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EVALUATION OF NITRIFICATION PROCESS IN CONSTRUCTED WETLANDS: A REVIEW ON NOVEL BIOLOGICAL NITROGEN REMOVAL PROCESSES

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Abstract: Constructed wetlands attracted the attention of researchers as a sustainable, economic and efficient wastewater treatment technique. Many papers showed the efficient performance of constructed wetlands to treat municipal, industrial, livestock, petroleum and other types of wastewater, effectively removing organic matters, phosphate, nitrogen and contaminants of emerging concern such as pharmaceuticals and antibiotics. There have been numerous reviews in the literature that studied nitrogen removal in constructed wetlands from different perspectives. However, the majority are concerned about the conventional nitrification process. It is worth mentioning that some biological nitrogen pathways other than the conventional nitrification process were implemented in constructed wetlands efficiently such as partial nitrification and denitrification, simultaneous nitrification and denitrification, anaerobic ammonium oxidation and completely autotrophic nitrogen removal over nitrite which have been reviewed in this study. The outcomes of this study showed that anaerobic ammonium oxidation is the most common pathway applied in constructed wetlands. Moreover, this review showed that the efficient performance of these novel pathways is constrained by the difficulty of controlling the operating parameters such as dissolved oxygen, temperature and pH.

Keywords: *Constructed wetlands; nitrification; ammonia; total nitrogen.*

1. Introduction

Historically, conventional centralized wastewater treatment facilities have been utilized successfully in urban areas. However, many economic and technical issues constrained the widespread implementation of these conventional processes in rural areas. Consequently, easily designed and operated alternatives with relatively low cost were the main concerns for researchers [1, 2]. Constructed wetlands are one of the suggested alternatives, especially in areas with relatively low land costs. Moreover, easy operation and maintenance of constructed wetlands do not require skilled labor. Furthermore, wetlands as a treatment process could be used alone or in combination with another treatment process [3].

Natural wetlands are terrestrial and aquatic ecosystems' interface, creating an ecosystem where a sophisticated ecological process occurs due to the interaction between soils, vegetation and soils [4, 5]. Constructed wetlands are designed to simulate the natural wetlands ability

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Corresponding Author: **kmshahot@elmergib.edu.ly** to treat wastewater [6]. The main components of these treatment units are the substrate (media), microorganisms, plants and water. The treatment process is accomplished by interacting with various physical, chemical and biological mechanisms to enhance the water quality[1] and remove a broad spectrum of contaminants such as nutrients, organic matter, pharmaceuticals, trace metals and pathogens [7].

Constructed wetlands have been used efficiently to treat a broad spectrum of wastewater such as food industry waste [8], pharmaceutical [9], saline wastewater [10], sulfur [11] hospital wastewater [12], industrial wastewater [12], agricultural runoff [13], petroleum refinery [14, 15], tannery wastewater [15], antibiotic [16], hydroponics [17] dye wastewater [18] olive mill wastewater [19] agro industry [20] high load wastewater [21], swine wastewater [22] livestock wastewater [23] textile wastewater [24], heavy metals [25], fertilizer manufacturing [26].

There have been many studies concerned with the performance of wetlands as wastewater nitrogen removal techniques. These studies were discussed in numerous review papers focused on specific issues related to the nitrogen removal process in wetlands. Vymazal and Kröpfelová [27] evaluated the performance of subsurface horizontal flow wetlands to remove different nitrogen compounds from different types of wastewater. Another review was conducted by Lee [28] to discuss the general concepts of the nitrogen removal process in wetlands and the factors affecting this process. The optimization of wetlands configuration and operation process in addition to the concepts of nitrogen removal mechanisms were discussed and analyzed by Zhuang [29]. The concepts of biological nitrogen removal in addition to the operating parameters of wetlands were reviewed by Saeed and sun [30]. Hybrid constructed wetlands and their performance in removing nitrogen compounds were studied by Vymazal [31]. Another review by Wu [32] highlighted the configuration of wetlands, expanded design and combinations with other techniques to improve nitrogen removal and organics from wastewater. The denitrification process in constructed wetlands using plant carbon source was reviewed by Hang [33]. The impact of oxygen and organic load on intensified constructed wetlands was reviewed by Ilyas and Masih [34]. Tang [35] discussed the microbial coupling mechanism for the removal of nitrogen. Martínez-Espinosa, Sauvage [36] conducted a meta-analysis to figure out the significant issues that attracted the attention of the researchers on the topic of nitrogen removal in wetlands. Rampuria, Kulshreshtha [37] reviewed the metabolic requirements of various species of microorganisms that participating in nitrogen removal routes.

