioremediation) has been a focus in global water research, with the aim of developing an environmentally friendly technology using biological entities that produce less or even no harmful byproducts [115]. Biological remediation of NO_3^- and other N-based contaminants has been proven to produce N_2, which is an environmentally friendly byproduct that is recycled back into the atmosphere [116]. Different biological entities, both eukaryotic (plants, fungi and algae) and prokaryotic (bacteria and archaea), have been proven to have N metabolisms that uptake NO_3^- from the environment and convert it into different forms [117]. Nitrate reduction by different biological entities can yield different N byproducts, such as NO_2, NH_3, NO, N_2O, and N_2, depending on the organisms and environmental conditions [118]. Biological denitrification is a stepwise enzymatic process dependent on enzymatic reactions, as the organisms' inherent proteins reduce the oxidized N into other forms [119]. In

biological systems, NO_3^- removal from water can be either assimilatory, where the N is incorporated into the biomass of the organism, or dissimilatory, where the oxidized N is reduced through catabolism to less oxidized forms based on the organisms and conditions in the environment [120]. Several eukaryotic microorganism, such as fungi, diatoms, and ciliates, can uptake NO_3- for storage, then when their environment becomes favorable for dissimilatory NO3- reduction, they can reduce it to N_2O or NH_3, NH_3, and NO_2, respectively [118]. These byproducts can be re-oxidized back to NO_3^- in the presence of oxygen, which makes the organism not ideal for sole application in a remediation system.

Phytoremediation is one of the green chemistry techniques applied to remove dissolved contaminants that can be absorbed by different plants in stagnant or slow flowing streams [121]. Plantation of aquatic plants such as water hyacinth (*Eichhorniacrassipes*), water lettuce (*Pistia stratiotes*), and water spinach (*Ipomoea aquatic*), which require high concentrations of N, can uptake up to 50 mg/L, hence their recommendable cultivation in phytoremediation sites to remove NO_3^- [122]. Aquatic plants grow faster (1 to 12 days) than other terrestrial plants, and certain varieties can sequester most of NO_3^-, up to 90% of 120 mg/L from NO_3^- contaminated water, lowering the concentration levels near to the acceptable limit of 10 mg/L [123]. This method is environmentally friendly and cheap to maintain, as it only needs a large wetland surface area. It requires no addition of chemicals into the water which could result in post remediation contamination. Application of the phytoremediation strategy in NO_3^- removal is limited to waters with lower metal concentrations as co-contaminants, as these may hinder plant growth. In certain wastewater systems, growing plants could take longer to grow due to the co-contaminants that hinder their growth, together with low bioavailability of nutrients besides NH_3^+ and NO_3^-, hence affecting the effectiveness of the system [124].

Photosynthetic high N-demanding microorganisms such as microalgae and cyanobacteria have been recognized as potential biological agents, and can remove up to 100 mg/L of NO_3^- from drinking and ground water with low metal concentrations [125]. The denitrification capacities of these microorganisms are limited by temperature, pH, metal concentrations, and sunlight, several of which could render this method futile for annual industrial wastewater remediation systems.

Amongst all modern NO_3^- remediation strategies developed for industrial wastewater, microbial application for denitrification is the most promising one which has been proven to be cost effective, durable, and effective with less to no secondary contamination [115,125]. Application of denitrifying bacteria for industrial wastewater remediation can be affected by various factors such as oxygen concentration, pH, temperature, carbon sources, metals, and microbial diversity in the environment [115]. For low NO_3^- concentrations in drinking water, a suitable carbon source at low levels of dissolved oxygen will accommodate the reduction of NO_3^- to N_2. Denitrifying bacteria used in this remediation process are categorized into two groups based on their energy sources for the reduction of oxidized N [125]. Heterotrophic denitrifiers use natural and chemical organic carbon sources to harvest energy from to perform denitrification in wastewater with high NO_3^- concentrations over 1000 mg/L [126,127].

The second category, the autotrophic denitrifiers, require inorganic electron donors such as hydrogen and sulfide as their energy source and inorganic carbon compounds to perform denitrification, with limitations of up to 500 mg/L [128]. The two groups of denitrifiers capable of heterotrophic and autotrophic denitrification can co-exist in a wastewater system, where they simultaneously facilitate over 95% of complete denitrification of 1000 mg/L of NO_3^- to N_2, as reported by [129,130]. This approach has been globally applied and extensively studied, with reports on how to optimize the technology based on different contamination conditions, mostly with pure bacterial isolates [131,132]. McCarty, 2018 [117] reviewed various approaches for N removal from industrial wastewater and concluded that a combination of anaerobic denitrification and ANAMMOX excels in the production of N_2 gas, which is the ideal desired byproduct. Below, details of different path-

ways by which bacteria metabolize N are discussed, with brief regulations and optimization of promoting different enzyme-facilitated stages.

5. Microbial Nitrogen Metabolism and Biochemical Pathways

Application of bacteria in NO_3^- remediation system requires knowledge and understanding of the biochemical pathways (Boxes 1–7) used to metabolize N at its different oxidation stages. The oxidation and reduction processes of N can be influenced by physical and chemical processes; mainly, they are catalyzed by microorganisms that use N as the terminal electron acceptors in their metabolic pathways [28,119]. Nitrogen and its oxidized species occur in nature in trace quantities; however, it can be concentrated in specific environments where certain bacterial groups dominate the biogeochemical processes that favor the production of either NO_3^-, NO_2^-, NO, N_2O, NH_3, or NH_4^+ [133]. Nitrate (NO_3^-), as the highest oxidized N species, can be reduced in various pathways facilitated by microorganisms, including bacteria, algae, fungi, and higher plants.

Nitrate reduction results in lowering of the oxidation state of N, such as in dissimilatory NO_3^- reduction, which predominantly yields NO_2^- and NH_3 [134,135]. Complete denitrification is the ultimate NO_3^- reduction pathway, where gaseous N is released into the atmosphere as the terminal byproduct. Denitrification is a stepwise process that can be facilitated by various microorganisms such as *Pseudomonas stutzeri* and *Paracoccus denitrificans*. *Pseudomonas stutzeri* was previously proposed as a complete denitrifying model organism which has the capability to reduce nitrate (NO_3) to nitrogen (N_2) gas [136]. Stepwise denitrification of NO_3^- to N_2 gas can be facilitated by other *Pseudomonas* strains such as *P.aeruginosa* [137], *P. fluorescens* [138], and *P. denitrificans* [139], which host all of the same denitrification genes as *P.stutzeri*. The two bacterial genera, *Pseudomoas* and *Paracoccus*, that include strains bearing all required genes to reduce NO_3^- to NO_2^-, NO and further to gaseous N_2O and N_2 gasses are used to benchmark denitrification kinetics under different conditions [119,135]. Denitrification kinetics are used to determine the efficiency of the microbial community or pure culture by monitoring the complete reduction of NO_3^-, which is determined by detection of intermediate N species and the production on N_2 gas for complete denitrifiers [19,119].

The N cycle in the environment is facilitated mainly by bacteria that possess genes encoding for enzymes that catalyze the reduction and oxidation of different N species [140,141]. As represented in Figure 3, the extensively studied processes that take place in the microbial N cycle are highlighted together with genes and enzymes involved in the processes. Nitrogen fixations, nitrification, nitrate reduction (assimilatory), dissimilatory nitrate reduction to ammonium (DNRA) and nitrogen (complete denitrification), and finally the anaerobic ammonium oxidation (ANNAMOX) process as an alternative N_2 gas production process, are depicted [140–143]. Each process in the N cycle can be applied separately by selection of specific bacterial groups in the environmental conditions that promote the facilitation of the desired process [144,145].

In the absence of oxygen, anaerobic respiration takes place, resulting in specialized microorganisms within the environment that utilize NO_3^-, SO_4^{2-} and Fe^{3+}as electron acceptors while breaking down organic compounds as energy sources. From these common contaminants, NO_3^- is an energetically favorable electron acceptor used by most bacteria. Bacteria acquire energy by oxidizing available electron donors such glucose, ethanol, acetate, glycerol, and other carbon sources. For electron acceptors, O_2 is commonly used up first, followed by NO_3^- in the absence of O_2, as it is used by microorganisms as a micronutrient, protein, and amino acid constituent for biomass while yielding more energy as the most electropositive electron acceptor after O_2. In an environmental setting where microorganisms with different metabolic activities are present, NO_3^-, with its high reduction potential, becomes the most preferable electron acceptor compared to other available contaminants such as Fe_3^+ and SO_4^{2-} that yield less energy from the oxidation of carbons sources [146]. Although there are denitrifiers which solely perform complete denitrification yielding N_2 gas, different bacterial species can partially perform

both assimilatory and dissimilatory NO_3^- reduction by reducing NO_3^- to NO_2^- or NH_3 and N_2 through synergistic metabolic activities where byproducts of one bacterial group serve as an electron acceptor of another to continue the N cycle in one environment. Sulphate reducing bacteria such as *Desulfovibrio* sp. are capable of reducing NO_3 to NH_3 in the absence of O_2 and SO_4^{2-} [147], while enterobacteria such as *Escherichia coli* can reduce NO_3^- to NO_2^- in the absence of oxygen [133,148]. The reduction of NO_3^- by other bacterial species is based on the environmental conditions that favor and promote its reduction as a microelement needed for the growth of different bacteria. This phenomenon of co-existence of different bacteria with different metabolisms has been exploited in bioremediation studies, where these bacteria are enriched and applied as consortia to remove different N species from water. Nevertheless, regulation factors of each denitrification step (highlighted in Figure 3) need to be understood in order to apply consortia aimed at performing complete denitrification, as changes in microbial diversity and environmental conditions can halt and terminate the denitrification pathway.

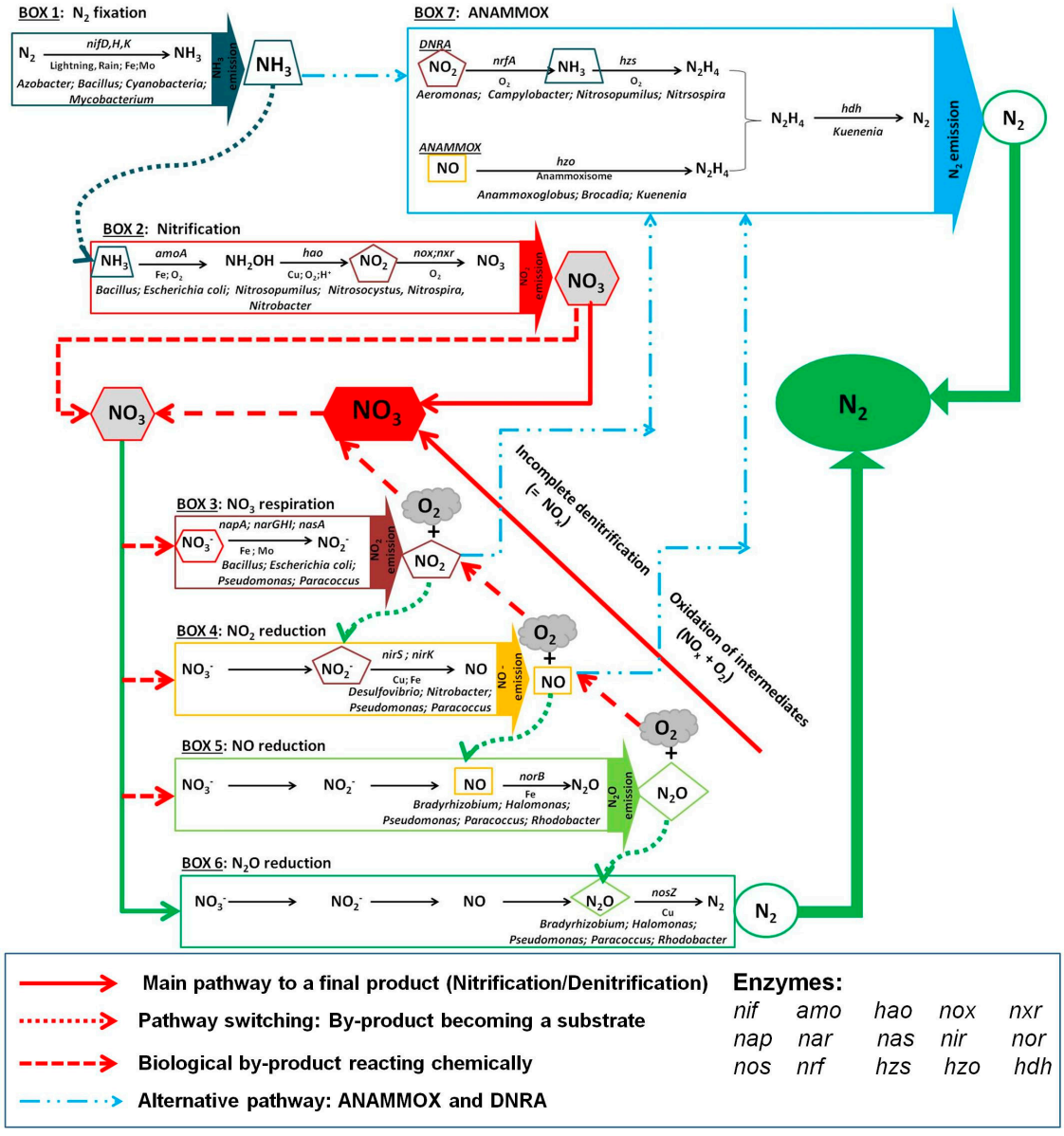

Figure 3. Biochemical pathways involved in the microbial nitrogen cycle facilitated by inherent enzymes and their co-factors.

