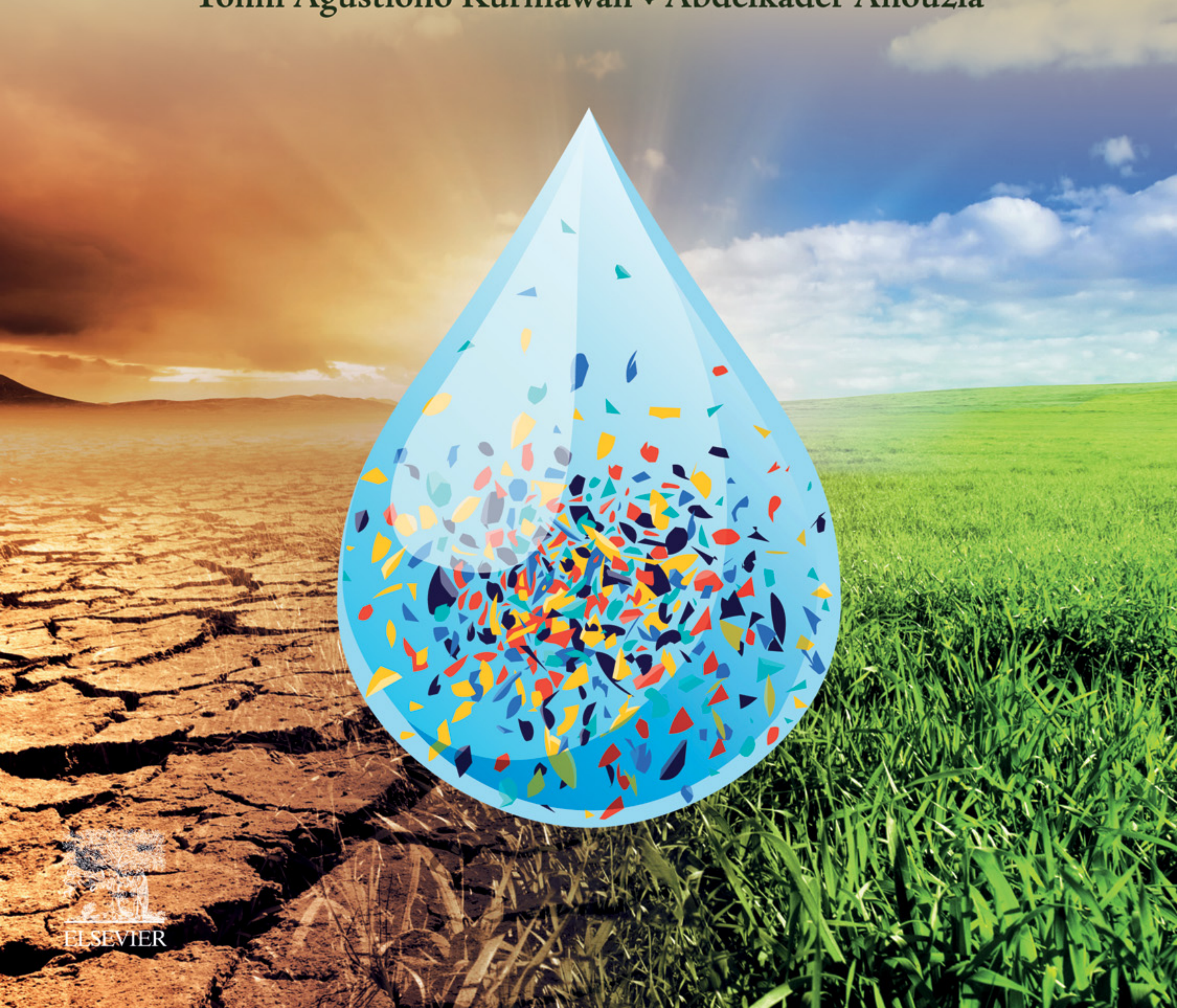
Point Source Nitrogen Pollution

Climate Mitigation and Sustainable Solutions for the Modern Era

EDITED BY

Tonni Agustiono Kurniawan ◆ Abdelkader Anouzla



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Climate Mitigation and Sustainable Solutions for the Modern Era

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Preface

Nitrogen is essential for sustaining life, forming the backbone of proteins, DNA, and many biomolecules that support the structure and function of ecosystems and human societies. Yet, the accelerated mobilization of reactive nitrogen due to anthropogenic activities has precipitated a profound and urgent environmental challenge. Of particular concern is point source nitrogen pollution, a concentrated and traceable origin of nitrogen discharge into natural systems—most notably from wastewater treatment plants, industrial outfalls, agricultural effluent tanks, and animal husbandry facilities.

This book brings together the scientific understanding, methodological advancements, case studies, and emerging innovations that define the state-of-the-art in identifying, managing, and mitigating nitrogen inputs from point sources. It is written with a multidisciplinary readership in mind—from environmental engineers and wastewater specialists to policymakers, chemists, ecologists, and graduate students navigating the complexities of nutrient management in a rapidly changing world.

The genesis of this book lies in the growing recognition that controlling point sources of nitrogen pollution is not merely a technical issue—it is a systemic challenge that interlinks water quality, climate change, energy usage, public health, and ecological integrity. Unlike diffuse or nonpoint source pollution, which arises from scattered and often stochastic origins, point sources are characteristically easier to monitor and regulate. However, the magnitude of nitrogen discharges from these sources and their cumulative impact on eutrophication, hypoxia, and ecosystem degradation in both freshwater and marine environments remain a critical problem globally.

Recent decades have seen significant strides in both understanding the biogeochemical behavior of nitrogen and developing technologies for nitrogen removal, such as nitrification–denitrification systems, anammox processes, membrane bioreactors, and biological nutrient removal systems. Despite these advances, implementation remains uneven across regions due to varying capacities, socioeconomic priorities, regulatory stringency, and technological maturity. Thus, there is a pressing need for a comprehensive resource that both synthesizes the core knowledge of nitrogen pollution and provides practical frameworks for designing and evaluating nitrogen mitigation strategies.

Throughout this book, the emphasis is placed not only on problem identification but also on solution orientation. Each chapter integrates theoretical exposition with applied knowledge, supported by diagrams, tabular data, and graphical insights that make the material accessible to both specialists and interdisciplinary practitioners.

The decision to focus explicitly on *point sources* stems from an analytical necessity: while much public discourse focuses on the diffuse problem of agricultural runoff and stormwater, point sources are often underestimated despite being highly amenable to intervention. Indeed, in many countries, targeted upgrades in municipal wastewater treatment alone could lead to substantial improvements in aquatic nitrogen loading. The relatively discrete nature of point sources also offers a laboratory-like setting to test innovative removal technologies and to refine emission controls that can later be adapted for broader applications.

Furthermore, this book emerges at a critical juncture. Global nitrogen cycles are becoming more complex due to intensified urbanization, climate variability, and changing consumption patterns. The nitrogen footprint of cities is increasing, and with it, the importance of strategic, scalable, and sustainable point source nitrogen reduction. Simultaneously, efforts to align with the Sustainable Development Goals (especially SDG 6 on water and sanitation, SDG 14 on life below water, and SDG 13 on climate action) hinge on effectively reducing reactive nitrogen emissions into the biosphere.

This book would not have been possible without the collaboration of dedicated researchers, engineers, field practitioners, and policy advocates who have worked tirelessly to unravel the challenge of nitrogen pollution. Their insights and experiences form the backbone of this volume. It is hoped that this book will serve as both a reference and a call to action—bridging the knowledge–practice gap and equipping stakeholders to more effectively confront the nitrogen crisis through science-based, technologically grounded, and policy-informed approaches.

We invite the reader to engage critically with the chapters ahead, to reflect on how point source nitrogen pollution intersects with their domain, and to contribute to shaping a more sustainable and nitrogen-smart future.

Tonni Agustiono Kurniawan
Abdelkader Anouzla

SECTION I

Potential solutions of nitrogen pollution

CHAPTER 8

Microbial niche tuning to track nitrate pollution in surface water/groundwater

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This chapter explores microbial niche tuning as a method to detect and mitigate nitrate pollution in surface and groundwater. It explains microbial niches in the nitrogen cycle, identifies microbial bioindicators, and evaluates bioaugmentation and biostimulation strategies. Additionally, it discusses challenges and real-world applications in environmental management.

Covering Grinnellian, Eltonian, and Hutchinsonian niche concepts, this chapter examines microbial roles in denitrification and anammox processes. It explores nitrate detection methods and strategies for optimizing microbial activity. Case studies from wastewater treatment, aquaculture, and constructed wetlands illustrate real-world applications.

The chapter is intended for environmental scientists, microbiologists, engineers, policymakers, and agricultural and industrial professionals, as well as academics and students in microbial ecology and environmental biotechnology.

Nitrate pollution from agriculture and industry is a growing issue. Traditional monitoring and remediation are costly, while microbial niche tuning offers a sustainable alternative by leveraging microbial communities for nitrate detection and removal.

This chapter identifies key microbial species and genetic markers, develops microbial biosensors, and evaluates bioaugmentation and biostimulation for nitrate removal. It also provides policy recommendations for sustainable wastewater management.

Considerations include ecosystem balance, biosafety, water quality monitoring, and ensuring public health safety in microbial-based treatments.

A research-based approach includes literature review, metagenomics, biosensor development, simulations, and case study analysis of microbial niche tuning in environmental applications.

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Challenges include ecosystem complexity, environmental variability, and high costs. Solutions involve bioinformatics, adaptive niche tuning, and integration with existing treatment systems for cost efficiency.

Microbial communities correlate with nitrate levels, proving their use as bioindicators. Bioaugmentation improved nitrate removal, biosensors enabled real-time detection, and case studies showed over 80% nitrate reduction in some systems.

Readers will understand microbial niche concepts, environmental applications, challenges, ethical considerations, and interdisciplinary approaches combining microbiology, ecology, and engineering for sustainable pollution management.

Microorganisms have specific niches, including preferences for certain environmental conditions and food sources. Some microbes have a high affinity for nitrate and utilize it in metabolic processes, such as denitrification (conversion of nitrate to nitrogen gas). By understanding and manipulating this microbial niche, we can utilize them as indicators or bioremediation agents of nitrate pollution. The presence and abundance of certain microbes specific to nitrate-rich environments can indicate pollution. For instance, an increasing denitrifying bacteria population in a water body may indicate high nitrate levels. Microbes utilizing nitrate from polluted sources will have a different isotopic signature than those utilizing natural sources. Analysis of microbial DNA and RNA can identify genes involved in nitrate metabolism (Zhou et al., 2015). The expression of these genes can measure microbial activity and the level of nitrate pollution. When exposed to nitrate, microbes can be genetically engineered to produce signals (e.g., fluorescence). These microbial biosensors can be used to detect and measure nitrate concentrations in real-time. The concept of manipulating the microbial niche can be applied to improve the effectiveness of nitrate tracking and remediation by performing bioaugmentation and biostimulation (Muter, 2023).

8.1 Microbial niche tuning concept

The term "niche" was introduced in 1913 by Grinnell and Swarth (1913) and was understood as a home for one species or a subspecies. The right definition of niche depends heavily on the perspective and intent of the scientist/researcher/writer, which can be ambiguous when applied for different purposes.

