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Review

Review on the Fate and Mechanism of Nitrogen Pollutant Removal from Wastewater Using a Biological Filter

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Abstract

Biological filter (biofilter) technology has developed rapidly and has been extensively employed to remove nitrogen pollutants originating from aquatic environments. Due to the different responses of nitrogen microorganisms to various operating parameters and environmental factors, achieving satisfactory nitrogen removal in biofilters remains a challenge. Hence, this review aims to provide useful information on the underlying nitrogen removal mechanisms in biofilters by giving a comprehensive review of traditional and newly discovered nitrogen transformation processes and microbial communities associated with nitrogen cycling. Firstly, a brief summary on overall performance of biofilters using traditional and newly discovered methods for nitrogen removal was presented. The detailed nitrogen transformation pathways and functional microbial communities associated with nitrogen cycling in biofilters were discussed. A brief overview is followed by a more detailed discussion of techniques for assessing nitrogen microbial population dynamics and community structure and function. Finally, conclusions and recommendations for future work are highlighted.

Keywords: biotransformation, nitrogen removal, biofilter, wasterwater, nitrogen microorganisms

Introduction

The discharge of excessive organic matter and nitrogen into water can cause serious ecological problems such as eutrophication, algae blooms, and habitat degradation in aquatic environments (i.e., rivers, lakes, reservoirs, and groundwater) [1]. As in many aquatic environments, pollution is a worldwide issue, especially in developing countries. Over the last few decades, intensive economic development in developing countries has led to excess nitrogen being discharged into aquatic environments due to burgeoning industrialization, civilization, and agricultural activities [2-4]. In particular, increased nitrogen pollutant

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inflows can stimulate excessive and unbalanced growth of plants and algae, leading to oxygen depletion and eventual eutrophication of the water body [5].

A biofilter is a type of wastewater treatment system. It consists of a fixed bed of rocks, lava, coke, gravel, slag, polyurethane foam, sphagnum peat moss, ceramic, or plastic media over which sewage or other wastewater flows downward and causes a layer of microbial slime (biofilm) to grow, covering the bed of media. The systems utilize complex removal mechanisms involving filtration, adsorption, and biological degradation to remove various contaminants or improve water quality [6]. Biofilters afford many considerable advantages, including simple design and operation, low capital and operating costs, and a low requirement for energy and maintenance inputs [7]. During the past two decades, use of biofilter systems has developed rapidly, and biofilters have been widely used to remove various pollutants originating from aquatic environments [1, 8-12].

However, how to achieve satisfactory NH_4^+-N and NO_3^--N removal has become an urgent issue and a research hotspot in the field of biofilters. On one hand, substrate types (i.e., plastic, mineral inert media, modified zeolite, and bio-ceramic) are a predominant influencing factor on nitrogen removal in biofilters. The substrates play an essential role in nitrogen adsorption and can provide optimal growing and enriching conditions for nitrogen microorganisms. On the other hand, nitrogen microbial activity is the primary reason for primary nitrogen removal. This has been a cornerstone of the technology almost from the beginning [13-14]. Due to the different responses of nitrogen microorganisms to operating parameters (free ammonia concentration, temperature, dissolved oxygen (DO), and pH, etc.) and the complex interrelationships among various nitrogen species, achieving satisfactory nitrogen removal in biofilters remains a challenge [1, 15]. Thus, understanding the underlying microbial nitrogen transformation mechanisms under different operating conditions has extended concurrently with the use of biofilters.

While the underlying microbial nitrogen transformation mechanisms have been intensively studied, there is still a knowledge gap in the understanding of these detailed underlying mechanisms that control nitrogen removal in biofilters. Meanwhile, there are complex interrelationships among various nitrogen species (i.e., NH_4^+-N, NO_3^--N, and nitrite (NO_2^--N)) and also different nitrogen removal mechanisms in biofilters. Hence, an overview focusing on microbial nitrogen transformations will be helpful for researchers with the choice of appropriate microbial nitrogen removal pathways to optimize the design, operation, and application of biofilters. This paper presents the first attempt to provide an overall review on the traditional method and new aspects of microbial nitrogen removal performance using biofilters. Besides, the paper also makes a comprehensive summary of the microbial nitrogen removal processes and pathways in biofilters. Techniques for assessing nitrogen microbial communities in biofilters will also be discussed.

