Chapter 12
Removal of Nitrogen Oxyanion (Nitrate) in Constructed Wetlands

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Abstract The increasing levels of nitrogen oxyanion pollution especially nitrate in water environments have become a critical issues of concern because of the potential risk on ecology and human health. Owing to its distinctive merits of sustainability, lesser operational and maintenance expenditure, the utilization of constructed wetland systems for the treatment of wastewater has turned out to be predominant worldwide. Its nitrogen oxyanion removal performance has received significant attention in the last two decades. This chapter presents a comprehensive outline of the application of constructed wetlands (CW) for nitrogen oxyanion removal from water

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and wastewater. The removal mechanisms and transformations of nitrogen are also discussed. In addition, the major factors that influence the removal performances in CWs are elucidated, especially the types of carbon sources commonly used, and how it affects the denitrification process. This chapter would be useful to engineers and researchers in the field of water and wastewater engineering.

Keywords Carbon sources · Constructed wetlands · Nitrate · Nitrogen pollution · Wastewater

12.1 Background

12.1.1 Nitrate in the Environment

Nitrate (NO_3^-) is one of the major generic forms of nitrogen oxyanions that exist naturally in moderate concentrations in different environmental media. The oxidation of nitrites (NO_2^-) majorly generates nitrates during nitrification process of the nitrogen cycle. The nitrogen cycle is the biogeochemical cycle by which organic protein from animals and plants origin is converted into ammonia (NH_3) and then NO_2^-, and NO_3^- in the environment. The transformations of the different nitrogen forms are carried out via physicochemical and biological processes (Fig. 12.1). Owing to its high solubility in water, the presence of NO_3^- has adverse effects on the environment, as it greatly accounts for the pollution of soil, surface water and the groundwater [1]. Several wastewater types such as urban drainage, landfill leachate, industrial and agricultural wastewater that contain nitrogenous compounds initiate undesirable phenomena (e.g. eutrophication and methemoglobinemia (i.e. blue baby syndrome)) when they are released into water bodies [2–4]. The concentrations of NO_3^- in these wastewaters vary from low to high, and thus, demand an appropriate technique for the removal. According to Rajmohan et al. [5], the usual NO_3^- level in polluted water ranges from 200 to 500 mg/L, based on the nature of the source (Table 12.1), but wastewater from nuclear industries contain up to 50,000 mg/L of NO_3^-.

Excess NO_3^-, discharged from the large-scale utilization of agricultural fertilizers, concentrated livestock feeding operations and disposal of partially treated sewage, that enters the groundwater, is among the priority pollutants of the groundwater system. Over 10,000 public water supply wells are estimated to have high levels of nitrate in the USA and thousands of wells were also ascertained with nitrate concentrations at or above the established health standards, across Western Europe and Asia [14]. Consequently, the maximum permissible concentration limit of NO_3^- in drinking water was set at 10 mg/L as nitrate-nitrogen ($NO_3–N$) by the US Environmental Protection Agency, while 50 mg/L NO_3^- was set by World Health Organization to address the concerns of methemoglobinemia in infants [14, 15]. High levels of NO_3^- is recognized to cause environmental and public health issues. The presence of this nitrogen oxyanion in water environments is a global challenge that needs urgent attention. To this end, various technological solutions, including electrodialysis, chemical
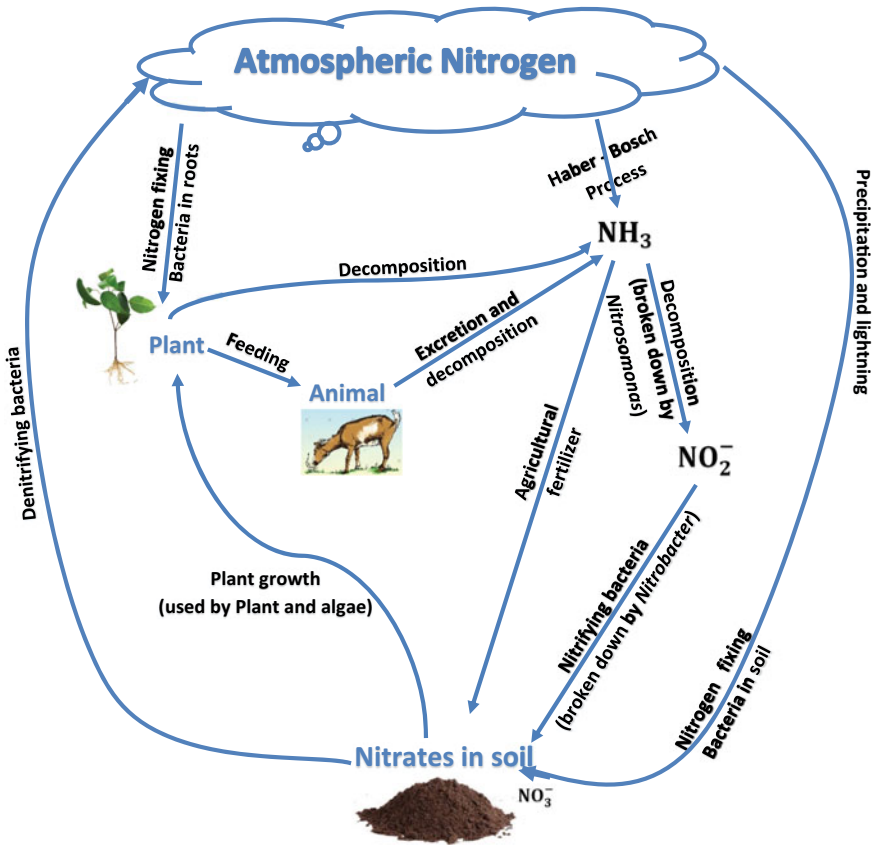
Fig. 12.1 Nitrogen cycle