Grinellian niche concept was introduced by American ecologist Joseph Grinnell, which describes species' niche based on the physical habitat or living space in which the species is found. Grinellian niches emphasize the place or physical environment (e.g., climatic conditions, vegetation, topography, etc.) where a species can survive and reproduce. Each species adapts to its environment, explaining why certain species can only be found in some geographical regions that match their Grinellian niche. The species adaptation can be morphological (physical form), physiological (body function), or behavioral, enabling the species to survive in a particular habitat. For example, Grinellian niches of cacti are in dry and hot desert areas, since they have adapted to these conditions through morphological modifications (thorns, thick stems to store water, and extensive roots to absorb water).

Two species can have overlapping Grinellian niche in the same habitat. However, resource competition can occur if their niches overlap too much. In short, the Grinellian niche explains a species' specific environmental requirements that affect its existence and distribution in a habitat. This concept is fundamental in understanding how species interact with their environment and how environmental factors shape biodiversity on Earth.

The Eltonian niche, found by British ecologist Charles Elton, is a concept that describes the functional role of a species in an ecosystem, focusing primarily on the food chain and food webs. Contrary to Grinellian niches, Eltonian niches do not pay much attention to a species' physical habitat or living space. Instead, it focuses on species interaction regarding the transfer of energy and matter through feeding activities.

This concept is closely related to the trophic level (nutrient level) in the food chain, where each species has a specific role in this hierarchy. Eltonian niches include various types of feeding interactions, such as predation (predator–prey), herbivory (plant-eating), saprophagy (eating dead organic matter), and parasitism (parasite–host). Each of these interactions forms part of a species' Eltonian niche. Eltonian niches also focus on how species obtain their food, including hunting techniques, eating behavior, and morphological adaptations that support their eating habits.

Ecologist George Evelyn Hutchinson developed a more comprehensive niche concept known as hypervolume niche or multidimensional niche. This concept applies to all organisms, including microbes. According to Hutchinson, species' niches are not limited to one or two dimensions (such as Grinnellian or Eltonian niches); instead, they can be described as *n*-dimensional spaces, where each dimension represents a different environmental factor. For microbes, these dimensions can be numerous and diverse, including various factors, for example, types of nutrient sources, physical conditions, water availability, and interactions with other microbes and the local abiotic environment.

Hutchinson also distinguishes between fundamental niche (the potential niches of a species under ideal conditions) and realized niche (the actual niches of a species under conditions of competition and environmental limitations). Hutchinsonian niche concept helps in understanding how microbes can live in very specific microhabitats within a seemingly homogeneous environment. These microhabitats can differ in terms of nutrient availability, chemical conditions, and other environmental factors. In summary, the differences between fundamental niches and niches of realization, according to Hutchinson, can be seen in Table 8.1.

Although Hutchinsonian niches do not directly focus on functional roles as Eltonian niches, they can still be used to understand how microbes play a role in biogeochemical cycles (including the nitrogen cycle) and microbial food webs. For example, microbes

Table 8.1 The fundamental niches and realized niches according to Hutchinson.

Feature	Fundamental niche	Realized niche
Definition	Niche potential without competition	Actual niche with competition
Competition	No competition	Competition and other interaction
Condition	Ideal condition	Actual environmental condition
Size	Wider	Narrower
Description	Maximum potential of species	Actual living conditions of species
Limitation	Nothing	Competition, predator, and environmental factors

that degrade organic matter have different niches from microbes that fix nitrogen (Ayuwaningsih et al., 2018). For example, intestinal *Escherichia coli* have multidimensional niches, including glucose availability, intestinal pH, body temperature, and interactions with other intestinal microbiota.

The Hutchinsonian niche concept also explains how microbial species coexist without direct competition. Since each species has different niches, they can minimize competition and share available resources. Microbes can separate their niches in time (e.g., some are active during the day, others at night) or space (e.g., some live on the surface, others in depth). This separation reduces competition and allows more species to coexist. For example, in one biofilm, different types of bacteria can coexist by utilizing different nutrients, producing different metabolic products, or occupying different layers in the biofilm.

Each type of microbe has a specific functional role in the ecosystem, for example, decomposition, nutrient cycling, nitrogen fixation, and methane production. In some cases, several microbial species may have the same function (i.e., functional redundancy), which can provide ecosystem stability. If one species is lost due to environmental change, another species with the same function can take over. For example, in the nitrogen cycle, various types of bacteria are involved in different processes, such as nitrogen fixation, nitrification, and denitrification. Each of these types of bacteria has different niches and environmental requirements.

Hutchinsonian niche concept helps us understand how environmental changes can affect microbial niches and cause changes in the composition and function of microbial communities. Environmental changes can cause some microbial species to expand their niches, while others shrink or even disappear. Understanding microbial niches can help predict the impact of environmental changes on ecosystems. By understanding microbial niches, we can better interpret data from microbial community analysis and understand why certain species are found in an environment.

Hutchinsonian multidimensional niche concept allows us to view niches as multidimensional spaces and better understand how microbes interact with their environment, how multiple microbial species can coexist, and how environmental factors shape the composition and function of microbial communities. This concept is very important in microbial ecology research, biotechnology, and understanding the role of microbes in maintaining ecosystem balance, including pollution control through "microbial niche tuning." "Tuning" here refers to the ability to modify or direct a specific microbial niche by changing environmental conditions or introducing certain microbes that can modify other microbial niches that are already present. Microbial niche tuning is a promising strategy to address nitrate pollution by harnessing microbial capabilities. A deep understanding of microbial ecology and environmental conditions is essential to implement microbial niche tuning.

8.2 Microbial niche tuning for nitrate pollution control

Various microbes play a role in microbial niche tuning for nitrate pollution control in water and soil. These microbes work through various metabolic pathways, mainly denitrification and nitrate assimilation. Here are some of the important microbial species and their roles:

1. Denitrifying bacteria are the most important group of microbes in nitrate pollution control. They perform the denitrification process, which converts nitrate (NO_3^-) into harmless nitrogen gas (N_2) to be released into the atmosphere. Some well-known denitrifying bacteria are the genus *Pseudomonas*, genus *Bacillus*, *Paracoccus denitrificans*, some *Achromobacter*, and some *Thiobacillus*.
2. Anaerobic ammonium oxidation (anammox) bacteria convert ammonium (NH_4^+) and nitrite (NO_2^-) into nitrogen gas (N_2). This process is more efficient and energy-efficient than conventional denitrification and is important in wastewater treatment. Some examples of this type of bacteria are from genera *Brocadia, Kuenenia, Scalindua*, and *Jettenia*.

The biological removal of nitrogen in wastewater generally occurs in two stages: nitrification and denitrification. These processes are facilitated by microorganisms, specifically ammonium oxidizing bacteria (AOB) for nitrification and nitrite oxidizing bacteria (NOB) for denitrification. The anammox process within the natural nitrogen cycle is illustrated in Fig. 8.1 (Wijaya & Putra, 2021).

In ammonium removal through nitrification, two groups of bacteria play a crucial role: AOB and NOB. AOB are bacteria capable of oxidizing ammonium into nitrite, while NOB oxidize nitrite into nitrate. In the anammox process, NOB act as inhibitors of anammox bacteria by competing for nitrite (Miao et al., 2017; Wang et al., 2015).

Anaerobic ammonium oxidation (anammox) is a nitrogen removal process that utilizes nitrite as an electron acceptor under anaerobic conditions. The anammox process consists of two stages: partial nitrification and anaerobic oxidation of ammonium

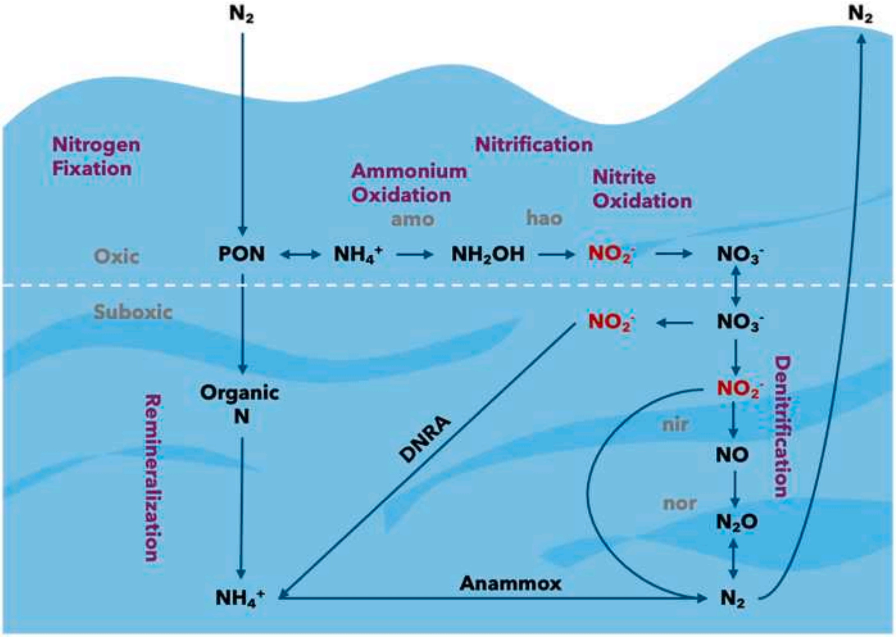

Figure 8.1 The anammox process in the nitrogen cycle in water.

and nitrite. This process is carried out by autotrophic bacteria, resulting in a very low organic carbon requirement. Anammox operates optimally at moderate temperatures (30°C–35°C) and under high ammonium concentrations. It is considered a promising alternative for nitrogen removal in wastewater treatment and has been widely implemented in developed countries for treating nitrogen-rich wastewater (Miao et al., 2017; Niu et al., 2016).

According to the reaction scheme of the anammox process within bacterial cells (Fig. 8.2), two distinct mechanisms govern the anammox reaction. In Scheme A, ammonium and hydroxylamine are converted into hydrazine by enzyme complexes. In Scheme B, ammonium and hydroxylamine undergo the same transformation, but hydrazine is further oxidized within the periplasm to produce nitrogen gas (Jetten et al., 1998).

The anammox process offers several advantages for biological nitrogen removal, including low oxygen demand, reduced operational costs, and no requirement for additional carbon sources (Hauck et al., 2016). The implementation of anammox technology can reduce aeration consumption by up to 64%, carbon source requirements by 100%, and sludge production by up to 90%.

1. The first step in denitrification in the nitrogen cycle is the reduction of nitrate into nitrite by bacteria, such as genera *Escherichia* and *Klebsiella*.

