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INNOVATIVE APPROACHES TO NITROGEN REMOVAL: EXPLORING THE MECHANISMS AND CHALLENGES OF HETEROTROPHIC NITRIFICATION AND AEROBIC DENITRIFICATION (HNAD) IN WASTEWATER MANAGEMENT

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Abstract

Nitrogen pollution in wastewater presents significant environmental and health hazards due to the presence of refractory substances, including phenolic compounds, polycyclic aromatic hydrocarbons, and heterocyclic nitrogenous compounds. Conventional treatment methods are often insufficient for successfully removing these pollutants. This review article provides a comprehensive overview of recent advancements in the treatment of nitrogen-rich wastewater, focusing on heterotrophic nitrification and aerobic denitrification (HNAD) processes. In spite of the potential of HNAD processes, they face challenges related to environmental influences and operational conditions. Conformist methods characteristically necessitate extensive capacity and precise conditions, often leading to inefficiencies. This review outlines the recent developments in HNAD processes and explores their mechanisms, metabolic pathways, and influencing factors, including carbon/nitrogen ratio, dissolved oxygen, temperature, pH, shaking speed, heavy metals, salinity, and antibiotics. The review also highlights the intricacy of treating coking wastewater due to its intractable nature and discusses how biological methods, especially bioaugmentation, can improve biodegradability. Integrated treatment methods are emerging as comprehensive solutions, combining various approaches to enhance efficiency and effectiveness. Furthermore, innovative technologies such as microwave irradiation show assurance for treating coking wastewater. Finally, this review identifies future research directions, emphasizing the need for further inquiry into the HNAD process and its application in real-world wastewater treatment facilities. By consideration the relations between various metabolic activities and optimizing environmental conditions, HNAD processes can be additional developed to offer sustainable and resourceful solutions for nitrogen removal from wastewater.

Keywords: Heterotrophic nitrification, Aerobic denitrification, Bioaugmentation, Refractory pollutants, Coking wastewater, Integrated treatment methods, Microwave irradiation, Environmental biotechnology.

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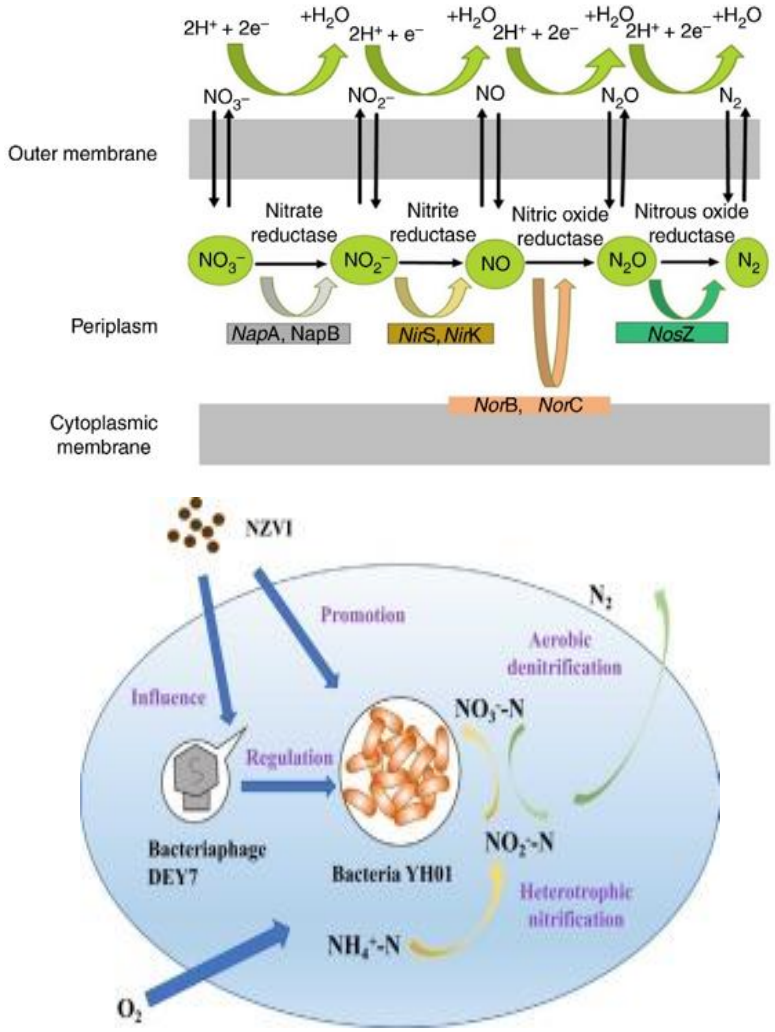

Fig 1: Graphical abstract

1. INTRODUCTION

A naturally occurring element, nitrogen found in the environment in an assortment of approaches. However, nitrate contamination, which has turned into a global ecological problem, is caused by an extreme rise in nitrate consolidation (>10 mgL-1 NO_3 - -N) in the environment [1]. Ammonia nitrogen and nitrate nitrogen pose significant hazards to both the environment and human health. Ammonia nitrogen, highly toxic to aquatic life even in low concentrations, contributes to air and water pollution, causing eutrophication and respiratory issues in humans. Nitrate nitrogen, notorious for leaching into groundwater and contaminating drinking water sources, can lead to methemoglobinemia, a potentially fatal condition in infants. Surface water pollution from agricultural runoff containing high nitrate levels exacerbates eutrophication and poses health risks like cancer. Chronic exposure to prominent nitrate intensities in consumption of water has

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been related to several health problems. The disruption of natural nitrogen cycles due to nitrate pollution further threatens biodiversity and ecosystem stability. Effective management and monitoring of ammonia and nitrate nitrogen levels are imperative to safeguard both environmental integrity and public health [2]. Ammonium nitrate (AN) is a broadly used fertilizer in agriculture due to its hygroscopic nature, oxidizing, and explosive houses. It is also used in the mining enterprise as a major component in ANFO explosive combos. Ammonium nitrate (AN) unsafe houses have brought about several historic accidents, such as the Texas catastrophe in 1947, the Toulouse disaster in 2001, and the Mihailesti explosion in 2004. Ammonium nitrate (AN) become brought to the Seveso II Directive and Directive 2003/105/EC to cowl dangers from storage and AN-based totally fertilizers. The storage, delivery, and managing of Ammonium nitrate (AN) are controlled within the European Union nations, and protection studies need to be advanced. The conduct of Ammonium nitrate (AN) is pretty complicated. Three primary dangers are linked to AN:

- Decomposition (which produces toxic smoke)
- Fire (because of AN oxidizing nature)
- Explosion

Particle size, density, porosity, purity, nitrate content material, ambient temperature, and strain are the maximum great elements influencing hazards [3]. Innovative techniques in environmental biotechnology, which include cardio denitrification and heterotrophic nitrification, are meant to dispose of nitrogen from specific wastewater streams. In assessment to traditional techniques, which often require large power inputs and specialized gadget, these processes use certainly going on microbes to convert nitrogenous substances into nitrogen gasoline that is secure. Heterotrophic nitrification is a sustainable method of ammonia removal in wastewater treatment flora. It entails heterotrophic bacteria oxidizing ammonia to nitrate in aerobic circumstances. In comparison, cardio denitrification makes use of cardio bacteria to convert nitrate to nitrogen gas, warding off the need for highly-priced anaerobic reactors and decreasing carbon necessities. Combined, those techniques offer effective and sustainable processes to nitrogen removal, supporting hold water exceptional and decrease eutrophication in aquatic environments. The implementation of these techniques has the potential to progress sustainable wastewater remedy methods and tackle nitrogen pollutants problems in numerous surrounding environments. In the manner of treating wastewater, both aerobic denitrification and heterotrophic nitrification are vital. By assisting within the removal of nitrogen from wastewater, they resource in stopping eutrophication.

2. BACKGROUND OFNITROGEN REMOVAL PROCESSES

2.1 Historical Perspective

In the late 19th century, nitrification and denitrification emerged as biological procedures. Autotrophs constantly catalyze the nitrification process in aerobic circumstances,

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although heterotrophs or autotrophs can catalyze the denitrification process in anoxic environments. As a consequence, for the conservative biological nitrogen removal procedure discrete anoxic and oxic components are required in both temporal and spatial terms. Still, prevalence of heterotrophic nitrification and the aerobic denitrification (HNAD) mechanism develops our understanding of the natural nitrogen confiscation process. [4]

The circumstances for this process might be both anaerobic and aerobic. On the other hand, nitrates and nitrites are transformed into nitrogen gas in the presence of oxygen through a process called aerobic denitrification[5]. Bacteria that specialize in aerobic denitrification conduct this process. In addition, they are more economical and environmentally beneficial than traditional nitrogen removal techniques. A rising number of people have been interested in using new technology and methods to enhance these processes' performance in the last several years[4]. The metabolic processes connected to heterotrophic nitrification involve various functions for different microorganisms. The HNAD method can be achieved through strains of Thiosphaera sp., Alcaligenes sp., Pseudomonas sp., Providencia sp., Acinetobacter sp., Bacillus sp., Rhodococcus sp., Agrobacterium sp., Halomonas sp., Aeromonas sp., Chryseobacterium sp., Serratia sp., Anoxybacillus sp., Vibrio sp., Diaphorobacter sp., Zobellella sp., Enterobacter sp., Arthrobacter sp., Gordonia sp., and Ochrobactrum sp. The boom and nitrogen elimination of HNAD bacteria may be prompted by way of numerous factors consisting of the carbon deliver, C/N ratio, temperature, salinity, pH stage, DO awareness, heavy metals, antibiotics, and shaking speed. Later studies and use of mixotrophic denitrification is tested in comparison to heterotrophic and autotrophic denitrification. Some of the disadvantages of the two denitrification procedures, such as the high carbon supply need for heterotrophic denitrification and the protracted embark period for autotrophic denitrification, can be addressed by mixotrophic denitrification. Additionally, low sludge production, high denitrification efficiency, and ease of start-up are benefits of mixotrophic denitrification[1]. Consequently, it has steadily drawn the attention of several scholars worldwide. The primary focus of current research on mixotrophic denitrification is on electron donors, influencing variables, and functioning denitrifying microorganisms.