Table 12.1 Nitrate levels at various sources as reported in the literature

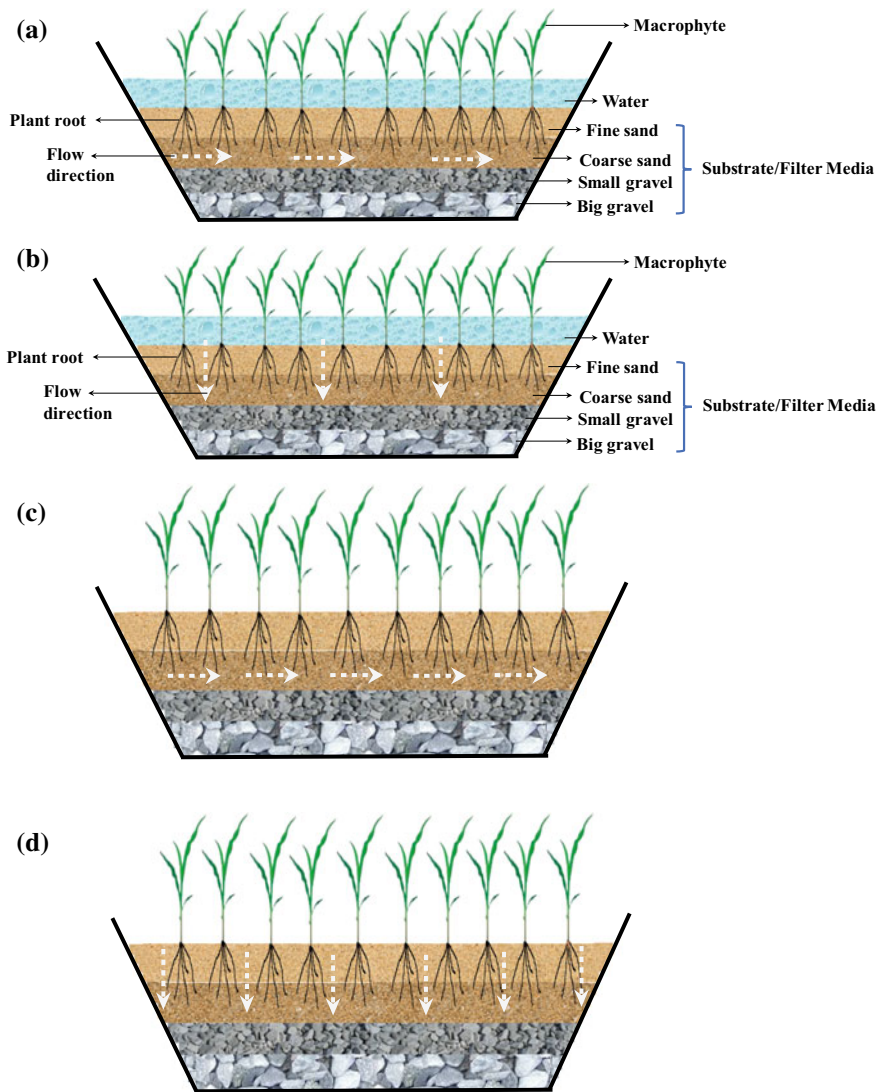| Wastewater source	Nitrate level (mg/L)	References
Domestic wastewaters/septic tanks	70–85	[6, 7]
Fertilizer, Diaries, metal finishing industries	200	[8]
Tannery, Pisa, Italy	222	[9]
Glasshouses waste	325	[10]
Brackish water	1000	[11]
Explosives factory, China	3600	[12]
Nuclear industry	50,000	[13]

Adapted from (Rajmohan et al. 2017)

reduction, membrane separation, adsorption, sequencing batch reactor, moving bed bioreactors, electrochemical denitrification, reverse osmosis; ion exchange, photo-catalytic degradation and membrane bioreactors have been developed to solve this menace [3, 16–27]. However, these technologies are always limited by their costly installation and high operational cost, secondary pollution, sludge production that need disposal, incomplete removal efficiency [28, 29]. In this chapter, constructed wetland (CW) systems, which are generally cost effective, simple, environmentally non-disruptive, ecologically sound, with relatively low maintenance cost, will be expounded in relation to NO_3^- removal.

12.1.2 Constructed Wetlands

Constructed wetlands, also referred to as treatment wetlands, are engineered systems that are designed and fabricated to treat several kinds of wastewater with relatively low external energy requirements and operationally simple technology and main-tenance (Fig. 12.2) [30–35]. Milani et al. [36] defined CW as a "sustainable and efficient solutions used around the world to treat wastewater as an alternative or a supplement to intensively engineered treatment plants". They are complex, inte-grated systems that involve the interaction of soil, water, plants, animals, microbes and the environment. The CWs have become an essential alternative wastewater treatment system since the method combines relatively high performance of pollu-tant removal with low maintenance and simple operation [37]. The CWs are planned methods designed and constructed to apply the natural procedures involving wetland vegetation, soils and the associated microbial assemblages to assist in wastewater treatment. It can effectively remove suspended solids, organic pollutants and nutri-ents from wastewater [38–40]. The CWs provide an inexpensive and reliable method for treating a variety of wastewaters such as sewage, landfill leachate, mine leachate, urban storm-water and agricultural run-off. This system of treatment is very efficient for nutrient removal and comparatively simple to construct, operate, maintain and suitable for advanced and polishing treatment if water reuse is an option [41]. The main NO_3^- removal mechanisms in wetlands are seepage loss, plant uptake and deni-trification [42] which are further expatiated in Sect. 12.2. Table 12.2 summarizes research studies on NO_3^- removal using CWs.

12.2 Nitrogen Transformation in Constructed Wetlands

As an ecological treatment technology, CWs have been largely utilized in recent decades in wastewater treatment plants. Before the arrival of CWs technology, conventional activated sludge-type wastewater treatment plants have been used for nitrogen removal but only minimal quantity is removed, via the consumption of the organic matter fraction of the wastewater.