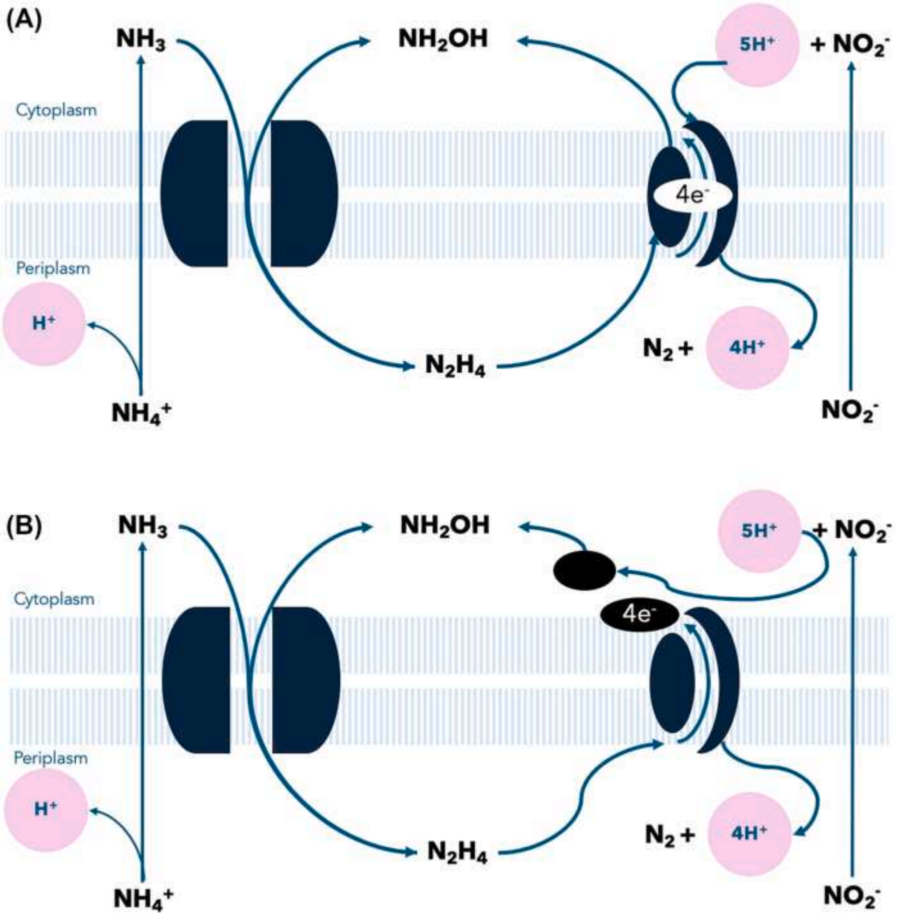

Figure 8.2 Anammox process mechanism inside bacterial cells. (A) shows the movement of NH4 plus from the cytoplasm to NH_3, which then converts to NH_2OH and N_2H_4. An electron transfer of 4e minus occurs, leading to the formation of N_2 and the release of 4H plus and NO_2 minus in the periplasm. Additionally, 5H plus is shown in the periplasm. (B) follows a similar process, with NH4 plus converting to NH_3, NH_2OH and N_2H_4. The electron transfer of 4e minus is depicted, resulting in N_2 formation and the release of 4H plus and NO_2^- in the periplasm. In this diagram, 5H plus is also shown in the periplasm.

2. Nitrate-assimilating bacteria use nitrate for growth and biomass formation and hel reduce nitrate in the environment.
3. Heterotrophic microbes, such as some types of bacteria and fungi, degrade organic matter and release carbon needed by denitrifying bacteria as an energy source. Various bacteria and fungi decompose organic matter, for example, *Cellulomonas*, *Flavobacterium*, *Aspergillus*, and *Penicillium*.

4. Although nitrification is not a nitrate reduction process, understanding nitrifying bacteria is still important because nitrifying bacteria affect nitrate availability in the environment. In some cases, niche tuning may focus on controlling nitrification to prevent nitrate accumulation.

- AOB include *Nitrosomonas*, *Nitrosospira*, and *Nitrosococcus*.
- NOB include *Nitrobacter* and *Nitrospira*.

Denitrifying bacteria have unique ecological niches characterized by several important environmental factors that optimize the denitrification process in microbial niche tuning. Some of the main environmental factors that affect the activity of denitrifying bacteria are are listed as follows:

1. Nitrate (NO_3^-) is the main substrate for denitrification. Sufficient nitrate concentration is required for the optimal denitrification process. An overly high nitrate concentration can inhibit the activity of denitrifying bacteria by causing toxic effects. Some denitrifying bacteria can also use nitrite (NO_2^-) as a substrate. The availability of the correct form of nitrogen will affect the rate and efficiency of denitrification.
2. Carbon is an electron donor and energy source for heterotrophic denitrifying bacteria. The type of carbon source available can affect the rate and efficiency of denitrification. Easily accessible carbon sources (e.g., glucose, acetate, etc.) are usually preferred over complex organic compounds. A proper carbon-to-nitrogen ratio is vital for denitrification.
3. Oxygen (O_2) is an electron acceptor. Low redox potential (reductive conditions) is required for denitrification. An increase in redox potential (oxidative conditions) will inhibit denitrification. Anaerobic conditions often occur in deeper soil layers, within soil aggregates, or in waterlogged areas. Some denitrifying bacteria are microaerophilic, which means they can denitrify at very low oxygen concentrations (Susana, 2016).
4. Each type of denitrifying bacteria has an optimal pH range for its growth and denitrification activity. Extreme changes in pH can denature enzymes and inhibit microbial metabolism.
5. The optimal temperature range that supports the growth and activity of denitrifying bacteria is required. Temperatures that are too low or too high can inhibit growth and denitrification activity. Sudden temperature changes can also affect the activity of denitrifying bacteria.
6. Balanced nutrient availability is essential. Lack of one nutrient can limit the growth and activity of denitrifying bacteria.
7. Presence of inhibitory substances such as heavy metals and toxic compounds (pesticides, herbicides, and antibiotics) affect the activity of denitrifying bacteria.

8. High salt concentrations can inhibit the growth and activity of denitrifying bacteria. Extreme changes in osmotic pressure can cause dehydration or lysis of bacterial cells, which inhibits denitrification.
9. Competition with other microbes for nutrients and space can affect the activity of denitrifying bacteria. Some microbes can aid the activity of denitrifying bacteria by providing carbon sources or other nutrients. Bacteriophages or protozoa can prey on denitrifying bacteria and reduce their population.

Controlling the aforementioned environmental factors is critical in designing an effective microbial niche tuning strategy. By manipulating or engineering the environmental factors

1. denitrification activity can be enhanced by optimizing environmental conditions for the growth and activity of denitrifying bacteria;
2. the denitrification process can be accelerated by providing an easily accessible carbon source and sufficient nutrients to accelerate the denitrification rate;
3. environmental conditions that can inhibit the activity of denitrifying bacteria can be avoided and
4. microbes, especially denitrifying bacteria, that are resistant to specific environmental conditions can be determined.

Changing environmental factors in microbial niche tuning can change microbial niches by the following explanation.

1. Temperature changes: The fundamental niches of microbes provide an optimal temperature range to grow and reproduce efficiently. Changes in global or localized temperature can alter microbial niches where species that are more adaptive to the new temperature will dominate. For instance, microbes tolerant to high temperatures (thermophiles) may expand their niches and microbes tolerant to low temperatures (psychrophiles) may experience niche shrinkage or local extinction. Changes in microbial communities can alter ecosystem function, for example, in organic matter decomposition or nutrient cycling.
2. Changes in pH: Microbes have particular pH preferences to function optimally. Environmental changes in the form of increased acidity (e.g., due to acid rain) or increased alkalinity (e.g., industrial pollution) can alter the niches by altering nutrient availability in the environment, affecting microbial growth. Microbial enzyme activity is susceptible to pH, where pH changes can inhibit or enhance microbial metabolism. For example, increased soil acidity may inhibit the growth of nitrogen-fixing bacteria and favor the growth of fungi.
3. Changes in oxygen availability: Microbes have different oxygen requirements, that is, aerobic (requires oxygen), anaerobic (does not require oxygen), or facultative anaerobic (can grow with or without oxygen). Changes in oxygen availability cause

niche shifts. Microbes can switch from aerobic to anaerobic metabolism, altering metabolic products and ecosystem function.

4. Changes in nutrient availability: Microbial fundamental niches are indicated by specific nutrient requirements, such as carbon, nitrogen, phosphorus, and other sources. Environmental changes due to increased fertilizers in waters (eutrophication) or organic matter pollution can increase nutrient availability, while deforestation or erosion can reduce nutrient availability in soil. Changes in nutrient availability lead to changes in competition, that is, microbes that are most efficient at utilizing available nutrients will have a competitive advantage, changing community composition. Microbes that were previously restricted due to nutrient deprivation may expand their niches. Increased nutrients can spur unbalanced microbial growth, causing population explosions of certain algae or bacteria. For example, eutrophication in lakes can trigger a population explosion of Cyanobacteria, forming a film on the water surface and causing ecosystem disruption.
5. Changes in water availability: Microbes require water for metabolic activities; some are more drought-tolerant than others. Changes in rainfall patterns, droughts, or floods can alter water availability. As a result, a niche shift occurs, where drought-tolerant microbes (xerophiles) will thrive in dry conditions while water-demanding microbes will be suppressed. For example, soil drought inhibits bacterial growth and increases populations of more drought-tolerant fungi.

Changes in environmental factors can significantly alter microbial niches by affecting resource availability, physical conditions, and competitive interactions. These changes can result in niche shifts, changes in microbial community composition, and changes in ecosystem function. Understanding how environmental factors affect microbial niches is critical to understanding microbial ecology and its environmental impact. Microbial niche tuning is sustainable, as it utilizes natural processes carried out by soil microbes.

Understanding microbial niches is not easy and is an interesting challenge. First, studying microbial ecology and its role in various ecosystem processes and biotechnology is essential. Overcoming these challenges requires combining innovative analytical techniques, sophisticated modeling approaches, and a deeper understanding of the interactions between microbes and their environments. Some of the major challenges facing scientists in microbial niche research can be described as follows:

1. Microbes are very small, and microbial environments are often very heterogeneous at the microscopic scale. It is difficult to map detailed environmental conditions at scales relevant to microbes. In one seemingly uniform environment, there may be many microhabitats with very different conditions (e.g., differences in nutrient concentration, pH, and oxygen) that are difficult to measure and analyze (Tribedi et al., 2018).

2. Microbes interact with various other species in complex communities. Mapping the network of microbial interactions within a community is very difficult due to the large number of species and dynamic interactions that may occur.
3. Most microbes cannot be cultured in the laboratory, making it difficult to study their properties and niches directly. Although metagenomics can provide information about the genes in a microbial community, linking these genes to specific microbial functions and niches is difficult.
4. Environmental conditions can change over time, affecting microbial niches. Sampling at a particular time may not reflect the overall condition of the microbial niche due to temporal variability.
5. Microbial niches are multidimensional, with many factors influencing their presence and function. Niche modeling requires accurate and comprehensive data, often difficult to obtain from the microbial environment.
6. Microbial niche analysis results are often very complex and difficult to interpret. Distinguishing correlation and causality in data analysis is complicated, making it difficult to determine what factors affect microbial niches. Some microbes may have the same function (functional redundancy), making it challenging to identify the specific role of each species in an ecosystem.
7. Manipulation or tuning microbes in the environment can raise ethical and sustainability questions, as changes to microbial niches can have long-term impacts on ecosystems.

8.3 Tracking nitrate pollution by microbes

Nitrate is one of the most common forms of nitrogen that pollute water. The pollutant source can be agricultural fertilizers or domestic and industrial waste (Hakiki et al., 2019; Wikaningrum et al., 2009). Some microbes living in a specific niche can convert nitrate into less harmful forms of nitrogen (e.g., nitrogen gas) through denitrification. Understanding the niche of denitrifying bacteria is essential for developing effective strategies to address nitrogen pollution in soil. This concept can be applied through microbial niche tuning, which manipulates (engineers) environmental conditions to optimize the activity of denitrifying bacteria. Some of the strategies that can be applied include

1. creating optimal conditions for growth and activity of denitrifying microbes (e.g., anoxic conditions, organic carbon availability);
2. biostimulation by adding nutrients or other materials triggers existing denitrifying microbes' growth and activity; and
3. bioaugmentation by introducing denitrifying microbes into the polluted environment.