Table 1: Morphological and Taxonomic Characteristics of Bacterial Isolate Strain

Sr. no	Characteristic	Description	References
1	Cell shape	Rod-shaped	[6]
2	Cell size	1.5-2.0 µm in length and 0.5-0.8 µm in width	[7]
3	Gram stain	Gram-negative	[8]
4	Flagella	Peritrichous	[9]
5	Colony morphology	Round, smooth, and white	[10]
6	Motility	Motile	[11]
7	Catalase test	Positive	[7]
8	Oxidase test	Negative	[6]
9	Nitrate reduction test	Positive	[12]
10	Citrate utilization test	Negative	[10]
11	Growth temperature range	25-37°C	[9]
12	Growth pH range	6.0-8.0	[8]
13	Growth in aerobic conditions	Yes	[7]
14	Growth in anaerobic conditions	No	[6]

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This paper discusses the supply, nitrogen deduction routes, and manipulating variables of HNAD bacteria, focusing on their metabolic pathways and the challenges they face in treating wastewater on a huge level. The review highlights that factors such as concentration, temperature, salinity, pH level, substantial metals, and antibiotics impact development of HNAD bacteria and their ability to remove nitrogen from the environment. The HNAD process, characterized by the simultaneous occurrence of many biochemical processes, makes its mechanism unclear. A careful study of the HNAD process is necessary to elucidate its method of occurrence. The evolution of the HNAD process is explained, and factors influencing the presentation of HNAD microorganisms are explained. The methodology of HNAD procedure is clarified by analyzing electron transfer between nitrogen conversion and organic matter metabolism. The paper concludes by discussing the issues and prognosis surrounding the HNAD procedure.

2.2 Heterotrophic Nitrification and Aerobic Denitrification (HN-AD)

The technique of heterotrophic nitrification changed into first recognized inside the 1910s and 1930s, and it could be achieved by microorganism and fungi. Four styles of bacteria do not produce spores that heterotrophically oxidize ammonia nitrogen (NH4 -N) to nitrite nitrogen (NO2 − -N) had been recognized through the analysis of many microorganism insulated after soil and a strainer bed used to acquire sugar beef effluent [13]. Heterotrophic nitrification, a biological process that mimics autotrophic nitrification, is a unique and beneficial process in bacteria. It involves the production of nitrogen gas (N_2) from nitrate (NO_3^-) by bacteria using oxygen as an electron acceptor. This process was first introduced by Seiser and Walz in 1925 when they discovered that Pseudomonas putida colonies containing nitrate, exposed to air, excreted significant quantities of nitrogen. Early studies by Verstraete provide valuable insights into this process.

This study highlights the importance of understanding and utilizing these bacteria for nitrification [13]. Nitrate used in the medium quickly vanished under aeration conditions, when Pseudomonas denitrificans and Pseudomonas fluorescens were used to perform denitrification. Nevertheless, the initial information of aerobic denitrification disregarded due to inadequate ventilation and an absence of accurate dissolved oxygen (DO) concentration detection methods, and the evidence of aerobic denitrification is not entirely conclusive[4]. Aerobic denitrification was demonstrated in the 1970s, with Alcaligenes sp. showing a 20% reduction rate under aerobic conditions. Mixotrophic denitrification methods can be heterotrophic, autotrophic, or mixotrophic depending on electron donors. Methanol and ethanol are commonly used as electron donors in HD practices, while hydrogen, elemental sulfur, compounds, and elemental iron are also used in AD processes. Mixotrophic denitrification based on sulfur is the most researched and used method, combining these electron donors in a specific ratio.[13]. The wastewater treatment system should include a consortium of heterotrophic microorganisms capable of both nitrification and denitrification. Microbial cultures enriched for ammonia-oxidizing bacteria and denitrifying bacteria can be used. Monitoring and optimizing conditions like pH, temperature, and oxygen levels enhances efficiency. Nitrogen produced during nitrification serves as a substrate for denitrifying microorganisms.[14].

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2.3 Metabolic Pathway of Heterotrophic Nitrification and Aerobic Denitrification (HN-AD) Procedure

The HNAD process is a metabolic pathway in bacteria that removes nitrogen over and is done with three pathways: the N_2O pathway, NO pathway, and NO_3 - /NO_2 pathway. The procedure includes the interest of numerous enzymes along with nitric-oxide reductase (NOS), haem-containing nitrite reductases (cd1-NIR), and periplasmic dissimilatory nitrate reductase (NAP) nitrite oxidoreductase (NXR), ammonia monooxygenase (AMO), hydroxylamine oxidoreductase (HAO), and nitrous oxide reductase (NOR).The concentrations of nitrogen within the HNAD method are easily decided, making it less complicated to determine the sports of those enzymes[14]. The NXR, NOR, and NOS enzymes' actions, however, have no longer been noted in any literature. The HAND approach become applied thru 3 pathways: the NO3-/NO2 - pathway, NO pathway, and N2O pathway, with the NO3-/NO2 - pathway being the most often used.

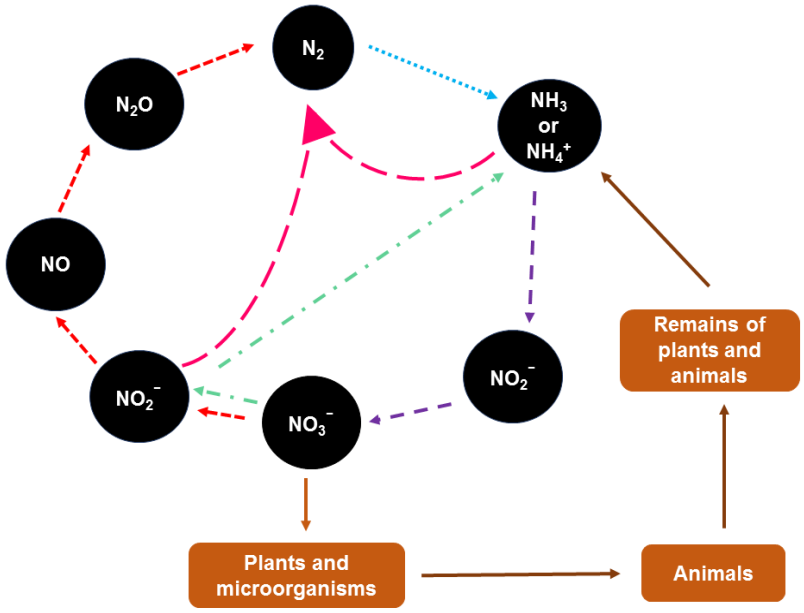

Fig 2: Metabolic pathway of heterotrophic nitrification and anaerobic denitrification [15]

The method known as Heterotrophic Nitrification and Aerobic Denitrification (HN-AD) involves putting off nitrate and ammonium from wastewater simultaneously. Usually, the NO_3 - /NO_2 route is used to do this. NH_3/NH_4+ is changed to NO_2− or NO_3− through NH_2OH, and NO_3− is subsequently denitrified to N_2 via NO_2, NO, and N_2O. Through this mechanism, most HNAD bacteria eliminate nitrogen. Nitrogen removal by this mechanism has been seen in bacteria such as Diaphorobacter polyydroxybutyrativorans SL-205, Ochrobactrum anthropic LJ81, Pseudomonas putida YH, Pseudomonas aeruginosa YL, and Pseudomonas stutzeri KTB. The absence of typical nitrification and denitrification enzyme activity, however, can occasionally be used to suggest the existence of

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alternative routes. Another process is the N_2O pathway, which involves oxidizing NH_3/NH_4+ to NH_2OH and then using N_2O to convert it to N_2. Over 90% of the products of denitrification are N_2, and the cells absorb about 50% of the original ammonium. A few bacteria use the N_2O route to carry out the HNAD process, including Alcaligenes faecalis No. 4. There are more metabolic routes for the HNAD process, and identifying them requires examining the known anaerobic denitrification and aerobic nitrification enzymes. Numerous bacteria have had their HNAD process evaluated and various strains' nitrogen removal properties have been investigated. For instance, the capacity to remove nitrogen has been investigated in Alcaligenes faecalis No. 4, Providencia rettgeri YL, Acinetobacter calcoaceticus HNR, and Bacillus sp. LY, Rhodococcus sp. CPZ24, and other bacteria. These strains have varying rates of assimilation and denitrification, which affects how well they remove nitrogen from the environment[14].