Nitrogen Removal Performance

Various biofilters have been designed to remove nitrogen pollution in wastewater. While there are complex interrelationships among various nitrogen species and the different responses of nitrogen microorganisms to operating parameters in biofilters, achieving high nitrogen removal performance remains a challenge. Hence, the evaluation of the removal and fate of nitrogen in various biofilters is imperative for the optimization of treatment processes. The operational adaptability and feasibility of biofilters are compared in an attempt to explore the traditional and newly discovered methods for nitrogen removal.

Traditional Methods for Nitrogen Removal

Traditional biofilters have been used to remove nitrogen pollution in aquatic environments. The traditional methods for nitrogen removal in aquatic environments have been the integration of nitrification-denitrification processes [16]. Ji et al. [8] used four different types of filter medium to treat wastewater with different ammonia levels: multimedia bio-ceramic, natural zeolite (diameter >2 mm), gravel (diameter >10 mm), and modified zeolite (diameter >10 mm). The multimedia biofilters reduced 88% of NH_4^+-N and 88% of TN. Similar observation was made by Wang et al. [1], who found that the biofilter filled with polyurethane foaming plastic and porous lava rock reduced 96±2.1% of chemical oxygen demand (COD) and 87-98% of NH_4^+-N throughout the phases of operation, and the filter medium adsorbed 22-31% of NH_4^+-N during the start-up stage. Zhang et al. [17] used an aerobic denitrification biofilter filled with reticulated polyurethane foam as the carrier material to remove nitrate (NO_3^--N) of groundwater. The TN and NO_3^--N removal efficiencies were 82.3-95.8% and 93.2-98.2%, respectively. Based on the above research, it is evident that NH_4^+-N adsorption by filter medium was the dominant mechanism in NH_4^+-N removal during the start-up stage of biofilters because chemoautotrophic nitrifying bacteria are slow-growing and need more time to function well. During the operational period, biodegradation was the key mechanism responsible for removing nitrogen in biofilters.

Most laboratory studies of biofilter performance have reported quite good removal for NH_4^+-N and NO_3^--N. Wang et al. [7] investigated a biofilter (working volume of 144 L) for improving drinking water. The influent concentration of COD, NH_4^+-N, NO_3^-N, and temperature were 6.0-25.0 mg/L, 1.2-15 mg/L, 4.9-5.0 mg/L, and 10.3-26.9°C, respectively. The biofilter was operated at the hydraulic loading rate of 2.0 $m^3/(m^2\ d)$. They noted that NH_4^+-N removal efficiencies ranged from 80.0% to 95.8%. The NO_3^--N effluent concentration increased from 5.3 to 12.1 mg/L during operational periods, suggesting more research was needed to further enhance nitrogen removal and polish effluent. Hasan et al. [18] studied an aerated biofilter for drinking water treatment. When the

Table 1. Biofilter traditional method for ammonium and nitrate remove from wastewater.