Biostimulation and bioaugmentation are two strategies in microbial niche tuning that aim to increase microbial activity in an environment. The main difference lies in their approach and mechanism of action:

8.3.1 Biostimulation

Biostimulation is modifying the environment to stimulate the growth and activity of indigenous microbes that already exist in the environment. Biostimulation works by increasing the availability of nutrients, oxygen, or other environmental factors that limit the growth and activity of native microbes by adding specific substrates, adjusting pH, or modifying other environmental conditions (Kanissery & Sims, 2011). Biostimulation aims to accelerate the degradation of pollutants or other desired biological processes by optimizing the performance of microbes already present at the site. For example, by

1. adding nitrogen compounds to the soil to stimulate the growth of native denitrifying bacteria;
2. adding an easily accessible carbon source to polluted water to increase the activity of pollutant-degrading microbes (Habibah et al., 2020); and
3. reducing soil aeration by minimizing tillage or good drainage arrangements to create anaerobic conditions that favor denitrification.

Biostimulation is more environmentally friendly and cheaper because it does not require isolation and culture of microbes. It can exploit the ability of native microbes that are adapted to local environmental conditions (Herrero & Stuckey, 2015). However, the effectiveness of biostimulation depends on the presence of suitable native microbes in the environment. It is less effective if the native microbes cannot degrade pollutants or perform the desired process. Therefore, a good understanding of environmental conditions and the needs of native microbes is required.

8.3.2 Bioaugmentation

Bioaugmentation introduces new microbes with specialized capabilities into a polluted environment to improve their ability to degrade pollutants or perform other desired biological processes. In general, the bioaugmentation process can be seen in Fig. 8.3.

Introduced microbes were selected or genetically engineered and are expected to perform biological processes more efficiently than the native microbes. The ideal microbial criteria for bioaugmentation are listed here:

1. High denitrification or anammox capability, that is, the microbes should also be able to reduce nitrate efficiently without producing harmful by-products (such as nitrite or nitric oxide). Anammox–anaerobic ammonium oxidation bacteria that convert ammonium and nitrite into nitrogen gas are preferable due to their efficiency and energy-saving options.

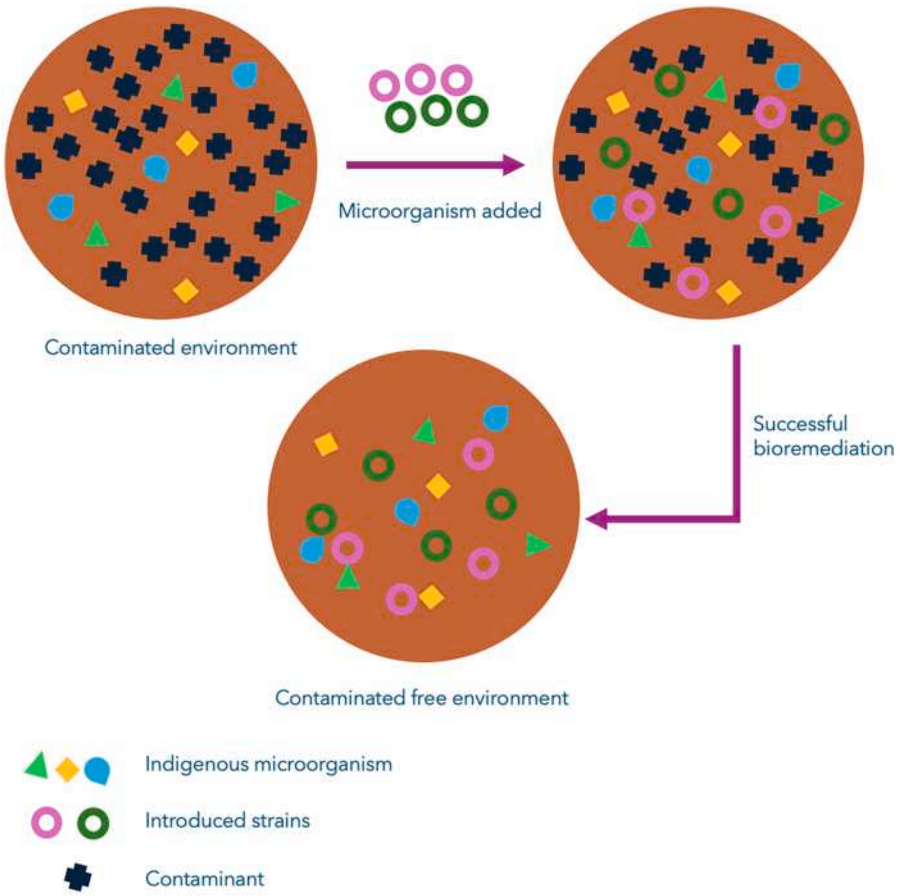

Figure 8.3 The bioaugmentation process.

2. Tolerant to environmental conditions, that is, the microbes must be able to adapt and remain active in the target environment. The microbes must be resistant to temperature fluctuations, pH, salinity, or inhibitory substances in the target environment (Karima et al., 2018).
3. Excellent growth rate, that is, the selected microbes should not require complicated and difficult-to-obtain nutrients in the target environment.
4. Nonpathogenic and harmless to humans, animals, and the environment, that is, the microbes do not produce harmful or toxic by-products during denitrification, are noninvasive, and do not disturb the ecosystem balance of the target environment.
5. The microbes are easily cultured on a large scale for mass production purposes and are stable and active in bioaugmentation products (e.g., liquid, solid, or granule). The microbial production process should be efficient and affordable.

6. The added microbes should not be overly competitive with the native microbes in the target environment. Ideally, the added microbes can positively interact with the native microbes to improve denitrification efficiency.

Bioaugmentation aims to introduce improved exogenous microbial capabilities into a polluted environment, especially if the native microbes lack the ability to perform the desired process or are not efficient enough. For example, by

- adding genetically engineered *Pseudomonas* to degrade persistent organic compounds in soil,
- adding specific denitrifying bacteria to wastewater to accelerate nitrate removal, and
- adding *Trichoderma* fungi to the soil to control plant diseases.

Several issues related to the real-scale application of bioaugmentation for the treatment of heavy metal-contaminated soil are summarized in Fig. 8.4, and the differences between biostimulation and bioaugmentation are presented in Table 8.2.

In practice, these two approaches can be combined to achieve optimal results to ensure that the exogenous microbes can survive and perform well in the new environment. The selection of the best method will depend on the specific conditions of the site and the objectives. Biostimulation is more suitable if the environment already has indigenous

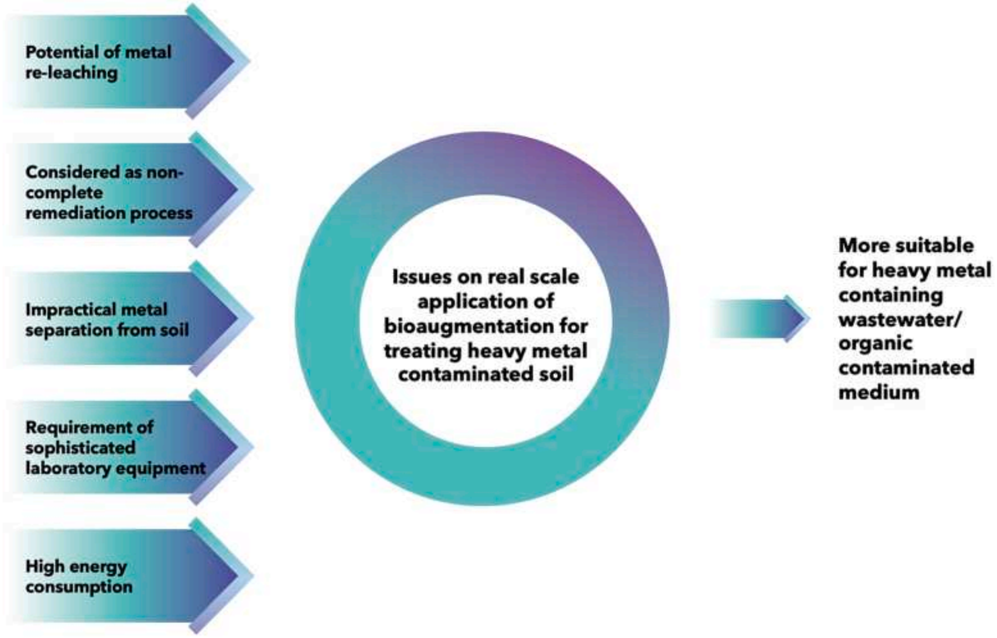

Figure 8.4 Challenges in bioaugmentation for real-scale applications.

Table 8.2 Key differences between biostimulation and bioaugmentation.

Definition	**Environment modification to stimulate the growth of indigenous microbes**	**Addition of exogenous microbes with specialized capabilities into the environment**
Objective	Increasing the activity of indigenous microbes in carrying out biological processes	Introducing new capabilities of exogenous microbes that are not present in indigenous microbes into a polluted environment
Mechanism	Optimizing environmental conditions to stimulate better growth of indigenous microbes	Adding exogenous microbes into the polluted environment
Main actor	Indigenous microbes	Exogenous microbes
Cost	Relatively cheaper	Relatively more expensive
Risk	More environmentally friendly	Potential risk of introducing exogenous microbes that are foreign, potentially causing changes in the food chain and ecosystem imbalances
Effectivity	Depends on the internal (physiological) ability of indigenous microbes to changes in stimulated environmental conditions	Can increase the efficiency of biological processes but depends on the ability of exogenous bacteria to adapt to the new environment

microbes that can perform the desired biological process, but environmental conditions limit their activity. In contrast, bioaugmentation is more suitable if the environment does not have indigenous microbes capable of performing the desired biological process, or if a significant increase in the efficiency of the biological process is required.

8.4 Microbial niche tuning engineering strategies

Several strategies can be used to engineer microbial niche tuning, particularly in the context of nitrate pollution control. The selection of the appropriate method will largely depend on the specific conditions of the environment, the type of target microbes, and the type of pollutant to be removed. The strategies can be outlined as follows:

1. Environmental engineering
   a. Oxygen is regulated by creating anoxic/anaerobic conditions (controlling water flow, adding oxygen-binding agents, or using specially designed bioreactors) and creating oxic and anoxic zones (i.e., setting up zones with different oxygen levels to facilitate various microbial processes).

   - **b.** pH is regulated by adding chemicals (e.g., lime to increase pH) to support the growth of desirable microbes and inhibit unwanted microbes.
   - **c.** Temperature is regulated by using heating or cooling systems, especially in bioreactors.
   - **d.** Nutrient availability is ensured by adding carbon sources (e.g., molasses, acetate, or other organic materials) to increase denitrification activity. Adding necessary nutrients (biostimulation) in the right amount can enhance the target microbes' growth.