Table 2: Metabolic pathways of HAND process

Metabolic Pathway	Description	Key Reactions	References
Pentose Phosphate Pathway (PPP)	Operates alongside glycolysis, converts glucose-6-phosphate to ribose-5-phosphate, generates NADPH for reductive biosynthesis and redox balance.	Glucose-6-phosphate → Ribulose-5-phosphate Ribulose-5-phosphate → Ribose-5-phosphate Glucose-6-phosphate + 2NADP+ → Ribulose-5-phosphate + 2NADPH + CO2 4. Ribulose-5-phosphate + ATP → Ribose-5-phosphate + ADP	[8]
Gluconeogenesis	Synthesizes glucose from non-carbohydrate precursors, crucial for maintaining blood glucose levels and providing glycolytic substrates.	Pyruvate + ATP + GTP + 2NADH → Glucose + ADP + GDP + 2NAD+	[9]
Fermentation	Partial oxidation of glucose or other organic compounds without external electron acceptors like oxygen; includes alcoholic, lactic acid, and mixed acid fermentation.	Glucose → 2 Ethanol + 2 CO2 (Alcoholic Fermentation) 2. Glucose → 2 Lactic Acid (Lactic Acid Fermentation) 3. Glucose → Acetic Acid + Formic Acid + Ethanol + CO2 (Mixed Acid Fermentation)	[11]
Tricarboxylic Acid (TCA) Cycle	Central metabolic pathway oxidizing acetyl-CoA from glucose, fatty acids, and amino acids, generating NADH, FADH2, and precursor molecules for biosynthesis.	Acetyl-CoA + 3NAD + + FAD + GDP + Pi → 2CO2 + 3NADH + FADH2 + GTP + 3H+ + CoA	[12]
Glyoxylate Shunt	Enables net synthesis of carbohydrates from C2 compounds, bypassing decarboxylation steps of the TCA cycle; important for organisms using acetate or fatty acids.	Isocitrate + NAD+ → Glyoxylate + CO2 + NADH + H+	[10]

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The identification of HNAD bacteria has created new opportunities for the treatment of wastewater. Numerous investigations have concentrated on the traits and limitations of HNAD bacteria as well as their capacity to remove nitrogen from a variety of sources, including wastewater with high salinity. With superb removal performance for NH4 -N, NO3--N, and TN, the use of HNAD microorganism in the control of wastewater with high salinity and high ammonia nitrogen has proven encouraging consequences. In end, the HNAD technique is a multifaceted organic mechanism that makes use of a lot of metabolic pathways to extract nitrogen from wastewater. The main channels via which nitrogen is removed are the NO3−/NO2− and N2O pathways, and numerous microorganism have various capabilities in relation to nitrogen elimination. The identification of HNAD bacteria has super promise for reinforcing wastewater treatment approaches, in particular in harsh environments with high salinity. To completely understand metabolic paths and possible uses of HNAD microorganism in wastewater remedy, more study on this area is imperative.

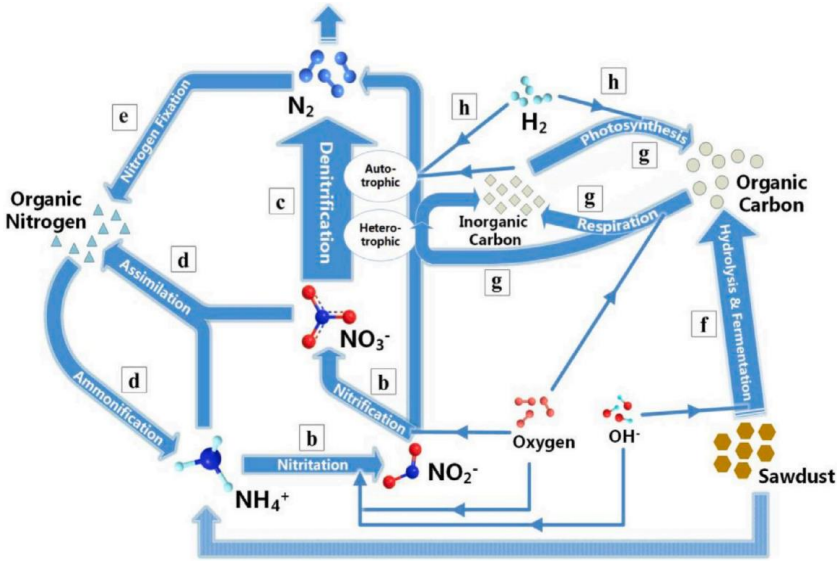

Fig 3: The pathway of HNAD [16]

2.4 Mechanism of Heterotrophic Nitrification and Aerobic Denitrification (HNAD) Process

HNAD process involves the simultaneous metabolic degree of organic depend and nitrogen alteration. Without an information of these simultaneous metabolisms, the system of the HNAD method cannot be comprehended. Energy-yielding reactions offer microorganisms with the energy they need to broaden, and a variety of enzymes, electron vendors, donors, and acceptors are intricate on this procedure[17]. When HNAD bacteria eat organic and inorganic remember, electrons from their metabolism can undertake electron passage chains thru electron transporters. Finally, those electrons are transferred to electron acceptors worried within the methods of aerobic denitrification, cardio respiration, and heterotrophic nitrification. In microorganisms, the electron sporting series made up ubiquinone, menaquinone, cytochrome, Complex I, II, III, and IV. NO2 −,

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NO3 −, and O2 are examples of electron acceptors, while reduced/oxidized flavin adenine dinucleotide (FADH2/FAD) and NADH/NAD are examples of electron vendors. The relationship between intracellular redox homeostasis and extracellular OPR is reflected in the NADH/NAD ratio[14].

Parts of the organic matter are digested during the degradation of the organic materials, while the remaining portion produces electrons transported by NADH and FADH2[18]. The electron transport chain receives the electrons that NADH is carrying. In addition, the production of poly-β-hydroxybutyrate (PHB) and nitrogen conversion may be achieved with NADH, which is generated during the metabolism of organic materials. The ubiquinone pool is a crucial node in the traditional nitrification process.

Within the ubiquinone pool, two of the electrons (4e−) are reimbursed ammonia monooxygenase (AMO) to maintain alteration NH_4 + to NH_2OH, although remaining two electrons (4e−) are moved to cytochrome bc1 (Complex III)[18]. The electrons from the oxidation of NH_2OH cross the threshold of electron transport chain in typical NH_4 + oxidation pathway at approximately +127 mV, excessively progressive to convert NAD+ to NADH directly. Therefore, Complex I (NADH oxidoreductase) cannot allow electrons liberated from NH_2OH oxidation in the typical nitrification process to join the electron transport chain[17].

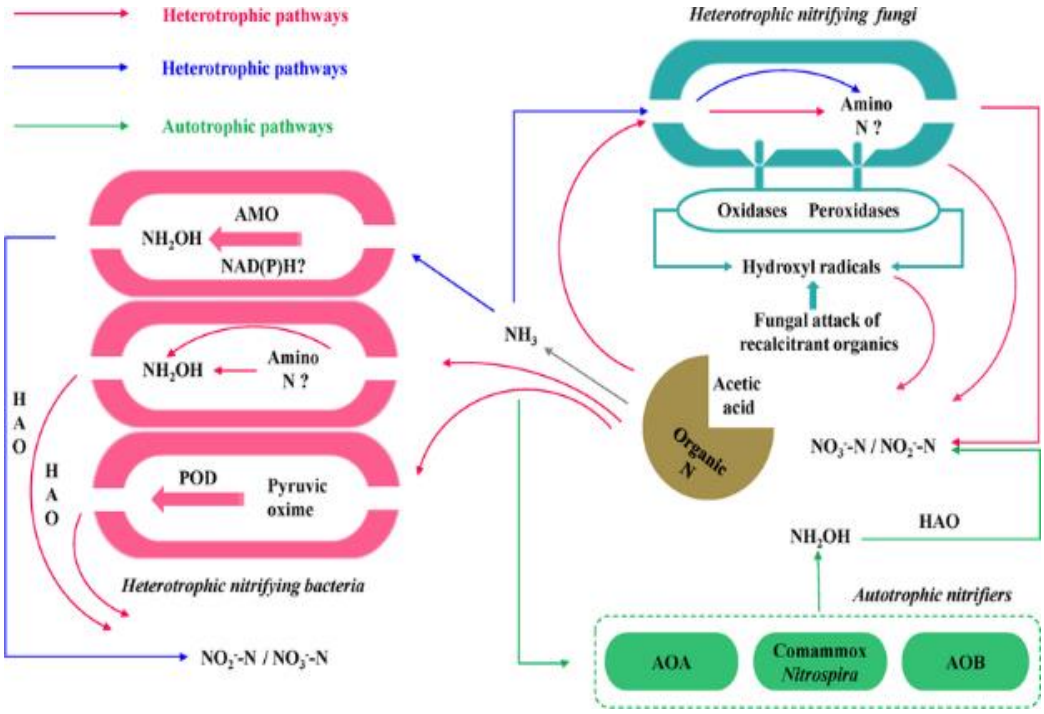

Fig 4: The putative pathway of oxidation [19]

Under aerobic conditions, NAP and CD1-NIR convert NO_3 -N and NO_2 -N to NO_2 -N and NO, correspondingly, for the aerobic denitrification process. In progression, electrons can be related to NAP via Complex I by NADH, which is created during the metabolism of

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organic materials. Furthermore, because the cytochrome chain has a "bottleneck" when electrons are transferred to it, jammed electrons must be transferred NAP or NIR to relieve “overloaded" cytochrome chain. As a result, the process of aerobic denitrification can take place.