Reactor	Carrier	Experiment condition	Removal efficiency	Reference
Denitrifying biofilter	Polycaprolacto-ne	NO_3^-- :17.6-33 mg/L; HRT: 0.2-0.8 h; Temperature: 17.5-24 ° C;	TN: 87.5-99.6%	[20]
Vertical flow trickling filter	Gravel and zeolite	COD: 98-417 mg/L; TN: 49-50 mg/L; NH_4^+: 48.6-49.8 mg/L; pH: 6.6-7.0	COD : 94.1%; NH_4^+: 96.1%; TN: 92.8%.	[21]
Lab-scale multimedia biofilter	Polyurethane foaming plastic, lava rock	COD: 6.0-25.0 mg/L; NH_4^+: 1.2-15.0 mg/L; Nitrogen loading rate: 1.7-20.8 g-N/m^2 d; CLR: 8.3-34.7 g-COD/m^2 d.	COD : 76-96%; NH_4^+: 76-98%.	[1]
Biotrickling filter	Light-weight filtration media	NO_3^--N :4,50-5.50 mg/L; NH_4^+-N : 0.01-0.3 mg/L; TN: 4.85-6.23 mg/L; DO:6.0-8.0 mg/L	TN removal rate: 3.5-10.2 mg/L·h; NO_3^--N removal rate: 3.2-10.2 mg/L· h	[22]
Denitrification biofilters	Polyurethane foam	NO_3^--: 30-100 mg/L; NH_4^+:15 mg/L; pH: 7.2	TN: 18.5-92.2%; NO_3^--: 42.9-99.5%; COD: 50.5-93.7%	[23]
Double-layer biofilter	Carbon-rich ceramic granules	COD: 26-209 mg/L; TN: 6.9-38.3 mg/L; NH_4^+: 2.2-35.8 mg/L; pH: 6.1-8.3.	COD: 82-91%; NH_4^+ : 83-99%; TN: 50-60%.	[24]
Multimedia biofilters	Pore mesh material, bio-ceramic, natural zeolite	TN: 2.5-121.8 mg/L; NH_4^+: 1.0-102.8 mg/L; NO_3^-: 1.5-19.0 mg/L; HL: 31.2-125.0 cm/d; Temperature: 18-25ºC.	NH_4^+ and TN: 88%.	[8]
Moving-bed biofilm reactors	Hacketten carrier and cylinder carrier	DO: 6.93-7.51 mg/L; pH: 7.27-7.52; COD: 2.1 mg/L; NH_4^+: 0.65-2.18 mg/L	NH_4^+: 63.1%	[25]
Subsurface wastewater filter systems	Gravel, modified zeolite	TN: 12.5-53.4 mg/L; NH_4^+: 4.2-50.8 mg/L; NO_3^-: 2.6-6.8 mg/L; COD: 22.6-165.2 mg/L; HL: 2.5-10.0 cm/d.	COD: 57-99%; NH_4^+ : 90-95%; TN: 65-90%.	[26]
Conventional biofilters	Volcanic rocks, bioceramsite	COD:255.4-450 mg/L; pH: 6.6-8.4	COD:50-86%	[27]

COD: chemical oxygen demand; HLR: hydraulic loading rate; HL: hydraulic loading; CLR: COD loading rate; HRT: hydraulic retention time

biofilter was operated with increasing organic loading rates (0.2-1.0 kg COD/m^3 d), aeration rates (0-2 L/min), and hydraulic retention times (6-24 h), the average removal of COD and NH_4^+-N increased to the range of 83.8-97.1% and 47.6-97.4%, respectively. Shi et al. [19] studied the long-term performance of a pilot-scale denitrification biofilter (working volume of 91 L) for the removal of NO_3^--N. The influent concentration of COD, NO_3^--N, pH, and dissolved oxygen (DO) were 20 mg/L, 11.2 mg/L, 6.9 mg/L, and 2.4 mg/L, respectively. They found that the removal efficiencies of NO_3^--N ranged from 55% to 88%. Ji et al. [10] studied an aerated multimedia biofilter with an effective volume of 9.6 L for synthetic wastewater treatment. The multimedia module media was filled with clinoptilolite and coal ash bioceramsite, and modified by metallic iron. The influent concentrations of COD, NH_4^+-N, and total phosphorus were 100-400 mg/L, 20-40 mg/L, and 4 mg/L, respectively. They found that the removal efficiencies of COD and NH_4^+-N were 81-94% and 82-95%, respectively. A summary of the studies on traditional biofilters for nitrogen removal is shown in Table 1.