2. Biostimulation
   - **a.** Specific nutrients required by the target microbes are provided.
   - **b.** Complex organic matter that can be broken down by microbes are added, which will then release the nutrients needed by the target microbes.
   - **c.** Enzymes that can accelerate certain biochemical reactions are added.
3. Bioaugmentation
   - **a.** The desired microbes are introduced into the polluted environment. These microbes can come from pure cultures or microbial consortia developed in the laboratory.
   - **b.** Genetically engineered microbes are introduced to degrade pollutants or convert compounds into harmless forms.
   - **c.** A combination of several microbes is introduced that interact and work together to degrade pollutants.
4. Environmental matrix modification
   - **a.** Support materials (e.g., zeolites, ceramics, or biomass) are used to provide growth sites for microbes and sinks for nutrients or pollutants.
   - **b.** The soil structure is changed by aeration or adding organic matter to create better conditions for microbial growth.
   - **c.** A combination of methods, such as some of the above methods, is used to achieve more effective results; for example, combining biostimulation with bioaugmentation, modifying environmental conditions, and adding supporting materials.

The selection of appropriate methods for microbial niche tuning requires an in-depth understanding of the objectives, environmental conditions, target microbes, and available methods. Niche tuning strategies should be tailored to the specific environmental conditions at each site, as the niches of denitrifying bacteria can vary from site to site. The following are the steps and considerations that need to be taken into account in method selection.

1. **Determining objectives**
   - **a.** The problem to be addressed are defined to determine the type of niche modification required. For example, is the primary objective to reduce nitrate pollution, degrade other pollutants, improve soil fertility, or something else?

   - **b.** The specific changes desired in the microbial community are determined. Is it to increase the population of certain microbes, inhibit the growth of others, or to change the microbes' metabolic activity?
   - **c.** The scale of implementation is determined, since it will affect the method chosen and the resources required. Will niche modification be done at laboratory, pilot, or field scales?

2. **Characterizing the environmental condition**
   - **a.** The environmental chemical and physical conditions, such as pH, temperature, oxygen content, nutrient availability, and types of pollutants, are analyzed to select a suitable method for the existing environmental conditions.
   - **b.** The microbial community (e.g., metagenomic or metatranscriptomic) is analyzed to determine the composition and activity of the native microbial community to understand the microbial interactions and predict the impact of niche modification.
   - **c.** Environmental variability, such as seasonality, rainfall, and temperature changes, is considered to select a flexible method for adapting to environmental changes.
3. **Considering the target microbe**
   - **a.** The target microbes are determined to select the appropriate method. It is necessary to examine the interactions between microbes (competition, mutualism) to optimize niche tuning.
   - **b.** The needs of the target microbes, such as nutritional needs, optimal environmental conditions, and the interaction of the target microbes with other microbes, are observed so that the methods selected meet their needs.
   - **c.** The adaptability of microbes to environmental changes is studied to choose the method that can promote the growth and activity of target microbes in the long term.
4. **Niche engineering method**
   - **a.** Engineering environmental conditions, such as
     - **i.** adjusting pH to suit the soil or water pH of the target microbes (Rousk et al., 2010),
     - **ii.** adjusting the environmental temperature to increase the activity of the target microbes (Allison & Martiny, 2008),
     - **iii.** increasing or reducing the oxygen availability according to the needs of the target microbes,
     - **iv.** adding specific nutrients to promote the growth of the target microbes, and
     - **v.** adding carbon sources that are easily accessible to denitrifying microbes.
   - **b.** Bioaugmentation
   - **c.** Biostimulation
   - **d.** Constructing genetically engineered microbes with enhanced ability in biological processes

   - **e.** Using nanomaterials to deliver nutrients or target microbes to desired locations, adding organic matter to improve soil structure and nutrient availability, and adding chemicals to engineer environmental conditions or microbial activity.

5. **Practical considerations**
   - **a.** the most cost-effective and efficient method,
   - **b.** the availability of necessary resources,
   - **c.** easy to implement and maintain; technical capabilities of the personnel,
   - **d.** resource sustainability, no secondary pollution, and negative impacts on the environment, and
   - **e.** safety for human health and the environment and potential risks associated with the use of microbes or chemicals.

By considering all these factors and conducting careful testing and evaluation, the most effective and efficient method can be selected to achieve the goal of microbial niche tuning.

8.5 Monitoring and evaluation

Continuous supervision and monitoring to ensure the effectiveness of the chosen method is crucial to ensure an effective denitrification process. Monitoring can be done by

1. conducting small-scale trials to evaluate the effectiveness and impact of the selected method and monitoring changes in the microbial community and environmental parameters (Hallin et al., 2018),
2. evaluating the method's effectiveness and using monitoring data to assess performance, and
3. adapt or adjust the method based on the evaluation results.

There are various technologies for in-field monitoring of microbial niche tuning effectivity. The selection of the appropriate technology will depend on the scale of the project, the budget, and the type of data to be collected. Some commonly used technologies are as follows.

1. Water/soil quality sampling and analysis
   - **a.** Water or soil sampler tools are automated at regular intervals to reduce human error and increase sampling frequency. Location-based sampling uses a global positioning system to ensure samples are taken from the exact location each time.
   - **b.** Chemical analysis is performed using a spectrophotometer to measure nitrate, nitrite, ammonia, and other chemical parameters in water or soil samples, to separate and measure the concentration of specific ions in water or soil samples

  with ion chromatography, and use portable chemical sensors to measure chemical parameters in real-time.
  - **c.** Physical analysis is performed using sensors to measure physical parameters of water or soil in real-time.
2. Molecular microbiology
   - **a.** High-throughput DNA sequencing is used to identify and characterize the entire microbial community. Functional analysis is performed to identify genes associated with denitrification or other biological processes.
   - **b.** High-throughput RNA sequencing is used to measure microbial gene activity. Gene expression analysis is used to monitor changes in microbial gene expression in response to environmental changes.
   - **c.** Quantitative polymerase chain reaction is used to measure the abundance of target-specific microbes and specific genes related to denitrification.
   - **d.** Denaturing gradient gel electrophoresis is performed to analyze the diversity of microbial communities.
3. Remote sensing
   - **a.** Multispectral/hyperspectral images are taken using satellites or drones equipped with multispectral or hyperspectral sensors to monitor environmental conditions, such as vegetation, soil moisture, and water quality. Vegetation index analysis is used to monitor plant health and nutrient availability. Remote sensing data can detect environmental changes related to the effectiveness of microbial niche tuning.
4. Microbial sensor or biosensor
   - **a.** Microbial sensors are used to detect the presence of nitrate or other compounds in the environment.
   - **b.** Enzyme-based sensors are used to detect nitrate or other compounds, and antibody-based sensors are used for target microbe detection.
5. **Data and modeling**
   - **a.** Geographic information system maps the distribution of microbes and environmental parameters in the field.
   - **b.** Mathematical modeling is used to predict how microbial niche tuning will affect microbial communities and nitrate removal effectiveness.
   - **c.** Machine learning automatically analyzes metagenomic, metatranscriptomic, and other sensor data.
6. **Important things that need to be considered**
   - **a.** Monitoring should be conducted periodically and regularly to identify changes in the microbial community and the effectiveness of niche tuning.
   - **b.** Parameters selected should be relevant and sensitive to changes in microbial communities and the environment.

- **c.** Integrating data from multiple sources provides a more comprehensive understanding of niche tuning effectiveness.
- **d.** Data validation is performed to ensure accuracy and reliability.

Combining these monitoring technologies can increase the effectiveness of microbial niche tuning to optimize strategies and achieve sustainable environmental management goals.

8.6 Challenges of microbial niche tuning implementation

Some of the key challenges in the field application of microbial niche tuning to address nitrate pollution can be explained as follows.

1. Microbial ecosystem complexity
   - Microbial ecosystems in nature are very complex and diverse, making it difficult to predict how changes in environmental conditions will affect interactions between microbes. Changing one aspect of the environment can trigger unexpected changes in microbial populations. Microbes can adapt quickly to environmental changes, meaning niche tuning that works initially may no longer be effective in the long run.
2. Environmental variability.
   - Environmental conditions can change significantly over time, affecting the effectiveness of niche tuning. Niche tuning needs to be customized for each site and adjusted according to seasonal changes (Eronen-Rasimus et al., 2017; Van Colen et al., 2014).
3. Technical and operational challenges.
   - Implementing niche tuning at the field scale can be very difficult and expensive. Intensive and continuous monitoring requires relatively expensive analytical costs. Setting consistent environmental conditions to maintain optimal conditions for target microbes is often tricky and requires sophisticated technology. Sustainable bioaugmentation by introducing exogenous microbes is not always successful. The long-term availability of sufficient carbon sources for denitrifying microbes is also challenging, especially in carbon-poor environments.
4. Regulatory and social challenges
   - Regulations on microbial niche tuning are not entirely clear, especially regarding genetically engineered microbes. Acceptance of microbial niche tuning-based technologies by farmers or industries is also a challenge, especially if significant changes in their usual practices are required.
5. Economic challenges
   - The start-up and operational costs of implementing microbial niche tuning are potentially very high, especially if sophisticated infrastructure is required. It is

   necessary to ensure that technologies that work on a small scale can be applied successfully on a larger scale at an affordable cost.

6. Research and development challenges
   - Research and development on microbial niche tuning requires a deeper understanding of microbial interactions in complex environments. A more effective, efficient, and affordable technology is needed to overcome the challenges of microbial niche tuning implementation.

Despite the challenges described above, microbial niche tuning remains a promising approach to address nitrate pollution. With continued research and development, many of these challenges can be overcome to achieve a more robust and reliable tool for environmental management. The complexity of microbial ecosystems in applying microbial niche tuning is a significant challenge. Here are some strategies and approaches to overcome the challenge of microbial ecosystem complexity.

1. Data-driven approach and in-depth analysis
   - **a.** Microbial community analysis using metagenomic (DNA analysis) and metatranscriptomic (RNA analysis) techniques are used to identify and characterize the entire microbial community present in the environment.
   - **b.** Bioinformatics is used to model interactions between microbes and predict how environmental changes will affect microbial communities.
   - **c.** Stable isotope analysis is performed to track nutrient and energy flows in microbial ecosystems to help understand the role of different microbes in biogeochemical cycles.
   - **d.** Database development stores information on microbes, metabolic pathways, and their interactions.
2. Microbial ecology approach
   - **a.** Understanding niche ecology is needed to determine the optimal environmental conditions for the growth and activity of target microbes.
   - **b.** Microbial consortia is used to degrade pollutants or perform other desired biological processes for more stable and efficient results than pure microbial cultures.
   - **c.** Microbial ecology/environmental engineering promotes the growth of desirable microbes and inhibits the growth of undesirable microbes. It is equally important to bioaugment or introduce exogenous microbes that play an important role in the desired biological process, such as denitrifying microbes.
3. Adaptive and flexible approach
   - **a.** Continuous monitoring of microbial communities is required to identify changes and effectiveness and adapt microbial niche tuning strategies.
   - **b.** Adaptive and flexible strategies are used to adjust to changing environmental conditions and microbial communities. Errors and failures can be the basis for continuously improving the microbial niche tuning strategy.

   - **c.** Predictive models can be used to predict how environmental changes will affect microbial communities and niche tuning effectiveness.
4. Technology and innovation approach
   - **a.** Microbial genetic engineering aims to obtain superior microbes with better capabilities in degrading pollutants or performing other desired biological processes.
   - **b.** Nanomaterials can be used to deliver nutrients or target microbes to the desired location. Microbial sensors can potentially used to monitor environmental conditions and microbial activity.
   - **c.** System automation for microbial niche tuning and monitoring microbial communities. Data processing using machine learning is very helpful for automatically processing metagenomic and metatranscriptomic data.