The aforementioned study did not focus on novel nitrogen removal pathways. Therefore, the main objective of this review is to summarize the research articles investigating the possibility of applying and controlling novel nitrogen removal pathways in constructed wetlands.

2. Types of Constructed Wetlands

Generally, three main types of constructed wetlands are known, free water surface, horizontal subsurface flow and vertical subsurface flow constructed wetlands. However, some emerging modifications were investigated [38-40]. Moreover, artificial aeration and effluent recirculation might reduce footprint or conduct nitrification and denitrification [41, 42].

2.1. Free Water Surface Constructed Wetlands

In this kind of constructed wetlands, the water flows horizontally above ground. Depending on the plants' species, this type of wetland could be subdivided into free-floating plants constructed wetlands Figure 1 (with a depth of 50 -100 cm in which aerobic, facultative and anaerobic zones occur, and plants roots act as a supporting media for the biofilm) and emergent plants constructed wetlands Figure 2 (a minimum of 20 -30 cm of soil must be available to support the plants' roots, the superior layer of the soil in addition to the submerged portion of the stems and leaves act as biofilm supporting media)

The low pollutants removal efficiency per unit volume for this type of constructed wetland imposes a high footprint, consequently high construction cost. Moreover, the free surface of polluted water encourages the insects to dominate and cause odors. However, these disadvantages are compensated by simple design, maintenance and operation. [43, 44]

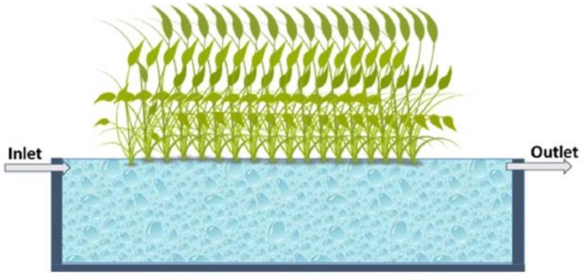

Figure 1. Free floating plants constructed wetlands [45]

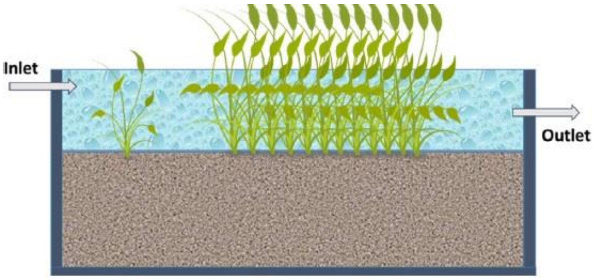

Figure 2. Emergent plants constructed wetlands [45].

a. Horizontal subsurface flow constructed wetlands

In this type of wetlands, the water flows in the bed horizontally as shown in Figure 3. Therefore, the required land area could be estimated as 5- 10 m^3/ PE. Usually aerobic and anaerobic processes occur in this type of wetlands. The aerobic process occurs near the plants' roots where oxygen production is expected [44, 45].

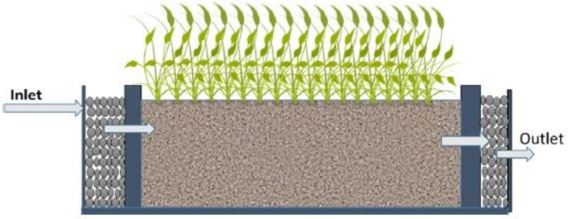

Figure 3. Horizontal subsurface flow constructed wetlands [45]

b. Vertical subsurface flow constructed wetland

In this type, the water is applied from the top and drained from the bottom Figure 4. Mainly, the aerobic condition occurs throughout the media presenting better removal efficiencies of pollutants; in addition, the required area is small (1 -3 m^2/PE). On the other hand, it requires more maintenance compared to the horizontal flow type [44, 45].

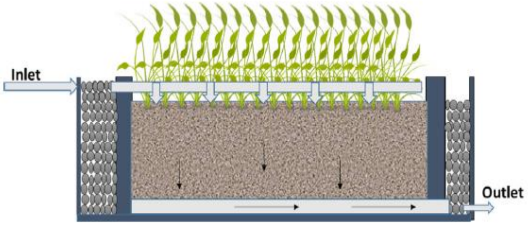

Figure 4. Vertical subsurface flow constructed wetlands [45]

3. Media of Constructed Wetlands

The media is a vital parameter in the design and operation of constructed wetlands. Water hydraulics in wetlands depends mainly on media characteristics. Moreover, the particle size (which determines the surface area) is another critical parameter, basically, the larger the surface area the more biomass grows. Furthermore, media sorption can play the main role in absorbing some pollutants such as phosphorous [1, 46].