A review reveals that the biofilter technology is capable of treating inorganic nitrogen pollution in wastewater with COD content from 2.5 to 400 mg/L, NH_4^+-N from 0.25 to 102.8 mg/L, and NO_3^--N from 1.5 to 100 mg/L. The biofilter used in those studies removed 37-99% of COD, 76-98% of NH_4^+-N, and TN 50-92% of TN. The pH in the biofilter systems ranged from 6.0 from 8.3 and the influents temperature varied between 10.3 and 26.9ºC. Thus, inorganic nitrogen pollution of wastewater having the above influent characteristics could be treated by biofilter systems.

Newly Discovered Methods for Nitrogen Removal

Newly discovered processes and technologies such as partial nitrification, anammox, simultaneous partial nitrification, anammox and denitrification (SNAD), single-reactor high activity ammonia removal over nitrite (SHARON), completely autotrophic nitrogen removal over nitrite (CANON), and oxygen-limited autotrophic nitrification-denitrification (OLAND) all have a high potential for nitrogen removal [16]. Guillén et al. [28] studied a partial nitrification biofilter for synthetic wastewater treatment. The biofilter removed the 31.7-76% of NH_4^+-N and 52-54% of TN, respectively. Chatterjee et al. [29] developed an anammox biofilter with a working volume of 10 L for removing nitrogen from wastewater.

Table 2. Biofilter new methods for ammonium and nitrate remove from wastewater.

Reactor	Carrier	Experiment conditions	Removal efficiency	Reference
Anammox biofilm reactor	Nylon ropes	COD: 36-48 mg/L; TN loading:0.07-0.20 kg TN/m^3 d; CLR:0.053-0.176 kg COD/m^3 d; HRT: 4.36-12 h; Temperature: 14-41ºC	COD: 78 ± 2%; NH_4^+: 95 ± 1%; TN: 79 ± 11%.	[29]
Anammox trickling filter	Polyurethane sponge	Temperature: 30.5 °C; pH: 7.89; NH_4^+: 49-50 mg/L; NO_2^-: 50-52 mg/L; HRT: 1.14-2.23 h; HLR: 3.4-13.7 m^3/m^{-2} d; Temperature: 20-30ºC;	TN: 74%-84%;	[35]
Partial nitritation trickling filter	Polyurethane sponge	Flow: 5.7-14.9 L/d; HRT: 1.6-4.2 h; HLR: 1.3-3.3 m^3/m^2 d; NH_4^+: 111.5-118.5 mg/L;	NH_4^+: 31.7%-76%; TN: 52%-54%.	[28]
SNAD biofilter	Volcanic rock	NH_4^+: 200 mg/L; Temperature: 25 °C; Aeration rate: 4.5 L/min; Influent C/N ratio: 0.2; HRT (h): 0.55-0.6.	TN: 65%-76%; COD: 81%.	[30]
Anammox biofilter	Volcanic rock	NH_4^+: 46.5 mg/L; Temperature: 15.3-23.2ºC.	TN: 76%.	[31]
CANON biofilter	Volcanic rock	NH_4^+: 100-400 mg/L; HRT (h): 0.52-11.70; pH: 7.87-8.20. Temperature: 24.3-27.6ºC; DO: 5.06-6.72 mg/L;	TN: 61%-67%.	[32]
SHARON biofilter	PVC	Oxygen demand: 1.5 mg/L; pH: 7.5; Temperature: 35ºC; HRT: 0.4-0.5 day; NH_4^+:600 mg/L.	NH_4^+: 60-100%.	[33]
OLAND biofilter	Polyvinylchloride	HRT (d): 0.77-0.91; NH_4^+: 476-846 mg/L; Loading rate: 525-1100 mg N/L d.	TN: 84%.	[34]

COD: chemical oxygen demand; HLR: hydraulic loading rate; CLR: COD loading rate; HRT: hydraulic retention time

The biofilter removed the 78±2% of COD, 95±1% of NH_4^+-N, and 79±11% of TN. Liang et al. [30] investigated a 40 L SNAD biofilter filled with volcanic rock as biofilm carrier for treating low C/N ratio synthetic wastewater. The results suggested that the biofilter simultaneously removed 70.5% of TN and 81% of COD. Zeng et al. [31] developed an upflow anammox biofilter for treating synthetic wastewater with low ammonia concentration (46.5 mg/L) at ambient temperature (15.3-23.2ºC), and the average nitrogen removal rate and removal efficiency were 2.26 kg/ (m^3 day) and 75.9%, respectively.