(a)

Macrophyte

Plant root

Flow
direction

Water

Fine sand
Coarse sand
Small gravel
Big gravel

Substrate/Filter Media

(b)

Macrophyte

Plant root

Flow
direction

Water

Fine sand
Coarse sand
Small gravel
Big gravel

Substrate/Filter Media

(c)

(d)

Fig. 12.2 Constructed wetlands **a** Horizontal flow free water surface constructed wetland **b** Vertical flow free water surface constructed wetland **c** Horizontal sub-surface flow constructed wetland **d** Vertical sub-surface flow constructed wetland

In wastewater treatment operations, nitrate is removed through a process known as denitrification. This is a process, where organic and ammonia nitrogen is converted, through a process known as nitrification, to NO_3^- in the absence of oxygen (an anaerobic environment). The NO_3^- produced through nitrification is further reduced to nitrogen gas in this same anoxic environment, thus completing the denitrification process [65, 66]. This process is carried out by several range of autotrophic and

Table 12.2 Nitrate removal performance from various constructed wetland types

Source of WW	NO$_3$–N Influent (mg/L)	CW types	Macrophyte type	Substrate type	HRT (days)	Carbon source	NO$_3$–N removal (%)	References
Sullage	14.40 ± 0.23	HSSFCW	Water hyacinth (*Eichhornia crassipes*), *Commelina cyanea*, *Phragmites australis*	Sandstone, gravel	6–12	–	50–94	[30]
Aquaculture	8.50 ± 0.06	VDFCW	*Sacciolepsis Africana*; *Commelina cyanae*	Charcoal, fine sand and gravel	5	–	2.50 ±0.09	[43]
Synthetic NO$_3^-$	21–47	VUFCW	*Phragmite australis*, *Commelina communis*, *Penniserum purpureum*, *Ipomoea aquatica*, *Pistia stratiotes*	Fine sand	4.2	Fructose	60–97	[44]
Synthetic	22 ± 2.89	VUFCW	*Phragmite australis*, *Commelina communis*, *Penniserum purpureum*, *Ipomoea aquatica*, *Pistia stratiotes*	Fine sand	4.2	Fructose	70–99	[45]
Industrial	13	Hybrid CW (FWS-VF)	*Schoenoplectus validus*	Sand, gravel	6	–	78	[46]

(continued)

Table 12.2 (continued)

Source of WW	NO_3–N Influent (mg/L)	CW types	Macrophyte type	Substrate type	HRT (days)	Carbon source	NO_3–N removal (%)	References
Secondary effluent from WWTP	15.12 ± 1.99	ICWS	*Vetiver zizanioides, Coix lacrymajobi L*	Sand, Steel slag, Peat	10	–	76	[47]
NO_3^- contaminated groundwater	12 ± 0.5	Hybrid (unsaturated VF – HSSFCW)	*Phragmites australis*	Cork, granitic gravel	2	–	80–99	[48]
Synthetic	20	VFCW	*Iris pseudacorus*	Iron scraps, biochar, sand, gravel	3	Sucrose	87	[49]
Synthetic	12.01 ± 1.32	HSSFCW	*Canna indica L*	Sand, gravel	5	–	83	[50]
Synthetic agricultural run-off	7.4	FWSCW	–	Hydrilla verticillate—GS, PS, gravel; Vallisneria—GS, PS, gravel	–	Glucose	90–100	[51, 52]
Synthetic	50	VFCW	*Acorus calamus L*	Gravel	3	SCSs; SCSs combined with NZVI; Without SCSs and NZVI	47–91	[53]
Synthetic	65/100/150	VFCW	-	Peat soil	–	–	49–83	[54]
Domestic	8.6	VFCW	Water hyacinth (*Eichhornia crassipes*),	hydroponic	–	–	100	[55]

Table 12.2 (continued)

Source of WW	NO$_3$–N Influent (mg/L)	CW types	Macrophyte type	Substrate type	HRT (days)	Carbon source	NO$_3$–N removal (%)	References
Synthetic	10.83 ± 1.61	IVCW	*Arundo donax, Canna indica*	Gravel	2.4	–	59–79	[56]
Hospital	0.9 ± 0.2(dry) 1.1 ± 0.5 (rainy)	HSSFCW	*Cattails (Typha domingensis), Cyperus papyrus, dark green bulrush (scirpus atrovirens) and sugar cane (Saccharum officinarum)*	Brick, gravel	4	–	80 (dry season) 81 (rainy season)	[57]
Domestic	14.4 ± 1.6	HSSFCW	*Cyperus papyrus, Canna indica, Hedychium coronarium*	Porous stone, sand, tepezil	3	–	44	[58]
Synthetic	10–30	VFCW	*Canna indica L*	Quartz sand, Sponge iron particles (S-Fe^0)	0.25–0.5	Glucose	16–87	[59, 60]
Synthetic	100	HSSFCW	*Phragmites australis, Typha angustifolia*	River gravel, breakstone	–	Rice husk, glucose	96	[61]
Synthetic	10.04 ± 1.29	VUFCW	*Typha orientalis*	Ceramsite	5	PHBV	93–99	[62]
Synthetic	15 (TN)	EFTW	*Canna indica L*	Styrofoam floating mat	7	Glucose	83–98	[63]

(continued)

Table 12.2 (continued)

Source of WW	NO$_3$–N Influent (mg/L)	CW types	Macrophyte type	Substrate type	HRT (days)	Carbon source	NO$_3$–N removal (%)	References
Municipal	5.7 ± 2.4	VPSCW	*Canna hybrids*, *Zantedeschia aethiopica*	Tezontle	–	–	82–84	[64]

HRT: Hydraulic retention time; WW: Wastewater; WWTP: Wastewater treatment plant; VDFCW: Vertical downward flow constructed wetland; FWSCW: Free water surface constructed wetland; ICWS: Integrated constructed wetland system; VPSCW: Vertically partially saturated constructed wetland; EFTW: Enhanced floating treatment wetland; VF: Vertical flow; VFCW: Vertical flow constructed wetlands; GS: Granitic sulphur; PS: Pretreated soil; HSSFCW: horizontal sub-surface flow constructed wetland; VUFCW: vertical upward flow constructed wetland; SCSs: Solid carbon sources; IVCW: Integrated vertical constructed wetlands; PHBV: Poly(3-hydroxybutane-hydroxyvalerate)

heterotrophic facultative anaerobic bacteria, which are capable of utilizing NO_3^- (and NO_2^-), under anoxic conditions, as an electron acceptor [67]. Some of these bacteria include *Pseudomonas, Micrococcus, Bacillus, Paracoccus denitrificans and Achromobacter.* For better nitrogen removal, an external organic carbon source is needed to act as an electron donor in the respiratory chain [38], and CWs are a better option to achieving this.

