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Review

Nitrogen removal in constructed wetland systems

Since the mid 1990s, constructed wetlands have been increasingly used as a low-energy 'green' technique, in the treatment of wastewater and stormwater, driven by the rising cost of fossil fuels and increasing concern about climate change. Among various applications of these wetlands, a significant area is the removal of nitrogenous pollutants to protect the water environment and to enable effective reclamation and reuse of the wastewater. This paper provides a review of the current state of nitrogen removal technology, focusing on existing types of wetlands, the mechanisms of nitrogen removal, major environmental factors relative to nitrogen removal, and the operation and management of the wetlands.

Keywords: Nitrogen removal / Reed bed / Subsurface flow / Wastewater treatment

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1 Introduction

Most wastewaters, such as industrial and agricultural wastewater, urban drainage, sewage, and landfill leachate, contain nitrogenous compounds that have given rise to various negative phenomena in water environments, *e.g.* damage to aquatic life, being toxic to fish and/or causing oxygen depletion in receiving water biota [1]. In wastewater treatment wetlands, the efficiency of organic matter removal often meets the specified design target, but the efficiency of nitrogen removal is mostly poor. In European systems, for example, typical removal percentages of ammoniacal-nitrogen in long-term operation is only 35%, or up to 50% after modifications are made specifically to improve nitrogen removal [2, 3]. Similarly, stormwater wetlands typically remove only around 45% of total nitrogen, most of which is made up of particulate organic nitrogen [1].

In order to understand and improve the performance of the wetlands, it is necessary to briefly look back at the history of this technology and look into the mechanisms of pollutant removals. In doing this, we aim to identify critical knowledge gaps, as well as potential areas worthy of future exploration and development.

1.1 Constructed wetland: a relatively new technique

The constructed wetland system is a cost-effective natural alternative to conventional wastewater treatment plants. A constructed wetland is defined as an engineered system designed to simulate a natural wetland for waste treatment or other purposes [4]. In Europe, a constructed wetland is also known as a reed bed. The evolution of constructed wetland technology has been comparatively short (the foundation was laid by early researchers, notably two German scientists: Dr. Käthe Seidel and Dr. Reinhold Kickuth). Studies in this field were rare until the second international conference on constructed wetlands was held in 1990 at Cambridge, UK [5]. Hence, the history of research development in constructed wetlands is relatively short, in comparison to steel-and-concrete wastewater treatment systems (*e.g.* the activated sludge process can be traced back to 1913).

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1.2 Increasing applications driven by cost of fossil fuels and climate change

Since the 1990s, the applications of constructed wetlands have expanded radically, due to the rising cost of fossil fuels and increasing concern about climate change, which provide a financial incentive, as well as public support, to the implementation of this low energy consumption 'green' technique. Constructed wetlands have now been successfully used in the treatment of several wastewaters such as domestic sewage, urban runoff and stormwater, industrial and agricultural wastewater, and leachate [6].

1.3 Constructed wetland: a complex bioreactor

A constructed wetland is considered to be a complex bioreactor. A number of physical, chemical, and biological processes with microbial communities, emergent plants, soil, and sediments accumulated in the lower layer take place in the systems.

Nitrogen concentration is often of concern because of its potential to cause adverse effects in receiving water systems [7, 8]. Among various nitrogen groups, dissolved inorganic nitrogen species like nitrate (NO_3^-), nitrite (NO_2^-), and ammonia (NH_3) or ammonium (NH_4^+) – rather than particulate organic nitrogen – have the greatest impact on aquatic systems, because they are easily available for uptake by microorganisms [9]. Many researches have shown the impact of excessive nitrogen loads on receiving waters [10]. The removal of organic substances, typically 80–90%, is now satisfactory in constructed wetlands because of gradual improvement over two decades. However, the nitrogen removal rates are often unsatisfactory. A variety of nitrogen forms in constructed wetlands can be removed through specific treatment processes, such as combined nitrification-denitrification and sedimentation, particularly at the sediment-water and water-plant interface [1].

1.4 Multiple benefits

Constructed wetlands provide a range of benefits in the treatment of wastewater. Wetland utilization generates economic savings, as it depends upon natural treatment routes which cost less in terms of electricity and human labor and have lower construction and maintenance costs, including chemicals, fuel, services and plant operation [11]. Moreover, they offer flexible site selection, easy operation and maintenance, a wildlife habitat as well as high stability under changing environmental conditions. Constructed wetlands are typically large passive systems with long residence times. Conversely, conventional wastewater treatment plants rely on energy-intensive operation with short residence times. Steel-and-concrete treatment systems have the disadvantage of excess sludge production, high energy demand, and high cost for operation and maintenance.