Box 1. Nitrogen fixation and ammonification.

Nitrogen gas (N_2) is abundant and non-reactive in the atmosphere until it is fixed into reactive nitrogen species (NH_4^+, NO_2^-, and NO_3^-) by lightning (2%) and through biological processes (98%), which are dominated by microbial metabolic activities [149]. N is naturally converted to NH_3 during rain and thunder, where it becomes readily available inthe soil to be used by plants and other biological forms. This process is termed N-fixation, and can be catalyzed by prokaryotes such as bacteria using the nitrogenase enzyme (nif) that catalyzes the conversion [38]. Bacterial groups such as Azotobacter, Cyanobacteria, Bacillus, and Mycobacteriaum are prominent in facilitating the process, mostly in soil [134]. Ammonification can occur through other processes where other N compounds than N_2 gas are converted to NH_3, such as dissimilatory nitrate reduction to ammonia (DNRA), which is the dissociation of NO_3 by bacteria to form NH_4 [66].The most common natural ammonification process happens during the decomposition of organic matter when animals and plants are degraded by bacteria, releasing most of the NH_3 into the soil. In the absence of O_2, NH_3 can be a terminal biological product and accumulates when N_2 gas is available for fixation, halting N cycling [119]. Ammonia in soil and water can be oxidized to other forms of N, such NO, N_2O, NO_2, and ultimately NO_3, which can accumulates in the environment; this process is called nitrification [149,150].

Box 2. Microbial nitrification.

Nitrification is the stepwise formation of NO_3^- from various nitrogen species that are mostly oxidized biochemically and chemically. This process is biologically facilitated, mostly by heterotrophic bacteria such as *Bacillus* sp. that are ubiquitous due to their ability to utilize different carbon sources to thrive in different temperature and pH ranges while oxidizing N [2]. Bacterial nitrification is more effective in aerobic conditions with temperatures between 25 and 28 °C [150,151]. Ammonia is oxidized to NO_2^-in two steps by autotrophic nitrifying microorganisms that first synthesize ammonia monooxygenase (*amo*), which converts NH_3 to hydroxylamine (NH_2OH). Further oxidation of NH_2OH is catalyzed by hydroxylamine oxidoreductase (*hao*), which completes the process by adding one O_2 molecule to form NO_2 [152,153], as depicted in Box 2, Figure 3. The process has been proven to not be limited to ammonium oxidizing bacteria, and is carried out by some archaea such as *Nitrosopumilus maritimus* which are capable of converting NH_3 to NO_2^- in the presence of O_2bysynthesizing the *amo* enzyme [153]. Nitrification by *hao* and *amo* enzymes takes place in aerobic conditions; when O_2isdepleted or when *hao* ceases to be synthesized, incomplete oxidation of NH_2OH will accumulate, which over time forms N_2O. Formation of N_2O under anaerobic conditions has been reported to be facilitated by a c-type heme of *hao* (cytochrome P460) in most closed systems when O_2 has depleted [89,152]. Continuous oxidation of N from NO_2 to NO_3 is facilitated by nitrite oxidase (*nox* and *nxr*) enzymes that are synthesized in the presence of oxygen. Nitrification can take place in the soil, deep oceans, and lakes, where chemolithotrophic bacteria such as *Nitrosomonas*, *Nitrosocystus*, *Nitrospira*, and *Nitrobacter* convert NH_3 first to NO_2^-, then to NO_3^- [154,155]. Nitrate formation from NH_3 has been observed in fungi such as *Asperigillus*, *Penicillium*, and *Absidiacylindrospora*, which are ubiquitous in the soil [156]. Most bacteria assimilate N for their growth when species such as NH_4^+, NO_2^-, and NO_3^-aredirectly transported through the cell membrane to be converted to metabolites for nucleic acid and protein formation [157]. Nitrogen assimilation in the environment results in the loss of N as it is incorporated in the biomass of different microorganisms, and hence not recovered in the geochemical cycle. For continuous cycling of N, NO_3 is reduced through different processes discussed below, including bacterial denitrification that is either assimilatory or dissimilatory depending on the fate of the reduced N specie [9].

Box 3. Nitrate to nitrite.

During denitrification by microorganisms, NO_3^- is taken up as a terminal electron acceptor to be converted into NO_2^-, which is transported intracellularly using nitrate reductase proteins (*Nar*). Nitrate reducers have either one of the two enzymes coded by the nitrate reductase genes *napA* and *narG*, which are transcribed and translated into nitrate reductases, then embedded in and on the periplasmic membrane [119]. Nitrate can be reduced to NO_2^- within the cell by respiratory nitrate reductase (*Nar*) and in assimilatory NO_3^- reduction by nitrate reductase (*nasA*) depending on the denitrification operon of the prokaryote [9]. Most bacteria found in the environment are not true denitrifiers; instead, they undergo NO_3^- respiration, where they use NO_3 as their growth factor for biomass and protein biosynthesis while producing NO_2 as a terminal byproduct [19,148]. Nitrate-respiring bacteria use one or two of the three nitrate reductases (respiratory nitrate reductase (*nar*), periplasmic nitrate reductase (*nap*), and/or assimilatory nitrate reductase (*nas*)), which have Fe and Mo as metal co-factors that promote their activity. These genes can be found in one micro-organism, and differ in their operon location, organization, and structure [9,134]. This process yields large amounts of energy in the form of adenosine tri-phosphate (ATP), as NO_2^- further serves as an electron acceptor when NO_3^- is depleted during respiratory ammonification (forming NH_4^+) or denitrification (forming NO, N_2O, or N_2) [158]. Several microorganisms, such as *Escherichia coli* and *Bacillus subtilis*, possess the *narG* gene, which facilitates NO_3^- respiration, converting it to NO_2^- as the terminal byproduct [136]. This serves as the first complete denitrification truncation step, forming NO_2 as a terminal byproduct mostly due to the absence of bacteria that contain genes that facilitate NO_2 reduction.

Box 4. Nitrite to nitric oxide.

Nitrite is one of the two inorganic signaling molecules in the N cycle, together with NO; they occur intracellularly during denitrification. Reduction of NO_2^- to NO is facilitated by bacteria containing one of the two well-characterized nitrite reductase genes *nirS* and *nirK*, which transcribe the cytochrome *cd-1* and copper-containing nitrite reductases, respectively. Both enzymes facilitate the conversion of NO_2^- to NO within a bacterial cell [119,159]. The two nitrite reductase genes are structurally different in the sense that *nirK* contains copper (Cu-Nir) while *nirS* contain iron (heme*c* and hemed_1 (*cd*-Nir) subunits; however, both code for the same nitrite reductases with the same function [159,160]. Expression of these genes is only activated when O_2 concentration is lower than intracellular NO_2^- concentration [119]. These genes are often used in biodiversity and microbial ecology studies to determine the abundance of NO_3^- and NO_2^- reducing bacteria, as they serve as biomarkers for denitrifying bacteria [159]. Lack of nitrite reductase genes results in the inability of the microorganism to reduce NO_2^-, which accumulates within the cell until it is converted to NH_4^+, rendering the micro-organism a nitrate-respiring bacteria instead of a denitrifying bacteria [19,161]. Nitric oxide does accumulate intracellularly, becoming toxic to the bacteria, and is then emitted into the environment to be oxidized into other N species.

Box 5. Nitric oxide to nitrous oxide.

Nitric oxide (NO) contains unpaired electrons, making it a free radical and a highly reactive nitrogen specie (RNS) that can be converted to NO_2, N_2O, N_2O_3, N_2O_4, and N_2O_5 depending on the concentration of the dissolved oxygen [162]. Nitric oxide has molecular fragmenting effects similar to reactive oxygen species (ROS), with a very short half-life of 10^{-9} s, hence its instability and capacity to react quickly. It is difficult to quantify, and hence is termed a reactive nitrogen specie (RNS) in the nitrogen cycle [163]. During biological NO_3^- reduction, NO is produced intracellularly; once released from the cell, it is converted to other forms of NO_x. For a system desiring to completely reduce NO_3^- to N_2, emission of NO is not an advantage, as it can be converted by oxidation back to $NO_2^-$and then NO_3^- or through its reduction to N_2O as a terminal product of a remediation system. Excessive emissions of RNS in the form of NO have harmful effects similar to ROS on bacterial cells, causing internal oxidative damage due to their high reactivity [164]. In the context of NO_3^- bioremediation, reduction of NO is facilitated by nitric oxide reductases (*norB*) to nitrous oxide (N_2O), which will remain intracellularly until reduced to nitrogen gas (N_2) or emitted by the cell if the nitrous oxide reductases are not expressed in order to facilitate its reduction [119,162]. The enzyme is hosted by bacteria such as *Bradyrhizobium*, *Halomonas*, and *Rhodobacter*, which are incomplete denitrifiers containing all denitrification genes except the *nosZ* gene, and hence unable to reduce N_2O, emitting it as their terminal product [135]. Nitrous oxide is a greenhouse gas, and in a denitrification system it is an indicator of an incomplete denitrification process. Once emitted, N_2O can either accumulate in the atmosphere, be re-oxidized back to NO, or be reduced by complete denitrifiers to N_2 gas that is environmentally friendly.

Box 6. Nitrous oxide to nitrogen.

Nitrous oxide (N_2O) is one of the gaseous intermediate byproducts of denitrification systems where denitrifying bacteria lack the nitrous oxide reductase (*nosZ*) gene to reduce N_2O to N_2. The gas is a potent greenhouse gas with a global warming potential 298 times greater than CO_2. While the gas is emitted naturally in the environment in lower quantities, incomplete denitrification systems that emit N_2O gases and burning of fossil fuels are major sources [152,165]. Of all the nitrogen species, N_2O and N_2 are the most volatile, accumulating in the atmosphere mostly from anthropogenic activities such as burning of fossil fuels, utilization of explosives, decomposition of organic matter, and other forms of microbial activity [9]. N_2O gas can be emitted during the second step of nitrification, when oxygen is depleted and hydroxylamine oxidoreductase (*hao*) oxidizes NH_2OH to N_2O instead of NO_3^-. Complete nitrate reduction results in N gas that is environmentally friendly, as it is continuously cycled from N_2 back to NH_3 and NO_3^- through the fixation and nitrification processes, respectively. Microorganisms lacking the *nosZ* gene are often referred to as incomplete denitrifiers, as their terminal denitrification product is either N_2O or NO instead of N_2 [119,140]. Bacteria containing the nitrous oxide reductase gene (*nosZ*) can facilitate the reduction of N_2O to N_2, which is dependent on Cu ions that serve as cofactors for the proteins that render the bacteria a complete denitrifier [119]. Biological denitrification is performed by mostly gram-negative Proteobacteria such as *Paracoccus*, *Agrobacterium*, *Pseudomonas*, and *Acinetobacter*, which possess all of the denitrifying enzymes that facilitate the reduction of NO_3^- to N_2 gas [152].A system that produces less or no N_2O is desirable in denitrification, and advances in achieving this have been a pinnacle focus in denitrification studies globally. Removal of N_2O has become a major focus in most N-based projects due to its environmental impacts, as natural and engineered denitrification ecosystems account for 40 to 85% of global N_2O emissions [152]. One suggested approach to achieving complete denitrification is to supplement the systems with metal ions such as Cu, Fe, and Mo, which are required for the activation and optimum functionality of nitrous oxide reductases and other denitrifying enzymes [166]. Complete denitrification is ideal for a wastewater bioremediation system with high NO_3^- concentration; the byproduct of the system would be environmentally friendly with no secondary contamination, as N_2 gas is restored to its inert gaseous state in the atmosphere to be incorporated back into the N cycle.

Box 7. Anammox: analternative denitrification pathway.

Anaerobic ammonium oxidation (ANAMMOX) is a microbial pathway where NH_3 is oxidized to hydrazine (N_2H_2) in anoxic environments [139]. This process is carried out by micro-organisms that contain genes that transcribe hydrazine synthase (*hzs* and *hzo*), facilitating the synthesis of N_2H_2 and hydrazine dehydrogensa (*hdh*),whichoxidize N_2H_2 to N_2 [152]. Excess NH_3 and the intermediate denitrification byproducts highlighted in Box 3 (NO_2) and Box 4 (NO) can be removed by anammox to N_2 under oxygen-limited conditions. In environments with excess NO_2 concentration and low O_2 concentration, microorganisms with a unique organelle called ananammoxisome and the *hzo* enzyme, such as *Brocodia*, *Kuenenia*, and *Anammoxoglobus*, facilitate the synthesis of N_2H_2, which is further converted to N_2 by *hdh* [140,159]. Bacteria such as *Nitrosomonas europaea* can perform both oxidation and reduction processes depending on the availability of oxygen. These bacteria oxidize NH_3 in the presence of oxygen, forming NO_3^-, and antithetically reduce NO_3^- in the absence of O_2 to NO_2, which can aid the initiation of anammox or DNRA and hence control the dominance of either of the two pathways [167,168]. Alternatively, excess NO_2 can undergo ammonification catalyzed by the *nrfA* enzyme, forming NH_3, which is later oxidized to N_2H_2, catalyzed by *hzs*, then to N_2 by *hdh.* The key enzyme in this N_2-producing pathway is *hdh*, which catalyzes the dehydrogenation of N_2H_2 to N_2. While rare, these processes are prevalent in O_2-limited environments, which serves as evidence that N cycling is a complex accomplished by synergistic metabolisms of different microorganisms in the environment [159]. Dominance of anammox and DNRA in water and soil can be promoted by excess of carbon source, limited O_2 and excess intermediate byproducts from the denitrification pathway [160].