Various materials have been used as wetlands media; however, the most common are silt, sand and gravel. Other alternative natural materials have been used successfully such as limestone, zeolite and dolomite [45, 47], biochar [48], steel slag aggregate [49], calcium-rich attapulgite [50], mine waste [51], drinking water treatment sludge [52], oyster shell [53] manganese oxide [54], construction waste [55], wood chip [56]. Mixtures of certain materials were used successfully to remove specific pollutants such as the mixture of sand and dolomite to remove phosphate[1].

4. Common Plants in Constructed Wetlands

Plants are considered the main parameter affecting the treatment performance of a wetland. They contribute to a variety of treatment processes, in addition, to directly uptake nutrients and heavy metals [57]. Floating, emergent and submerged plants are used frequently in constructed wetlands. *Macrophytes* are the most common plants, more than 150 species were examined in constructed wetlands. However, a limited number of species are used in reality.

Plants play a significant role in removing nitrogen and phosphorous, their contribution is expected to range from (15 – 80%) and (24 -80%) for nitrogen and phosphorous respectively, however, less than the expected removal was also reported in some studies. In addition, the uptake capacity of a certain plant species differs based on many factors such as retention time, climate, wastewater characteristics, loading rate,….etc. [1].

In cold climates, the designer should select certain species of plants that show high tolerance to low temperatures. Moreover, some species can help keep an appropriate level of performance in cold climates by insulating the water in cold environments and preventing falling snow [58].

5. Operation Parameters of Wetlands

Three main operation parameters should be controlled and optimized to efficiently operate wetlands, water depth, retention time, hydraulic load and wastewater feeding mode.

Water depth is vital in determining the plant species that would grow. Moreover, it affects the dissolved oxygen level and the chemical reactions. Finally, water depth directly affects the contribution of the various metabolic processes [59].

On the other hand, the hydraulic loading rate plays a significant role in the performance of wetlands. A high hydraulic loading rate stimulates the water to pass fast through the media, consequently, reducing the hydraulic retention time. Basically, the longer the retention time, the higher the removal efficiency and many studies showed a direct effect between the retention time and the removal efficiency. However, this effect varies from one wetland to another according to the plant species and ambient temperature [59, 60].

Feeding mode is another crucial operation parameter directly related to the oxidation process. Continuous, intermittent and batch are the common feeding mode in wetlands. Generally, less oxidation opportunity coincides with continuous feeding mode leading to a significant deterioration in removal efficiency.

Many studies revealed that batch mode operation results in a noticeable improvement in contaminants removal efficiency compared to continuous flow. Moreover, other studies proved the intermittent operation mode's superiority over the continuous flow mode [59, 60].

6. The Removal Efficiency of Pollutants in Constructed Wetlands

Constructed wetlands showed a high level of organic matter removal, more than 50 and 70 % removal for COD and BOD respectively were accomplished in most constructed wetlands. However, the relatively long hydraulic retention time (HRT) required to achieve this removal (around 24 h) is a significant concern regarding the feasibility of constructed wetlands. Therefore, many modifications were suggested to enhance the performance and reduce the HRT, Introducing artificial aeration and modifications in the wetlands configuration reduced the HRT to around 12 h [61].

On the other hand, ammonia removal in constructed wetlands varies (within a wide range of 20 -99%) depending on the wastewater characteristics namely carbon/nitrogen ratio and operating conditions such as HRT and wastewater feeding mode. Moreover, the high growth rate of heterotrophic bacteria compared to ammonia oxidizing bacteria may result in the domination of heterotrophic bacteria in the biofilm community preventing the ammonia oxidizing bacteria from conducting the nitrification process, consequently, low nitrogen removal occurs [43, 61].

A wide range of removal efficiencies was reported regarding phosphorous removal (6 – 99) % depending on the design, loading rate and environmental conditions. The major mechanism in phosphorous removal is the plant uptake process which requires a relatively long contact time. However, low removal efficiencies of phosphate (40 -60%) are common in constructed wetlands treating domestic wastewater [43, 62].

Other contaminants such as surfactants, pesticides and herbicides have been removed fairly in constructed wetlands [61].