In addition to the aforementioned strategies, mathematical modeling can simplify the complexity of microbial systems, make predictions, test hypotheses, and optimize biotechnological processes. Mathematical modeling helps us to understand how microbes interact with their environment and how we can manipulate microbial systems to our advantage. Here are some of the ways mathematical modeling can help understand microbial niches.

1. Hutchinson niches are multidimensional, which means they are affected by many environmental factors. Mathematical modeling can include these multifactors in equations and graphs, so microbial niches can be visualized and analyzed in a multidimensional space. Mathematical models can help to understand how microbes respond to changing environmental conditions.
2. Mathematical modeling can model microbial population growth based on specific environmental conditions. These models can help us predict how microbial populations will change over time.
3. Mathematical modeling can help explain symbiotic interactions between microbes and predict how these interactions affect each species' niche by developing a complex web of interactions. Models can incorporate positive and negative feedback effects between microbes and the environment, affecting microbial niche dynamics (Coyte et al., 2015; Goldford et al., 2018).
4. Mathematical modeling allows hypothesis testing on how environmental factors affect microbial niches. Experimenting "in silico" (on a computer) can be done to test scenarios that are difficult or impossible to do in the laboratory and to identify key factors affecting microbial niches.
5. Mathematical models can predict how climate change affects microbial niches. Models can be used to predict how pollution will affect the composition and function of microbial communities. Modeling can help us predict the impact of biotechnological interventions on microbial niches.

6. Mathematical models can integrate data from multiple sources (e.g., genomics, transcriptomics, metabolomics) to provide a more comprehensive picture of microbial niches. Modeling can help us conduct sensitivity analysis to identify the factors most affecting the model results.

   Several types of mathematical models used are

1. population growth models, such as logistic models and Lotka–Volterra models, to describe the growth and interaction of microbial populations,
2. reaction–diffusion models to describe the distribution of nutrients and metabolites in the microbial environment,
3. agent-based models to simulate the behavior of individual microbes and how this behavior affects population dynamics, and
4. network models to visualize the complex web of interactions between different microbial species.

Many things must be considered when implementing microbial niche tuning in addressing nitrogen pollution; therefore, various data will be needed to create a mathematical model. The following are the main types of data needed:

1. Environmental data include physicochemical parameters. Nutrient availability includes concentrations of organic carbon, nitrogen compounds, phosphorus, and other nutrients important for microbes. In addition, data on dissolved oxygen concentration, soil moisture, water activity (aw), and light intensity and spectrum are needed. Data on the concentration of toxic compounds or inhibitors that can inhibit microbial growth, such as antibiotics, heavy metals, or organic pollutants, are also needed.
2. Microbiological data
   - **a.** Microbial community composition:
     - **i.** DNA/RNA sequence data: This data can be in 16 S rRNA (for bacteria and archaea) or ITS (for fungi).
     - **ii.** Metagenomic data from DNA sequencing provide information on microbial communities' genetic potential and function.
     - **iii.** Metatranscriptomic data from RNA sequencing provide information on gene expression and metabolic activities of microbes.
     - **iv.** Metabolomics data of metabolite profiling provide information on microbial metabolic products.
   - **b.** Physiological characteristics of microbes:
     - **i.** Microbial growth rate under different environmental conditions.
     - **ii.** Microbial nutrient rate consumption.
     - **iii.** Microbial metabolite production rate.

        - **iv.** Environmental conditions tolerated by microbes.
        - **v.** Nutrient type and concentration needed by microbes to grow.
- **3.** Microbial interaction data:
    - **a.** Symbiosis data that provides information on symbiotic interactions between different microbial species.
    - **b.** Competition data from microbes competing for the same resources.
    - **c.** Predation data between microbes.
    - **d.** Interaction networks between different microbial species in a community.
- **4.** Spatial and temporal data:
    - **a.** Spatial data, that is, the geographical location of the sample, and the spatial structure of the microbial environment (e.g., nutrient concentration gradient in soil or biofilm).
    - **b.** Temporal data, that is, changes in environmental conditions and microbial community composition over time.
- **5.** Additional data:
    - **a.** Genomic data on the DNA sequence and genetic potential of the microbes involved.
    - **b.** Proteomic data on the proteins expressed by the microbes.
    - **c.** Phenotypic data on the physical and physiological characteristics of the microbes.

Data quality is critical to creating an accurate model. Data should be collected by appropriate methods and analyzed carefully. Data from various sources should be integrated to provide a comprehensive picture of the microbial niche. The data type required will vary depending on the type of microbial niche being modeled and the research questions. Validation of a mathematical model is also important to ensure its accuracy, reliability, and meaningful predictions.

8.7 Microbial niche tuning case studies

Some examples of the application of microbial niche tuning to address nitrate pollution, along with a brief explanation of the approach used, are as follows.

8.7.1 Case study on constructed wetlands

Constructed wetlands are designed with different zones: aerobic (oxygenated) zones and anaerobic (no oxygen) zones. The aerobic zone supports nitrification, while the anaerobic zone supports denitrification. Organic matter such as sawdust or straw is added to the anaerobic zone to provide a carbon source for denitrifying microbes (Hang et al., 2016; Wang et al., 2016). Water flow is regulated in a way that allows water to pass through the zones sequentially, maximizing the nitrate removal process. Studies show that artificial wetlands designed with the principle of microbial niche tuning can significantly reduce nitrate concentration in water. The nitrate removal efficiency can reach more than 80% in some cases (Jingyu et al., 2020; Liu et al., 2024; Tazkiaturrizki, 2016).

The microbial niche tuning approach in this case involves manipulating the artificial wetland environment to optimize the activity of denitrifying microbes. Denitrification is an anaerobic process, so the wetland environment must be designed to have sufficient anaerobic zones. This can be achieved by regulating water depth and flow, as well as the use of media that has the ability to retain water. Denitrifying microbes require organic carbon as an energy source. The addition of organic materials such as straw, sawdust, or other organic materials can increase denitrification activity.

Several studies in Europe, North America, and Indonesia have shown the success of artificial wetlands in addressing nitrate pollution from agricultural runoff and domestic wastewater. Artificial wetlands can also provide other benefits such as increased biodiversity, flood control, and environmental education. According to Kesarwani et al. (2023), constructed wetland microbial fuel cells (CW-MFCs) are an innovative alternative technology that integrates microbial fuel cells within constructed wetlands. Plant roots release oxygen and rhizodeposits, which aerate the rhizosphere and enhance microbial activity. Jingyu et al. (2020) said that AOB and NOB play a crucial role in nitrogen transformation, converting NH_4^+ (ammonium) into NO_2^- (nitrite) and NO_2^- into NO_3^- (nitrate). In this system, nitrite (NO_2^-) or oxygen (O_2) acts as the final electron acceptor at the cathode (Fig. 8.5). The key reactions occurring in CW-MFCs are illustrated in Fig. 8.6.

Wastewater treatment in constructed wetlands occurs through various physico-chemical and biological processes, including filtration, sedimentation, adsorption, bioaccumulation, and microbial denitrification in different wetland zones (Jingyu et al., 2020; Minarti et al., 2024). Several studies conducted in Europe, North America, and Indonesia have demonstrated the effectiveness of constructed wetlands in mitigating nitrate pollution from agricultural runoff and domestic wastewater. Additionally, constructed wetlands offer multiple co-benefits, such as enhancing biodiversity, flood control, and providing environmental education opportunities.

8.7.2 Case study on groundwater bioreactor

The bioreactor was built in the ground at a nitrate polluted site (in situ system). Bioaugmentation was carried out, where denitrifying microbes that have been cultured in the laboratory are injected into the bioreactor. The process was followed by biostimulation, by adding molasses or other organic carbon sources to increase the activity of denitrifying microbes. The system was designed to maintain anoxic conditions in the denitrification zone. The study showed a significant reduction in nitrate concentration in the groundwater around the bioreactor. The nitrate removal

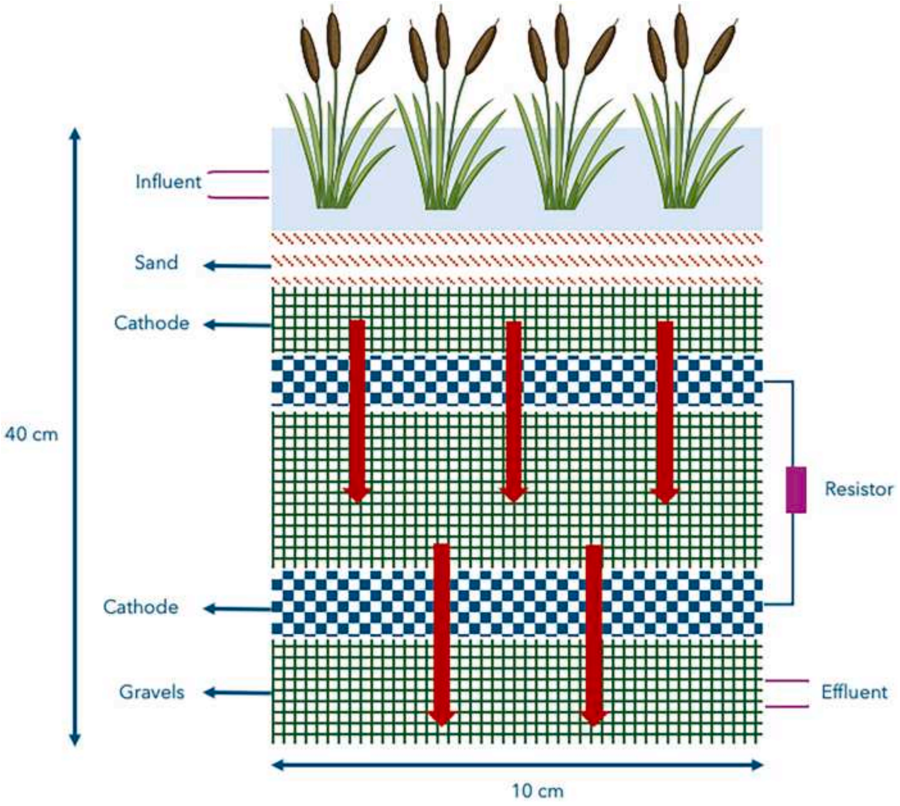

Figure 8.5 Constructed wetland microbial fuel cell (vertical subsurface flow).

rate achieved was quite high, even under heterogeneous soil conditions. Several studies in China and the United States have successfully applied groundwater bioreactors to address nitrate pollution from agricultural and industrial activities.