Industrial denitrification using indigenous bacteria is classically performed by the addition of a carbon source in an O_2 limited environment, which promotes denitrification [169,170]. The oxidation of these organic substrates is coupled to the reduction of NO_3^- to lesser oxidized forms of N [171]. The complete denitrification process is controlled by various factors given its multiple steps, of which any could be final, thereby truncating the process to yield intermediate denitrification products (NO_2, NO, NH_3, N_2O) in addition toN_2gas, as discussed above [160]. Removal of NH_3 and NO_3^- to N_2 restores the N cycle, allowing nitrification to take place once again and thus keeping the ecosystem balanced [119,169]. In a denitrification system, environmental conditions such as temperature, pH, and dissolved oxygen concentration and their fluctuations affect the effectiveness of the system. Changes in C:N ratio and NO_2^- concentration can hinder the expression of the *nosZ* gene, resulting in the accumulation of N_2O in the system [152,165]. A complete denitrification pathway aimed at removing NO_3^- waste has been studied and widely applied in industrial wastewater treatment; this approach has been shown by batch experiments to be efficient, with the prospects of low operational cost and no secondary contamination [172,173].

6. Application of Denitrification in Bioreactors

A bioreactor refers to any device or system that creates an environment that supports optimal bacterial growth and activity according to bacterial kinetics [174]. A bioreactor provides a stable and controllable environment that allows colonization of biofilm-forming bacteria or any other microorganisms in the reactor. There are various factors to consider when operating bioreactors with bacteria, as changes in the environment can affect bacterial growth and activity. Changes in factors such as the matrix used, seeding culture diversity, and carbon source as well as growth conditions such as pH, temperature, and ORP can either boost or hinder the bacterial activity, hence the need for stable conditions within the reactor. Optimizing the above in a reactor with a defined bacterial community can promote bioremediation efficiency.

6.1. Use of aMatrix as a Bacterial Support in Bioreactors

The matrix is commonly a water-resistant material used as a surface for the growth of microbial biofilms. The matrix can be made out of various water-insoluble materials such as plastic, wood, and different forms of rocks [175,176]. A matrix material is cut into smaller particles and packed in the bioreactor to serve as an adhesion material for the

targeted bacterial colonization and formation of biofilms. The matrix serves as a filter to trap and remove insoluble contaminants from the wastewater during remediation. Different materials used as matrix are applied in different reactors for different contributions to the system other than bacterial adhesion. However, certain materials are biodegradable, such as wood chips, which are broken down to release organic substances that bacteria can use as additional carbon sources and growth factors [176]. Matrix materials can be eroded by water after a specific period of time, leading to the leaching of chemicals from the matrix, which can change the chemistry of the water. Changes in the environmental condition exerted by chemicals from the matrix could be beneficial or detrimental to the system; hence, knowledge of the geochemistry of matrix is vital. In bioremediation of industrial acid mine drainage, rocks such as dolomite and dolerite are used as a matrix and are beneficial to the system as they slowly dissolve, leaching carbonates and thereby increasing the pH of the water [177]. A remediation system favored by higher pH conditions such as denitrification is established rapidly in such conditions, as denitrifying bacteria grow optimally near neutral pH [176,178]. Certain matrix materials are biotechnologically engineered from polymer (beads) and are porous, with the capacity to contain chemicals that can slowly leach out into the water in order to optimize the desired conditions for bacteria activities [179]. Utilization of expanded clay aggregate matrix is beneficial in systems where pH needs to be kept constant, as this matrix serves as a pH and redox potential buffer, allowing a constant pH condition for bacteria that are sensitive to pH changes [151]. Knowledge of the type of matrix material needed for reactors is a vital factor that can render the NO_3^-bioremediation system ineffective due to chemicals introduced from the matrix.

6.2. Importance of SeedingBioreactor Culture

Microbial communities drive the biochemical processes that take place in all bioremediation systems. In a denitrification system with a consortium of bacteria targeting the reduction of NO_3^-, establishment of anaerobic conditions will promote denitrification, hindering the growth of most nitrifying bacteria that could produce $NO_3^-$and rendering the system futile and ineffective [2]. In other systems, a synergistic relationship is established where denitrifiers and other bacteria that consume oxygen create an anoxic environment for denitrifiers to grow and reduce NO_3^- optimally [180]. However, having other anaerobic bacteria other than denitrifiers thriving in a denitrification system will create competition for carbon sources and other nutrients, lessening the growth and metabolic activity of denitrifiers [181]. To combat nutritional competition in a denitrifying system, a pure culture or defined denitrifying consortium can be inoculated in the bioreactor and be incubated to acclimatize and colonize the system, forming biofilms for effective removal of NO_3^-. Bacteria such as *Pseudomonas*, *Paracoccus*, *Alcaligenes*, *Acinetobacter*, and *Micrococcus* have been well studied, and arereported to have the capacity to completely reduce NO_3^- to N_2 in systems that contain high concentrations of NH_3^+ and NO_3^- [180,181]. Bacteria such *Thiosphaera pantotropha*, a heterotrophic nitrification–aerobic denitrifier, has the capacity to facilitate nitrification and denitrification based on the environmental conditions it is exposed to [180,182]. This bacterium has the capability to switch metabolisms to prevent accumulation of NH_3^- and NO_2^- in a denitrification system by using them as electron acceptors to produce N_2 [183]. Selection and enrichment of a suitable inoculum for the denitrification system is vital, as complete denitrification requires the expression of all denitrifying functional genes in order to be successful. Expression of all functional genes and activity of expressed proteins requires specific environmental settings such as previously determined optimum pH, temperature, metals that act as co-factors, dissolved oxygen concentration, and a suitable carbon source, all of which can affect the effectiveness of the system [142]. Characterizing the bacterial culture used for a denitrification system can aid in the determination of the controllable conditions of reactors.

6.3. Choice of Carbon Sources in Denitrification

Various carbon sources have been tested and applied for biological NO_3^- reduction based on the bacterial groups used in the system [184]. Commonly-used carbon sources such as acetate, acetic acid, aspartate, ethanol, glucose, glycerol, methanol, and succinate are among the readily-available electron donors used for bacteria when directly added in a denitrification system [89,160,181]. Selecting a suitable carbon source for the inoculum used as well as defined temperature and pH will improve the effectiveness of the system, as bacteria will grow optimally for their effective metabolic activities [185]. Energy yields for bacterial growth depend on the breaking down of the carbon source in the system. The lower the ration of C:N, the more suitable the carbon source, as the loading rate of the organic content will remain low, and hence there will be fewer or no chemical changes in the composition of the medium [181]. A carbon source is regarded as suitable for a remediation system when the bacteria can easily and quickly break it down in order to harvest energy in the form of electrons while removing N in the form of ammonia or any oxidized N species (N_2O, NO, NO_2 and NO_3) to N_2 gas [181].A suitable electron donor is crucial to the process, as denitrification ceases once the donor is depleted [151]. In O_2-limited environments, the ratio of carbon source to nitrogen (C:N) is mostly three or lower, with lower carbon source demand for bacterial metabolism of ethanol and nitrates [160]. The balance between the C and N sources is vital in remediation systems, as higher consumption of N by bacteria requires greater accumulation of biomass, which in turn requires a sufficient C-source to proliferate [186]. The effectiveness of a bacterial system in removing N from water depends mostly on the preference bacteria has for the supplied C-source, which is needed to accumulate enough biomass to remove the NO_3^- in the system. The C:N ratio will increase due to higher carbon source demands if the C-source used is less preferred by the bacteria. The ratio will decrease if the bacteria can easily and quickly use the C-source for growth, making the C and N sources directly proportional in their uptake, which then results in greater bacterial biomass yields [161].

Depletion of C-sources during denitrification leads to truncation of the process, which yields intermediate nitrogen species such as NO_2, NO and N_2O that accumulate in the system as terminal products of NO_3^- metabolism [19,151]. A limited electron donor supply will be depleted quickly during microbial growth, in turn hindering the reduction of NO_2^- as the first intermediate product of denitrification [151]. Intracellular accumulation of NO_2^- within the cell is eventually pumped out of the cell temporarily until the NO_3^- or NO_2^- reducing conditions are restored and favorable for denitrification to proceed. The presence of a suitable electron donor or NO_3^- lowered concentrations will allow continuation of denitrification and aid cells in taking up NO_2^- to be further reduced to gaseous nitrogen species [19]. Biological reduction of high NO_3^- concentrations has been successful in most industrial wastewater treatment plants, where NO_3^- concentrations are balanced with the carbon source concentration (C:N). Although achievable, high NO_3^- and C-source concentrations have toxic effects on bacteria when converted to other forms both within the cells and in the denitrification system. When the NO_3^- influx in the cell is not controlled due to high extracellular concentrations, the NO_3^- reduced products, including NO_2^- within the cell, will not be metabolized, halting NO_3^- reduction; this effect can be lethal to the bacteria [19,151].

Heterotrophic and autotrophic denitrification metabolisms take place in various microorganisms such as *Pseudomonas* depending on the source of electrons used in the system [185,187]. In heterotrophic metabolism, bacteria use various C-sources as their electron donors for growth. For example, the breakdown of acetate used as a sole C-source in a denitrification system produced 216 KJ/mol from eight electrons to be used in the bacterial metabolism, as shown in Table 2, below; the same breakdown occurs with other carbon sources used by heterotrophic denitrifiers. In autotrophic metabolism, where hydrogen (H_2) is used as an electron donor, 185 KJ/mol of energy is produced, which is lower compared to the energy produced when a carbon source such as acetate is utilized (over 200 KJ/mol) [187,188]. Utilization of electron acceptors by microorganisms has been

exploited in bioremediation, where the electron donors are used to aid removal of contaminants such asNO_3^-, SO_4, Fe, and other chemical contaminants [185]. Utilization of NO_3^- in denitrification has been shown to be the most electron shuffling process, with over 500 KJ/mole compared to other electron acceptors that produce less energy for the microorganisms. The half-reactions of electron acceptors in Table 2 represent the metabolic activities where most bacteria use NO_3^- as their terminal electron acceptor [185].

Table 2. Half-reactions of electron donors and electron acceptors with their energy productions in anoxic environments (Used with permission from [187]).

Metabolism	Electron Donating/Accepting Half-Reaction	e^- don/acc	ΔG (Kj mol^{-1})
Autotrophy	$CH_4COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^-$	e^- donating	−216
Hydrogenotrophy	$4H_2(aq) \rightarrow 8H^+ + 8e^-$	e^- donating	−185
Denitrification	$8e^- + 8/5\ NO_3^- + 48/5H^+ \rightarrow 4/5N_2(aq) + 24/5H_2O$	e^- accepting	−550

7. Factors That Inhibit and Promote Bacterial Denitrification

There are several metabolic catalysts and inhibitors in the environment that affect and regulate bacterial activity and growth. Depletion or excess of either electron acceptor or donor is mostly a limiting factor in microbial denitrification, amongst the other factors described above [189]. Temperature is the most vital environmental factor affecting denitrification, followed by the amount of dissolved oxygen (DO), C and N ratio (C:N), pH, NO_3^- concentration in the influent water, and hydraulic retention time (HRT) in the bioreactor [89]. Enzymes involved in denitrification have various regulation factors, one being metal cofactors that promote the catalytic activity of enzymatic reduction or oxidation process, while others suppress and inhibit activity [190].

7.1. Effects of Temperature on Denitrification

Temperature plays a vital role in all biological systems, including those orchestrated by bacteria. The effectiveness of bacterial nitrification and denitrification processes can be slowed down by 60% in temperatures below 20 °C, even in closed systems [150]. Lowering the temperature of the denitrification system, such as in bioreactors, to below 25 °C will result in the lowering of the metabolic activity as well as minimal gene expression of the proteins needed to facilitate denitrification. In systems where consortia of both nitrifying and denitrifying bacteria are present, lower temperatures between 1 and 10 °C will halt denitrification, promoting nitrification, which results in conversion of most dissolved N species to NO_3^- [150]. Denitrification as a biochemical process is highly dependent on temperature; while a few known denitrifying microorganisms, such as *Pseudomonas aeruginosa* and *Bacillus*, can thrive in high temperatures such as 30 °C, none can survive beyond 40 °C while reducing NO_3^- to N_2 [191,192]. Studies conducted by [193] reported that lowering temperatures from 25 to 15 °C in a closed denitrifying system lowered the denitrification rate by 50%, showing the impact of lower temperatures on the metabolic activity and growth of denitrifying bacteria. Denitrifying bacteria such as *Paracoccus denitrificans* and *Thiobacillus denitrificans* were found to be more sensitive and less active, with insignificant denitrification activity at temperatures below 16 °C; however, they attained over 77% reduction of 250 mg/L of NO_3^- at temperatures between 28 and 30 °C [194,195]. While denitrification takes place optimally at temperatures between 25 and 30 °C, removal of NH_3^+ and NH_4^+ to N_2 through the anammox pathway was reported to optimally occur between 15 and 20 °C which is lower than reported optimum temperature for denitrification [194]. With annual seasonal changes, temperature fluctuates, which could affect a denitrifying system, hence the need to determine and regulate the optimum temperature for the bacteria used in the system and devise a strategy to maintain it during season changes.