Also, the purified water produced in constructed wetlands is suitable for reuse. For instance, irrigation reuse is practiced at about 30% of Australian constructed wetlands [12]. They can provide opportunities for environmental education, recreational and exercise activities, whilst acting as a hydrological buffer or a reservoir and removing pathogenic organisms [13, 14]. In a typical steel-and-concrete wastewater treatment system, the removal rate of pollutants is generally 80∼95% for biological oxygen demand (BOD), 70∼80% for suspended solids (SS), 20∼30% for total nitrogen (TN), and less than 20% for total phosphorus (TP) [15, 16]. In contrast, nitrogen removal efficiency in constructed wetlands is relatively high. Constructed wetland is beneficial compared with the conventional treatment plant as shown Table 1 [17, 18].

1.5 Constructed wetland performance and processes: the big unknowns

Since nitrogen-rich discharges into receiving water systems are responsible for a variety of environmental problems, optimizing nitrogen removal is a critical objective. So far, activated sludge and biofilm processes have been the main focus for biological nitrogen removal. However, these processes are expensive, particularly when employed in medium and small communities. Constructed wetlands have proven potential for nitrogen removal, but nitrogen removal efficiency has been inconsistent, due to inadequate observation of nitrogen transformation and removal mechanisms [19]. There are still many unknown parts related to constructed wetlands performance, diverse driving operations, and nitrogen constraints [20, 21]. Therefore, it is necessary to explore explicit nitrogen transformation mechanisms based on consideration of kinetics and the interactions between microbial communities and emergent plants.

2 Types of constructed wetlands

Constructed wetlands are classified into two major types according to hydraulic water flow characteristics in the system: surface flow (SF) and subsurface flow (SSF) systems.

2.1 Surface flow systems

The water depths of SF systems typically vary between 0.2 and 0.6 m, and these systems are densely vegetated [22]. The wetland base may be permeable, allowing exfiltration of water.

Table 1. Comparison of characteristics of constructed wetlands and conventional wastewater treatment plants.

		Economical considerations			Removal efficiency, %				
Type of treatment system	Treatment capacity	Construction cost, $	Management cost, $/year	Facility size, m^3	BOD	SS	TN	TP	Remarks
Constructed wetland	Sewage 100 m^3/day	220 000	300	800	80–90	80–90	40–50	50–60	Remove some heavy metals, *E. coli*
Conventional treatment plant		300 000	2000	450	80–99	70–80	20–30	<20	

In this system, wastewater passes over the support medium, between the stems of plants and through any surface debris. Sunlight permeates into the bottom through a shallow water basin within the system so that it can trigger a faster rate of algal growth and active photosynthesis reaction. SF systems are frequently used in North America [23]. SF wetland systems offer low construction cost, but they generally have a lower contaminant removal efficiency compared with SSF systems. There has been a recent attempt to develop an open-water zone, without vegetation, to improve the nitrogen removal efficiency, promote better inflow flux, and provide wildlife habitats [24].

2.2 Subsurface flow systems

SSF systems typically consist of a ditch or a bed, sealed by an impermeable substance to block leakage, and media that assist the growth of emergent plants. The media are typically composed of rock or crushed gravel of 10–15 mm diameter, and different soils, or in various combinations [23, 25]. This system is broadly recognized for its ability to remove various contaminants such as BOD, chemical oxygen demand (COD), SS metals, nitrogen, and phosphorus as well as pathogens [26]. SSF systems are primarily used in Europe, and South-Africa.

SSF systems are subdivided into horizontal and vertical flow systems according to the flow direction of the wastewater. The combination of subsurface vertical and horizontal flow wetlands, namely a hybrid system, has been used to improve the treatment performance, especially for nitrogen. Among various types of SSF wetland systems, the horizontal SSF type has been most commonly used. In a horizontal SSF wetland, primary treated wastewater flows horizontally through the wetland matrix consisting of plant roots, gravel and/or sand. The matrix hosts layers of attached microorganisms [12].

Currently, some researchers are developing new wetland systems, applying features of different wetland types in order to achieve higher efficiency of pollutant removal, e.g. by stimulating tidal flow [27, 28] or using a modified wetland equipped with a flow-shift module to enhance microbial decomposition of organic matter [29].

Selection of the most appropriate wetland type depends on the targeted pollutants, the available land, and the acceptable level of maintenance and management. Other issues such as interaction with groundwater may also need to be considered.