Although the eutrophication and the toxic effects of NO_3^- on aquatic organisms of both vertebrate and invertebrate species are sources of concerns [68], it also boosts plants' growth, which sequentially promotes the environmental biogeochemistry in the wetlands. The circulation of nitrogen in wetlands involves composite processes, while very straightforward chemical conversion of this element still poses a great task in environmental engineering. Such processes, which include bacterial actions, plant/microbial uptake, adsorption (interaction between ionized NH_3 and the media in sub-surface horizontal flow, (SSHF) CWs), and volatilization (i.e. transformation of aquatic NH_4^+ to gaseous NH_3, within the operating pH regime of the surface flow CW), mostly achieved nitrogen removals in wetlands [68–70].

Nitrogen transformation involves some processes and mechanisms, which lead to the transference of wetland nitrogen from one point to the other without any consequential molecular alteration [69]. As earlier noted, the physical processes of management of nitrogen oxyanion in CW include, settling of particles and re-suspension, dissolution and diffusion, plant translocation, litterfall, volatilization and sorption [68, 69]. Generally, nitrogen oxyanion removal in CW occurs through two processes that include biological and physicochemical treatment processes. The five major biological treatment process include denitrification, nitrification, ammonification (mineralization), assimilation and decomposition [69, 71, 72]. The physicochemical processes include, sedimentation, NH_3 stripping, breakpoint chlorination and ion exchange [70, 73]. It was suggested that low oxygen and organic matter contents in the root zone offers restriction to nitrification and denitrification processes [69]. However, an integration of partial nitrification and anaerobic NH_4^+ oxidation has equally been recommended to be resourceful in removing nitrogen from constructed wetlands. This is largely due to the autotrophic nature of anaerobic ammonia oxidation (Anammox) process, in which NH_4^+ is completely converted into nitrogen gas in the presence of NO_2^- and without the addition of organic matter [69].

12.2.1 Ammonification

The ammonification refers to the process by which the organic nitrogen fraction is transformed to NH_3, through a biological process [71]. The first stage of nitrification in sub-surface flow CW (SSFCW) systems is initiated by ammonification, if the inbound wastewater is highly loaded with organic nitrogen [74]. This biochemical process, where the amino acids fractions are exposed to oxidative deamination yielding NH_3 is acomplex and exergonic process, (Eq. 12.2.1) [74, 75].

$$\text{Amino acids} \;\rightarrow\; \text{Imino acids} \;\rightarrow\; \text{Keto acids} \;\rightarrow\; \text{NH}_3 \tag{12.2.1}$$

Since the process of occurrence decreases with depth, it shows that ammonification is quickest within the upper zone of the wetlands, where the aerobic condition is prominent. It is time-consuming within the lower zone, where the environment moves from facultative anaerobic condition to obligate anaerobic condition [71, 76]. In CWs, the inorganic ammoniacal-nitrogen is mostly removed by nitrification–denitrification processes, but ammonification kinetically progresses faster than nitrification [71]. Kadlec and Knight [70] suggested that the ammonification process progresses quicker in higher temperature, doubling the rate with a temperature rise of 10 °C. The pH range observed to be ideal for ammonification is 6.5–8.5 [74, 77, 78]. The ammonification process is therefore generally affected by pH, temperature, carbon-to-nitrogen (C/N) ratio, soil structure and available nutrient [76]. Furthermore, processes such as adsorption, plant uptake and volatilization are suggested to be resourceful in ammonia–nitrogen removal [38], though the effectiveness of nitrification–denitrification processes is, in general, suggested to be the most resourceful in NH_4^+ removal [71].

12.2.2 Nitrification

Nitrification is the major transformation mechanism by which the level of ammonia nitrogen is reduced. This reduction is achieved through the conversion of the ammonia nitrogen into oxidized form of nitrogen (i.e. NO_2^- and NO_3^-). Graaf et al. [79] defined nitrification as the biological formation of nitrate or nitrite from compounds containing reduced nitrogen with oxygen (O_2) as their terminal electron receptor. Lee et al. [71] defined it as the chemolithoautotrophic oxidation of NH_3 to NO_3^- in the presence of adequate O_2, occurring in two successive oxidative steps, namely ammonia oxidation (NH_3 to NO_2^-) and nitrite oxidation (NO_2^- to NO_3^-), carried out by nitrifying bacteria. These bacteria use NH_3 or NO_2^- as an energy source, O_2 as the terminal electron recipient and carbon dioxide as the carbon source [71]. The first stage is the oxidation of NH_3 to NO_2^-, by ammonium oxidizing bacteria such as *Nitrosomonas* or *Nitrospira* or *Nitrosococcus* (Eq. 12.2.2) [71, 74].

$$\text{NH}_4^+ + 1.5\text{O}_2 \xrightarrow{\text{Nitroso - genus}} \text{NO}_2^- + \text{H}_2\text{O} + 2\text{H}^+ \tag{12.2.2}$$
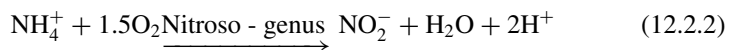
The above first stage is succeeded by the second stage which is the oxidation of NO_2^- by nitrite-oxidizing bacteria such as *Nitrobacter* or *Nitrospira*. The second stage is described by Eq. 12.2.3 [71, 74].

$$\text{NO}_2^- + 0.5\text{O}_2 \xrightarrow{\text{Nitro - genus}} \text{NO}_3^- \tag{12.2.3}$$