7.2. Effects of pH Changes in a Denitrification System

Acidic and basic conditions affect the bacterial metabolism differently in various stages of denitrification. Complete denitrification takes place optimally at near neutral to alkaline pH, between 7.5 and 8.5. At pH conditions above 9, denitrification rates decrease, with great accumulation of NO_2^- leading the system to operate on NO_3^- respiration state and yielding other intermediate by-products such as NO_2 and NO [19]. Acidic pH below 7 favors reduction of NO_3^-, with accumulation of NO_2^- exerting the same metabolic pressure as pH above 9. Over time, accumulation of NO_2^- and NH_4^+ in a closed system such as a bioreactor with a constant carbon source will stimulate growth of nitrifiers, which will favor and promote formation of NO_3^- through nitrification [196]. Microbial nitrification produces two hydrogen molecules that continue to lower the pH, making the environment more acidic, as outlined in Equation (4) below [197].

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + H_2O + 2H^+ \quad (4)$$

Restoration of denitrification conditions to further reduce NO_2^- with overlapping utilization of NO_2^- by other microbial groups was achieved by increasing pH beyond 8.5 through the addition of a base [19]. At neutral pH, accumulation of nitrous acids and NO_2^- exert inhibitory effects on the denitrifying bacteria, reducing the rate of denitrification until the pH is raised above 8. A pH between 7 and 8 is crucial to create an optimum environment for denitrification to dominate over NO_3^-respiration and nitrification in a closed system [176,197]. During denitrification, bacterial cells breakdown the carbon source, donating electrons that produces carbonates. The carbonate ions contribute to raising the pH of the water in most remediation systems, hence supporting the reduction of all intermediate N species, as outlined in Equation (5) below [19]:

$$0.625CH_3COO^- + 1NO_3^- + 0.375H^+ \rightarrow 1.25HCO_3^- + 0.5N_2 + 0.5H_2O \quad (5)$$

7.3. Oxidation Reduction Potential (ORP) as a Redox Indicator in Denitrifcation

Introduction of O_2 in a denitrification system can suppress reduction of intermediate N species by hindering the expression of the required reductase genes [140]. Sequential expression of reductase genes is dependent on the presence of the N species to be reduced [140]. Denitrifying bacteria such as *Pseudomonas* and *Arthrobacter* have been reported to be capable of reducing NO_3^- in both aerobic and anaerobic conditions [89,198]. Microbial diversity in denitrification systems has a huge impact on reduction of NO_3^-, as both nitrifiers and denitrifiers can coexist with opposite metabolic activities. The anammox gene (*hzo*) is less sensitive to O_2 introduction compared to denitrification genes such as *narG*, *nirS*, *nirK*, and *nosZ*, which cease expression as O_2 concentrations increase in the system [140]. Increasing ORP in a system can be an indication of O_2 intrusion, which is succeeded by a lowering of denitrification activity. Lower ORP can thus serve as an indication of an anoxic environment that promotes denitrification.

7.4. Influence of Metals on Denitrification

Metals, as common industrial wastewater contaminants, have various effects on microorganisms and their metabolic activities. Presence of heavy metals such as Cd, Co, Pb, Zn, Ni, Mn, Fe, Cu, and Mo in the environment can be beneficial or toxic to the bacteria, depending on their concentrations [190,199]. Heavy metals have different effects on the microbial denitrification pathway, and simultaneously affect the microbial respiration, biomass yields, N-mineralization, nitrification, and microbial community structure [199]. Certain heavy metals have no significant effects on the denitrification process, while others halt and hinder denitrification at different concentrations, exerting inhibitory effects on growing microorganisms, while lower concentrations can be used by microorganisms to boost and promote their metabolic activities. Studies conducted by [198] revealed that 0.25 mg/L of Cd and Mn enhanced the denitrification capacities of the aerobic denitrifying bacterium *Arthrobacter arilaitensis*, while concentrations beyond 0.5 mg/L hindered

denitrification. As for Co, denitrification and microbial growth ceased immediately after it was added, showing its toxicity even at low concentrations (0.25 mg/L). However, the same heavy metals that denitrifiers use to promote denitrification can be toxic, as Mn was observed to inhibit denitrification at concentrations above 30 mg/L [198]. Metals such as Fe, Cu, and Mo play crucial roles in the denitrification pathways facilitated by enzymes, as these metals are required by different proteins as co-factors in different concentrations [200]. All NO_3^- reductases expressed by *napA* and *narG* are molybdopterin enzymes, which require molybdenum to bind to the catalytic heart of the enzyme to induce high NO_3^- affinity [9]. Other metals, such as Cd, Cu, Zn, and Pb, were found to hinder and lower the metabolic processes of denitrifying bacteria in most wastewaters [201]. Metals such as organic Cu can play a dual role in denitrification; it promotes the reduction of N_2O to N_2 at concentrations below 0.5 mg/L, while negatively affecting microbial activity when present in higher concentrations beyond 1 mg/L. It has been reported that the presence of Cu in high concentrations above 0.7 mg/L can prevent the expression of key functional denitrification genes such as *nirS*, *nirK*, and *nosZ* slowing down the denitrification process [165,190]. Control of metal concentrations in a denitrification system can induce denitrification when added with caution based on pre-determined metal concentrations for the defined bacterial diversity.

8. Recommendations for Optimizing Denitrification for Wastewater Remediation

Industrial wastewater bioremediation using indigenous bacteria is predominantly performed in closed systems with anaerobic conditions [160]. Denitrification kinetics are used to determine the efficiency of the microbial community or pure culture by monitoring the reduction of NO_3^- [19]. Higher consumption of C-source is directly proportional to N uptake, which then results in bacterial biomass yields. Utilization of controlled systems such as bioreactors, where bacterial growth conditions are adjustable, has created a platform for optimization of denitrification by various microbial groups for bioremediation applications, which is the focus of this study: to explore microbial denitrification and its regulation, optimization, and limitations. With the data gathered in this, complete denitrification can confidently be proposed as an option in the treatment of NO_3^- contamination from both mining and farming activities. Platforms such as bioreactors applied for bioremediation can be used on different scales depending on the wastewater volumes involved, with the discussed modifications and environmental regulations enabling attainment of the desired result, namely, emitting N_2 gas as the terminal denitrification product.

Below (Figure 4) is a schematic flow chart proposed as an approach to treating industrial wastewaters with NO_3^- concentrations above WHO acceptable limits. Industrial wastewater can result from different continuous industrial activities that use or produce nitrogen-based products. In this review, NO_3^- contamination in water due to the application of N-based explosives and fertilizers in mining and farming, respectively, was the primary focus. The bioremediation approach proposed here would begin with water sample collection from the contaminated water for chemical and biological analysis in order to identify the different contaminants and characterize indigenous bacterial groups. Application of metagenomic approaches to determine the metabolic activities of the indigenous bacteria can serve as a useful tool to predict the outcomes of biological systems with different bacteria. This is followed by the batch experiments to enrich microbial communities to facilitate N removal depending on the form of N in the water (NH_3 or NO_3). Denitrification kinetics experiments can then aid optimization in batch reactors to adjust bacterial growth factors such as the carbon source, nitrogen source, and other nutrients. Modification of denitrification conditions such as temperature, pH, ORP, dissolved oxygen, and hydraulic retention time allow the bacteria adequate contact time with the N species to facilitate complete denitrification. Finally, recommendations to test the feasibility of the bioremediation system include analyzing the gas emissions to determine whether the terminal product is N_2 gas, and if not, recycling of the effluent back into the reactor, adjusting of *nosZ* cofactors (such as Cu), and/or increasing the HRT to promote denitrification at this

stage and circumvent N_2O emissions. This proposed approach requires extensive research, as various control factors could affect the bacteria which are the dependent variable in any environmental and chemical manipulations of the system. The approach can be further tested for other wastewater contaminants using indigenous bacteria, thereby avoiding any change in the microbiome and microbial ecosystem in the environment though introduction of genetically modified organisms.

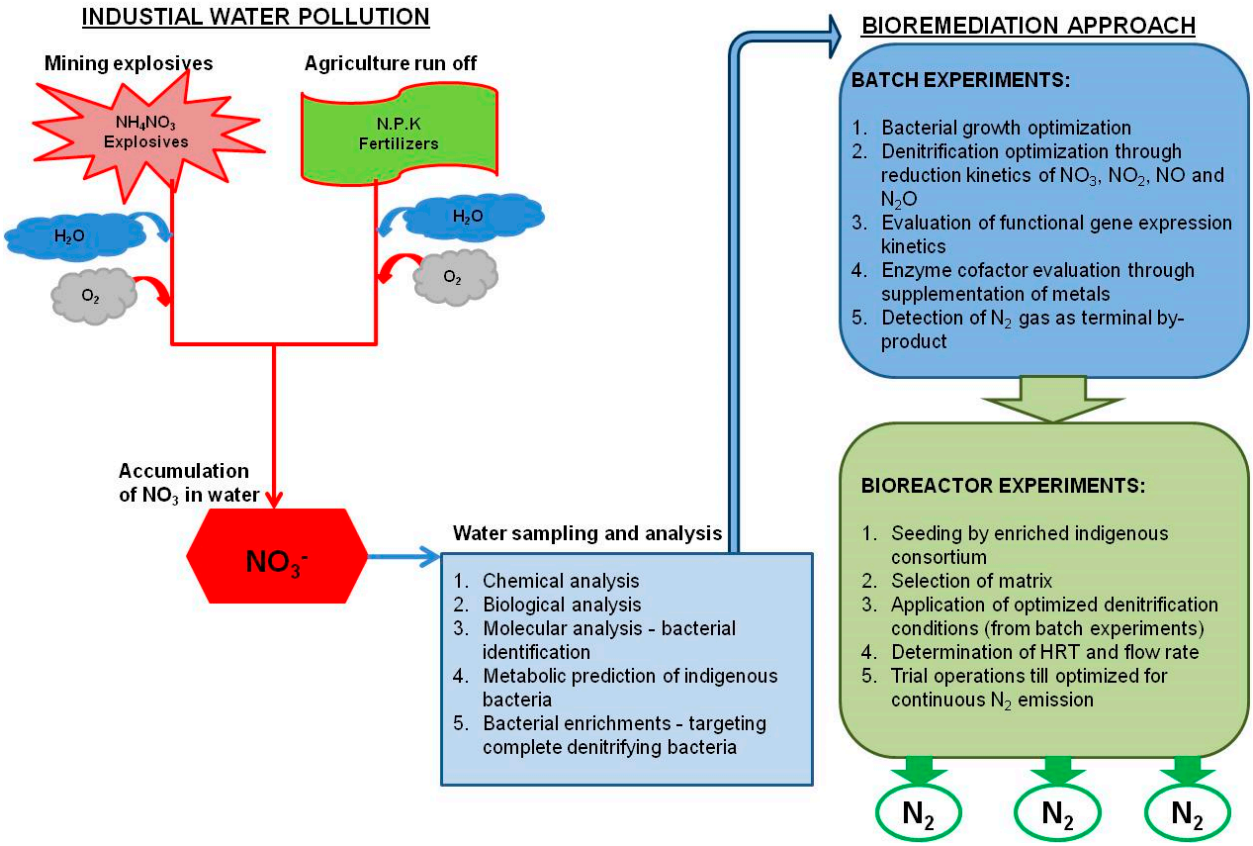

Figure 4. Proposed approach for nitrate bioremediation of industrial wastewater optimizing for complete denitrification with indigenous bacteria from the contaminated wastewater.

9. Conclusions

Nitrate contamination has become a global challenge as urbanization and industrialization increases in most developing countries. NO_3 contamination in water poses various health concerns to all forms of life, either directly or indirectly. A few effective, yet expensive remediation techniques are available for NO_3 remediation; however, these are not practical or sustainably applicable for treating large amounts of industrial wastewater. Several known remediation methods result in secondary contamination which is difficult to remove from the environment. Microbial denitrification has been identified as a strategy for industrial wastewater management, with many discoveries in the biochemical and metabolic processes involving denitrifiers. Application of microorganisms that are capable of removing NO_3^- from the environment and converting it to environmentally friendly N gas have been explored extensively, mostly in pure cultures. Few microorganisms with complete denitrification capabilities have been identified and applied in benchmarking denitrification dynamics and kinetics on a biochemical and molecular level. Intermediate byproducts of microbial NO_3^- reduction other than N_2, such as NO and N_2O, are harmful to most microbial communities around the ecosystem and detrimental to the ozone layer, causing global warming. Successful development and maintenance of a system that reduces N_2O emission and produces N_2 gas is desirable for N remediation. In this review, we have explored the metabolic synergisms of different bacteria as an approach

to treating wastewaters from mines and farms contaminated with NO_3^-. This green technology further explores the capabilities of bacterial to perform complete denitrification in a closed and controlled bioremediation system, producing no secondary contamination. With experiments to study denitrification promoters and inhibitors using NO_3^- reduction kinetics, the development of such a system could make feasible complete denitrification. With limited knowledge and application of microbial consortia to perform these complex yet possible processes, more studies are needed in order to apply the discussed factors that could promote the complete denitrification of industrial wastewaters. The proposed approach to remediating NO_3^- can help to prevent the health hazards that result from using water contaminated by waste from mining and farming activities, as well as other industries that produce NO_3^- as a contaminant in wastewater. This technology has the prospect of being effective while demanding lower startup, operational, and maintenance costs compared to the discussed chemical and physical methods. The system is sustainable over time, with no secondary contamination even post-remediation.

Author Contributions: Conceptualization: K.M.M., E.v.H.; Writing—Original Draft Preparation: K.M.M.; Writing—Reviewing & Editing: K.M.M., E.D.C., J.C.C.; Formal Analysis: K.M.M., Z.P.K., E.v.H., E.D.C., J.C.C.; Project Administration: E.v.H., E.D.C.; Funding Acquisition: E.v.H., Z.P.K.; Validation: K.M.M., Z.P.K., E.D.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by National Research Foundation (NRF-South Africa), Central University of Technology, Technology Innovation Agency (TIA-SABDI16/1070).