8.7.3 Case study on wastewater treatment plants

- Wastewater treatment includes
  - modifying the wastewater treatment process to maximize nitrate removal efficiency by setting up a nitrification-denitrification process;
  - adding anoxic zones to the treatment system to provide ideal conditions for denitrifying microbes (Gaimster et al., 2018);
  - using activated sludge rich in denitrifying microbes to accelerate the nitrate removal process (Nielsen et al., 2004); and

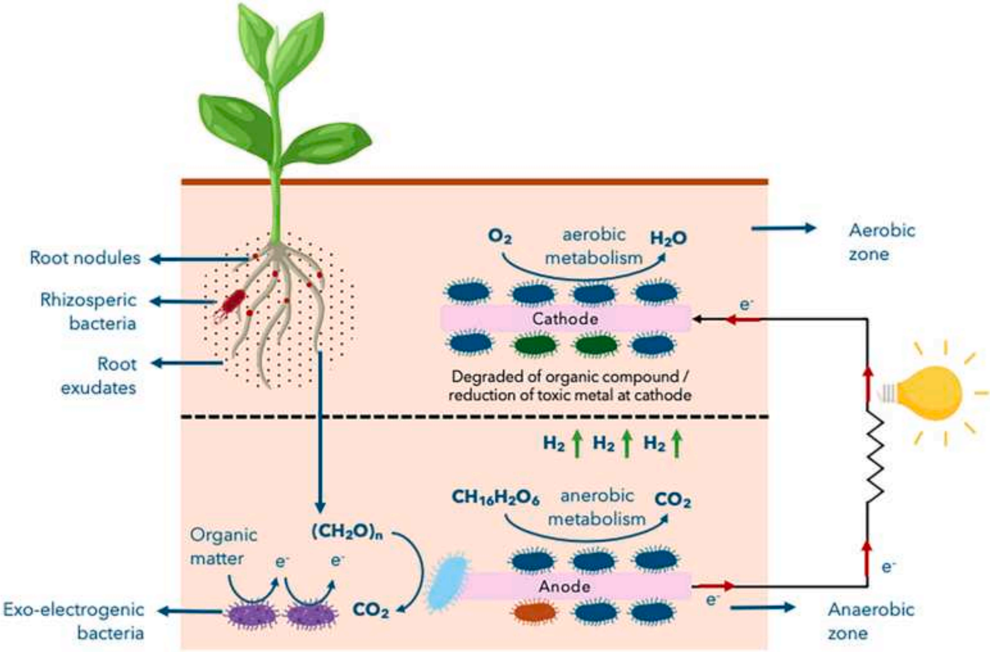

Figure 8.6 Principle reactions occurring in the anodic and cathodic chambers of a constructed wetland microbial fuel cell.

- adjusting aeration to create different conditions between the nitrification and denitrification zones.

The results showed an increase in nitrate removal efficiency from wastewater after the application of microbial niche tuning. Some wastewater treatment plants were able to achieve very low nitrate levels in the output water. Many wastewater treatment plants around the world have adopted this method, especially in countries that have strict regulations on output water quality.

8.7.4 Case study on aquaculture

- Heterotrophic microbes are maintained (Ebeling et al., 2006) in the form of bioflocs in the aquaculture system to assimilate nitrate and other wastes (Avnimelech, 2007).
- The water carbon-to-nitrogen ratio is adjusted to promote the growth of beneficial microbes and reduce nitrate levels (Ebeling et al., 2006).
- Probiotic microbes that help in controlling pathogenic microbial populations and improving nitrate removal efficiency are added.

The study showed that aquaculture systems with a microbial niche tuning approach can reduce nitrate levels and improve water quality, thereby supporting healthier fish growth. Several studies in Southeast Asia have demonstrated the success of biofloc systems in addressing nitrate pollution in aquaculture ponds.

Generally, successful case studies apply a combination of several microbial niche tuning methods and rely on a good understanding of the microbial ecology of the environment in question. The methods used need to be tailored to local conditions, such as soil type, climate, and pollution sources. Continuous monitoring is essential to ensure that the tuning process is effective, and nitrate pollution is under control. These case studies provide evidence that microbial niche tuning is a promising approach to address nitrate pollution in a sustainable and environmentally friendly manner (Ding, 2023; Kanissery & Sims, 2011; Li et al., 2023)

The recirculating aquaculture system is a sustainable approach that can be implemented due to its numerous advantages. In this system, the biofilter is the most critical component, serving as the heart of the system by purifying and improving water quality. The biofilter plays a crucial role as a breeding ground for bacteria (Helfrich & Libey, 2003), commonly referred to as chemotrophic bacteria. In freshwater aquaculture, these bacteria are known as Nitrosomonas and Nitrobacter, which are essential for neutralizing dissolved ammonia produced by fish farming activities (Figs. 8.7 and 8.8). In marine aquaculture, similar bacteria, known as Nitrosococcus and Nitrococcus, perform the same function of ammonia neutralization in saltwater environments (Fig. 8.9).

8.8 Government's role in microbial niche tuning implementation

The government has a crucial role in supporting the application of microbial niche tuning to address nitrate pollution and other environmental issues. Government support can be in the form of policies, regulations, funding, research, and socialization. Here are some important roles that the government can play (Sitawati et al., 2022).

1. Formulation of policies and regulations
    - **a.** set strict water and soil quality standards to limit nitrate pollution and other pollutants and encourage the use of environmentally friendly technologies, including microbial niche tuning, to meet the standards;
    - **b.** provide fiscal incentives (e.g., tax breaks or subsidies) for industries and farmers that apply microbial niche tuning technologies and provide awards or recognition for those who successfully apply these technologies; and
    - **c.** simplify the licensing process for the implementation of microbial niche tuning projects and facilitate the development of a certification system for technologies and products that use microbial niche tuning.

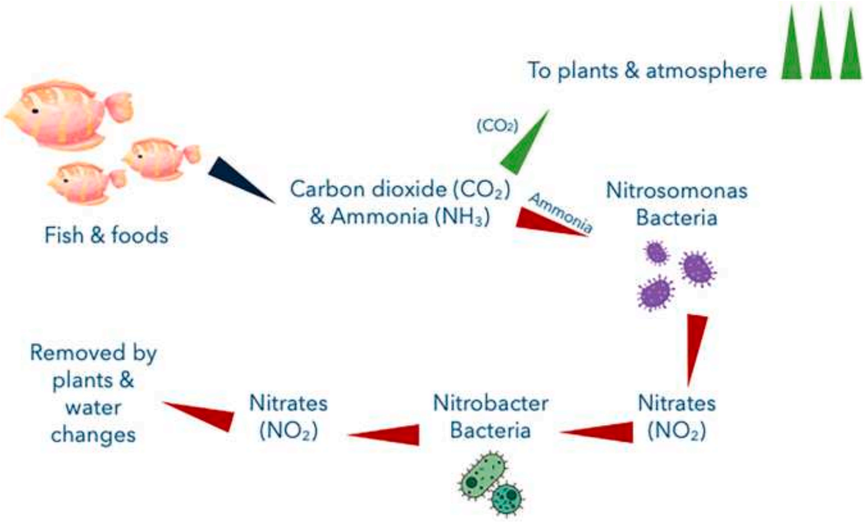

Figure 8.7 The nitrogen cycle in freshwater and saltwater.

Figure 8.8 The nitrification process in freshwater.

Figure 8.9 The nitrification process in saltwater.

2. Funding and investment
   a. allocation of funds for research and development of microbial niche tuning technology;
   b. funding large-scale demonstration projects to show the effectiveness of microbial niche tuning in the field; and
   c. facilitate investment in infrastructure required for microbial niche tuning implementation.

3. Research and development
    - **a.** support basic research to improve understanding of microbial ecology and denitrification processes and support research on the development of more effective genetically engineered microbes;
    - **b.** support the development of cheaper, easy-to-use, and accurate monitoring; and
    - **c.** establishing centers of excellence for microbial niche tuning research and development in various regions.
4. Socialization and education
    - **a.** develop a public education program to raise awareness about the benefits and potential of microbial niche tuning and organize seminars, workshops, and training for communities, industries, and farmers;
    - **b.** provide open access to data and information on microbial niche tuning;
    - **c.** collaborate with mass media to disseminate information on microbial niche tuning; and
    - **d.** organizing training for experts in microbial niche tuning and supporting formal and informal education in the field of biotechnology and microbiology.
5. Coordination and collaboration
    - **a.** establish good coordination between various government agencies related to the environment, agriculture, and industry;
    - **b.** build partnerships with the private sector to encourage investment and implementation of microbial niche tuning technology and provide support for start-up companies developing this technology; and
    - **c.** collaborate with other countries that have experience in implementing microbial niche tuning and facilitate knowledge and technology development with other countries.
6. Evaluation and monitoring
    - **a.** monitor and evaluate the implementation of policies and programs related to microbial niche tuning and
    - **b.** evaluate the impact of microbial niche tuning on the environment, human health, and economy, utilizing monitoring and evaluation data to improve program effectiveness.

With an active role and comprehensive support from the government, the application of microbial niche tuning can be accelerated and expanded, thus providing significant benefits to the environment and society. Based on the above, the successful application of microbial niche tuning to track nitrate pollution in surface water or soil is greatly influenced by

1. an integrative approach to various approaches and disciplines, including microbiology, ecology, biochemistry, bioinformatics, and engineering;

2. collaboration with various experts and research institutions to gain a more comprehensive understanding of the microbial ecosystem; and
3. continuous research and development to improve understanding of microbial ecosystems and develop more effective and efficient microbial niche tuning methods.

AI disclosure

During the preparation of this work, the author(s) used Kakak.ai in order to prepare this manuscript. AI was used solely as a tool to expand perspectives and assist in structuring the framework or outline. The final content was carefully refined, rewritten based on the authors' critical thinking, and supported by credible and validated references to ensure accuracy and reliability. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

References

Allison, S. D., & Martiny, J. B. H. (2008). Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences of the United States of America, 105*(1), 11512–11519. https://doi.org/10.1073/pnas.0801925105, http://www.pnas.org/content/105/suppl.1/11512.full.pdf.

Avnimelech, Y. (2007). Feeding with microbial flocs by tilapia in minimal discharge bio-flocs technology ponds. *Aquaculture (Amsterdam, Netherlands), 264*(1–4), 140–147. https://doi.org/10.1016/j.aquaculture.2006.11.025.

Ayuwaningsih, I. P., Fachrul, M. F., Rinanti, A., Abdullah, A. G., & Dani Nandiyanto, A. B. (2018). Increasing lipid content from biomass of microalgae to produce biofuels with optimization of nitrogen source. *MATEC Web of Conferences, 197*, 13011. https://doi.org/10.1051/matecconf/201819713011.

Coyte, K. Z., Schluter, J., & Foster, K. R. (2015). The ecology of the microbiome: Networks, competition, and stability. *Science (New York, N.Y.), 350*(6261), 663–666. https://doi.org/10.1126/science.aad2602, http://www.sciencemag.org/content/350/6261/663.full.pdf.

Ding, J. (2023). Soil nitrogen transformation and functional microbial abundance in an agricultural soil amended with biochar. *Revista Brasileira de Ciência do Solo, 47*, e0220156. https://doi.org/10.36783/18069657rbcs20220156.

Ebeling, J. M., Timmons, M. B., & Bisogni, J. J. (2006). Engineering analysis of the stoichiometry of photoautotrophic, autotrophic, and heterotrophic removal of ammonia-nitrogen in aquaculture systems. *Aquaculture (Amsterdam, Netherlands), 257*(1–4), 346–358. https://doi.org/10.1016/j.aquaculture.2006.03.019.

Eronen-Rasimus, E., Luhtanen, A. M., Rintala, J. M., Delille, B., Dieckmann, G., Karkman, A., & Tison, J. L. (2017). An active bacterial community linked to high chl-a concentrations in Antarctic winter-pack ice and evidence for the development of an anaerobic sea-ice bacterial community. *ISME Journal, 11*(10), 2345–2355. https://doi.org/10.1038/ismej.2017.96, http://www.nature.com/ismej/marketing/aims_scope.html.