Liang et al. [32] used a CANON biofilter (working volume: 40 L) packed with volcanic rock as biofilm carrier for treating different ammonia levels in wastewater. The influent of NH_4^+-N, DO, and pH were 100-800 mg/L, 5.06-6.72mg/L, and 7.87-8.20. The maximum nitrogen removal rate ranged from 0.5 to 3.7 kg/ (m^3 d). They found that the removal efficiencies of NH_4^+-N were 55-85%. González-Martínez et al. [33] constructed a submerged SHARON biofilter (working volume: 3 L) with PVC carriers for treating high concentrations of NH_4^+-N and low COD wastewater. The influent of NH_4^+-N, DO, and pH temperature were 600 mg/L, 1.5 mg/L, 7.5, and 35ºC. They found that the removal efficiencies of NH_4^+-N were 100% at a hydraulic retention time (HRT) of 0.5 day and 65% at a HRT of 0.4 day. Windey et al. [34] constructed a biological contact reactor (working volume: 50 L) operated under OLAND conditions treating high-salinity wastewater. The influent of NH_4^+-N was 476-846 mg/L, and the HRT was 0.77-0.91 day. They found that the removal efficiencies of NH_4^+-N and TN were 70% and 57%-93%, respectively. The OLAND process consumes 63% less oxygen and 100% less biodegradable organic carbon compared to the conventional nitrification-denitrification process and has, therefore, a lower operating cost. A summary of the studies on novel biofilters for nitrogen removal is shown in Table 2.

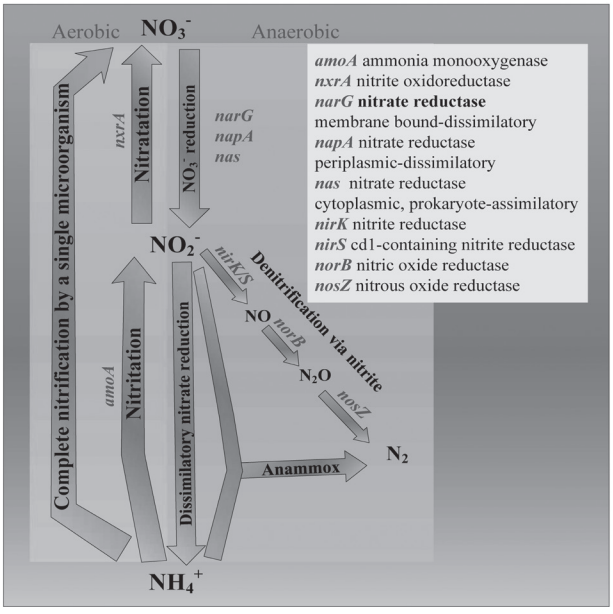

Fig. 1. The major biological nitrogen transformation pathways in biofilters are linked by their associated enzymes [adapted from 37]. Genes encoding enzymes that conduct the important transformations include those for various nitrate reductases (*nas*, *narG*, *napA*), nitrite reductases (*nirK*/*S*), nitric oxide reductase (*norB*), nitrous oxide reductase (nosZ), ammonium monooxygenase (*amoA*), nitrite oxidoreductase (*nxrA*), and anaerobic ammonium oxidation (anammox).