Institutional Review Board Statement: This study was conducted in accordance with the Declaration of South Africa, and the protocol was approved by the Ethics Committee of the University of the Free State (UFS), Ethics Clearance number: UFS-ESD2020/0165/1011.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Howard, G.; Calow, R.; Macdonald, A.; Bartram, J. Climate Change and Water and Sanitation: Likely Impacts and Emerging Trends for Action. *Annu. Rev. Environ. Resour.* **2016**, *41*, 253–276. [CrossRef]
2. Khanichaidecha, W.; Nakaruk, A.; Ratananikom, K.; Eamrat, R.; Kazama, F. Heterotrophic nitrification and aerobic denitrification using pure-culture bacteria for wastewater treatment. *J. Water Reuse Desalination* **2019**, *9*, 10–17. [CrossRef]
3. Santucci, L.; Carol, E.; Tanjal, C. Industrial waste as a source of surface and groundwater pollution for more than half a century in a sector of the Río de la Plata coastal plain (Argentina). *Chemosphere* **2018**, *206*, 727–735. [CrossRef] [PubMed]
4. Seckler, D.; Barker, R.; Amarasinghe, U. Water scarcity in the twenty-first century. *Int. J. Water Resour. Dev.* **1999**, *15*, 29–42. [CrossRef]
5. Payus, C.; Huey, L.A.; Adnan, F.; Rimba, A.B.; Mohan, G.; Chapagain, S.K.; Roder, G.; Gasparatos, A.; Fukushi, K. Impact of extreme drought climate on water security in North Borneo: Case study of Sabah. *Water* **2020**, *12*, 1135. [CrossRef]
6. Kihampa, C.; Wenaty, A. Impact if mining and farming activities on water and sediment quality of the Mara river basin, Tanzania. *Res. J. Chem. Sci.* **2013**, *3*, 15–24.
7. Pimentel, D.; Berger, B.; Filiberto, D.; Newton, M.; Wolfe, B.; Karabinakis, E.; Clark, S.; Poon, E.; Abbett, E.; Nandagopal, S. Water Resources: Agricultural and Environmental Issues. *BioScience* **2004**, *54*, 909–918. [CrossRef]
8. Fagiya, A.O.; Ipinmoroti, M.O.; Chirenje, T. Environmental pollution in Africa. *Environ. Dev. Sustain.* **2018**, *20*, 41–73. [CrossRef]
9. Sparacino-Watkins, C.; Stolz, J.F.; Basu, P. Nitrate and periplasmic nitrate reductases. *Chem. Soc. Rev.* **2013**, *43*, 676–706. [CrossRef]
10. Meng, Q.; Feng, Q.; Wu, Q.; Meng, L.; Cao, Z. Distribution characteristics of nitrogen and phosphorus in mining induces subsidence wetland in Panbei coal mine, China. *Procedia Earth Planet.* **2009**, *1*, 1237–1241.
11. Maherry, A.; Tredoux, G.; Clarke, S.; Pengelbrecht, P. State of Nitrate Pollution in Groundwater in South Africa. Available online: http://researchspace.csir.co.za/dspace/bitstream/handle/10204/4288/Maherry_2010_P.pdf;jsessionid=C3B27245662E9D7865F7FDB24E1F19FF?sequence=1 (accessed on 17 January 2020).
12. Mahvi, A.H.; Nouri, J.; Babaei, A.A.; Nabizadeh, R. Agricultural impact of groundwater nitrate pollution. *Int. J. Sci. Technol.* **2005**, *2*, 41–47. [CrossRef]
13. Stadler, S.; Talma, A.; Tredoux, G.; Wrabel, J. Identification of sources and infiltration regimes of nitrate in the semi-arid Kalahari: Regional differences and implications for groundwater management. *Water SA* **2012**, *38*, 213–224. [CrossRef]

14. Negovanović, M.; Kričak, L.; Milanović, S.; Dokić, N.; Simić, N. Ammonium nitrate explosion hazards opasnosti od eksplozijeko-jepoticu od amonijumnitrata. *Undergr. Min. Eng.* **2015**, *27*, 49–63.
15. Dwivedi, U.N.; Misha, S.; Singh, P.; Tripathi, R.D. Nitrate pollution and its remediation. In *Environmental Bioremediation Technologies*; Springer: Berlin/Heidelberg, Germany, 2007.
16. Liu, L.; Zheng, X.; Wei, X.; Kai, Z.; Xu, Y. Excessive application of chemical fertilizer and organophosphorus pesticides induced total phosphorus loss from planting causing surface water eutrophication. *Sci. Rep.* **2021**, *11*, 23015. [CrossRef]
17. Agrawal, A.; Pandey, R.S.; Sharma, B. Water Pollution with Special Reference to Pesticide Contamination in India. *J. Water Resour. Prot.* **2010**, *2*, 432–448. [CrossRef]
18. Ward, M.H.; Jones, R.R.; Brender, J.D.; de Kok, T.M.; Weyer, P.J.; Nolan, B.T.; Villanueva, C.M.; van Breda, S.G. Drinking water nitrate and human health: An updated review. *Int. J. Environ. Res. Public Health* **2018**, *15*, 1557. [CrossRef]
19. Glass, C.; Silverstein, J. Denitrification kinetics of high nitrate concentration water: pH effect on inhibition and nitrite accumulation. *Water Res.* **1998**, *32*, 831–839. [CrossRef]
20. Huno, S.K.M.; Rene, E.R.; van Hullebusch, E.D.; Annachhatre, A.P. Nitrate removal from groundwater: A review of natural and engineered processes. *J. Water Supply: Res. Technol.-Aqua* **2018**, *67*, 885–902. [CrossRef]
21. Tagma, T.; Hsissou, Y.; Bouchaou, L.; Bouragba, L.; Boutaleb, S. Groundwater nitrate pollution in Souss-Massa basin (South-west Morocco). *Afr. J. Environ. Sci. Technol.* **2009**, *3*, 301–309.
22. Feng, W.; Wang, C.; Lei, X.; Wang, H.; Zhang, X. Distribution of Nitrate Content in Groundwater and Evaluation of Potential Health Risks: A Case Study of Rural Areas in Northern China. *Int. J. Environ. Res. Public Health* **2020**, *17*, 9390. [CrossRef]
23. Beutel, M.W.; Horne, A.J.; Taylor, W.D.; Losee, R.F.; Whitney, R.D. Effects of oxygen and nitrate on nutrient release from profundal sediments of a large, oligo-mesotrophic reservoir, Lake Mathews, California. *Lake Reserv. Manag.* **2008**, *24*, 18–29. [CrossRef]
24. Schaider, L.A.; Swetschinki, L.; Campbell, C.; Rudel, R.A. Environmental justice and drinking water quality: Are there socioeconomic disparities in nitrate levels in U.S. drinking water? *Environ. Health* **2019**, *18*, 3. [CrossRef] [PubMed]
25. Coronel, G.; Chang, M.; Rodríguez-Delfín, A. Nitrate Reductase Activity and Chlorophyll Content in Lettuce Plants Grown Hydroponically and Organically. In Proceedings of the International Symposium on Soilless Culture and Hydroponics 843, Lima, Peru, 25–28 August 2008; pp. 137–144.
26. Coffey, M.; Pal, M.; Stamp, J.; Hamilton, A.; Johnson, T. A review of water quality responses to air temperature and precipitation changes 2: Nutrients, algal blooms, sediment, pathogens. *J. Am. Water Resour. Assoc.* **2018**, *55*, 844–868. [CrossRef] [PubMed]
27. Cammack, R.; Joannou, C.; Cui, X.-Y.; Martinez, C.T.; Maraj, S.R.; Hughes, M.N. Nitrite and nitrosyl compounds in food preservation. *Biochim. et Biophys. Acta* **1999**, *1411*, 475–488. [CrossRef]
28. Xue, Y.; Song, J.; Kong, F.; Wen, M.; Zhang, G. Nitrate pollution and preliminary source identification of surface water in a semi-arid river basin, using isotopic and hydrochemical approaches. *Water* **2016**, *8*, 328. [CrossRef]
29. Panagiotaras, D.; Koulougliotis, D.; Nikolopoulos, D.; Kalarakis, A.N.; Yiannopoulos, A.C.; Pikios, K. Biogeochemical cycling of nutrients and thermodynamic aspects. *J. Thermodyn. Catalysis.* **2015**, *6*, 1–9.
30. Stein, L.Y.; Klotz, M.G. The nitrogen cycle. *Curr. Biol.* **2016**, *26*, 94–98. [CrossRef]
31. Stüeken, E.E.; Kipp, M.; Koehler, M.C.; Buick, R. The evolution of Earth's biogeochemical nitrogen cycle. *Earth-Sci. Rev.* **2016**, *160*, 220–239. [CrossRef]
32. Park, Z.S.; Kim, J.; Woo, Y.Y.; Lee, H.; Kim, J.H.; Moon, Y.H. Forced circulation of nitrogen gas for accelerated and eco-friendly cooling of metallic parts. *Appl. Sci.* **2019**, *9*, 3679. [CrossRef]
33. Kuypers, M.M.M.; Marchant, H.K.; Kartal, B. The microbial nitrogen-cycling network. *Nat. Rev. Microbiol.* **2018**, *16*, 263–276. [CrossRef]
34. Pajares, S.; Bohannan, B.J.M. Ecology of nitrogen fixing, nitrifying and denitrifying microorganisms in tropical forest soils. *Front. Microbiol.* **2016**, *7*, 1045. [CrossRef] [PubMed]
35. Scolow, R.H. Nitrogen management and the future of food: Lessons from the management of energy ad carbon. *Proc. Natl. Acad. Sci. USA* **1999**, *96*, 6001–6008. [CrossRef] [PubMed]
36. Song, W.; Liu, X.-Y.; Hu, C.-C.; Chen, G.-Y.; Walters, W.W.; Michalski, G.; Liu, C.-Q. Important contributions of non-fossil fuel nitrogen oxides emissions. *Nat. Commun.* **2021**, *12*, 243. [CrossRef] [PubMed]
37. Skiba, U.M.; Rees, R.M. *Nitrous Oxide, Climate Change and Agriculture*; CABI: Wallingford, UK, 2014.
38. Pidwirny, M. "The Nitrogen Cycle". In *Fundamentals of Physical Geography*, 2nd ed.; Available online: http://www.physicalgeography.net/fundamentals/9s.html (accessed on 17 January 2022).
39. Stefanakis, A.I.; Zouzias, D.; Marsellos, A. Groundwater pollution: Human and natural sources and risks. *Environ. Sci. Eng.* **2017**, *4*, 82–102.
40. Withers, P.J.A.; Neal, C.; Jarvie, H.P.; Doody, D.G. Agriculture and Eutrophication: Where Do We Go from Here? *Sustainability* **2014**, *6*, 5853–5875. [CrossRef]
41. Bosman, C. The hidden dragon: Nitrate pollution from open-pit mines—A case study from the Limpopo province, South Africa. In Proceedings of the International Mine Water Conference, Pretoria, South Africa, 19–23 October 2009.
42. Cyplik, P.; Marecik, R.; Piotrowska-Cyplik, A.; Olejnik, A.; Drozdzynska, A.; Chrzanowski, L. Biological denitrification of high nitrate processing wastewaters from explosives production plant. *Water Air Soil Pollut.* **2012**, *223*, 1791–1800. [CrossRef]
43. Shen, J.; He, R.; Han, W.; Sun, X.; Li, J.; Wang, L. Biological denitrification of high-nitrate wastewater in a modified anoxic/oxic-membrane bioreactor (A/O-MBR). *J. Hazard. Mater.* **2009**, *172*, 595–600. [CrossRef]