Oxygen supply plays a vital role in the treatment process. Oxygen in constructed wetlands is supplied mainly as a product of the photosynthesis process (oxygen that can be released from plant roots and leaves) or by atmospheric re-aeration [63].

7. Disadvantages of Constructed Wetlands

Many advantages are known for constructed wetlands, such as low operation cost, suitable for various types of wastewater, can remove organic materials and nutrients, various materials could be used as wetland media and being a decentralized wastewater treatment process, it could be used for small communities.

Despite of the aforementioned advantages, numerous disadvantages were reported for the constructed wetlands such as the relatively long HRT required for the treatment. Moreover, the high growth rate of planted species implies a necessity for the harvesting process which increases the operation cost. Moreover, different plant species have different abilities to remove pollutants. Consequently, limited species could be planted and not all pollutants are expected to be removed. Furthermore, treatment efficiency depends mainly on the climate, in summer the performance of many species improves leading to an enhancement of the treatment process [45].

8. Biological Nitrogen Removal Pathways

Unconventional nitrogen removal processes have been applied in various treatment processes. However, their application was limited due to a lack of knowledge about optimum operation conditions and environmental parameters. In addition, the interaction of many environmental parameters and treatment techniques in constructed wetlands complicated the consistent application of these unconventional methods. However, some attempts were highlighted in Table 1.

8.1. Simultaneous Nitrification and Denitrification

In this process, nitrification and denitrification coincide in the reactor. The oxygen concentration gradient in activated sludge floc or biofilm is the reason behind this phenomenon, while the surface layer of bacteria can get the required oxygen, the inner layer finds itself in a limited oxygen situation, consequently, the nitrifying bacteria flourish on the surface layer and the denitrifying bacteria dominate the inner layer [29]. The advantage of this process is that nitrification and denitrification occur simultaneously and in the same tank, which implies omitting the denitrification tank. Moreover, this process works efficiently even with low C/N wastewater implies saving the cost of external carbon source [64]

8.2. Shortcut Nitrification and Denitrification

In contrast to the conventional nitrification process, the shortcut (partial) nitrification-denitrification process does not comprise the full oxidation of ammonia. Oxidation process of ammonia is stopped at nitrite due to the limiting oxygen situation, and nitrite is reduced to nitrogen gas. This process saves 25% of oxygen requirement and around 40% of organic requirements [30, 65].

8.3. Anaerobic Ammonium Oxidation (ANAMMOX)

In this novel process, ammonia is directly oxidized to nitrogen by nitrite by means of the planctomycete group under an anaerobic environment. This process requires less oxygen and energy and does not rely on external carbon.. This process is relatively sensitive and dependent on numerous parameters such as the ratio of ammonium to nitrite (should be 1.32), the low growth rate of this kind of bacteria (0.04 -0.06 /day) and low biomass yield (0.11- 0.13 g VSS/ g NH4)[28, 30].

Anaerobic ammonium oxidation is a very sensitive process, and a high concentration of certain substrates (such as ammonium, sulfide and nitrite) can hinder the process. The ANAMMOX process is optimized at a pH range of 6.7 – 8.3 and a temperature range of 30 -37 ^{o}C [30, 37].

8.4. Completely Autotrophic Nitrogen Removal Over Nitrite (CANON)

Simultaneous anammox and partial nitrification processes stimulate nitrogen removal in one reactor in a process known as CANON. The coexistence of these two processes could be established by controlling the oxygen concentration [30, 35]. Since it is an entirely autotrophic process, no external carbon source is needed. Moreover, it requires less oxygen (63% less than the conventional nitrification process), these two aforementioned advantages promise feasibility cost saving [64].

Table 1. Removal efficiencies and operational conditions for the investigated constructed wetlands Using different biological nitrogen removal pathways