3 Mechanisms of nitrogen removal

Nitrogen removal is achieved by two major processes, physicochemical and biological treatment techniques. Traditional biological nitrogen removal from water and wastewater, primarily composed of a combination of aerobic nitrification and anaerobic denitrification, is usually considered to accomplish optimal and economic nitrogen treatment. However, there are still many unresolved issues, such as the requirement of an extra carbon source in wastewater with low C/N ratio, the requirement for large treatment areas, and high maintenance cost [30, 31]. Most wastewaters do not have enough biodegradable carbon and an external organic source to carry out heterotrophic denitrification. In nitrogen removal treatment, biological processes frequently have several obstacles because of lower energy consumption and high cost in the wastewater treatment plant. Consequently, many studies on the mechanisms of the nitrogen cycle – not only nitrification and denitrification but also new sustainable processes – are being conducted [32].

The removal process of pollutants in the SSF wetland system is complex and dynamic, with many variables. The forms of nitrogen in natural ecosystems are illustrated in Fig. 1. Total nitrogen in the natural state can fall into two basic groups, total Kjeldahl nitrogen (TKN) and oxidized nitrogen (NO_x). Organic nitrogen is subdivided into particulate organic nitrogen (PON) and dissolved organic nitrogen (DON). Nitrate and nitrite are soluble inorganic nitrogen and, coupled with ammonia and ammonium, form the dissolved inorganic nitrogen (DIN). Total dissolved nitrogen (TDN) builds up; DON, NH_3/NH_4^+, and NO_x are known to be highly bio-available [1, 33].

Many studies reported that water purification mechanisms in constructed wetlands are achieved by hydrophytes and microorganisms around the plant root zone, along with physical precipitation [22, 34]. The major nitrogen treatment mechanisms of constructed wetlands include microbial interactions with nitrogen, sedimentation, chemical adsorption, and plant uptake [26]. The central pathways for nitrogen removal in constructed wetlands are nitrification followed by denitrification [35]. In constructed wetlands, nitrogen removal ranges from 25 to 85% [36].

In constructed wastewater wetlands, the denitrification process may remove 60~70% of the total removal nitrogen and 20~30% of that is derived from plant uptake [35, 37]. In stormwater wetlands, the proportion of nitrogen removed by wetlands is considered to be considerably lower [38]. The mass budget for nutrients indicates that 14% is originated by physical treatment process and 8.6% by plant uptake; *i.e.*, the absorbed amount of nitrogen into the plant itself is

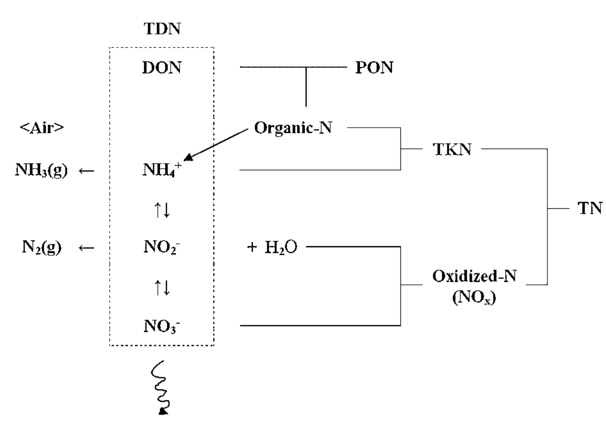

Figure 1. Different forms of nitrogen and nitrogen flow in natural ecosystems.

small, but the absorbed nitrogen stimulates diverse ecological activities [39].

3.1 Biodegradation: classic routes

The nitrogen removal mechanisms in constructed wetlands are known to involve ammonification, nitrification-denitrification, plant uptake, and physicochemical methods such as sedimentation, ammonia stripping, breakpoint chlorination, and ion exchange [22, 40]. Figure 2 shows a nitrogen conversion diagram for constructed wetlands.

3.1.1 Ammonification

Ammonification is the process where organic N is biologically converted into ammonia. Pollutants containing nitrogen are readily degraded in both aerobic and anaerobic zones of reed beds, releasing inorganic ammoniacal-nitrogen (NH_4–N). The inorganic NH_4–N is mainly removed by nitrification-denitrification processes in constructed wetlands. Kinetically, ammonification proceeds more rapidly than nitrification. The rates of ammonification are fastest in the oxygenated zone and then decrease as the mineralization circuit changes from aerobic to facultative anaerobic and obligate anaerobes. The rates are influenced by temperature, pH, C/N ratio, available nutrients, and soil structure [41]. NH_4–N in SSF systems can be reduced by other processes, which include adsorption, plant uptake and volatilization [42]. However, it is generally believed that the contribution of these processes to the NH_4–N removal is very limited compared with nitrification-denitrification.