44. Soomro, F.; Rafique, T.; Michalski, G.; Ali, S.A.; Naseem, S.; Khan, M.U. Occurrence and delineation of high nitrate contamination in the groundwater of Mithi sub-district, Thar Desert, Pakistan. *Environ. Earth Sci.* **2017**, *76*, 355. [CrossRef]
45. Alex, R.; Kitalika, A.; Mogusu, E.; Njau, K. Sources of Nitrate in Ground Water Aquifers of the Semiarid Region of Tanzania. *Geofluids* **2021**, *2021*, 6673013. [CrossRef]
46. Tredoux, G.; Engelbrecht, P.; Israel, S. Nitrate in Groundwater. In *Why Is It a Hazard and How to Control It? Report to Water Research Commission*; Water Research Commission: Pretoria, South Africa, 2009; pp. 1–22. ISBN 978-1-77005-873-6.
47. Zendehbad, M.; Cepuder, P.; Loiskandl, W.; Stumpp, C. Source identification of nitrate contamination in the urban aquifer of Mashhad, Iran. *J. Hydrol. Reg. Stud.* **2019**, *25*, 100618. [CrossRef]
48. Laursen, A.E.; Carlton, R.G. Response to atrazine of respiration, nitrification, and denitrification in stream sediments measured with oxygen and nitrate microelectrodes. *FEMS Microbiol. Ecol.* **1999**, *29*, 229–240. [CrossRef]
49. Lee, C.; Beauchemin, K.A. A review of feeding supplementary nitrate to ruminant animals: Nitrate toxicity, methane emissions, and production performance. *Can. J. Anim. Sci.* **2014**, *94*, 557–570. [CrossRef]
50. Avisar, O.; Kronfeld, J.; Talma, A.S. Amelioration of groundwater nitrate contamination following installation of a central sewage system in two Israeli villages. *Environ. Earth Sci.* **2008**, *58*, 515–520. [CrossRef]
51. Verlicchi, P.; Grillini, V. Surface Water and Groundwater Quality in South Africa and Mozambique—Analysis of the Most Critical Pollutants for Drinking Purposes and Challenges in Water Treatment Selection. *Water* **2020**, *12*, 305. [CrossRef]
52. Schoeman, J.J. Nitrate-nitrogen removal with small-scale reverse osmosis, electrodialysis and ion-exchange units in rural areas. *Water SA* **2009**, *35*, 5. [CrossRef]
53. Amatya, I.M.; Kansakar, E.B.; Tare, E.V.; Fiksdal, L. Impact of temperature on biological denitrification process. *J. Inst. Eng.* **2015**, *7*, 121–126. [CrossRef]
54. Clark, C.M.; Bell, M.D.; Boyd, J.W.; Compton, J.E.; Davidson, E.A.; Davis, C.; Fenn, M.E.; Geiser, L.; Jones, L.; Blett, T.F. Nitrogen-induced terrestrial eutrophication: Cascading effects and impacts on ecosystems services. *Ecosphere* **2017**, *8*, e01877. [CrossRef]
55. Glibert, P.M.; Harrison, J.; Heil, C.; Seitzinger, S. Escalating Worldwide use of Urea—A Global Change Contributing to Coastal Eutrophication. *Biogeochemistry* **2006**, *77*, 441–463. [CrossRef]
56. Basada, H.; Werner, K. An assessment of the effects of Africa's water crisis on food security and management. *Int. J. Water Resour. Dev.* **2015**, *3*, 120–133. [CrossRef]
57. Rahman, K.M.A.; Zhang, D. Effects of fertilizer broadcasting on the excessive use of inorganic fertilizers and environmental sustainability. *Sustainability* **2018**, *10*, 759. [CrossRef]
58. Yousaf, M.; Li, J.; Lu, J.; Ren, T.; Cong, R.; Fahad, S.; Li, X. Effects of fertilization on crop production and nutrient-supplying capacity under rice-oilseed rape rotation system. *Sci. Rep.* **2017**, *7*, 1270. [CrossRef] [PubMed]
59. Zavale, H.; Matchaya, G.; Vilissa, D.; Nhemachena, C.; Nhlengethwa, S.; Wilson, D. Dynamics of the Fertilizer Value Chain in Mozambique. *Sustainability* **2020**, *12*, 4691. [CrossRef]
60. Heffer, P.; Gruere, A.; Roberts, T. Assessment of Fertilizer Use by Crop at the Global Level. Available online: https://www.fertilizer.org/images/library_downloads/agcom.13.39%20-%20fubc%20assessment%202010.pdf (accessed on 17 January 2022).
61. Li, Z.; Zhang, R.; Xia, S.; Wang, L.; Liu, C.; Zhang, R.; Fan, Z.; Chen, F.; Liu, Y. Interactions between N, P and K fertilizers affect the environment and the yield and quality of satsumas. *Glob. Ecol. Conserv.* **2019**, *19*, e00663. [CrossRef]
62. Kochakinezhad, H.; Peyvast, G.; Kashi, A.K.; Olfati, J.A.; Asadii, A. A comparison of organic and chemical fertilizers for tomato production. *J. Org. Syst.* **2012**, *7*, 14–25.
63. Liu, C.-W.; Sung, Y.; Chen, B.-C.; Lai, H.-Y. Effects of Nitrogen Fertilizers on the Growth and Nitrate Content of Lettuce (*Lactuca sativa* L.). *Int. J. Environ. Res. Public Health* **2014**, *11*, 4427–4440. [CrossRef]
64. Khan, M.N.; Mobin, M.; Abbas, Z.K.; Alamri, S.A. Fertilizers and their contaminants in soils, surface and groundwater. *Encycl. Anthr.* **2018**, *5*, 225–240.
65. Fuadi, M.; Sutiarso, L.; Radi; Virgawati, S.; Nugraheni, P.H.T. Design of liquid fertilizer applicator based on Variable Rate Application (VRA) for Soybean. *IOP Conf. Ser. Earth Environ. Sci.* **2019**, *355*, 012009. [CrossRef]
66. Damashek, J.; Francis, C.A. Microbial nitrogen cycling in estuaries: From genes to ecosystems. *Estuaries Coasts* **2018**, *41*, 626–660. [CrossRef]
67. Hirel, B.; Tetu, T.; Lea, P.J.; Dubois, F. Improving Nitrogen Use Efficiency in Crops for Sustainable Agriculture. *Sustainability* **2011**, *3*, 1452–1485. [CrossRef]
68. Ye, L.; Zhao, X.; Bao, E.; Li, J.; Zou, Z.; Cao, K. Bio-organic fertilizer with reduced rates of chemical fertilization improves soil fertility and enhances tomato yield and quality. *Sci. Rep.* **2020**, *10*, 177. [CrossRef]
69. Hasnain, M.; Chen, J.; Ahmed, N.; Memon, S.; Wang, L.; Wang, Y.; Wang, P. The Effects of Fertilizer Type and Application Time on Soil Properties, Plant Traits, Yield and Quality of Tomato. *Sustainability* **2020**, *12*, 9065. [CrossRef]
70. Kakar, K.; Xuan, T.D.; Noori, Z.; Aryan, S.; Gulab, G. Effects of Organic and Inorganic Fertilizer Application on Growth, Yield, and Grain Quality of Rice. *Agriculture* **2020**, *10*, 544. [CrossRef]
71. Carvalho, F.P. Mining industry and sustainable development: Time for change. *Food Energy Secur.* **2017**, *6*, 61–77. [CrossRef]
72. Ngcofe, L.; Cole, D.I. The distribution of the economic mineral resource potential in the Western Cape Province. *S. Afr. J. Sci.* **2014**, *110*, 1–4. [CrossRef]
73. Africa Mining IQ (2019). Available online: www.projectsiq.co.za (accessed on 17 January 2022).

74. Mattila, K.; Zaitsev, G.; Langwaldt, J. Biological removal of nutrients from mine waters. In *Biologinen ravinteiden poisto kaivosvedestä*; Finnish Forest Research Institute: Rovaniemi, Finland, 2007.
75. Suceska, M.; Tumara, B.S.; Skrlec, V.; Stankovic, S. Prediction of concentration of toxic gases produced by detonation of commercial explosives by thermochemical equilibrium calculations. *Def. Technol.* **2021**. [CrossRef]
76. Mikić, M.; Rajković, R.; Jovanović, M.; Maksimović, M. The impact of blasting on the environment in the open pit mining. *Min. Metall. Inst. Bor* **2017**, *3–4*, 165–170. [CrossRef]
77. Crosson, C.C.; Tonking, M.J.H.; Paterson, G. The development of drilling and blasting practice at Palaborwa mining company limited. *J. S. Afr. Inst. Min. Metall.* **1976**, *76*, 325–340.
78. Suppajari, P.; Elie, M.; Baron, M.; Gonzalez-Rodriguez, J. Classification of ANFO samples based on their fuel composition by GC–MS and FTIR combined with chemometrics. *Forensic Sci. Int.* **2019**, *301*, 415–425. [CrossRef]
79. Brochu, S. *Assessment of ANFO on the Environment*; Technical Investigation; Defence Research and Development Canada Valcartier (QUEBEC): Quebec, QC, Canada, 2010.
80. Biessikirski, A.; Kuterasinski, L.; Dworzak, M.; Pyra, J.; Twardosz, M. Comparison of structure, morphology, and topography of fertilizer-based explosives applied in the mining industry. *Microchem. J.* **2019**, *144*, 39–44. [CrossRef]
81. Turcotte, R.; Badeen, C.M.; Goldthorp, S. Minimum Burning Pressures of Water-based Emulsion Explosives. *J. Vis. Exp.* **2017**, e56167. [CrossRef]
82. Ignarro, L.J. After 130 years, the molecular mechanism of action of nitroglycerin is revealed. *Proc. Natl. Acad. Sci. USA* **2002**, *99*, 7816–7817. [CrossRef] [PubMed]
83. Purwantini, E.; Mukhopadhyay, B. Conversion of NO_2 to NO by reduced coenzyme F_{420} protects mycobacteria from nitrosative damage. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 6333–6338. [CrossRef] [PubMed]
84. Dutta, M.; Khare, P.; Chakravarty, S.; Saikia, D.; Saikia, B.K. Physico-chemical and elemental investigation of aqueous leaching of high sulfur coal and mine overburden from Ledo coalfield of Northeast India. *Int. J. Coal Sci. Technol.* **2018**, *5*, 265–281. [CrossRef]
85. Dold, B. Evolution of Acid Mine Drainage Formation in Sulphidic Mine Tailings. *Minerals* **2014**, *4*, 621–641. [CrossRef]
86. Wei, W.; Ma, R.; Sun, Z.; Zhou, A.; Bu, J.; Long, X.; Liu, Y. Effects of Mining Activities on the Release of Heavy Metals (HMs) in a Typical Mountain Headwater Region, the Qinghai-Tibet Plateau in China. *Int. J. Environ. Res. Public Health* **2018**, *15*, 1987. [CrossRef]
87. Saha, S.; Sinha, A. Review on treatment of acid mine drainage with waste materials: A novel approach. *Glob. NEST J.* **2018**, *20*, 512–528.
88. Song, X.; Yang, X.; Hallerman, E.; Jiang, Y.; Huang, Z. Effects of Hydraulic Retention Time and Influent Nitrate-N Concentration on Nitrogen Removal and the Microbial Community of an Aerobic Denitrification Reactor Treating Recirculating Marine Aquaculture System Effluent. *Water* **2020**, *12*, 650. [CrossRef]
89. Sangwan, S.; Dukare, A. Microbe-mediated bioremediation: An Eco-friendly sustainable approach for environmental clean-up. In *Advances in Soil Microbiology: Recent Trends and Future Prospects*; Springer: Singapore, 2018. [CrossRef]
90. Wang, J.; Chu, L. Biological nitrate removal from water and wastewater by solid-phase denitrification process. *Biotechnol. Adv.* **2016**, *34*, 1103–1112. [CrossRef]
91. Murphy, A.P. Chemical removal of nitrate from water. *Nature* **1991**, *350*, 223–225. [CrossRef]
92. Gologan, D.; Popescu, M.E. The optimization of coagulation-flocculation process in a conventional treatment water plant. *IORS J. Environ. Sci. Toxicol. Food Technol.* **2016**, *10*, 33–39. [CrossRef]
93. Archna; Sharma, S.K.; Sobti, R.C. Nitrate removal from ground water: A review. *Environ. J. Chem.* **2012**, *9*, 154616. [CrossRef]
94. Christianson, L.E.; Schipper, L. Moving Denitrifying Bioreactors beyond Proof of Concept: Introduction to the Special Section. *J. Environ. Qual.* **2016**, *45*, 757–761. [CrossRef] [PubMed]
95. Rosen, T.; Christianson, L. Performance of Denitrifying Bioreactors at Reducing Agricultural Nitrogen Pollution in a Humid Subtropical Coastal Plain Climate. *Water* **2017**, *9*, 112. [CrossRef]
96. Widory, D.; Petelet-Giraud, E.; Negrel, P.; Ladouche, B. Tracking the sources of nitrate in groundwater using coupled nitrogen and boron isotopes: A synthesis. *Environ. Sci. Technol.* **2005**, *39*, 539–548. [CrossRef]
97. Petrounias, P.; Rogkala, A.; Giannakopoulou, P.P.; Tsikouras, B.; Lampropoulou, P.; Kalaitzidis, S.; Hatzipanagiotou, K.; Lambrakis, N.; Christopoulou, M.A. An Experimental Study for the Remediation of Industrial Waste Water Using a Combination of Low Cost Mineral Raw Materials. *Minerals* **2019**, *9*, 207. [CrossRef]
98. Tugaoen, H.O.; Garcia-Segura, S.; Hristovski, K.; Westerhoff, P. Challenges in photocatalytic reduction of nitrate as a water treatment technology. *Sci. Total Environ.* **2017**, *599–600*, 1524–1551. [CrossRef]
99. Liu, G.; You, S.; Ma, M.; Huang, H.; Ren, N. Removal of nitrate by photocatalytic denitrification using nonlinear optical material. *Environ. Sci. Technol.* **2016**, *50*, 11218–11225. [CrossRef]
100. Shrimali, M.; Singh, K. New methods of nitrate removal from water. *Environ. Pollut.* **2001**, *112*, 351–359. [CrossRef]
101. Santos, A.S.G.G.; Restivo, J.; Orge, C.A.; Pereira, M.F.R.; Soares, O.S.G.P. Nitrate Catalytic Reduction over Bimetallic Catalysts: Catalyst Optimization. *C* **2020**, *6*, 78. [CrossRef]
102. Tokazhanov, G.; Ramazanova, E.; Hamid, S.; Bae, S.; Lee, W. Advances in the catalytic reduction of nitrate by metallic catalysts for high efficiency and N_2 selectivity: A review. *Chem. Eng. J.* **2019**, *384*, 123252. [CrossRef]
103. Werth, C.J.; Yan, C.; Troutman, J.P. Factors Impeding Replacement of Ion Exchange with (Electro)Catalytic Treatment for Nitrate Removal from Drinking Water. *ACS EST Engg.* **2020**, *1*, 6–20. [CrossRef]