Complex I, Complex III, and Complex IV are the pathways by which electrons from organic materials are transferred to oxygen during aerobic respiration. NADH serves as the electron carrier during this action. It follows that NADH, which is engaged in all three metabolic pathways, is crucial to the HNAD process. Furthermore, as the electron transport chain is responsible for moving electrons, the HNAD process should also take the electron transport chain's saturation into account. The HNAD process is characterized by a frequent characteristic. The electron transport chain may become saturated at this point, which would prevent the HAND bacteria from performing better at removing nitrogen from the medium even if the influent C/N ratio is at its optimal level. Despite the sufficient amount of NADH, the electrons are unable to go across the electron transport chain to the electron acceptor.

Variations in the C/N ratio during the HNAD process can alter the ATP level, electron transport chain activity, and intracellular NADH/NAD+ ratio; as a result, HNAD bacteria's capacity to remove nitrogen is affected. Intracellular redox potential varies in response to alterations in the NADH/NAD+ ratio. In addition to being used up by the processes of heterotrophic nitrification, aerobic respiration, and aerobic denitrification, the accumulated electrons also exhausted by production of PHB. As a result, control of the intracellular NADH/NAD+ ratio is possible. Acetyl coenzyme A, or Acetyl-CoA, is the link between the Krebs cycle and PHB production and breakdown. The NADH/NAD+ ratio and the acetyl-CoA/CoA ratio may be used to control this process. The HNAD procedure entails the simultaneous prevalence of the PHB synthesis, aerobic breathing, denitrification, and heterotrophic nitrification strategies.

These tactics are linked to electron delivery, further complicating the HNAD manner. It may be assumed that the numerous metabolic activities taking place in HNAD microorganism might also interrelate with one another.

2.5 Gene Sequencing

Gene sequencing is a fundamental tool in know-how the mechanisms inside the back of heterotrophic nitrification and anaerobic denitrification techniques, which can be vital components of the nitrogen cycle. Heterotrophic nitrification is a microbial-driven method wherein microorganism use organic carbon compounds to oxidize ammonia to nitrite and nitrate, which takes place in environments like soil, sediment, and wastewater treatment systems. Understanding the genetic make-up of organisms concerned in this system offers insights into the metabolic pathways and regulatory mechanisms governing this device. Gene sequencing techniques, which encompass metagenomics and amplicon sequencing, permit researchers to analyze the genetic material present in environmental samples containing microbial groups engaged in heterotrophic nitrification. By extracting DNA from these samples and sequencing unique genetic markers, researchers can come

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to be aware of and represent the range of microorganisms involved in nitrification. Advanced sequencing generation additionally allow the reconstruction of complete genomes, facilitating the have a look at of metabolic pathways and the genetic basis underlying heterotrophic nitrification

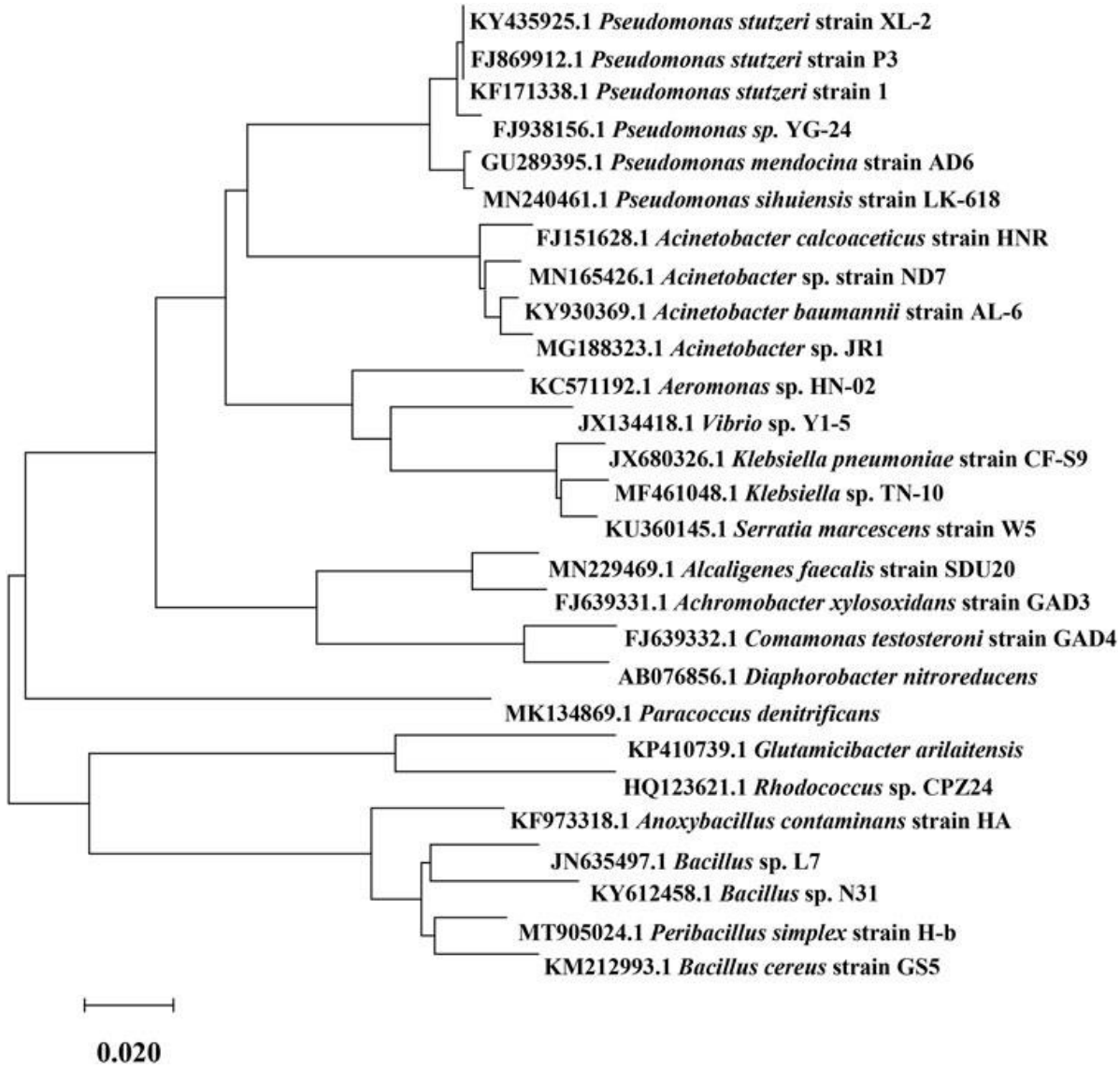

Fig 5: Bacterial Sequence [12]

Anaerobic denitrification is each different vital process in the nitrogen cycle, wherein microorganism use nitrate or nitrite as electron acceptors within the absence of oxygen, changing them into nitrogen fuel or nitrous oxide. By sequencing genes encoding key enzymes involved in denitrification, researchers can discover and signify denitrifying bacteria in diverse environmental niches. In end, gene sequencing era are critical in advancing our know-how of heterotrophic nitrification and anaerobic denitrification

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strategies, allowing researchers to unravel the complexity of microbial ecosystems, pick out novel metabolic pathways, and increase techniques for environmental manage and bioremediation.

Table 3: Enzyme and genes involved in denitrification reaction

Sr no.	Enzyme	Genes	Denitrification reaction	References
1	Nitrate reductase	narG, narH, narI, narJ	NO3- → NO2-	[7]
2	Nitrite reductase	nirS, nirK	NO2- → NO	[8]
3	Nitric oxide reductase	norB, norC, norD	NO → N2O	[9]
4	Nitrous oxide reductase	nosZ	N2O → N2	[10]

3. FACTORS AFFECTING THE PROCESS OF HETEROTROPHIC NITRIFICATION AND AEROBIC DENITRIFICATION (HN-AD)

Diversity, structure and community of nitrification and denitrification bacteria were pretentious by different environmental factors such as organic matter, water contents, temperature, carbon source, and PH.