Gaimster, H., Alston, M., Richardson, D. J., Gates, A. J., & Rowley, G. (2018). Transcriptional and environmental control of bacterial denitrification and N2O emissions. *FEMS Microbiology Letters, 365*(5), fnx277. Available from: http://femsle.oxfordjournals.org/ https://doi.org/10.1093/femsle/fnx277, http://femsle.oxfordjournals.org/.

Goldford, J. E., Lu, N., Bajić, D., Estrela, S., Tikhonov, M., Sanchez-Gorostiaga, A., Segrè, D., Mehta, P., & Sanchez, A. (2018). Emergent simplicity in microbial community assembly. *Science (New York, N.Y.), 361*(6401), 469–474. https://doi.org/10.1126/science.aat1168, http://science.sciencemag.org/content/361/6401/469/tab-pdf.

Grinnell, J., & Swarth, H. S. (1913). *An account of the birds and mammals of the San Jacinto area of southern California with remarks upon the behavior of geographic races on the margins of their habitats*. Berkeley: University of California Press 10.5962/bhl.title.15778. An account of the birds and mammals of the San Jacinto area of southern California with remarks upon the behavior of geographic races on the margins of their habitats.

Habibah, R., Iswanto, B., & Rinanti, A. (2020). The significance of tropical microalgae chlorella sorokiniana as a remediate of polluted water caused by chlorpyrifos. *International Journal of Scientific and Technology Research, 9*(1), 4460–4463. http://www.ijstr.org/final-print/jan2020/The-Significance-Of-Tropical-Microalgae-Chlorella-Sorokiniana-As-A-Remediate-Of-Polluted-Water-Caused-By-Chlorpyrifos.pdf.

Hakiki, R., Astuti, M. P., & Wikaningrum, T. (2019). Comparison of global warming potential-impact on the handling of the hazardous-sludge from the centralized industrial-wastewater treatment plant. *Indonesian Journal of Urban and Environmental Technology, 3*(1), 84–102. https://doi.org/10.25105/urbanenvirotech.v3i1.5530.

Hallin, S., Philippot, L., Löffler, F. E., Sanford, R. A., & Jones, C. M. (2018). Genomics and ecology of novel N2O-reducing microorganisms. *Trends in Microbiology, 26*(1), 43–55. https://doi.org/10.1016/j.tim.2017.07.003, www.elsevier.com/locate/tim.

Hang, Q., Wang, H., Chu, Z., Ye, B., Li, C., & Hou, Z. (2016). Application of plant carbon source for denitrification by constructed wetland and bioreactor: Review of recent development. *Environmental Science and Pollution Research, 23*(9), 8260–8274. https://doi.org/10.1007/s11356-016-6324-y.

Hauck, M., Maalcke-Luesken, F. A., Jetten, M. S. M., & Huijbregts, M. A. J. (2016). Removing nitrogen from wastewater with side stream anammox: What are the trade-offs between environmental impacts? *Resources, Conservation and Recycling, 107*, 212–219. https://doi.org/10.1016/j.resconrec.2015.11.019, www.elsevier.com/locate/resconrec.

Helfrich, L.A., & Libey, G.S. (1991). Fish farming in recirculating aquaculture systems (RAS). https://www.semanticscholar.org/paper/Fish-farming-in-recirculating-aquaculture-systems-Helfrich-Libey/80d42a4cc5dfd309b2aadac4e38b0f13fa6e13d0.

Herrero, M., & Stuckey, D. C. (2015). Bioaugmentation and its application in wastewater treatment: A review. *Chemosphere, 140*, 119–128. https://doi.org/10.1016/j.chemosphere.2014.10.033.

Jetten, M. S. M., Strous, M., Van De Pas-Schoonen, K. T., Schalk, J., Van Dongen, U. G. J. M., Van De Graaf, A. A., Logemann, S., Muyzer, G., Van Loosdrecht, M. C. M., & Kuenen, J. G. (1998). The anaerobic oxidation of ammonium. *FEMS Microbiology Reviews, 22*(5), 421–437. https://doi.org/10.1016/S0168-6445(98)00023-0, http://femsre.oxfordjournals.org/.

Jingyu, H., Miwornunyuie, N., Ewusi-Mensah, D., & Koomson, D. A. (2020). Assessing the factors influencing the performance of constructed wetland-microbial fuel cell integration. *Water Science and Technology, 81*(4), 631–643. https://doi.org/10.2166/wst.2020.135, https://iwaponline.com/wst/article/81/4/631/73286/Assessing-the-factors-influencing-the-performance.

Kanissery, R. G., & Sims, G. K. (2011). Biostimulation for the enhanced degradation of herbicides in soil. *Applied and Environmental Soil Science, 2011*, 1–10. https://doi.org/10.1155/2011/843450.

Kesarwani, S., Panwar, D., Mal, J., Pradhan, N., & Rani, R. (2023). Constructed wetland coupled microbial fuel cell: A clean technology for sustainable treatment of wastewater and bioelectricity generation. *Fermentation, 9*(1), 6. https://doi.org/10.3390/FERMENTATION9010006.

Karima, A., Silalahi, M. D. S., & Rinanti, A. (2018). Increasing content of lipid in tropical microalgae *Chlorella sorokiniana* and *Closterium* sp. with variation of nitrogen content and extraction temperature. *MATEC Web of Conferences, 197*, 13019. 10.1051/matecconf/201819713019, http://www.matec-conferences.org/.

Li, S., Wei, W., & Liu, S. (2023). Long-term organic amendments combined with nitrogen fertilization regulates soil organic carbon sequestration in calcareous soil. *Agronomy, 13*(2), 291. https://doi.org/10.3390/agronomy13020291.

Liu, S., Zhang, Y., Feng, X., & Pyo, S.-H. (2024). Current problems and countermeasures of constructed wetland for wastewater treatment: A review. *Journal of Water Process Engineering, 57*, 104569. https://doi.org/10.1016/j.jwpe.2023.104569.

Miao, Y., Zhang, L., Li, B., Zhang, Q., Wang, S., & Peng, Y. (2017). Enhancing ammonium oxidizing bacteria activity was key to single-stage partial nitrification-anammox system treating low-strength sewage under intermittent aeration condition. *Bioresource Technology, 231*, 36–44. https://doi.org/10.1016/j.biortech.2017.01.045.

Minarti, A., Rinanti, A., Fachrul, M. F., Tazkiaturrizki, & Fadhila, R. (2024). Circular economy for biodiesel production by managing wastewater using microalgae. *Environmental Science and Engineering, 2024*, 463–521. https://www.springer.com/series/7487, https://doi.org/10.1007/978-981-97-2371-3_17, https://www.springer.com/series/7487.

Muter, O. (2023). Current trends in bioaugmentation tools for bioremediation: A critical review of advances and knowledge gaps. *Microorganisms, 11*(3), 710. https://doi.org/10.3390/microorganisms11030710.

Nielsen, P. H., Thomsen, T. R., & Nielsen, J. L. (2004). Bacterial composition of activated sludge - importance for floc and sludge properties. *Water Science and Technology, 49*(10), 51–58. https://doi.org/10.2166/wst.2004.0606.

Niu, Q., Zhang, Y., Ma, H., He, S., & Li, Y. Y. (2016). Reactor kinetics evaluation and performance investigation of a long-term operated UASB-anammox mixed culture process. *International Biodeterioration and Biodegradation, 108*, 24–33. https://doi.org/10.1016/j.ibiod.2015.11.024, www.elsevier.com/inca/publications/store/4/0/5/8/9/9.

Rousk, J., Brookes, P. C., & Bååth, E. (2010). Investigating the mechanisms for the opposing pH relationships of fungal and bacterial growth in soil. *Soil Biology and Biochemistry, 42*(6), 926–934. https://doi.org/10.1016/j.soilbio.2010.02.009.

Sitawati, A., Taki, H. M., & Andajani, R. D. (2022). The influence of environmental policies on selecting investment locations. *Indonesian Journal of Urban and Environmental Technology, 5*(3), 266–280. https://e-journal.trisakti.ac.id/index.php/urbanenvirotech/article/download/14448/8496, https://doi.org/10.25105/urbanenvirotech.v5i3.14448, https://e-journal.trisakti.ac.id/index.php/urbanenvirotech/article/download/14448/8496.

Susana, T. (2016). Tingkat Keasaman (pH) Dan Oksigen Terlarut Sebagai Indikator Kualitas Perairan Sekitar Muara Sungai Cisadane. *Indonesian Journal of Urban And Environmental Technology, 5*(2), 33–39. https://doi.org/10.25105/urbanenvirotech.v5i2.675.

Tazkiaturrizki (2016). Pengaruh penambahan glycine max pada penyisihan nitrogen dalam constructed wetland tipe subsurface horizontal flow. *Indonesian Journal of Urban And Environmental Technology, 8*(1), 117–124. https://doi.org/10.25105/URBANENVIROTECH.V8I1.724.

Tribedi, P., Goswami, M., Chakraborty, P., Mukherjee, K., Mitra, G., Bhattacharyya, P., & Dey, S. (2018). Bioaugmentation and biostimulation: A potential strategy for environmental remediation. *Journal of Microbiology & Experimentation, 6*(5), 223–231. https://doi.org/10.15406/jmen.2018.06.00219.

Van Colen, C., Underwood, G. J. C., Serôdio, J., & Paterson, D. M. (2014). Ecology of intertidal microbial biofilms: Mechanisms, patterns and future research needs. *Journal of Sea Research, 92*, 2–5. https://doi.org/10.1016/j.seares.2014.07.003, www.elsevier.com/inca/publications/store/6/0/0/3/1/8.

Wang, G., Xu, X., Gong, Z., Gao, F., Yang, F., & Zhang, H. (2016). Study of simultaneous partial nitrification, ANAMMOX and denitrification (SNAD) process in an intermittent aeration membrane bioreactor. *Process Biochemistry, 51*(5), 632–641. https://doi.org/10.1016/j.procbio.2016.02.001.

Wang, S., Peng, Y., Ma, B., Wang, S., & Zhu, G. (2015). Anaerobic ammonium oxidation in traditional municipal wastewater treatment plants with low-strength ammonium loading: Widespread but overlooked. *Water Research, 84*, 66–75. https://doi.org/10.1016/j.watres.2015.07.005.

Wijaya, I. M. W., & Putra, P. E. D. (2021). Anaerobic ammonium oxidation (anammox) pada penyisihan nitrogen air limbah domestik. *Jurnal Ecocentrism, 1*(2), 113–122. https://doi.org/10.36733/jeco.v1i2.2425.

Wikaningrum, T., Hakiki, R., Astuti, M. P., Islmail, Y., & Sidjabat, F. M. (2009). The eco enzyme application on industrial waste activated sludge degradation. *Indonesian Journal of Urban And Environmental Technology, 5*(2), 33–39. https://doi.org/10.25105/URBANENVIROTECH.V5I2.675.

Zhou, J., He, Z., Yang, Y., Deng, Y., Tringe, S. G., & Alvarez-Cohen, L. (2015). High-throughput metagenomic technologies for complex microbial community analysis: Open and closed formats. *mBio, 6*(1), e02288-14. http://mbio.asm.org/content/6/1/e02288-14.full.pdf, https://doi.org/10.1128/mBio.02288-14, http://mbio.asm.org/content/6/1/e02288-14.full.pdf.