3.1.2 Nitrification

Decomposition processes in the wetlands are believed to convert a significant part of the organic nitrogen to ammonia [43]. Biological nitrification, which is performed by nitrifiers such as *Nitrosomonas*, *Nitropira*, *Nitrosococcus* and *Nitrobacter*, followed by denitrification is believed to be the major pathway for ammonia removal in both SF and SSF constructed wetlands [22, 44]. In traditional nitrogen treatments, the biological nitrogen removal requires a two-step process: nitrification followed by denitrification. Nitrification implies a chemolithoautotrophic oxidation of ammonia to nitrate under strict aerobic conditions and is performed in two sequential oxidative stages: ammonia to nitrite (ammonia oxidation) and nitrite to nitrate (nitrite oxidation). Each stage is performed by different bacterial genera which use ammonia or nitrite as an energy source and molecular oxygen as an electron acceptor, while carbon dioxide is used as a carbon source. The most commonly recognized genus of bacteria is that of *Nitrosomonas* for the ammonia oxidation process and *Nitrobacter* for the nitrite oxidation process. The overall equations for these two reactions can be represented as follows [45].

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + H_2O + 2H^+ \quad (1)$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \quad (2)$$

The nitrification process is very oxygen demanding. Oxygen consumed in this process is 3.16 mg O_2/mg NH_4-N oxidized and 1.11 mg O_2/mg NO_2-N oxidized. Moreover, yields produced by *Nitrosomonas* and *Nitrobacter* are 0.15 mg cells/mg NH_4–N oxidized and 0.02 mg cells/mg NO_2-N oxidized, respectively. In addition, alkalinity is needed as 7.07 mg $CaCO_3$/mg NH_4-N oxidized [46]. However, the alkalinity reduction by the acid made in the nitrification process can cause a deep pH reduction. The pH value is very important in the nitrification reaction since nitrification rates swiftly decline where the pH drops to lower than 7.0. Thus, the appropriate chemicals such as lime should be replenished when the alkalinity in the process is reduced by the acid produced in the nitrification reaction [46].

The doubling time of nitrifying bacteria is reported as 2–6 days [47]. The nitrifying bacteria of the autotroph group have much lower respiration rates than the heterotrophs, which are responsible for BOD removal. Accordingly, in the SSF systems, significant nitrification generally does not take place before substantial BOD reduction [48, 49]. The rate of nitrification is influenced by temperature, pH, alkalinity, inorganic carbon source, moisture, microbial population, and concentrations of ammonium–N and dissolved oxygen. The ammonia uptake rate (AUR) varies with reactor configuration, substrate type, and influent ammonium concentration.

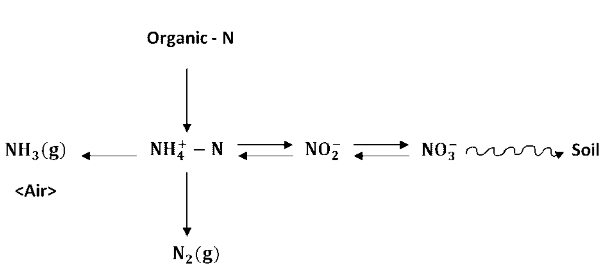

Figure 2. Nitrogen conversion diagram in constructed wetlands.

3.1.3 Denitrification

The biological denitrification mechanism makes use of nitrate as the terminal electron acceptor in low-oxygen environments. In this process, denitrifying bacteria decrease inorganic nitrogen such as nitrate and nitrite into innocuous fundamental nitrogen gas [50, 51]. Denitrifying bacteria (denitrifiers) can be classified into two major species, heterotrophs and autotrophs. Heterotrophs are microbes that need organic substrates to obtain their carbon source for growth and evolution, and get energy from organic matter. In contrast, autotrophs utilize inorganic substances as an energy source and CO_2 as a carbon source [52]. So far, the heterotrophic denitrification process has been mainly engaged in conventional wastewater treatment plants, while autotrophic denitrification has only recently been studied [53].

The second step, denitrification, is conducted by a heterotrophic microorganism (such as *Psuedomonas*, *Micrococcus*, *Achromobactor* and *Bacillus*) under anaerobic or anoxic conditions. The proportion of total nitrogen removal by denitrification is typically 60–95%, in comparison to 1–34% assimilated by plants and algae. Heterotrophic microorganisms utilize an oxidized form of nitrogen, NO_2^-, NO_3^-, as terminal electron acceptor and organic carbon as electron donor under anoxic conditions [45]. Consequently, the denitrification provides energy to denitrifiers and it is also affected by the organic matter of the electron donor. This process is shown in the following [54].