The oxygen consumption of nitrification process is estimated to be 3.16 mg O_2 per mg $NH_4 - N$ oxidized, and 1.11 mg O_2 per mg $NO_2 - N$ oxidized, while *Nitrosomonas* and *Nitrobacter* produce 0.15 mg cells per mg $NH_4 - N$ oxidized and 0.02 mg cells per mg $NO_2 - N$ respectively [71]. Furthermore, alkalinity is necessary as 7.07 mg $CaCO_3$ per mg $NH_4 - N$ oxidized [80]. The acid formation (i.e. low pH value) during nitrification process causes alkalinity reduction and a deep reduction in pH [68, 80–82], and a swift decline in the nitrification rate below the neutral pH value [81]. Hence, it is important to replenish the alkaline level with lime during the process, when there is a drop in alkalinity [80]. Though nitrification is basically attributed to chemoautotrophic bacteria, it is suggested that heterotrophic nitrification takes place, which can be significant [68]. Aside from autotrophic nitrification, heterotrophic nitrifying bacteria are also capable of producing $NO_3 - N$. Some of these species (in bacteria, algae and fungi) are *Actinomycetes, Arthrobacter globiformis, Aerobacter aerogenes, Bacillus, Mycobacterium phlei, Streptomyces griseus, Theosphaera and Pseudomonas* [38, 74, 83]. Gerardi [83] affirmed that although these heterotrophic nitrifiers are resourceful, the nitrification rates achieved by *Nitrosomonas* and *Nitrobacter* groups are significantly greater (relatively greater by 1000 to 10,000 times) [74]. However, owing to constraints against nitrification and denitrification processes, offered by low oxygen and organic matter concentration in SSF, it has been affirmed that a combination of partial nitrification and Anammox is a resourceful means of removing nitrogen from CWs [69]. Moreover, since the Anammox process is autotrophic, the transformation of NH_4^+ to nitrogen could be possible without adding organic matter [69].

12.3 Denitrification

Kadlec and Wallace [68] defined denitrification as the process by which NO_3^- is transformed to dinitrogen (N_2) via intermediates such as NO_2^-, nitric oxide, and nitrous oxide, and finally nitrogen (Eq. 12.2.4). The denitrification process is also called NO_3^- dissimilation, and it is accomplished by facultative heterotrophic organisms that can use NO_3^- as the terminal electron receptor, and organic carbon as an electron donor under anoxic condition [71]. During the transformation, inorganic nitrogens such as NO_2^- and NO_3^- are usually reduced to harmless nitrogen gas by denitrifying bacteria [71, 84, 85]. Some denitrifiers require organic substrates to get their carbon source for growth and evolution, whereas others use inorganic substances as their energy sources and CO_2 as their carbon source [86]. Therefore, denitrifying bacteria are categorized into two main species, namely autotrophs and heterotrophs [71]. However, earlier studies have focussed on the heterotrophic denitrification process, due to its frequency in conventional wastewater treatment plants [71, 87], while the autotrophic denitrification process started gaining attention in recent studies [88–94].

Moreover, denitrification is led by some heterotrophic microorganisms like *Pseudomonas, Micrococcus, Achromobacter and Bacillus,* under anaerobic or low-oxygen conditions. Denitrificating microbes can be grouped as: organotrophs (e.g.

Pseudomonas, Alcaligenes, Bacillus, Agrobacterium, Flavobacterium, Propionibacterium and *Vibrio*), chemolithotrophs (e.g. *Thiobacillus, Thiomicrospira, Nitrosomonas*), photolithotrophs (e.g. *Rhodopsuedomonas*), diazotrophs (e.g. *Rhizobium, Azospirillum*), archaea (e.g. *Halobacterium*) and other microorganisms such as *Paracoccus* or *Neisseria* [68]. The fraction of total nitrogen removal through denitrification is normally 60–95%.

$$2NO_3^- \rightarrow 2NO_2^- \rightarrow 2NO \rightarrow 2N_2O \rightarrow N_2 \qquad (12.2.4)$$

12.3.1 Assimilation Process of Nitrogen

The uptake of nitrogen by plants or microbes is regarded as the assimilation process. Masclaux-Daubresse et al. [95] and Xu et al. [96] asserted that the usage of nitrogen by plants encompasses numerous stages, including uptake, assimilation, translocation and, when the plant is ageing, recycling and remobilization. The assimilation process occurs via the formation of organic nitrogen compounds such as amino acids from inorganic nitrogen compounds available in the environment. Organisms like plants, fungi and specific bacteria that cannot fix nitrogen gas (N_2) rely on the ability to assimilate NO_3^- or NH_3 for their needs. Animals also depend fully on the organic nitrogen form for their food. Several studies have affirmed the significance of the removal of NH_3 from water by wetland plants [97–104]. However, many of these studies are commonly seen to portray the measurement of gross nitrogen uptake, without deduction for consequential losses due to plant death and decomposition, with associated leaching as well as re-solubilization of nitrogen [68].

For nitrogen removal from the wetland water, the attention is usually on the net influence of the macrophytes (macroflora) on the water phase concentrations [68]. When discussing plant uptake as a process of nitrogen removal from wetland water, terms such as phytomass (the totality of vegetative materials, living and dead), biomass (all living vegetative materials) and necromass (all dead vegetative materials) are often used [68]. Macrophytes are vital in enhancing nitrogen removal from wetlands due to their functions such as providing surfaces and O_2 for the growth of microbes within the rhizosphere, thus improving nitrification [99, 105–107], and providing carbon from root secretions (due to photosynthetically fixed carbon, within a range of 5–25% C), enhancing organics removal and denitrification process [97, 108–111]. Various relative researches between unplanted and planted wetlands indicated good nitrogen and organics removal, with the latter yielding more significant results, hence indicating the necessity of macroflora for enhancing nitrogen removal operations in CWs [74].

Inorganic nitrogen forms are usually transformed into organic compounds through the uptake of NH_3 and NO_3^- by macrophytes. This serves as the building blocks for cells and tissues [78]. The ability of rooted plants to utilize sediment nutrients partly describes their massive yield in comparison with planktonic algae in many

systems [112]. Different plant species have varying ability in their ideal nitrogen forms absorbed, and the nutrient concentration of plants tissues also influences the uptake and storage rate of nutrient [71]. However, NH_4^+ preference is conventional in macroflora within NH_4^+ -rich environments where restricted nitrification occurs [113]. In general, the uptake of nitrogen by plants varies along with system configurations, loading ranges, type of wastewater and environmental conditions [74]. In nitrogen removal, plants contribution is affirmed to be about 0.5–40.0% of the total nitrogen removal [74, 103, 104]. Plant biomass accumulates 60% of total nitrogen thus, enhancing nitrogen removal significantly [103]. For efficient nutrient assimilation and storage, plants with features such as high tissue nutrient content, rapid growth and ability to achieve high-standing crops are preferably desired. On the contrary, plants with immense biomass accumulation during autumn and winter have a likelihood of releasing a considerable amount of their stored nitrogen back into the water during the winter season [38]. Brodrick et al. [114] equally suggested that decaying plant materials could also raise the concentration of nutrients in the effluent through leaching [74].