3.1 Carbon source

The overall performance of HNAD bacteria is notably stimulated by means of the carbon deliver. Huang et al. (2015) determined that the use of sodium acetate, 78.86% of the nitrate was removed in 72 hours for Zoogloea. According to reviews, Pseudomonas aeruginosa, Pseudomonas putida, and Acinetobacter junii prefer each succinate and acetate as carbon resources. Because organic acid salts like succinate and acetate have tiny, simple molecular systems that lead them to smooth to absorb, heterotrophs like Agrobacterium sp. Have been shown to choose them over carbohydrates like glucose. Food waste fermentation liquid has been applied as a C-supply in bioreactors to dispose of nitrogen. Moreover, inspired sludge utilized as a carbon source (C-source) in bioreactors for the elimination of nitrates. It proves, this method decreased the nitrate content to much less than 1 mgL-1. Denitrification approaches have made use of residual carbon resources, along with effluent from candy factories, dairy residue, and tender drink industry residue. It became observed that those carbon assets high in sugar outperformed methanol in terms of denitrification. Associated with different carbon assets, which can be pretty luxurious, the usage of these waste merchandise as a carbon source has a worthwhile benefit. Currently, some strong carbon assets are being investigated for denitrification. Solid-segment denitrification is the method of using resources of strong carbon [14].

Solid carbon resources have covered both herbal and synthetic resources, together with wood chips, composted leaves, rice straw, polylactic acid, and polybutylene succinate. The stable carbon resources must be obviously biodegradable and insoluble. These resources function a substrate for the development of biomass similarly to being electron donors. When a strong carbon supply is utilized in a denitrification machine, microbial have a look at exhibits a higher range in biodiversity than while a liquid carbon source is used. The complexity of the solid carbon supply and variations in niches in heterogeneous

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structures have been blamed for this considerable version in biodiversity. It is surprisingly perfect to employ plant-based totally carbon assets to put off nitrate from wastewater due to their low cost and clean availability. To recognize their involvement in denitrification, the synthetic wetlands had been planted with withered plant life consisting of Pontederia cordate and a mixture of flora like Pontederia cordata and Arundo donax. High ammonium elimination and nitrate nitrogen (NO3-N) had been discovered in every of these investigations. A plant-primarily based carbon source was additionally proposed by the have a look at as an inexpensive alternative for high priced carbon assets. Woodchips are the most almost beneficial natural carbon supply because of their plentiful availability and occasional emission of dissolved organic carbon. In addition to natural carbon sources, decomposable synthetic polymers like polylactic acid (PLA), poly-three-hydroxybutyric acid (PHB), and polycaprolactone alsoutilized as per carbon bases[14]. Their use is costly, but they have a high denitrification effectiveness and a significantly lower release of liquified organic carbons.

Table 4: Heterotrophic nitrification and aerobic denitrification strain and growth

No.	Strains (source)	Optimal or cultivated rate of nitrification	Optimal or cultivated rate of denitrification	End-result of nitrification	End-References result of denitrification
1	Providencia rettgeri YL	_	_	NO2-	NO [9]
2	Alcaligenes faecalis NR	_	Undetectable	_	_ [10]
3	Acinetobacter sp. Y16	_	0.43	NO2-	N2O [8]
4	Klebsiella pneunomiae CF-S9	_	0.0074	NO2-	N2O [11]
5	Paracoccus versutus LYM	_	0.0156	NO2-	N2 [12]
6	Acinetobacter sp. Y16	0.0024	0.03	NO2-	N2O [7]
7	Acinetobacter junii YB	_	0.0195	NO2-	N2O [9]
8	Acinetobacter sp. Y1	_	0.0081	NO2-	N2O [10]
9	Alcaligenes faecalis C16	_	0.019	NO2-	N2O [12]
10	Zobellella taiwanensis DN-7	_	0.498	NO2-	N2O [19]
11	Acinetobacter junii YB	_	0.0095	NO2-	N2O [11]
12	Pseudomonas putida YH	_	0.0186	NO2-	N2O [16]
13	Pseudomonas aeruginosa YL	_	0.0117	NO2-	N2O [9]
14	Diaphorobacter polyydroxybutyrativorans SL-205	_	0.124	NO2-	N2O [10]
15	Klebsiella sp. TN-10	_	0.0283	NO2-	N2O [8]

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16	Pseudomonas sp. JQ-H3	0.081	0.054	NO2-	N2O [11]
17	Acinetobacter sp. JR1	_	0.034	NO2-	N2O [19]
18	Pseudomonas putida NP5	_	0.021	NO2-	N2O [15]
19	Pseudomonas putida ZN1	_	0.158	NO2-	N2O [12]
20	Pseudomonas aeruginosa P-1	0.127	0.016	NO2-	N2O [7]
21	Acinetobacter sp. ND7	_	0.00051	NO2-	N2O [9]
22	Bacillus subtilis JD-014	_	0.04315	NO2-	N2O [10]
23	Gordonia amicalis UFV4	_	0.5238	NO2-	N2O [15]
24	Pseudomonas sp. JQ-H3	_	0.04571	NO2-	N2O [16]
25	Pseudomonas putida NP5	_	0.03453	NO2-	N2O [19]
26	Pseudomonas putida ZN1	_	0.03446	NO2-	N2O [12]
27	Acinetobacter baumannii AL-6	_	0.558	NO2-	N2O [11]
28	Acinetobacter sp. YS2	_	0.3758	NO2-	N2O [9]
29	Pseudomonas aeruginosa P-1	_	0.1275	NO2-	N2O [8]
30	Acinetobacter sp. ND7	_	0.565	NO2-	N2O [7]

3.2 Carbon / Nitrogen Ratio

In the denitrification process, the carbon-to-nitrogen (C/N) ratio is a measurement of the electron donor-to-acceptor ratio. Using the right amount of carbon source is necessary for efficient denitrification to occur. Less carbon means less electron flow, which impacts both denitrification and cell development by insufficiently supplying the energy needed for cell growth. However, there won't be much of a denitrification rate rise if the amount of carbon is higher than the ideal concentration. Consequently, it becomes essential to keep the right (C/N) ratio[20]. According to Prasetyo et al. (2018), who investigated impact of various C/N ratios on Pseudomonas sp. nitrate removal, the strain demonstrated 98.35% nitrate removal efficiency at a C/N ratio of 10. Bacillus sp. and Pseudomonas taiwanensis with glucose had the maximum nitrate removal rate, as reported by Zhao et al. (2010) and He et al. (2018), with a C/N ratio of 15. After examining the effects of various C/N ratios, Zhang et al. (2019) found that a C/N ratio of 16, which is significantly greater than that of other heterotrophs, was best for nitrogen removal when citrate was used as the carbon source. This implies that these species are suitable for use in high-organism wastewater treatment plants. Bacillus cereus was employed by Rout et al. (2017) to extract nitrogen from household wastewater, with a C/N ratio of 7.5 being ideal. According to the study, low C/N ratios (2.5 and 5) led to poor development and nutrient removal capacity. Ji et al. (2015) also noted that although high carbon concentration inhibits bacterial growth, low carbon deliberation causes low bacterial growth yield. Because the

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effectiveness of denitrification declines at very high or low carbon concentrations, crucial to continue a certain C/N ratio for the heterotrophic organism being used to achieve high denitrification.

3.3 Dissolved Oxygen

Denitrification, initially thought to be anoxic, is influenced by higher shaking speed and dissolved oxygen concentration. Microorganisms prefer oxygen as their terminal electron acceptor due to its higher energy yield. Studies have shown that some denitrifiers can accept high oxygen considerations, while others, like Hyphomicrobium X, can tolerate very low oxygen concentrations. While Paracoccus denitrificans can grow in aerobic environments, it can only denitrify in an oxygen-free environment. Therefore, proper control of dissolved oxygen concentration is crucial for effective nitrogen removal in aerobic denitrification[20]. Citrobacter diversus has the best-dissolved oxygen concentration of 5 mgL-1. Pseudomonas aeruginosa co-respires using NO3 and O2, and aerobic denitrification is carried out by Achromobacter, Acinetobacter, and Pseudomonas at dissolved oxygen concentrations of 3-10 mgL-1. Agrobacterium denitrification efficiency is limited by dissolved oxygen concentrations of 7-8 mgL-1.Pseudomonas stutzeri accumulates nitrite at high dissolved oxygen, but lowering oxygen concentration increases nitrite subtraction from 62.37% to 100%. Pseudomonas putida has oxygen acceptance levels of 5-6 mgL-1. Enterobacter cloacae, anovel aerobic denitrifying bacterium, shows 100% total nitrogen removal at a dissolved oxygen level of 6.08 mgL-1. Hocaoglu et al. (2011) discovered that dissolved oxygen concentrations between 0.15-0.35 mgL-1 were ideal for nitrogen elimination. The effectiveness of removing nitrate decreases significantly as the concentration of oxygen rises to 0.5 mgL-1. When the dissolved oxygen content was 1.5 mgL-1, a high nitrogen removal efficiency was seen in an aerobic granular sludge reactor. The microorganisms under consideration for denitrification determine how dissolved oxygen concentration affects them, and same species of aerobic denitrifiers have varying denitrification capacities at various dissolved oxygen concentrations.