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \quad (3)$$

Denitrification can only take place in the anoxic zones of the systems, as the presence of dissolved oxygen suppresses the enzyme system required for this process [45]. High concentrations of nitrate in the inlet zones can lead to more vigorous and robust populations of denitrifiers within the inlet sediments [55]. In constructed wetlands, it is believed that microsites with steep oxygen gradients can be established, which allow nitrification and denitrification to occur in sequence, in very close proximity to each other. Sufficient organic carbon is needed as an electron donor for nitrate reduction, which provides an energy source for denitrification microorganisms [56, 57]. This carbon source can be available in reed beds from organic pollutants of wastewater or cell materials of microorganisms. The rate of denitrification is influenced by many factors, including nitrate concentration, microbial flora, type and quality of organic carbon source, hydroperiods, different plant species residues, the absence of O_2, redox potential, soil moisture, temperature, pH value, presence of denitrifiers, soil type, water level, and the presence of overlying water [55, 58, 59].

Numerous studies have shown that the denitrification rate in organic carbon-restricted water and wastewater can be improved continually by supplementing any carbon sources [60], even though there are some issues regarding external organic carbon sources in heterotrophic denitrification [61]. Currently, there is much attention towards biological nitrogen removal, whilst the denitrification process is generally time-consuming, especially for industrial wastewaters involving much nitrate [62]. Also, a number of researchers have studied denitrification systems, including the application of granular activated carbon, packed beds, and rotating biological contractors. These attempts are developing and some new systems such as membrane biofilm reactors have been established [63].

3.2 Biodegradation: Anammox Routes

The Anammox (anaerobic ammonium oxidation) process provides a potential alternate process for improving total nitrogen removal. The recent discovery of Anammox bacteria [64] opened up a new avenue in the study of nitrogen transformations. Denitrification by Anammox bacteria is now proven to be partly responsible for the transformation of ammonia into nitrogen gas within the nitrogen cycle. In this process, ammonium is autotrophically oxidized to nitrogen gas while nitrite is employed as an electron acceptor under anaerobic conditions. Thus, there is no demand for aeration and addition of an external carbon source, resulting in a cost saving and preventing insufficient conversion of organic substances [65].

The Anammox bacteria, such as *Candidatus brocadia anammoxidans, Planctomycetes spp., Thiobacillus senitrificans, Thiomicrospira denitrificans, Thiosphaera ponotropha*, and *Paracoccus denitrificans*, are autotrophic, in contrast to classic denitrifiers which are mostly heterotrophic and thus need organic carbon for their carbon and energy supply. Therefore, stimulating Anammox bacteria in a wastewater treatment system reduces the need for an organic carbon source, which is required in the conventional denitrification process [66].

In these processes with partial nitrification in one reactor, such as Anammox (anaerobic ammonium oxidation), Sharon (single-reactor high-activity ammonia removal over nitrite), and Oland (oxygen-limited autotrophic nitrification-denitrification), single-stage autotrophic nitrogen removal is accomplished through assistance between aerobic ammonia-oxidizing bacteria (AOB) and Anammox bacteria [67, 68]. However, a strict controlled environment and reactor arrangement are needed, as Anammox bacteria have a slow growth rate [69]. In reality, the Anammox process has been reported to produce higher removal efficiency of total nitrogen [70, 71], and to save up to 90% of operation cost, due to a reduction of the input of organic matter [72]. When the Anammox bacteria co-function with autotrophic nitrosobacteria *via* the following route in a single reactor, the removal pathway is known as 'completely autotrophic nitrogen removal over nitrite' (CANON) [73]:

$$NH_4^+ + 1.32NO_2^- + 0.13H^+ \rightarrow 1.02N_2 + 0.26NO_3^- + 2.03H_2O \quad (4)$$

The key operating factors of partial nitrification processes (*i.e.* Anammox and CANON) include temperature, pH, free ammonia, free nitrous acid, hydraulic residence time (HRT), dissolved oxygen, salt, organic compounds, and hydroxylamine [74–76]. Further research on the selection of Anammox bacteria species and optimal operating parameters is needed to stimulate novel nitrogen removal routes in constructed wetlands.

3.3 Plant uptake

The uptake of ammonia and nitrate by macrophytes converts inorganic nitrogen forms into organic compounds, as building blocks for cells and tissues [58]. The capability of rooted plants to use sediment nutrients partly explains their extensive yield compared with planktonic algae in many systems [77]. Various plant species differ in their favored forms of nitrogen absorbed, depending on the forms available in the wetland. The NH_4^+ preference is common in macrophytes living in environments with limited nitrification, where NH_4^+ is abundant [78]. The uptake and storage rate of nutrients by plants depend on the nutrient concentration of their tissues. Thus, desirable features of a plant used for nutrient assimilation and storage include fast growth, high tissue nutrient content, and the ability to obtain a high-standing crop. Conversely, plants that have great biomass accumulation during autumn and winter may release much of their accumulated nitrogen back into the water during the winter season [42].