Some selected plants have been employed in constructed wetlands; however, *Phragmites australis* remains the most typical plant used in SSFCW due to its capability to pass O_2 from its leaves through the stems and rhizomes and out of from its fine hair roots into the rhizosphere [115]. Reports from literature about the ability of the plant to convey oxygen (thereby fostering microbial conversion and nitrification) express various illustrations [74, 116–118]. Armstrong et al. [116] noted O_2 release (per unit wetland area) by phragmites species to be in the range of 5–12 g O_2 per square metre per day, while the O_2 release by phragmites in a study by Brix and Schierup [117] gives a record of only 0.02 g O_2 per square meter in soil substrate. The oxygen released by phragmites species recorded by Bavor et al. [118] is about 0.8 g O_2 per square meter in gravel substrate [74]. Figure 12.3 represents the major typical routes for nitrogen removal in SSFCWs.

12.4 Factors Affecting Nitrogen Removal Efficiency in CWs

Nitrogen oxyanion removal efficiency, especially NO_3^-, in CWs has been discussed to involve various biological and physicochemical processes. Therefore, various environmental factors are bound to affect the efficiencies of these processes, thereby limiting the oxyanion removal efficiency. Some of such factors include pH, temperature, hydraulic residence time (HRT), NO_2^- concentration, oxygen concentration, vegetation type (wetland plant species) and density, activity of microorganism, distribution of wastewater, climate, and attributes of influent[71, 74, 119–122]. It should be noted that most of these factors are interdependent; hence, a variation of one factor often leads to a consequent change in other factors [121]. Furthermore, Kuschk et al. [123] stressed that the two major factors affecting the nitrogen removal from CWs are temperature and HRT [71]. The following subsections give a concise analysis of the key factors influencing nitrogen removal efficiency in CWs.
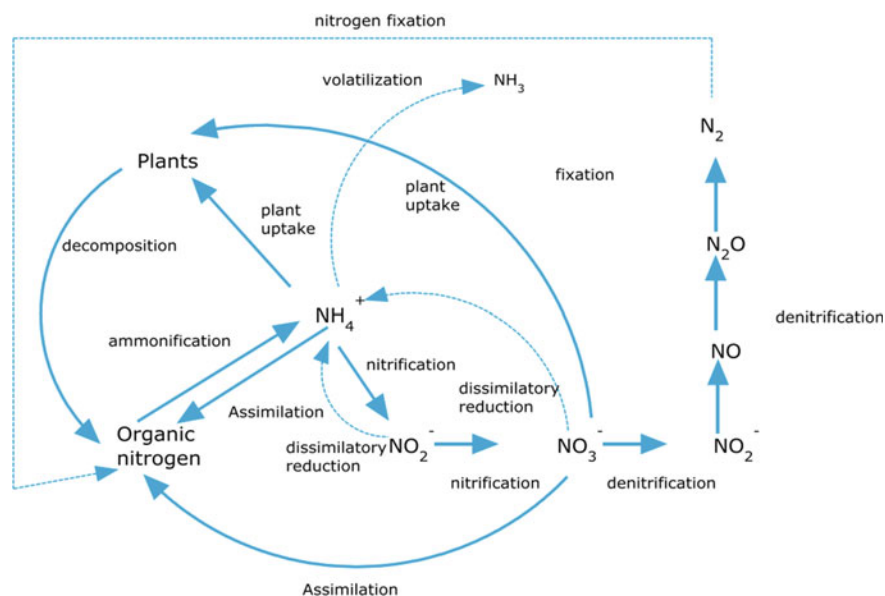
Fig. 12.3 Key classical nitrogen removal routes in sub-surface flow wetlands [74]

12.4.1 Carbon Source

Amidst all the aforementioned factors, the carbon source is one of the known dominant external factors which has disreputed the nitrogen oxyanion removal efficiency of CWs [56, 124]. Other factors to be considered in the choice of the carbon source include cost, handling and storage safety/stability, denitrification rate, degree of utilization, kinetics, sludge production, the content of unfavourable/toxic compounds. A commonly used carbon source, which is readily available, and with a high denitrification rate is methanol. Other closely related examples are ethanol and acetic acid. Although the nitrogen removal efficiency, using the methanol carbon source is desirable, the existence of NO_2^- accumulation in wastewater with high NO_3^- concentration often results in bacteria growth suppression [125]. Furthermore, the danger of overapplication of easily biodegradable materials of these liquid carbon sources through aerobic degradation can adversely impact nitrogen oxyanion removal [126]. Considering the aforementioned shortcomings, plant-based carbon sources have been considered [124, 127], and used to treat different wastewater, such as domestic sewage, agricultural run-off and industrial effluent. At present, most of the denitrifying bacteria in CWs are heterotrophic which require organic carbon sources for the substantive effect on denitrification process for nourishment and NO_3^- reduction [128]. In a study by Zhao and Chen [129], it was discovered that ammonia nitrogen, nitrous nitrogen and total nitrogen (when alkali-treated corn stover was used as additional carbon source material) were removed in the upper and middle layers, while nitrate is removed mainly at the bottom layer. Xiao et al. [130] showed

the addition of solid carbon source to the vertical flow CW. In the system, there was almost 100% nitrification reaction, when the addition position of the carbon source was at the lower layer, thus giving the total nitrogen removal rate at the highest.

The type of exogenous carbon sources that are widely used for CWs are of three classes, including natural organic matters, low molecular carbohydrates and biodegradable macromolecule polymers.