Biological Nitrogen removal process	Water used in the experiments	Scale of the experiments	Flow rate, HRT or HLR	Horizontal or vertical flow	Feed type (intermittent, continuous or batch)	Species of grown plants	Influent nitrogen compounds concentration	% Removal	Ref.
Anammox process	Synthetic wastewater	Lab scale (1.5 L)	48 h	Subsurface horizontal	Continuous flow	Unplanted	TN loading = 58 g N/m^3.d	75 %	[66]
Anammox process	Primary treated domestic sewage	Full scale	29 h	Subsurface horizontal	Continuous flow	Canna indica	TN = 76±7.8 mg/L	57 %	[67]
Anammox process	Hospital wastewater	Full scale	28 h	Subsurface horizontal	Continuous flow	Canna indica	TN = 76±5.9 mg/L	50 %	[67]
Anammox process	Livestock waste	Full scale	---	Subsurface horizontal	Continuous flow		NH_3 - N= 140 mg/L	------	[68]
Anammox process	Synthetic wastewater	Lab scale (8 L)	1.5 L/d	Vertical	Continuous flow	Scirpus acutus	NH_3 – N = 100 mg/L	25±7 %	[69]
Anammox process	Swine wastewater	Full scale	0.18 m^3/d	Surface water flow	Intermittent flow	Myriophyllum aquaticum	TN = 380-650 mg/L.	86.2 - 97.8 %	[70]
Anammox process	Surface water	Lab scale (L=50 cm, W= 20 cm, H = 30 cm)	2- 4 d	Subsurface horizontal	Continuous flow	Iris pseudacorus	TN = 15 mg/L	90 %	[71]
Partial-nitrification / anammox	Domestic wastewater	Full scale	First stage 1.15 ±0.13 d and second stage is 5 d	1st stage is vertical and 2nd stage is subsurface horizontal	Intermittent (feeding 3.5 d with 7 days intervals)	Phragmites	TN = 31-78 mg/L	53.94 - 54.83 %	[72]
Anammox process	Domestic wastewater	Lab scale (L, W and H of 1500, 400 and 700 mm) ,	4 d	Subsurface horizontal	Continuous flow	Calamus	TN = 25.77±7.58 mg/L	48 %	[73]
Anammox process	Secondary treated sewage	Full scale	0.5 - 1.5 ML/ d.	Surface water flow	Intermittent flow (2 weeks flood / 2 weeks dry)	Unplanted	_____	_____	[74]

Table 1. (continued)

Biological Nitrogen removal process	Water used in the experiments	Scale of the experiments	Flow rate, HRT or HLR	Horizontal or vertical flow	Feed type (intermittent, continuous or batch)	Species of grown plants	Influent nitrogen compounds concentration	% Removal	Ref.
Partial nitrification/ denitrification	Synthetic wastewater	Lab scale (diameter = 20 cm and depth = 30 cm)	0.03 $m^3/m^2.d$	Vertical	Continuous flow	Unplanted	TN load = 0.57 g $N/m^2.d$	55–90 %	[75]
CANON process	Livestock wastewater	Lab scale (1 m height, 9.3 cm in diameter)	________	Vertical	Tidal flow (10 min feeding, variable flood time , 10 min drain and variable rest time)	Common reeds.	TN load = 15 g $N/m^2.d$	80 %	[76]
CANON process	Digested swine wastewater	Lab scale (diameter of 20 cm and depth of 80 cm).	0.02 $m^3/m^2.d$	Vertical	Continuous flow	Reeds	TN = 487.60 ± 38.74 mg/L	76.74 ± 7.30 %	[77]
Anammox process	low polluted domestic wastewater	Lab scale (600 L)	------------	Vertical	Intermittent flow (48 hr. interval)	Canna indica	TN = 3.91 ± 1.71 mg/L	66 ± 0.9 to 88 ± 2.4 %	[78]
Anammox process	Rural wastewater	Lab scale (dia. = 41.5 cm and height = 80 cm)	20 L/d	Vertical followed by subsurface horizontal	Intermittent flow (feeding 2 days/ rest 5 days)	Reeds.	TNK = 202 ± 35.2	48 -53%	[79]
Simultaneous nitritation, anammox and denitrification	Anaerobic digested dairy manure	Lab scale (diameter of 15.2 cm and a total height of 75 cm)	1 d in each saturated layer	Vertical	Batch mode (fill/ 7 days reaction/drain)	Cyperus alternifolius	NH_3 - N= 450 mg N/L	9.1 ± 0.6 (g $N/m^2.d$)	[80]

Table 1. (continued)