104. González-Martínez, A.; de Simón-Martín, M.; López, R.; Táboas-Fernández, R.; Bernardo-Sánchez, A. Remediation of Potential Toxic Elements from Wastes and Soils: Analysis and Energy Prospects. *Sustainability* **2019**, *11*, 3307. [CrossRef]
105. McAdam, E.; Judd, S. A review of membrane bioreactor potential for nitrate removal from drinking water. *Desalination* **2006**, *196*, 135–148. [CrossRef]
106. Park, I.-K.; Hou, J.; Yun, J.; Lee, H.-D.; Lee, C.-H. A Study to Enhance the Nitrate-Nitrogen Removal Rate without Dismantling the NF Module by Building a PFSA Ionomer-Coated NF Module. *Membranes* **2021**, *11*, 769. [CrossRef]
107. Mahvi, A.H.; Malakootian, M.; Fataheizadeh, A.; Ehrampoish, M.H. Nitrate removal from aqueous solutions by nanofiltration. *Desalination Water Treat.* **2017**, *29*, 326–330. [CrossRef]
108. Mogheir, Y.K.H.; Albahnasawi, A.M.; Abuhabib, A.A. Optimal use of nanofiltration for nitrate removal from Gaza strip municipal wells. *IUG J. Nat. Eng. Stud.* **2014**, *22*, 45–60.
109. Hoinkis, J.; Valero-Fretag, S.; Caporgno, M.P.; Patzold., C. Removal of nitrate and fluoride by nanofiltration—A comparative study. *Desalination Water Treat.* **2011**, *30*, 278–288. [CrossRef]
110. Zou, L.; Zhang, S.; Liu, J.; Cao, Y.; Qian, G.; Li, Y.-Y.; Xu, Z.P. Nitrate removal from groundwater using negatively charged nanofiltration membrane. *Environ. Sci. Pollut. Res.* **2018**, *26*, 34197–34204. [CrossRef]
111. Bottas, A.; Gaidoumi, A.E.; Ksakas, A.; Kherbeche, A. Adsorption study for the removal of nitrate from water using local clay. *Sci. World J.* **2019**, *2019*, 9529618. [CrossRef]
112. Naidu, L.D.; Saravanan, S.; Chidambaram, M.; Goel, M.; DAS, A.; Babu, J.S.C. Nanofiltration in Transforming Surface Water into Healthy Water: Comparison with Reverse Osmosis. *J. Chem.* **2015**, *2015*, 26869. [CrossRef]
113. Kurama, H.; Poetzschke, J.; Haseneder, R. The application of membrane filtration for the removal of ammonium ions from potable water. *Water Res.* **2002**, *36*, 2905–2909. [CrossRef]
114. Wu, J.; Cao, M.; Tong, D.; Finkelstein, Z.; Hoek, E.M.V. A critical review of point-of-use drinking water treatment in the United States. *npj Clean Water* **2021**, *4*, 40. [CrossRef]
115. Mohseni-Bandpi, A.; Elliott, D.J.; Zazouli, M.A. Biological nitrate removal processes from drinking water supply—A review. *J. Environ. Health Sci. Eng.* **2013**, *11*, 35. [CrossRef] [PubMed]
116. He, T.; Ye, Q.; Sun, Q.; Cai, X.; Ni, J.; Li, Z.; Xie, D. Removal of Nitrate in Simulated Water at Low Temperature by a Novel Psychrotrophic and Aerobic Bacterium, *Pseudomonas taiwanensis* Strain J. *BioMed Res. Int.* **2018**, *2018*, 4984087. [CrossRef] [PubMed]
117. McCarty, P.L. What is the best biological process for nitrogen removal: When and why? *Environ. Sci. Technol.* **2018**, *52*, 3835–3841. [CrossRef] [PubMed]
118. Kamp, A.; Hogslund, S.; Risgaard-Petersen, N.; Stief, P. Nitrate storage and dissimilatory nitrate reduction by eukaryotic microbes. *Front. Microbiol.* **2015**, *6*, 1492. [CrossRef]
119. Nogales, B.; Timmis, K.N.; Nedwell, D.B.; Osborn, A.M. Detection and Diversity of Expressed Denitrification Genes in Estuarine Sediments after Reverse Transcription-PCR Amplification from mRNA. *Appl. Environ. Microbiol.* **2002**, *68*, 5017–5025. [CrossRef]
120. Huang, X.; Weisener, C.G.; Ni, J.; He, B.; Xie, D.; Li, Z. Nitrate assimilation, dissimilatory nitrate reduction to ammonium, and denitrification coexist in *Pseudomonas putida* Y-9 under aerobic conditions. *Bioresour. Technol.* **2020**, *312*, 123597. [CrossRef]
121. Kinidi, L.; Salleh, S. Phytoremediation of Nitrogen as Green Chemistry for Wastewater Treatment System. *Int. J. Chem. Eng.* **2017**, *2017*, 1961205. [CrossRef]
122. Sundaralingam, T.; Gnanavelrajah, N. Phytoremediation Potential of Selected Plants for Nitrate and Phosphorus from Ground Water. *Int. J. Phytoremediation* **2013**, *16*, 275–284. [CrossRef]
123. Shyamala, S.; Manikandan, N.A.; Pakshirajan, K.; Tang, V.T.; Rene, E.R.; Park, H.S.; Behera, S.K. Phytoremediation of nitrate contaminated water using ornamental plants. *J. Water Supply Res. Technol.-Aqua* **2019**, *68*, 731–743. [CrossRef]
124. Li, Y.; Luo, J.; Yu, J.; Xia, L.; Zhou, C.; Cai, L.; Ma, X. Improvement of the phytoremediation efficiency of *Neyraudiareynaudiana* for lead-zinc mine-contaminated soil under the interactive effect of earthworms and EDTA. *Sci. Rep.* **2018**, *8*, 6417. [CrossRef] [PubMed]
125. Rezvani, F.; Sarrafzadeh, M.-H.; Ebrahimi, S.; Oh, H.-E. Nitrate removal from drinking water with a focus on biological methods: A review. *Environ. Sci. Pollut. Res.* **2017**, *26*, 1124–1141. [CrossRef] [PubMed]
126. Xu, Y.; Qiu, T.-L.; Han, M.-L.; Li, J.; Wang, X. Heterotrophic Denitrification of Nitrate-Contaminated Water Using Different Solid Carbon Sources. *Procedia Environ. Sci.* **2011**, *10*, 72–77. [CrossRef]
127. Rajta, A.; Bhatia, R.; Setia, H.; Pathania, P. Role of heterotrophic aerobic denitrifying bacteria in nitrate removal from wastewater. *J. Appl. Microbiol.* **2019**, *128*, 1261–1278. [CrossRef]
128. Zhou, W.; Sun, Y.; Wu, B.; Zhang, Y.; Huang, M.; Miyanaga, T.; Zhang, Z. Autotrophic denitrification for nitrate and nitrite removal using sulfur-limestone. *J. Environ. Sci.* **2021**, *23*, 1761–1769. [CrossRef]
129. Kim, S.; Jung, H.; Kim, K.S.; Kim, I.S. Treatment of High Nitrate-Containing Wastewaters by Sequential Heterotrophic and Autotrophic Denitrification. *J. Environ. Eng.* **2004**, *130*, 1475–1480. [CrossRef]
130. Liu, F.; Wang, S.; Zhang, X.; Qian, F.; Wang, Y.; Yin, Y. Nitrate Removal from Actual Wastewater by Coupling Sulfur-Based Autotrophic and Heterotrophic Denitrification under Different Influent Concentrations. *Water* **2021**, *13*, 2913. [CrossRef]
131. Casella, S.; Payne, W.J. Potential of denitrifiers for soil environment protection. *FEMS Microbiol. Lett.* **1996**, *140*, 1–8. [CrossRef]
132. Zhang, Y.; Wang, X.; Wang, W.; Sun, Z.; Li, J. Investigation of growth kinetics and partial denitrification performance in strain *Acinetobacter johnsonii* under different environmental conditions. *R. Soc. Open Sci.* **2019**, *6*, 191275. [CrossRef]

133. Zehr, J.P.; Ward, B.B. Nitrogen Cycling in the Ocean: New Perspectives on Processes and Paradigms. *Appl. Environ. Microbiol.* **2002**, *68*, 1015–1024. [CrossRef] [PubMed]
134. Martin-Rodríguez, A.J.; Rhen, M.; Melican, K.; Richter-Dahlfors, A. Nitrate Metabolism Modulates Biosynthesis of Biofilm Components in Uropathogenic *Escherichia coli* and Acts as a Fitness Factor during Experimental Urinary Tract Infection. *Front. Micrbiol.* **2020**, *11*, 1–12. [CrossRef]
135. Moreno-Vivián, C.; Cabello, P.; Martínez-Luque, M.; Blasco, R.; Castillo, F. Prokaryotic nitrate reduction: Molecular properties and functional distinction among bacterial nitrate reductases. *J. Bacteriol.* **1999**, *181*, 6573–6584. [CrossRef] [PubMed]
136. Zumft, W.G. Cell biology and molecular basis of denitrification. *Microbiol. Mol. Biol. Rev.* **1997**, *61*, 533–616. [PubMed]
137. Line, L.; Ealhede, M.; Kolpen, M.; Kühl, M.; Ciofu, O.; Bjarnsholt, T.; Moser, C.; Toyofuku, M.; Nomura, N.; Høiby, N.; et al. Physiological levels of nitrate support anoxic growth by denitrification of *Pseudomonas aeruginosa* at growth rates reported in cystic fibrosis lungs and sputum. *Front. Microbiol.* **2014**, *5*, 554. [CrossRef] [PubMed]
138. Tang, M.; Jiang, J.; Lv, Q.; Yang, B.; Zheng, M.; Gao, X.; Han, J.; Zhang, Y.; Yang, Y. Denitrification performance of *Pseudomonas fluorescens* Z03 immobilized by graphene oxide-modified polyvinyl-alcohol and sodium alginate gel beads at low temperature. *R. Soc. Open Sci.* **2020**, *7*, 191542. [CrossRef]
139. Chen, Z.; Jiang, Y.; Chang, Z.; Wang, J.; Song, X.; Huang, Z.; Chen, S.; Li, J. Denitrification characteristics and pathways of a facultative anaerobic denitrifying strain, *Pseudomonas denitrificans* G1. *J. Biosci. Bioeng.* **2020**, *129*, 715–722. [CrossRef]
140. Dalsgaard, T.; Stewart, F.J.; Thamdrup, B.; De Brabandere, L.; Revsbech, N.P.; Ulloa, O.; Canfield, D.E.; DeLong, E.F. Oxygen at Nanomolar Levels Reversibly Suppresses Process Rates and Gene Expression in Anammox and Denitrification in the Oxygen Minimum Zone off Northern Chile. *mBio* **2014**, *5*, e01966-14. [CrossRef]
141. Foglar, L.; Briški, F.; Sipos, L.; Vuković, M. High nitrate removal from synthetic wastewater with the mixed bacterial culture. *Bioresour. Technol.* **2005**, *96*, 879–888. [CrossRef]
142. Heylen, K.; Vanparys, B.; Wittebolle, L.; Verstraete, W.; Boon, N.; De Vos, P. Cultivation of Denitrifying Bacteria: Optimization of Isolation Conditions and Diversity Study. *Appl. Environ. Microbiol.* **2006**, *72*, 2637–2643. [CrossRef]
143. Erbanová, E.; Palarčík, J.; Slezák, M.; Mikulášek, P. Removing of nitrates from waste water by using pond culture. *Procedia Eng.* **2012**, *42*, 1552–1560. [CrossRef]
144. Larose, C.; Dommergue, A.; Vogel, T.M. Microbial nitrogen cycling in Arctic snowpacks. *Environ. Res. Lett.* **2013**, *8*, 035004. [CrossRef]
145. Ward, B.B.; Jensen, M.M. The microbial nitrogen cycle. *Front. Microbiol.* **2014**, *5*, 553. [CrossRef]
146. Strohm, T.O.; Griffin, B.; Zumft, W.G.; adSchink, B. Growth yields in bacterial denitrification and nitrate ammonification. *Appl. Environ. Microbiol.* **2007**, *73*, 1420–1424. [CrossRef]
147. Marietou, A. Nitrate reduction in sulfate-reducing bacteria. *FEMS Microbiol. Lett.* **2016**, *363*, fnw155. [CrossRef] [PubMed]
148. Cole, J. Nitrate reduction to ammonia by enteric bacteria: Redundancy, or a strategy for survival during oxygen starvation. *FEMS Microbiol. Lett.* **1996**, *136*, 1–11. [CrossRef]
149. Van der Hoek, J.P.; Duijff, R.; Reinstra, O. Nitrogen Recovery from Wastewater: Possibilities, Competition with Other Resources, and Adaptation Pathways. *Sustainability* **2018**, *10*, 4605. [CrossRef]
150. Palacin-Lizarbe, C.; Camarero, L.; Catalan, J. Denitrification temperature dependence in remote, cold, and N-poor lake sediments. *Water Resour. Res.* **2018**, *54*, 1161–1173. [CrossRef]
151. Rodziewicz, J.; Ostrowska, K.; Janczukowicz, W.; Mielcarek, A. Effectiveness of Nitrification and Denitrification Processes in Biofilters Treating Wastewater from De-Icing Airport Runways. *Water* **2019**, *11*, 630. [CrossRef]
152. Thakur, I.S.; Medhi, K. Nitrification and denitrification processes for mitigation of nitrous oxide from waste water treatment plants for biovalorization: Challenges and opportunities. *Bioresour. Technol.* **2019**, *282*, 502–513. [CrossRef]
153. Yin, Z.; Bi, X.; Xu, C. Ammonia-oxidizing archaea (AOA) play with ammonia-oxidizing bacteria (AOB) in nitrogen removal from wastewater. *Archaea* **2018**, *2018*, 8429145. [CrossRef] [PubMed]
154. Daims, H.; Wagner, M. Nitrospira. *Trends Microbiol.* **2018**, *26*, 462–463. [CrossRef] [PubMed]
155. Subbarao, G.V.; Ito, O.; Sahrawat, K.L.; Berry, W.L.; Nakahara, K.; Ishikawa, T.; Watanabe, T.; Suenaga, K.; Rondon, M.; Rao, I.M. Scope and Strategies for Regulation of Nitrification in Agricultural Systems—Challenges and Opportunities. *Crit. Rev. Plant Sci.* **2006**, *25*, 303–335. [CrossRef]
156. Zhu, T.; Meng, T.; Zhang, J.; Zhong, W.; Muller, C.; Cai, Z. Fungi-dominant heterotrophic nitrification in a subtropical forest soil of China. *J. Soil Sediments* **2015**, *15*, 705–709. [CrossRef]
157. Karl, D.M.; Michaels, A.F. Nitrogen cycle. In *Encyclopedia of Ocean Sciences*; Elsevier: Amsterdam, The Netherlands, 2001. [CrossRef]
158. Yoon, S.; Sanford, R.A.; Löffler, F.E. Nitrite Control over Dissimilatory Nitrate/Nitrite Reduction Pathways in *Shewanellaloihica* Strain PV-4. *Appl. Environ. Microbiol.* **2015**, *81*, 3510–3517. [CrossRef]
159. Braker, G.; Zhou, J.; Wu, L.; Devol, A.H.; Tiedje, J.M. Nitrite Reductase Genes (*nirK* and *nirS*) as Functional Markers to Investigate Diversity of Denitrifying Bacteria in Pacific Northwest Marine Sediment Communities. *Appl. Environ. Microbiol.* **2000**, *66*, 2096–2104. [CrossRef]
160. Priemé, A.; Braker, G.; Tiedje, J.M. Diversity of Nitrite Reductase (*nirK* and *nirS*) Gene Fragments in Forested Upland and Wetland Soils. *Am. Soc. Microbiol.* **2002**, *68*, 1893–1900. [CrossRef]