3.4 Effect of Temperature

Temperature is a crucial parameter in denitrification, with most studies indicating that the optimal temperature range for aerobic denitrification is 25-37 ◦C. On the other hand, psychrophilic and thermophilic microorganisms have also been discovered for their potential in nitrogen removal[20]. Pseudomonas taiwanensis, an aerobic denitrifier, showed 100% nitrate removal efficiency at 15◦C and 51.61% at 5◦C. Low temperatures can reduce denitrification efficiency, as reported in Pseudomonas mandelli when developed at 10◦C. Thermophilic bacteria can consume ammonia and nitrate at temperatures as high as 50◦C. Various microorganisms carry out aerobic denitrification at different temperatures. The growth, reproduction, and metabolism of bacteria are all significantly influenced by temperature. Proteins or nucleic acids become denatured at high temperatures, whereas enzyme activity is inhibited at low temperatures. Acinetobacter sp. HA_2 grows prosperous in an extensive temperature range, with 20 ◦C being the ideal temperature. The mainstream of isolated HNAD strains are mesophiles.

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While certain bacteria, such as Aeromonas sp. HN-02 and Acinetobacter sp. Y16 may survive in a variety of environments, 20 ◦C is ideal for growth and 5 ◦C for nitrification.The five classes of microorganisms are mesophiles, thermophiles, hyperthermophiles, psychrophiles, and psychrotrophic. Though Acinetobacter haerbinensis HITLi7T, Pseudomonas tolaasii Y-11, and Pseudomonas migulae AN-1 fit into the psychrophiles, most HNAD bacteria are classified as mesophiles. The ideal growing temperature for these strains is 15 ◦C. Thermophiles, such as Anoxybacillus contaminans HA, whose ideal growth temperature is 55 ◦C, were identified from a BioDeNOx reactor. Contrary to popular belief, microbes are more complexly affected by temperature. Different psychrophile, psychrotrophic, mesophilic, thermophilic, and hyperthermophilic cellular membrane architectures exist, and temperature influences the production of proteins, enzymes, intracellular and extracellular materials, and enzyme activity[20]. Mutant strains of temperature-sensitive bacteria can also be produced by temperature.

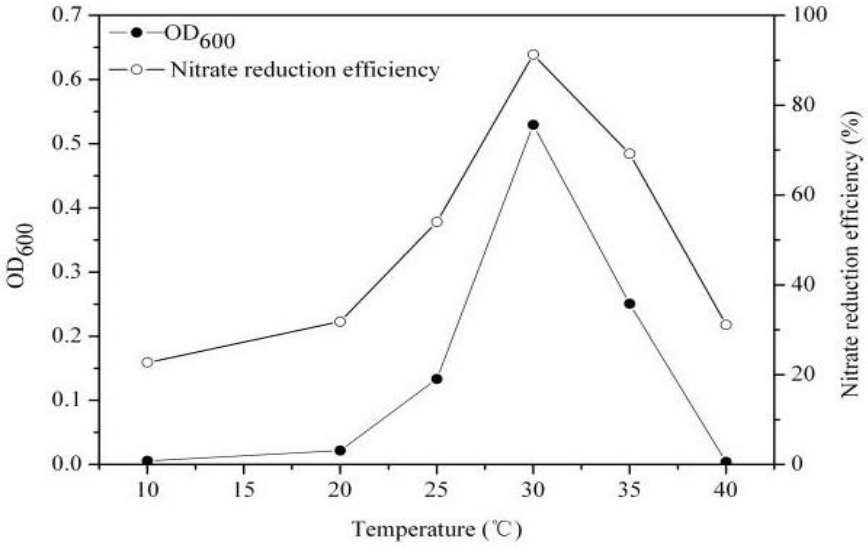

Fig 6: Effect of temperature [21]

3.5 Effect of pH

The process of denitrification causes nitrate depletion, which raises pH and causes the generation of hydroxide ions (OH−). Neutral to alkaline (7-8) is the ideal pH range for denitrification, which lowers the effectiveness of bacteria in denitrification. PH values greater than 8.75 or lower than 6.25 have an adverse effect on Enterobacter cloacae's overall nitrogen removal efficiency. Strong acidic or alkaline environments inhibit the development of Acinetobacter junii. Pseudomonas mandelli's capacity for denitrification is negatively impacted by pH 5. Few species, nevertheless, such Aeromonas and Halomonas campestris, can carry out denitrification at pH values greater than 9, which is 11. According to the study, High-Nitrogen-Acid (HNAD) bacteria are widely distributed and have a significant environmental adaption since they can thrive in acidic, neutral, and alkaline environments. Aeromonas sp. HN-02 achieves around 70% of its NH_4+-N

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removal efficiency pH ratio of 4.0–10.0, while Zobellella taiwanensis DN-7 multiplies in pH range of 5.0–11. More than 70% of NH_4+-N is removed by Acinetobacter junii YB, Pseudomonas putida YH, and Pseudomonas aeruginosa YL at pH values between 5.0 and 11.0.

Three categories can be used to categorize microbial communities: alkaliphiles, neutralophiles, and acidophiles. Since alkaline settings allow HNAD bacteria to generate more NH_3, which is a direct nitrogen source for bacterial development, most HNAD bacteria prefer slightly alkaline surroundings. Nonetheless, pH can impact the microbial colonies by influencing nutrient uptake, as it is the main regulator of microbial breakdowns[20]. Nonetheless, pH can influence the microbial communal by influencing salinity, nutrient ease of use in the soil, donating and accepting processes, and the makeup of aqueous solutions. pH is a major regulator of microbial metabolisms. Phyph may influence redox processes, reactions that produce or use protons, and energy yields both directly and indirectly. pH variations can harm DNA and proteins, alter metabolisms, and affect how naturally occurring populations metabolize their resources. Under acidic and alkaline circumstances, different species of bacteria require pH homeostasis to maintain proper cellular activity. The membrane potential of microorganisms, which is connected to the intracellular redox status (NADH/NAD), can also alter in response to changes in the medium's pH. The production of ATP, transport reactions, and other processes all need proton motive force (pmf). Proton motive force (pmf) is used in the establishment of ATP, transport reactions, and other energy-requiring reactions, affecting metabolic processes. Concerning the bacterial conversion of NH4+ to NH3 and other physiological processes, HNAD bacteria prefer an alkaline environment. To comprehend the influencing mechanism that is most common in HNAD bacteria, more research is required [21].

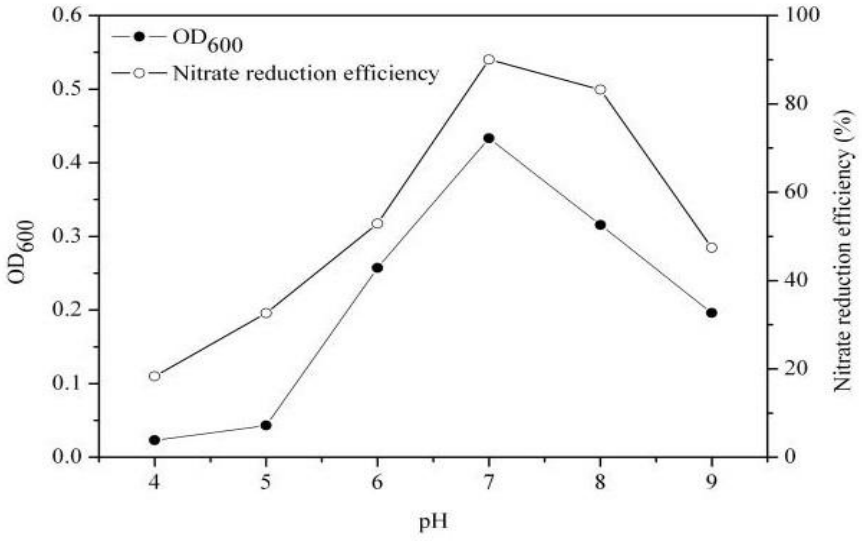

Fig 7: Effect of pH [21]

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3.6 Shaking Speed (DO Concentration)

The development of Heterotrophic Nitrogen Depletion (HNAD) bacteria depends on shaking and air supply. Different HNAD bacteria always have the ideal DO concentrations, with Pseudomonas stutzeri KTB favoring a high shaking speed of 160 rpm. However, the rates of NH4+-N removal are reduced at both greater and lower DO concentrations. Under high shaking speeds, Pseudomonas stutzeri T_{13} can obtain 100% removal efficiency for NH_4+-N and NO_3−-N, but only 23.47% because of significant NO2−-N buildup. Since dodecium (O_2) is an electron acceptor in both aerobic denitrification and heterotrophic nitrification processes, it is necessary for the oxic state of HNAD microbes. The ideal DO values for each kind of HNAD bacterium vary, and the removal of nitrogen is adversely affected by DO concentrations that are either greater or lower. For a very long time, DO's impacts on intracellular redox homeostasis have been disregarded. The concentration of DO and ORP has a linear connection, and intracellular redox is influenced by OPR in the bulk liquid. DO concentration has an intracellular effect. Because DO concentration alters the external environment's pH (ORP) in which HNAD bacteria are grown, it affects internal redox. Although NADH plays a significant role in the HNAD process, intracellular redox and electron transport pathways are mostly ignored in favor of research that primarily concentrates on nitrogen conversion and carbon oxidation.