Biological Nitrogen removal process	Water used in the experiments	Scale of the experiments	Flow rate, HRT or HLR	Horizontal or vertical flow	Feed type (intermittent, continuous or batch)	Species of grown plants	Influent nitrogen compounds concentration	% Removal	Ref.
Anammox process	Synthetic wastewater	Lab scale (1.5 L)	48 h	Subsurface horizontal	Continuous flow	Unplanted	TN loading = 58 g N/m^3 day	77.5%	[81]
Partial nitrification-anammox process	Low ammonium concentration synthetic wastewater	Lab scale (diameter of 20 cm, height of 130 cm)	16 h	Vertical	Batch mode (15 min. fill/ variable reaction time/15 min drain)	Typha latifolia	TN = 25-30 mg/L.	81.11 ± 2.92 %.	[82]
Nitritation / anammox	Synthetic wastewater	Lab scale (68 L)	34 L/week	Surface water flow	Batch mode (fill/7days reaction/drain)	T. latifolia	TIN = 367.9 ± 12.6 mg N/L	29 ± 6 %	[83]
CANON	Synthetic domestic wastewater.	Lab scale (diameter of 10 cm and a length of 100 cm)	3.5 h	Vertical	Tidal flow (7 min feeding, 3.5 h flood, 7 min drain and 30 min rest)	Phragmites australis	TN = 16.7 ± 0.29 mg / L	67 %	[84]
Anammox process	Treated domestic wastewater	Full scale	30 m^3/day	Surface water flow	Continuous flow	Rice	NH_3 -N =7.6 mg/L	50 %	[85]
Anammox process	Domestic sewage	Lab scale (L=1500, W= 400 and h= 700mm)	3 days	Subsurface horizontal	Continuous flow	Calamus	TN = 29.66 ± 3.73 mg/L	90%	[86]
CANON	Domestic sewage	Lab scale (diameter of 20, depth of 80 cm)	4 h	Vertical	Tidal flow (10 min feeding, 240 min flood, 10 min drain and 100 min rest)	Reeds	TN = 42.97 ± 2.85 mg/L.	127 ± 13.78 mg/L. d	[87]

Table 1. (continued)

Biological Nitrogen removal process	Water used in the experiments	Scale of the experiments	Flow rate, HRT or HLR	Horizontal or vertical flow	Feed type (intermittent, continuous or batch)	Species of grown plants	Influent nitrogen compounds concentration	% Removal	Ref.
Simultaneous heterotrophic and autotrophic denitrification	Synthetic wastewater	Lab scale (700 mm height, 160 mm in diameter)	24 h	Vertical	Continuous flow	Canna indica	———	TN removal 68.8 ± 7.9%	[88]
partial denitrification -anammox	Secondary effluent domestic sewage	Lab scale (1.2 m × 0.8 m × 0.6 m),	10 h	Vertical	Tidal flow (1 h flood/ 2 h drain)	Unplanted	TN = 34.23 ± 5 mg/L	81.18 %	[89]
Anammox process	Synthetic wastewater	Lab scale (1.0 m× 1.0 m× 1.0 m)	12 h	Vertical	Batch mode (12 h fill/ 12 h drain)	Reeds	_____	55 -64%	[90]

8.5. Oxygen limited Autotrophic Nitrification–Denitrification (OLAND)

This process is another nitrogen removal technique using a single reactor. In this process, ammonia oxidizing bacteria oxidize a portion of ammonia to nitrite using oxygen while the other portion of ammonia is used to reduce the nitrite to nitrogen gas. The main advantages of OLAND are 63% less oxygen requirement and no need for an external alkalinity source

9. Conclusion

Constructed wetlands have been used successfully to remove nitrogen through the non-conventional nitrogen removal pathways such as partial nitrification, CANON and ANAMMOX. However, the widespread implementation is constrained by the difficulty of controlling operating parameter such as dissolved oxygen, carbon availability, temperature and pH. Moreover, this review revealed that the most common non-conventional nitrogen removal process implemented in constructed wetlands is ANAMMOX, however, simultaneous nitrification and denitrification, partial nitrification and CANON have been implemented successfully. Furthermore, the review showed that the research experiments used both lab scale and full scale constructed wetlands, different wetlands configurations (free surface wetland, horizontal and vertical subsurface wetlands) and different feeding strategies (batch, intermittent and continuous flow) granting more confidence for the application of this methods. Finally, although the novel non-conventional nitrogen removal pathways have been applied successfully and can reduce the requirements of oxygen and carbon compared to the conventional process of operation, there are still some challenging points that should stimulate more studies that aim to facilitate operation, lower costs, and enhance nitrogen removal.

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Conflict of interest

The publication of this article causes no conflict of interest.

Abbreviations

ANAMMOX	Anaerobic ammonium oxidation
CANON	Completely autotrophic nitrogen removal over nitrite
HRT	Hydraulic retention time
OLAND	Oxygen limited autotrophic nitrification–denitrification
TN	Total nitrogen
TIN	Total inorganic nitrogen

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