3.4 Physicochemical processes

The contribution of physicochemical processes to overall nitrogen removal is generally high in newly built wetlands, but decreases with time. Although many physicochemical processes can take place in constructed wetlands, the major mechanisms for nitrogen removal are ammonia adsorption and sedimentation.

3.4.1 Ammonia adsorption

In constructed wetlands, adsorbed ammonia is bound loosely to the substrates and can be released easily when water chemistry conditions change. When the ammonia concentration in the water column is reduced as a result of nitrification, some ammonia will be adsorbed to regain equilibrium with the new concentration. If the ammonia concentration in the water column is increased, the adsorbed ammonia will also increase [42]. If the wetland substrates are exposed to oxygen, adsorbed ammonium may be oxidized to nitrate by periodic draining [27, 28, 79]. The ammonium ion is generally adsorbed as an exchangeable ion on clays, and adsorbed by humic substances. The rate and extent of these reactions are reported to be influenced by several factors, such as the type and amount of clay, alternating submergence and drying patterns, characteristics of soil organic matter, submergence period, and the presence of vegetation.

3.4.2 Sedimentation

Most particulate organic nitrogen in constructed wetlands is removed by sedimentation [1]. Particulates may settle on the wetland floor or may adhere to plant stems. The decomposed materials such as TN, TP, and organics of low molecular weight are used by microorganisms and plants [22]. In nitrogen removal in wetlands, combined physical and chemical processes can be employed. An enhanced sedimentation method using magnesium-ammonium-phosphate (MAP), as added precipitation reagent, has been developed for the removal of nitrogen and phosphorus in wastewater treatment and has the potential to be applied in constructed wetlands.

4 Environmental factors affecting nitrogen removal efficiency

Considering that pollutants are removed by a variety of physicochemical and biological processes in constructed wetlands, numerous environmental factors can influence the removal of nitrogen. Major factors include temperature, HRT, type and density of vegetation, the characteristics of microbial communities, climate, the distribution of wastewater and influent characteristics, *etc.* These factors are often related, and a change in one factor can cause a change in the others [55]. Among these, two of the most significant factors are temperature and HRT [80].

4.1 Temperature

Temperature, as a key environmental factor, is important in relation to the activities of nitrifying bacteria and the denitrification potential in treatment wetlands [81]. Biological nitrogen removal is most efficient at 20∼25°C, and temperatures affect both microbial activity and oxygen diffusion rates in constructed wetlands [82]. The microbial activities related to nitrification and denitrification can decrease considerably at water temperatures below 15 or above 30°C, and most microbial communities for nitrogen removal function at temperatures greater than 15°C [80].

Several studies have shown that the activity of denitrifying bacteria in constructed wetland sediments is generally more robust in spring and summer than in autumn and winter [83], and the overall removal rate of nitrate is higher in summer than in winter [57]. While denitrification is commonly believed to cease at temperatures below 5°C, some studies have demonstrated denitrification activity at 4°C or lower, albeit at lower rates [84]. Vymazal [42] reported that the optimum temperature range for nitrification is 30–40°C in soils, and the optimal ammonification temperature is 40∼60°C, while the optimal pH is between 6.5 and 8.5. At low temperature, nitrification can be insufficient to prevent a net increase in ammonia concentration due to ammonification [85].

4.2 Hydraulic residence time

HRT plays a critical role in nitrogen removal efficiency. Huang *et al.* [86] described that ammonium and TKN concentrations in treated effluent decrease dramatically with increase in wastewater residence time. In most wetland systems, nitrogen removal requires a longer HRT compared with that required for BOD and COD removal. Accordingly, nitrogen removal efficiency varies greatly with flow conditions and residence time [1]. Akratos and Tsihrintzis [85] reported that in an SSF wetland, an 8–day HRT at above 15°C is required, with 14∼20° days being recommended as optimal.

4.3 Types of vegetation

The roots of macrophytes provide surface areas for microbial growth and aerobic zones in constructed wetlands. The rhizosphere is the most energetic reaction zone in a constructed wetland. The root zone facilitates various physical and biochemical processes caused by the relationship of plants, microbial communities, soil and contaminants. To improve treatment performance and optimize the design of constructed wetlands, it is fundamental to understand the capability of diverse plant species, the peculiarities of microorganism groups, and the relations between biogenic matters and particular components in pollutants. Helophyte species perform the principal role in wastewater treatment systems due to their growth physiology, which assures their viability even under severe environments [87]. Wissing [88] argued that there are three central groups, an aquaculture system, a hydrobotanical system, and a soil system, in the natural system of a constructed wetland. The soil matrix provides the substance for plant growth and microbial films. In vertical flow systems, oxygen mostly enters the soil filter by water suction, whereas in horizontal flow systems oxygen primarily enters the soil by helophytes.