161. Dong, L.F.; Sobey, M.N.; Smith, C.J.; Rusmana, I.; Phillips, W.; Stott, A.; Osborn, A.M.; Nedwella, D.B. Dissimilatory reduction of nitrate to ammonium, not denitrification or anammox, dominates benthic nitrate reduction in tropical estuaries. *Am. Soc. Limnol. Oceanogr.* **2011**, *56*, 279–291. [CrossRef]
162. Skalska, K.; Miller, J.S.; Ledakowicz, S. Kinetics of nitric oxide oxidation. *Chem. Pap.* **2010**, *64*, 269–272. [CrossRef]
163. Griendling, K.K.; Touyz, R.M.; Zweier, J.L.; Chilian, W.; Chen, Y.-R.; Harrison, D.G.; Bhatnagar, A. Measurement of Reactive Oxygen Species, Reactive Nitrogen Species, and Redox-Dependent Signaling in the Cardiovascular System. *Circ. Res.* **2016**, *119*, e39–e75. [CrossRef]
164. Di Meo, S.; Reed, T.T.; Venditti, P.; Victor, V.M. Role of ROS and RNS Sources in Physiological and Pathological Conditions. *Oxid. Med. Cell. Longev.* **2016**, *2016*, 1245049. [CrossRef] [PubMed]
165. Magalhães, C.M.; Machado, A.; Matos, P.; Bordalo, A.A. Impact of copper on the diversity, abundance and transcription of nitrite and nitrous oxide reductase genes in an urban European estuary. *FEMS Microbiol. Ecol.* **2011**, *77*, 274–284. [CrossRef] [PubMed]
166. Granger, J.; Ward, B.B. Accumulation of nitrogen oxides in copper-limited cultures of denitrifying bacteria. *Limnol. Oceanogr.* **2003**, *48*, 313–318. [CrossRef]
167. Schmidt, I.; van Spanning, R.J.M.; Jetten, M.S.M. Denitrification and ammonia oxidation by *Nitrosomonas europaea* wild-type, and NirK- and NorB-deficient mutants. *Microbiology* **2004**, *150*, 4107–4114. [CrossRef]
168. Zhu, X.; Burger, M.; Doane, T.A.; Horwath, W.R. Ammonia oxidation pathways and nitrifier denitrification are significant sources of N_2O and NO under low oxygen availability. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 6328–6333. [CrossRef]
169. Nelson, M.B.; Berlemont, R.; Martiny, A.C.; Martiny, J.B.H. Nitrogen Cycling Potential of a Grassland Litter Microbial Community. *Appl. Environ. Microbiol.* **2015**, *81*, 7012–7022. [CrossRef]
170. Ruiz, B.; Le Scornet, A.; Sauviac, L.; Remy, A.; Bruand, C.; Meilhoc, E. The Nitrate Assimilatory Pathway in *Sinorhizobiummeliloti*: Contribution to NO Production. *Front. Microbiol.* **2019**, *10*, 1526. [CrossRef]
171. Maintinguer, S.I.; Sakamoto, I.K.; Adorno, M.A.T.; Varesche, M.B.A. Evaluation of the microbial diversity of denitrifying bacteria in batch reactor. *Braz. J. Chem. Eng.* **2013**, *30*, 457–465. [CrossRef]
172. Kong, L.; Jing, H.; Kataoka, T.; Buchwald, C.; Liu, H. Diversity and Spatial Distribution of Hydrazine Oxidoreductase (*hzo*) Gene in the Oxygen Minimum Zone Off Costa Rica. *PLoS ONE* **2013**, *8*, e78275. [CrossRef]
173. Wilson, R.W.; Murphy, K.L.; Sutton, P.M.; Lackey, S.L. Design and cost comparison of biological nitrogen removal processes. *J. Water Pollut. Control. Fed.* **1981**, *53*, 1294–1302.
174. Tekere, M. Microbial Bioremediation and Different Bioreactors Designs Applied. In *Biotechnology and Bioengineering*; IntechOpen: London, UK, 2019.
175. Langwaldt, J.H.; Puhakka, J.A. On-site biological remediation of contaminated groundwater: A review. *Environ. Pollut.* **2000**, *107*, 187–197. [CrossRef]
176. Klein, R.; Tischler, J.S.; Műhling, M.; Schlömann, M. Bioremediation of mine water. *Adv. Biochem. Eng./Biotechnol.* **2013**, *141*, 109–172.
177. Williams, P.; Botes, E.; Maleke, M.; Ojo, A.; DeFlaun, M.; Howell, J.; Borch, R.; Jordan, R.; van Heerden, E. Effective bioreduction of hexavalent chromium–contaminated water in fixed-film bioreactors. *Water SA* **2014**, *40*, 549. [CrossRef]
178. Sun, P.; Zhuge, Y.; Zhang, J.; Cai, Z. Soil pH was the main controlling factor of the denitrification rates and N_2/N_2O emission ratios in forest and grassland soils along the Northeast China Transect (NECT). *Soil Sci. Plant Nutr.* **2012**, *58*, 517–525. [CrossRef]
179. Sharma, S.; Bhattacharya, A. Drinking water contamination and treatment techniques. *Appl. Water Sci.* **2017**, *7*, 1043–1067. [CrossRef]
180. Zhang, Q.-Y.; Yang, P.; Liu, L.-S.; Liu, Z.-J. Formulation and Characterization of a Heterotrophic Nitrification-Aerobic Denitrification Synthetic Microbial Community and its Application to Livestock Wastewater Treatment. *Water* **2020**, *12*, 218. [CrossRef]
181. Khanitchaidecha, W.; Sumino, T.; Kazama, F. Influence of carbon source on biological nitrogen removal by immobilised bacteria. *J. Water Resour. Prot.* **2010**, *2*, 527–531. [CrossRef]
182. van Niel, E.W.J.; Arts, P.A.M.; Wesselink, B.J.; Robertson, L.A.; Kuenen, J.G. Competition between heterotrophic and autotrophic nitrifiers for ammonia in chemostat cultures. *FEMS Microbil. Ecol.* **1993**, *102*, 109–118. [CrossRef]
183. Robertson, L.A.; Kuenen, J.G. Combined heterotrophic nitrification and aerobic denitrification in *Thiosphaera pantotropha* and other bacteria. *Antonie van Leeuwenhoek* **1990**, *57*, 139–152. [CrossRef]
184. Lee, N.M.; Welander, T. The effect of different carbon sources on respiratory denitrification in biological wastewater treatment. *J. Ferment. Bioeng.* **1996**, *82*, 277–285. [CrossRef]
185. Xu, D.; Xiao, E.; Xu, P.; Lin, L.; Zhou, Q.; Xu, D.; Wu, Z. Bacterial community and nitrate removal by simultaneous heterotrophic and autotrophic denitrification in a bioelectrochemically-assisted constructed wetland. *Bioresour. Technol.* **2017**, *245*, 993–999. [CrossRef]
186. Gou, J.; Hong, C.U.; Deng, M.; Chen, J.; Hou, J.; Li, D.; He, X. Effect of Carbon to Nitrogen Ratio on Water Quality and Community Structure Evolution in Suspended Growth Bioreactors through Biofloc Technology. *Water* **2019**, *11*, 1640. [CrossRef]
187. Bethke, C.M.; Sanford, R.A.; Kirk, M.; Jin, Q.; Flynn, T. The thermodynamic ladder in geomicrobiology. *Am. J. Sci.* **2011**, *311*, 183–210. [CrossRef]
188. Huang, S.; Zheng, Z.; Wei, Q.; Han, I.; Jaffé, P.R. Performance of sulfur-based autotrophic denitrification and denitrifiers for wastewater treatment under acidic conditions. *Bioresour. Technol.* **2019**, *294*, 122176. [CrossRef]

189. Wlodarczyk, T.; Stepniewaska, Z.; Brzinska, M. Denitrification, organic matter and redox potential transformations in Cambisols. *Int. Agrophysics* **2003**, *17*, 219–227.
190. Zou, G.; Ylinen, A.; Di Capua, F.; Papirio, S.; Lakaniemi, A.M.; Puhakka, J. Impact of heavy metals on denitrification of simulated mining wastewaters. *Adv. Mater. Res.* **2013**, *825*, 500–503. [CrossRef]
191. Hollocher, T.C.; Kristjánsson, J.K. Thermophilic denitrifying bacteria: A survey of hot springs in Southwestern Iceland. *FEMS Microbiol. Ecol.* **1992**, *101*, 113–119. [CrossRef]
192. Khan, M.A.; Asaf, S.; Khan, A.L.; Jan, R.; Kang, S.; Kim, K.; Lee, I. Thermotolerance effect of plant growth-promoting *Bacillus cereus* SA1 on soybean during heat stress. *BMC Microbiol.* **2020**, *20*, 175. [CrossRef]
193. Canion, A.; Kostka, J.E.; Gihring, T.M.; Huettel, M.; van Beusekom, J.E.E.; Gao, H.; Lavik, G.; Kuypers, M.M.M. Temperature response of denitrification and anammox reveals the adaptation of microbial communities to in situ temperatures in permeable marine sediments that span 50° in latitude. *Biogeosciences* **2014**, *11*, 309–320. [CrossRef]
194. Carrera, J.; Vicent, T.; Lafuente, F.J. Influence of temperature on denitrification of an industrial high-strength nitrogen wastewater in a two-sludge system. *Water SA* **2003**, *29*, 11–16. [CrossRef]
195. Saleh-Lakha, S.; Shannon, K.E.; Henderson, S.L.; Goyer, C.; Trevors, J.T.; Zebarth, B.J.; Burton, D.L. Effect of pH and Temperature on Denitrification Gene Expression and Activity in Pseudomonas mandelii. *Appl. Environ. Microbiol.* **2009**, *75*, 3903–3911. [CrossRef]
196. Prinčič, A.; Mahne, I.; Megušar, F.; Paul, E.A.; Tiedje, J.M. Effects of pH and oxygen and ammonium concentrations on the community structure of nitrifying bacteria from wastewater. *Appl. Environ. Microbiol.* **1998**, *64*, 3584–3590. [CrossRef]
197. Hoffmann, H.; da Costa, T.B.; Wolff, D.B.; Platzer, C.; da Costa, R.H.R. The potential of denitrification for the stabilization of activated sludge processes affected by low alkalinity problems. *Braz. Arch. Biol. Biotechnol.* **2007**, *50*, 329–337. [CrossRef]
198. He, T.; Xie, D.; Ni, J.; Li, Z.; Li, Z. Effect of Cobalt, Cadmium and Manganese on Nitrogen Removal Capacity of *Arthrobacter arilaitensis* Y-10. *Water* **2020**, *12*, 1701. [CrossRef]
199. Magalhães, C.; Costa, J.; Teixeira, C.; Boralo, A. Impact of trace metals on denitrification in estuarine sediments of the Douro River estuary, Portugal. *Mar. Chem.* **2007**, *107*, 332–341. [CrossRef]
200. Zhu-Barker, X.; Steenwerth, K.L. Chapter Six—Nitrous Oxide Production from Soils in the Future: Processes, Controls, and Responses to Climate Change. *Dev. Soil Sci.* **2018**, *35*, 131–183. [CrossRef]
201. Rivett, M.; Buss, S.R.; Morgan, P.; Smith, J.W.N.; Bemment, C.D. Nitrate attenuation in groundwater: A review of biogeochemical controlling processes. *Water Res.* **2008**, *42*, 4215–4232. [CrossRef]