12.4.1.1   Natural Organic Matters

Plant is a naturally degradable material that is rich in lignin, cellulose, hemicellulose and many more. In CWs, it is the most vital composition because of its ability to absorb nitrogen as nutrient and also to provide suitable environment for nitrification and denitrification [131]. In recent years, the application of natural material (especially plant biomass) as a carbon source for maximum nitrogen removal efficiency has gained substantial ground in CWs, because of the economic viability and practicability [132]. Their effects in nitrogen oxyanion removal vary, due to the diversity in the composition of lignin, cellulose, hemicellulose and other components in plants. Some natural organic matters (mostly plant biomass) have been studied to assess the effectiveness and efficiency of plant carbon source in CW denitrification rate [133] (Table 12.3). Conversely, these natural materials (especially for plant biomass) have some demerits, which include unstable carbon supply and discharge of coloured matter [134], which sometimes affect their applications.

12.4.1.2   Low Molecular Carbohydrate

Low molecular organic carbon sources have some desirable properties, which have also gained them recognition as an external carbon source. They are rich in carbon, which can easily be used up during decomposition. If classified in terms of physical form, they are liquid organic substances, which are termed liquid carbon sources. Examples are glucose [142–144], fructose [44], ethanol, methanol [145–147] and acetic acid [148].

12.4.1.3   Biodegradable Macromolecule Polymers

In recent time, a wide range of external carbon sources, which by the physical classification are solid organic substances, were checked in some laboratory studies, to function as physical support for biofilm formation in solid-phase denitrification system [149]. Some of the polymers were even blended. Examples of these polymer/polymer blends used so far are polybutylene succinate [150], polycaprolactone [151, 152], polyhydroxyalkanoates [153], polyvinyl alcohol [154], starch [155], starch/polyvinyl alcohol [156], poly(3-hydroxybutyrate-co-3-hydroxyvalerate)/poly(lactic acid) [157] and PHBV/starch [149].

Table 12.3 Plant biomass used as carbon source in CWs and their denitrification rates and removal efficiencies. Adapted from [133]

Carbon Source	Wastewater Treatment	Denitrification Rate (g m^{-3} d^{-1})	NO$_3^-$ − N		Removal efficiency (%)	References
			Influent (mg L^{-1})	Effluent (mg L^{-1})		
Wheat straw	Drinking water	32–53	≈ 20	0	100	[135]
Sawdust	Groundwater	0.24–3.36	NA	NA	>95.0	[136]
Wheat straw	Simulated sewage	NA	200	>170	>15	[121]
G.*verrucosa*	Simulated sewage	13.2	100	0	100	[137]
Giant reed	Simulated sewage	3.36	100	0	100	[137]
Liquorice	Synthetic brackish water	20.64	100	0	100	[138]
Giant reed	Synthetic brackish water	85.92	100	13	87	[138]
Giant reed	Synthetic brackish water	101.52	100	0–13	87–100	[138]
Cotton wool	Aquaculture wastewater	NA	>200	<10	>95	[139]
Pine bark	Landfill leachates	33.6	600	0	100	[140]
Pine woodchip	Simulated sewage	2.4	17.2	11.6	32.6	[141]
Maize cobs	Simulated sewage	6.24	17.2	4.9	71.5	[141]
Wheat straw	Simulated sewage	4.56	17.2	8.9	48.3	[141]
Green waste	Simulated sewage	5.04	17.2	5.8	66.3	[141]
Sawdust	Simulated sewage	4.32	17.2	8.6	50.0	[141]
Eucalyptus	Simulated sewage	3.6	17.2	10.3	40.1	[141]
Maize cobs	Municipal portable water	19.8	141	0	100	[119]

Table 12.3 (continued)

Carbon Source	Wastewater Treatment	Denitrification Rate (g m^{-3} d^{-1})	NO$_3^-$ − N		Removal efficiency (%)	References
			Influent (mg L^{-1})	Effluent (mg L^{-1})		
Wheat straw	Municipal portable water	10.5	141	NA	NA	[119]
Softwood	Municipal portable water	5.8	141	NA	NA	[119]
Hardwood	Municipal portable water	3.0	141	NA	NA	[119]

NA: No data available

Hitherto, there have been some investigations of denitrification performance and microbial community structure in both liquid and solid carbon sources supported denitrification systems but their differences are scarcely studied. Srinandan et al. [158] reported that both liquid and solid organic carbon sources influence the nitrate removal activity, biofilm architecture and community structure although molecular weight and chemical structure of the biodegradable polymers were generally higher and more complicated when compared with the liquid carbon sources. Furthermore, denitrification performance and microbial diversity using starch/PCL and ethanol as an electron donor for nitrate removal were also investigated through comparison. The outcome revealed that the ethanol system displayed a higher denitrification rate while the blended starch/PCL system had richer microbial diversity [159].

Generally, when blended polymers and other external carbon sources were utilized, they yielded a good denitrification effect [160, 161] but the water of the solution of the blended polymer carbon source took some ample of time, causing some lag period. In addition, the morphology of the blended materials, surface properties and particle size posed great influence on the denitrification rate, with its biodegradability and denitrification performance decreasing with increasing molecular weight [162]. Thus, polymer/polymer blended carbon sources are not commonly used because of some factors such as high market price and slow release of carbon sources which requires a lot of time.

12.4.2 Selected Operating Parameters

12.4.2.1 pH

It has been established that nitrification process consumes alkalinity. Vymazal [38] affirmed that a pH >8.0 is capable of decreasing nitrification and denitrification

processes to an insignificant level, with denitrification process occurring slowly at pH 5. Moreover, previous studies have suggested that high pH leads to a decline in dissolved oxygen (DO) in substrate [163], thus influencing nitrification and denitrification processes [32]. Also, some studies suggested that pH <6.0 and >8.0 hinder denitrification [32, 38, 81, 163], while the peak rate is observed at a pH range 7.0–7.5 [74, 164].

12.4.2.2 Temperature

Temperature is a significant environmental factor that controls the solid-phase denitrification process by hindering the activity of the associated enzymes in both hydrolysis of the solid substrate and reduction of NO_3^- [165]. In other words, temperature affects both microbial activities and diffusion rate of O_2 in constructed wetlands [166]. A temperature range between 16.5 and 32 ℃ is favourable for nitrification in CWs [74, 167], while the most efficient removal occurs at temperature that ranged between 20 and 25 °C [164, 166]. The nitrification- and denitrification-associated microbial activities decreased significantly at temperatures below 15 °C and above 30 °C [123].