Macrophytes, which are adapted to anoxic rhizospheres, can survive due to an ability to supply their roots with oxygen from the atmosphere. Consequently, the relationship between different environmental conditions in the rhizosphere and the biochemical system can lead to anatomical alterations in the plants [89]. The input of ambient air with enough oxygen into the inside of a plant under anoxic conditions in the rhizosphere can be used for respiration. An oxidative protecting film on the root surface is made by oxygen release [90]. The interest of many studies is focused on the correlation between utilization of the rhizosphere and continuous oxygen release to treat wastewater in constructed wetlands. Many researches have shown that the redox potential of the rhizosphere has a key impact on the rates of oxygen release *via* the roots of helophytes [91–93].

Microphytes also play an important role in wastewater treatment through uptake of nutrients, surface bed stabilization, and other mechanisms [22]. The type of macrophytes in constructed wetlands has a greater influence on nitrogen removal than the removal of organic matter [85, 94]. Common macrophytes used in constructed wetlands are reed (*Phragmites australis*), cattail (*Typha* spp.) and bulrush (*Scirpus* spp.), all characterized as water-tolerant macrophytes that are rooted in the soil but emerge above the water surface [95]. Their growth changes with temperature and dissolved oxygen concentrations in sediment and water. Wetland systems with vegetation typically remove greater amounts of total nitrogen than non-vegetated systems [1, 38].

Nutrient removal by the emergent plants is achieved by two processes: absorption of the plant itself and microorganism activity around the rhizome [96]. Reed dead matter accumulated in the soils may cause eutrophication, increasing the BOD of fresh water in constructed wetlands for a long time. Thus, the plant harvest should be controlled at an appropriate time. If not, nutrients within the dead plants are re-discharged into the receiving water, so that a variety of adverse effects occur in constructed wetlands, because the ambient CO_2 gas influx is increased in the wetland. In general, the main role of hydrophytes in constructed wetlands is to promote microbial growth within media surfaces, and to assist the permeation velocity of the wastewater for pollutant treatment efficiency.

Several operational factors such as water depth, water level, and uneven bed surfaces can be used to control vegetation populations and manage colonization [97]. Hammer [98] indicates that the optimum plant species in constructed wetlands have the following functions: autogenous species suitable for regional climate and soil, resistance against pollution source, perennial and fast growth and easy production, good viewing location, and habitat to wildlife. Breen [99] found that routine plant harvesting may optimize the nutrient removal potential. However, Kadlec and Knight [22] pointed out that the regular harvest of plants from wetland treatment systems has not been successful in full-scale application due to high cost and low sustainability, even though many studies have shown that plant uptake is a significant route for nitrogen removal in constructed wetlands [100, 101]. To date, the relationship between the nitrogen in the plant rhizosphere and plant growth and harvest has not been convincingly demonstrated. In fact, the nitrogen uptake into the plant biomass is typically less significant because harvesting would remove only 5∼10% of the nitrogen [102]. Given the comparatively low nutrient content, plant biomass is commonly not harvested in Europe [103]. The experimental results of plant harvesting have shown very different results, case by case. Hence, more research is required on the optimal amount and the appropriate time of plant harvest to improve treatment performance.

4.4 Other factors

Another important regulating element of denitrification is diffusive transport from the aerobic water and substrate to the anaerobic substrate layers [104]. As such, wetland bathymetry, water depth variation and sediment type can all play a role in nitrogen removal.

The rates of nitrification and denitrification depend upon water pH, the presence or depletion of dissolved oxygen (DO), hydraulic loading rate (HLR), and the hydroperiod of the wetland. They could be limited seasonally because of the wide range of O_2 release, $0.02\sim12\,g/m^2/day$ by *P. australis* [105], $0.126\,mmol\,O_2/h$ g root dry mass by *Juncus ingens* [106], and $0.12\sim0.20\,mmol\,O_2/h$ g root dry mass by *T. latifolia* [107]. At low DO concentration, nitrification occurs in the aerobic zone, but denitrification occurs in the anoxic zone [22, 108].

The biofilm may improve the denitrification rates because periphytic algae provide a desirable carbon source for denitrifiers [109]. Under nutrient-rich conditions, well-developed periphytic biofilms show high rates of denitrification [110]. Thus, constructed wetland practitioners should consider the interaction between nutrient levels, hydraulic conditions, dissolved oxygen concentrations, and the quantity and function of the periphyton. It is necessary to provide suitable biotic environments for microbial communities. Consequently, in order to maintain and improve nitrogen removal and water quality in constructed wetlands, attention must be paid to factors that promote the growth rates of macrophytes and bacteria, such as planting depth, harvest of the hydrophyte flora, optimization of temperature, pH, dissolved oxygen concentration, and HRT.