3.7 Heavy Metals

Because they may complement enzymatic systems and decrease microbial activity owing to toxicity, heavy metals have diverse impacts on microorganisms. Cu_2+ sensitivity Aeromonas sp. HN-02 demonstrated by nearly ceasing NH_4+-N removal at 1.5 mg/L concentration. Zn_2+ deliberation upsurges to 8.0 mg/L and NH_4+-N elimination ranges 94.58%, indicating that the strain is not susceptible to Zn_2+. When Cu_2+ and Zn_2+ coexist on Aeromonas sp. HN-0_2, the toxicity greater, while they do so separately.0.5 mM Zn_2+ (32.5 mg/L) and 0.5 mM Cu_2+ (31.8 mg/L) have no effect on NH_4++-N elimination or Bacillus sp. PK15 growth. When 0.2 mM and 0.4 mM Fe_2+ are added, the amount of NO_2− that accumulates during the aerobic denitrification method that Paracoccus versutus catalyzes can be significantly reduced. The heterotrophic nitrification capacity of Serratia marcescens CL1502 is unaffected by the concentration of Cr_3+ and is only reduced by approximately 10% when the concentration is increased to 150 mg/L. The addition of Pb_2+ completely suppresses N_2 production of Alcalingenes sp. TB.

Pseudomonas putida ZN_1 growth and the NH_4 +– N exclusion mechanism are essentially unaffected by occurrence of 80.0 mg/L of Cu_2+ and Zn_2+. Pseudomonas putida ZN_1 has superior metal-resistant properties. Pseudomonas aeruginosa P-1, on the other hand, is more susceptible to Ni_2+. When 20.0 mg/L Ni_2+ is present, the heterotrophic nitrification capacity is completely gone. Pseudomonas aeruginosa P-1's capacity for heterotrophic nitrification may be inhibited by Cu_2+. Acinetobacter baumannii AL-6 may decrease Cr_6+ to Cr_3+, which is less hazardous, by conducting Cr balancing. Additionally, Acinetobacter baumannii AL-6 may decrease Cr_6+ to Cr_3+, not as much of hazardous, by accompanying Cr balancing. The pH value and C/N ratio have an impact on the decline of Cr_6+ by Acinetobacter baumannii AL-6. For elimination Cr_6+ and NH_4 +-N, neutral conditions and

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a high C/N ratio are ideal. The strain Pseudomonas aeruginosa P-1 has a strong resistance to Cr_6+. Pseudomonas aeruginosa P-1 is capable of removing 80% of NH_4 + -N when exposed to 50.0 mg/L of Cr_6+. Nonetheless, Pseudomonas aeruginosa P-1 almost does not react negatively to 50.0 mg/L of Zn_2+, Cd_2+, and Pb_2+.The toxicity of heavy metals may be explained by several factors, such as atomic mass of metals, their average electrode potential, and the relative stabilities of the chelate complexes that are generated among the metals and different biological ligands. The harmfulness of heavy metals can be influenced by ion interactions, pH levels, and environmental elements. The enzymatic reactions, metal-binding proteins, and energy-dependent efflux of harmful ions can provide toxicity resistance in bacteria. Conditional on the kind of metal and the strains examined, different heavy metals have different toxicities on HNAD bacteria. Because some metals, including Co_2+, Mn_2+, Fe_2+, and so on, are involved in enzymatic system, the development HNAD bacteria n't adversely impacted by quantities these metals. However, because of the intrinsic toxicity of heavy metals to microorganisms, HNAD bacterial growth will be negatively impacted by excessive metal concentrations. However, nearly all research done so far has failed to identify the mechanisms behind the toxicity caused by dense metals in HNAD bacteria.

3.8 Salinity

Salinity poses challenge for HNAD bacteria. The secretion of marine heterotrophic nitrifying organism benefits from appropriate salinity; nevertheless, excessive salinity raises the cell's osmotic pressure, which might impact microbial activity. Considering various HNAD strains, salt has fairly varied effects on the HNAD process. Even yet, the effects of similar salinity on aerobic denitrification and heterotrophic nitrification vary within the same strain. The ideal salinity for Bacillus methylotrophicus L7 during the heterotrophic nitrification procedure is 0.0 g/L NaCl; conversely, the ideal salinity for the aerobic denitrification process is 10.0 g/L NaCl. Halomonas campisalis ha3, which was inaccessible from the slush of a saline-alkali lake in Erods, Inner Mongolia, China, has an optimal salinity of 4% (w/v). This strain is capable of growing in a medium containing 20% (w/v) NaCl. Aeromonas sp. HN-02 is tolerant of up to 20.0 g/L of salinity, and its ability to remove NH4 + -N is only slightly impacted by 10.0 g/L of salinity. If it is between 1.0 and 5.0, Vibrio diabolicus SF16 may remove more than 90% of the NH_4 + -N. Additionally, Pseudomonas sp. AND-42 have salinity acceptance. The NaCl concentration does not significantly affect the rate of NH4 + -N removal when it is changed from 0.0 g/L to 10.0 g/L. NaCl concentrations ranging from 0.0 g/L to 30.0 g/L did not affect Zobellella taiwanensis DN-7 development; nevertheless, NH_4 +-N removal degrades with continuous concentration increases. The salinity tolerance of Serratia marcescens CL1502 is good; when the salinity is between 1.0 and 5.0%, more than 90% of the NH_4 + -N removal efficacy may attained. An appropriate, and essential for HNAD bacteria. At 3% salinity, Bacillus litoralis N31 obtains maximum NH_4 + -N deduction; removal effectiveness decreases by increasing or decreasing salinity. When the concentration of NaCl in the culture medium is between 30.0 and 180.0 g/L, Halomonas sp. B01 can grow to its maximum, which it does at 60 g/L. In situations when the salinity is less than 3%, Pseudomonas balearica UFV3 can remove all of the NH_4 +-N; in contrast, Gordonia

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amicalis UFV4 has a better NH_4 +-N removal competence under 3% compared to 0.0% salinity. When it is less than 20 g/L, Acinetobacter sp. JR1 can remove more than 96.0% of the NH_4 +-N; however, NH_4 +-N is scarcely eliminated when 20.0 g/L. exceeds As it rises to 3.0%, Pseudomonas putida NP_5 growth and NH_4 + -N removal, significantly reduced, even though they were able to colonies at salinities between 0.0 and 2.0%. Pseudomonas putida NP_5 growth and NH_4 + -N removal are inhibited when salt levels are continuously raised to 5.0%. High salinity levels are also tolerable to pseudomonas aeruginosa P-1. Under 24 hours, when less than 40.0 g/L, more than 90% of the NH_4 + -N may be eliminated.

Inorganic salt that has dissolved is connected to salinity. The internal molecules of microorganisms, structure, metabolic enzyme activity, and osmosis pressure are all affected by changes in salinity. Plasmodial activity takes place in highly salinized environments. Salt-tolerant mechanisms include the uptake of K+ and ejection of Na+ via K+/Na+ pumps, Na+/H+ reversed transport proteins, and K+ channels. Yet, bulk research to date has only examined how salt affects HNAD bacteria's performance; the mechanism behind the salinity suppression of HNAD bacteria is still unclear. Furthermore, the mechanism that may be used to explain why salt-tolerant HAND bacteria exist is currently elusive.

3.9 Antibiotics

The use of antibiotics has been linked to various water environments. The category and meditation of antibiotics have an impact on the efficiency of nitrogen removal from HNAD bacteria; low concentrations have little effect on this process, while high concentrations are toxic to microorganisms. The system's nitrogen removal rate (NRR) and organics removal rate (ORR) in a sequence batch reactor inoculating Klebsiella pneumoniae y6, an HNAD bacteria, at a concentration of 5 mg/L of ampicillin are 0.79 and 7.17 kg/(m3·d), respectively. These values are in the neighborhood of 0.80 and 7.60 kg/(m3·d) when ampicillin is not present. However, when the ampicillin concentrations are 25 mg/L and 50 mg/L, respectively, NRR and ORR decrease by 23.8% 13.4%, 48.6%, and 50.9%. 5 mg/L of sulfamethoxazole (SMX) and tetracycline (TEC) treatment did not affect the aerobic NH4 +-N removal efficiency in an MBBR inoculating Achromobacter sp. JL9; however, the addition of trimethoprim and ciprofloxacin results in a decrease in the NH4 +-N removal efficiency. The co-expression of nitrogen and carbon metabolism is impacted by antibiotics. Antibiotics impact electron transport chains, genes linked to antibiotic resistance, and the co-expression of carbon and nitrogen metabolism. Genes linked to denitrification (narG, narH, narI, and nirK), glycolysis, the TCA cycle, and the electron transfer chain (nuoA, nuoB, nuoD, nuoF, nuoG, nuoI, and nuoL) are all downregulated after exposure to 2 mg/L of SMX. The antibiotics' concentration affects how resistant ARGs are to them. Additionally, there is a discernible upsurge in the production of reactive oxygen species (ROS) in response to elevated antibiotic concentrations. Since reactive oxygen species (ROS) may kill cells, aerobic bacteria naturally develop an antioxidant defense system to fend against ROS's harmful effects. The high dose of antibiotics causes an imbalance between antioxidant defense and ROS production. Although the

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effects of antibiotics on HNAD bacteria are now receiving less attention than other variables, further study on the bacterium's antibiotic response will ultimately be made public.