A review of these novel biofilter systems reveals that the biofilter technology is capable of treating specific wastewater (i.e., low C/N ratio, high NH_4^+-N) or reducing operational cost. Research focusing on the CANON biofilters is intense at present due to its several advantages, including less aeration demand, no organic carbon consumption, and less sludge production. The SNAD biofilter can be used for treating low C/N ratio sewage due to its high TN removal efficiency. The SHARON biofilter can be used for treating high concentrations of NH_4^+-N and low of COD wastewater. The OLAND and partial nitrification biofilter is a feasible alternative for treating highly NH_4^+-N-loaded wastewater.

Nitrogen Removal Process

The main biodegradation processes in a biofilter includes nitrification, denitrification, anammox, dissimilatory/assimilatory nitrate reduction to ammonium, and complete oxidation of ammonia to nitrate in one organism (comammox) (Fig. 1) [7-8, 36]. Fortunately, with the discovery of the anammox pathway, new nitrogen removal techniques using biofilter systems have opened up. This section reviews what is known about biological nitrogen transformation pathways in biofilters.

Nitrification Process

Nitrification is a predominant microbiological process in which NH_4^+-N is converted to NO_3^--N under aerobic environmental conditions in biofilters (Table 3). Autotrophic nitrification is a two-step aerobic process in which NH_4^+-N is oxidized to NO_2^--N by ammonium-oxidizing bacteria (*AOB*, *Nitroso*-) and NO_3^--N by nitrite-oxidizing bacteria (*NOB*, *Nitro*-) [38]. *Nitrosomonas europea*, *Nitrosomonas mobilis strains*, *Nitrosomonas eutropha*, and *Nitrosomonas nitrosa* were considered the main microorganisms in ammonium oxidation. *Nitrobacter* spp. and *Nitrospira* spp. were considered the predominant players in nitrite oxidation in biofilters [39]. Detection of nitrification bacteria in biofilters has generally targeted *amoA* and *nxrA* genes [8, 37]. The *amoA* gene has been utilized as a molecular marker for quantitative studies of *AOB* and archaea (*AOA*) in biofilters [40]. The nitrite oxidase coding gene *nxrA* has been utilized as a marker of NO_2^--N oxidation [41]. Wang et al. [1] reported that the functional roles of *AOB*, *AOA*, and *NOB* have been analyzed by building quantitative relationships between NH_4^+-N and NO_2^--N transformation rates and functional genes in a multimedia biofilter. The results revealed that *amoA*/archaea had the prominent contribution on the NH_4^+-N removal rate (54.6%). Bagchi et al. [42] investigated the decrease of NH_4^+-N accumulation in nitrifying biofilters by enhancing rapid conversion to NO_3^--N via NO_2^--N. Nitrogen balances analysis suggested that active nitrification accounted for ≥81-86% of TN conversion.

The nitrification process was determined to be a pivotal pathway responsible for the robust NH_4^+-N treatment performance in biofilters. The primary factors in controlling nitrification process include free ammonia, temperature, dissolved oxygen (DO), and pH. Free

Table 3. Simplified equations for selected microbial nitrogen transformation processes [16, 43].