Many studies have investigated the activities of denitrifiers in CW sediments during various climatic conditions and found that their activities are generally more robust in spring and summer than in autumn and winter [74, 168–171]. Oostrom and Russell [172] affirmed that, in general, the degree of removal of NO_3^- is greater around summer than during winter [71]. Denitrification is usually believed to terminate at temperatures below 5 °C [71]. In soils, the optimal temperature limits for nitrification and ammonification are 30–40 °C and 40–60 °C, respectively [38].

12.4.2.3 Hydraulic Residence Time and Hydraulic Loading

Hydraulic residence time (HRT) is an important factor in nitrogen removal. The nitrogen removal efficiency is highly influenced by the flow condition and the residence time [4]. An increase in wastewater residence time leads to an intense decrease of ammonium and total Kjeldahl nitrogen concentrations in treated effluent [71]. This is because of the lengthier time of contact of nitrogen pollutant with microorganisms that gives advantage to the microbe to play a significant catabolic activity [74]. Lee et al. [71] also stated that lengthier HRT is necessary in nitrogen removal from wetlands than for BOD and COD removal. An eight-day HRT at a temperature above 15 °C is needed in SSFCWs [173]. However, if anaerobic conditions dominate in the wetlands, there is likelihood that an increase in HRT will not facilitate NO_3^- removal [174]. About 3–4 h HRT is required when NO_3^- concentration is not more than 40 mg L^{-1} and a minimum HRT of 6 h is necessary when NO_3^- concentration is more than 70 mg L^{-1} [175]. Hydraulic loading is also important in this regard, especially in SSFWs. Saeed and Sun [74] affirmed that the greater the hydraulic loading the faster the passage of wastewater through the media.

12.4.2.4   Dissolved Oxygen (DO)

As earlier discussed, most denitrifiers are facultative anaerobic organisms that use nitrate as a terminal electron recipient in the absence of oxygen or under anoxic condition. DO is a great and energetic electron recipient, and for that reason, it exhibits direct competition or inhibition of enzymes, which consequently results to suppression of the denitrification process [165]. Denitrification could happen at DO concentration to the level of 4.0–5.0 mg L^{-1} [165], though the denitrification rate declined with increase in DO levels [153]. Furthermore, it is vital to note that the presence of DO promotes upsurge in carbon source consumption as a portion of predisposed organic carbon is used up by aerobic respiration instead of denitrification [153, 176]. Since enzymatic actions in reducing nitrate can be inhibited by DO, there may occur nitrate accumulation [165]. The lower the oxygen concentration, the higher the denitrification becomes [177].

Denitrification rate of *Diaphorobacter nitroreducens* strain NA10B decreased as the DO concentration increased, when using poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) powders as carbon substrate, even when more than 3 mg $NO_3 - N\ g^{-1}\ h^{-1}$ is maintained under complete aerobic conditions [178]. Gutierrez-Wing et al. [153] reported that the denitrification rate decreased from 5.5 to 0.5 g $NO_3 - N\ L^{-1}\ d^{-1}$ when the DO concentration increased from 0.5 to 4.0 mg L^{-1} in a circulating aquaculture water system filled with polyhydroxybutyrate (PHB). At the DO levels of 4–5 mg L^{-1}, a least denitrification rate of 0.18 g $NO_3 - N\ L^{-1}\ d^{-1}$ was noticed for 6 days and thereafter declined to zero. Xu et al. [179] also reported that the nitrate removal increased to more than 85% with increasing DO levels in the influent from 1.5 to 4.0 mg L^{-1}, and decreased to 50% at DO levels > 4.0 mg L^{-1} in a solid-phase denitrification system, using corncobs as carbon source. Wang and Chu [165] thus suggested that controlling the DO levels in the denitrification reactor appeared to be needless, but then it could promote the efficiency of the process.

12.4.3   Vegetation Type

Macrophytes (also known as macroflora, phytoremediators, hydrophytes, wetland plants and aquatic plants) are those plant species naturally found thriving in wetlands of all sorts, either in or on the water. They play a significant role in CWs and have been extensively used for decontamination of water bodies. For instance, their roots provide surface areas for microbial activities and aerobic zones in the wetlands. The rhizosphere is the most active reaction zone in a CW as it promotes the relationship benefits that exit amongst plants, microbes, soil and contaminants, thereby enhancing physical and biochemical processes [71]. Studies revealed that parts (the above-ground and below-ground) of the macrophytes enhanced microbial diversity and offer enormous surface areas for biofilm development which is accountable for the majority of the microbial activities occurring in the CWs [180, 181]. The categories of macrophytes commonly used in CW are emergent plants (Arundo donax L.,

Juncus spp., Phragmites spp., Typha spp., Iris spp., and *Eleocharis spp),* submerged plants (*Myriophyllum verticillatum, Hydrilla verticillata, Ceratophyllum demersum, and Vallisneria natans*), floating leaved plants (water spinach (*Ipomoea aquatica*), water lettuce (*Pistia stratiotes*) *Nymphaea tetragona, Nymphoides peltata, Trapa bispinosa and Marsilea quadrifolia*), free-floating plants (Water hyacinth (*Eichhornia crassipes*), *Lemna minor*, *Hydrocharis dubia* and *Salvinia natans*) and other large wetland grass-like plants like Bulrushes (e.g. *Scirpus luviatilis*, *Scirpus validus*, *Scirpus cyperinus*). It has been substantiated that planting of more than one species of macrophytes enhances the removal performance of CWs because the presence of diverse kind of plant species offers a more favourable microbial activities and longer retention time [182, 183]. For optimum treatment efficiency and favourable CW design, a detailed understanding of plant species, uniqueness of microorganism groups, and the associations between biogenic matters and particular components in contaminants are required.

12.5   Conclusion

Nitrate pollution remains a vital problem in the pursuit of environmental sustainability in water environments. This chapter has shed light on the viable means of treating nitrate contaminated water using an ecologically based technology called constructed wetland. CWs have been proved to be a beneficial and promising technique in wastewater treatment because of their low-cost, environmental quality preservation and easy maintenance. This chapter also summarizes several factors responsible for nitrogen removal in CW treatment systems from water and wastewater, including the various transformations of nitrogen with a focus on nitrogen oxyanion (nitrate).

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