4. APPLICATIONS AND CASE STUDIES OF HETEROTROPHIC NITRIFICATION AND AEROBIC DENITRIFICATION (HNAD)

Autotrophic nitrifies develop slowly, and their sensitivity to environmental influences and operating circumstances might impair the effectiveness of nitrogen elimination through conventional nitrification and denitrification processes. This is frequently resolved by establishing heterotrophic denitrifies and autotrophic nitrifies in separate tanks, which involves an extensive capacity for their installation. In a single tank, heterotrophic nitrification-aerobic denitrification (HN-AD) demonstrates the ground-breaking possibilities of wastewater treatment methods. To increase their contribution, it was not known how to enrich HN-AD bacteria-activated sludge. In a halophilic aerobic granular sludge (HAGS) arrangement, effects of feast/famine (F/F) ratio were investigated concerning progression of autotrophic ammonia-oxidizing bacteria (AOB) and HN-AD bacteria. The performance of removing total inorganic nitrogen (TIN) dropped dramatically when F/F ratio dropped from 1/9 to 1/15. The percentage of bacteria that are heterotrophic reduced from 79.0 to 33%. In consequence, copy number of the napA gene dropped from 2.2 × 1010 copies/g HAGS to 8.1 × 109, and the relatively large quantity of Paracoccus declined from 70.8% to 25.4% for the same reason [22].

Another study established a heterotrophic nitrification-aerobic denitrification (HN-AD) approach in mustard tuber wastewater after 105 days of acclimation in a sequencing assemblage reactor. Using manual wastewater, the effectiveness of simultaneous carbon and nitrogen removal (SCNR) was examined in salinity ranges of 1% to 6%. High salinity has a major impact on nitrogen removal but no effect on carbon removal. Heterotrophic nitrification rates were 2.83 and 3.96 times greater than autotrophic nitrification at 1% and 3% salinities, respectively. Autotrophic nitrification was greatly reduced at 6% salinity, although anoxic denitrification was only marginally impacted. The acclimated wastewater sludge's microbial community structure showed that autotrophic nitrification-causing genera were eventually reduced. Pseudomonas, Bacillus, Alcaligenes, and Paracoccus were the four main taxa associated with the HN-AD process. Investigations were conducted into the ideal SCNR functioning parameters. [23].

Another study related to our review was conducted by [24]. The study presents a cost-effective microalgae-assisted heterotrophic nitrification-aerobic denitrification (HNAD) method for well-organized nutrient removal from high-salinity wastewater. The system, comprising ammonia stripping, microalgae absorption, biological removal, and biologically induced phosphate precipitation (BIPP), provided outstanding nutrient removal (~100.0%). Of the nitrogen and phosphorus removed, biofilm clearance accounted for 55.3–71.8% and 45.6–51.8%, respectively. Theoretically, the symbiotic system might attain energy neutrality, offering fresh perspectives on effective and low-carbon nutrient removal techniques from wastewater with high salinity.

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To improve the quality of the water reuse effluent, the study investigates the use of a biological nitrogen removal system that uses pure-culture Bacillus licheniform is as an internal treatment unit in an aquarium. With a nitrification rate of 0.84 mg/L-h and a denitrification rate of 0.62 mg/L-h, the system showed evidence of both heterotrophic nitrification and aerobic denitrification. At an influent NH_4-N of 30 mg/L, the maximum NH_4-N and TN removal efficiencies were around 73%. On the other hand, Pseudomonas sp.'s other competitive heterotrophs caused lower efficiency as well as higher nitrogen removal and carbon consumption. Despite having a poorer overall performance than systems that used mixed-culture nitrifying and denitrifying microorganisms, the system's benefits include being land-limited and being simple to operate. [18].

The study's objective was to treat COD and reject water ammonium concurrently in Porsgrunn, Norway's primary wastewater treatment stream using two pilot-scale sequential moving bed biofilm reactors (MBBR). Biofilm carriers with a 60% filling ratio had a protected surface area of 650 m2/m3. The two reactors' combined ammonia removal efficiency (ARE) was 65.9%, whereas their respective nitrate production rate (NPR) and nitrite accumulation rate (NAR) were 19.8% and 80.2%. In both reactors, COD from rejected water was reduced by more than 28%. Heterotrophic nitrification and oxygen-tolerant aerobic denitrification were identified as the two main biological processes for ammonium elimination. Alcaligenaceae, a bacterial family that can simultaneously undergo heterotrophic nitrification and denitrification, was the predominant family in both reactors. There were also discovered other microbial groups that have an equivalent capacity for heterotrophic nitrification and aerobic denitrification to occur at the same time[25].

5. DISCUSSION AND FUTURE PERSPECTIVES

Coking wastewater poses significant environmental challenges due to its complex composition and high levels of pollutants. Therefore, the need for an effective and sustainable treatment method is paramount to mitigate the detrimental impacts on the environment and public health. Currently, treatment methods for coking wastewater include biological, chemical, and physical processes. For instance, conventional biological treatments, such as aerobic and anaerobic digestion, have been widely used but often require long treatment times and may not fully remove all contaminants. Additionally, chemical methods, such as advanced oxidation processes (AOPs), are effective in degrading refractory pollutants but come with high operational costs and potential secondary pollution [26]. However, recent advances in aerobic denitrification and heterotrophic nitrification show promise in treating nitrogen-rich wastewater. Nonetheless, these techniques face challenges such as the need for specific environmental conditions and the potential for incomplete nitrogen removal. Moreover, the integration of anaerobic, anoxic, and oxic processes has improved treatment efficiency but still requires optimization to reduce treatment time and enhance pollutant degradation [27]. Despite these advancements, the practical implementation of these methods at a full scale remains limited. Most studies have been conducted at laboratory or pilot scales, and only a few have shown successful scaling up. Furthermore, the

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complexity and cost of advanced treatment technologies, along with the need for skilled operation, hinder widespread adoption [28].

As a whole, finding a cost-effective treatment for coking wastewater is challenging due to its complex nature [28]. Single treatment methods are ineffective, as evidenced by recent studies focusing on integrated approaches to improve efficiency [29]. Conventional biological treatments have been replaced with integrated anaerobic, anoxic, and oxic processes, though treatment time remains high [30]. Bioaugmentation shows promise for degrading toxic pollutants, but full-scale application needs more in-depth studies on microorganism fate [28]. Advanced Oxidation Processes (AOPs) are efficient for removing refractory pollutants but face challenges like technical complexity, high costs, secondary pollution, and harsh reaction conditions, limiting full-scale use [27]. Despite many studies on coking wastewater treatment, most focus on efficiency at laboratory or pilot scales, with few showing practical full-scale application [31].

5.1 Future Perspective

Emerging technologies like microwave irradiation and bioaugmentation need further study for coking wastewater treatment [32]. As integrated methods are the only viable solutions, future research should focus on efficiency, cost-effectiveness, and technical feasibility. Technical and economic viability studies should aim for sustainable and cost-effective wastewater treatment [33].

6. CONCLUSION

The purpose of this review was to highlight the advancements in the field of heterotrophic nitrification and aerobic denitrification (HNAD) for nitrogen exclusion from wastewater. Important progress has been made, demonstrating the potential of HNAD processes in addressing nitrogen pollution. However, a variety of challenges remain, predominantly regarding the stability and efficiency of these processes under different environmental conditions. Biological methods, particularly bioaugmentation, have shown prominent improvements. Bioaugmentation has emerged as a promising technique, but the long-term fate of bioaugmented bacteria and their impact on the overall system remain controversial issues. Other difficult techniques such as advanced oxidation, reverse osmosis, and nanotechnology offer potential by high costs and the production of secondary pollutants. Integrated treatment methods have been identified as the most feasible solutions for managing complex and refractory wastewater. These approaches merge various treatment methods to develop efficiency and effectiveness. However, achieving a balance between cost and effectiveness is critical for the convenient application of these methods at an industrial scale. Future research should focus on optimizing these integrated approaches, exploring emerging technologies such as microwave irradiation, and ensuring the technical and economic feasibility of large-scale applications. As new contaminants persist to emerge and treatment technologies advance, there are plentiful opportunities for further innovation in the field of wastewater treatment. The development of sustainable, cost-effective solutions will be necessary in addressing the ongoing challenges posed by nitrogen pollution.

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