5 Operation and management for optimal nitrogen removal

Optimization of the nitrogen removal process relies on finding the most suitable operational variables that will create maximum efficiency. The pivotal prerequisite to optimal biological wastewater treatment depends on understanding the microorganisms and plants involved and how they respond to diverse operational conditions. Many studies have been performed to elucidate species and structure of the related microbes by traditional microorganism cultivation techniques and biogenetical approaches for the improvement of nitrogen removal [111]. The biotechnological approaches using 16S rDNA-based molecular technologies, including PCR-denaturing gradient gel electrophoresis (DGGE), fluorescent *in situ* hybridization (FISH), clone libraries, and terminal restriction fragment length polymorphisms (T-RFLP), have demonstrated their suitability for monitoring the dynamics and organization of microbes in wastewater treatment systems [112].

In operating constructed wetlands, the operator can encounter some common problems: oxygen depletion, clogging or occlusion by sludge sediment accumulation, destruction of wetland layers, clogging of the filter media, difficult transplanting, and unidirectional circuit or short-circuit flow, *etc.* There are some new attempts to resolve all the operational obstacles, including setting up aeration devices to supply oxygen, special sludge-extracting equipment, efficient media development, and a pre-flow circuit establishing another pipeline at inflow and outflow [113].

Successful wetland performance depends primarily on the growth of macrophytes, wetland design, and operation and maintenance. Good operation and maintenance will prolong the lifetime of constructed wetlands. The most significant operational variables that can be utilized to influence treatment performance of constructed wetlands are water level and flow rate. The water level can affect HRT, atmospheric oxygen diffusion, and plant diversity. When water levels are reduced to their lowest mark during the summer or dry season, the water temperature is often elevated, maximizing plant productivity and oxygen diffusion rates [22]. Flow rates can influence hydraulic and pollutant loading, and can be controlled by pretreatment.

Maintaining the desirable plant density is a primary aim of operation and maintenance. Plant species can be selected based on various criteria [26]. Whilst a monoculture of plants may be desirable to achieve the optimal nutrient removal rates, greater diversity may provide a 'buffer' against changing local conditions, allowing the vegetation community to respond and adapt more readily.

Site-specific constraints, such as climate, the presence and/or lack of groundwater, the location, and surface water type, will all affect wetland operation and maintenance. Therefore, monitoring as an essential part of an operation and maintenance is required to assess the wetland performance.

Simulation-based analyses provide an approximation of the real system behavior. Considering the great uncertainty that still surrounds the wetland treatment mechanism, it is most prudent to interpret the simulation output based on information obtained from comparison of competing alternatives. Simulation-based design offers tremendous flexibility to the user. Under the circumstances, simulation approaches can contribute considerably to improve decision-making in the design and operation of constructed wetlands [114]. Simulation-based procedures have been used to acquire insight into the behavior of complex water resource systems.

Practically, constructed wetlands receive various wastewater loadings, random inputs of atmospheric moisture, and energy. Non-ideal mixing can cause large errors in rate constant estimation. Recently, there have been many tracer studies for treatment wetlands, but technical methods using tracers have seldom been applied in wetland data analysis and management. Instead, a constant-flow variant of the first-order model has been a universal option in data analysis and has been accepted naturally in constructed wetlands design [115]. It is thus required to develop verification processes for constructed wetlands designed using tracers.

6 Future research

The newly discovered Anammox and CANON processes offer significant potential for improved nitrogen removal efficiency and treatment performance in various aquatic systems; however, more research is needed to investigate and explore this process in constructed wetlands. Specifically, studies are needed to identify the growth conditions of Anammox bacteria and to determine design parameters for promoting the conditions.

Further research is also required on the predominant microbial species and hydrophytes having a specific gene for nitrogen removal, using biogenetical techniques through gene modification of microbial-planted systems in order to improve process performance and the efficiency of the nitrogen removal in constructed wetlands. Advances in this area may help to optimize nitrogen removal and may reduce the drawbacks and imbalances with the natural ecosystem.

To optimize nitrogen removal, the kinetics study, based on mass balance analysis for components of nitrogen transformation processes occurring within the constructed wetland treatment cells using the isotope-tracking ^{15}N technique, will provide mechanistic information for nitrogen transformations. This pattern of details will be necessary to develop the design and better management of aquatic environments.

Further investigations are needed to evaluate the sustainable removal performance by long-term monitoring for water quality, development of scale-up techniques, and an actual proof test based on the experimental results at pilot scale. Studies on these fields will contribute greater insights into the nutrients treatment process in constructed wetlands.

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Conflict of interest

The authors have declared no conflict of interest.

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