No.	Process	Biochemical conversion
1	Nitritification	$NH_4^+ + 1.5\ O_2 + 2\ HCO_3^- \rightarrow NO_2^- + 2\ CO_2 + 3\ H_2O$
2	Nitratation	$NO_2^- + 0.5\ O_2 \rightarrow NO_3^-$
1+2	Nitrification	$NH_4^+ + 2\ O_2 + 2\ HCO_3^- \rightarrow NO_3^- + 2\ CO_2 + 3\ H_2O$
3	Denitratation	$2\ NO_3^- + C \rightarrow 2\ NO_2^- + CO_2$
4	Denitrification via nitrite (Denitritification)	$4\ NO_2^- + 3\ C + 2\ H_2O + CO_2 \rightarrow 2\ N_2 + 4\ HCO_3^-$
3+4	Denitrification	$4\ NO_3^- + 5\ C + 2\ H_2O \rightarrow 2\ N_2 + 4\ HCO_3^- + CO_2$
5	Partial nitrification (50% conversion)	$NH_4^+ + 0.75\ O_2 + HCO_3^- \rightarrow 0.5\ NO_2^- + 0.5\ NH_4^+ + CO_2 + 1.5\ H_2O$
6a	Anammox (without cell synthesis)	$NH_4^+ + NO_2^- \rightarrow N_2 + 2\ H_2O$
6b	Anammox (with cell synthesis)	$NH_4^+ + 1.32\ NO_2^- + 0.066\ HCO_3^- \rightarrow 1.02\ N_2 + 0.26\ NO_3^- + 0.66\ CH_2O_{0.5}N_{0.15} + 2.03\ H_2O$
1+2+3+4	Traditional nitrification denitrification	$NH_4^+ + 8O_2 + 5\ C + 4\ HCO_3^- \rightarrow 2\ N_2 + 9\ CO_2 + 10\ H_2O$
3+5+6	SNAD	-
1+6	CANON	$NH_3 + 0.85\ O_2 \rightarrow 0.11\ NO_3^- + 0.44\ N_2 + 0.14\ H^+ + 1.43\ H_2O$
7	OLAND	$NH_4^+ + 0.75\ O_2 \rightarrow 0.5\ N_2 + H^+ + 1.5\ H_2O$
8	SHARON	$NH_4^+ + HCO_3^- + 0.75\ O_2 \rightarrow 0.5\ NH_4^+ + 0.5\ NO_2^- + CO_2 + 1.5\ H_2O$
9	Complete ammonia oxidation	$NH_4^+ + 2\ O_2 \rightarrow NO_3^- + H_2O + 2\ H^+$

ammonia has a great influence on *AOB* and *NOB*. *NOB* are more sensitive than the *AOB* to free ammonia at high pH of 7.5-8. The inhibition of aerobic ammonia oxidizers and nitrite oxidation in aquatic environments is observed at NH_3-N concentrations of 10-150 mg /L and 0.08-0.82 mg/L [44]. However, Kim et al. [45] reported that *Nitrobacter* spp. and *Nitrospira* spp. were inhibited at 30~50 mg NH_3-N/L and 0.04~0.08 mg NH_3-N/L, respectively, suggesting a different threshold for *Nitrobacter* spp. and *Nitrospira* spp. Another study by Vadivelu et al. [46] reported that 100% inhibition of *Nitrobacter* sp. anabolism and 12-25% inhibition of *Nitrobacter* sp. catabolism were observed at NH_3-N concentrations of 1-9 mg/L.

Temperature has a strong influence on nitrifying activity. Nitrifying activity would be limited at less than 8-10ºC or higher than 35-45ºC, and the maximum growth rate of *AOB* and *NOB* are observed at 35ºC, and the thermal death points of *Nitrosomonas* spp. are observed at 54-58ºC [47-48]. The DO concentration is very important for both *AOB* and *NOB*. Kim et al. [49] reported that low ammonium conversion and low NO_2^--N and NO_3^--N accumulation are observed at DO less than 1.0 mg/L. Garrido et al. [50] reported that stable and 100% nitrite accumulation was observed at DO of 1.0 mg/L, and 50% of ammonium conversion to nitrite could also be obtained at DO of 1.5 mg/L. Full nitrification could also be obtained at DO higher 2.5 mg/L, and NH_4^+-N removal depended on applied ammonium load. The nitrite oxidizers are more sensitive to oxygen limitation than that of ammonium oxidizers [44]. Euiso et al. [47] also reported that the inhibition of ammonium and nitrite oxidizers was observed at temperature higher than 35-45ºC or lower than 8-10ºC. The pH of influent water showed significant impact on nitrifying bacteria activity. An optimum range of pH for ammonia oxidizers (*Nitrosomonas* spp.) and nitrite oxidizers (*Nitrobacter* spp.) were 7.9-8.2 and 7.2-7.6, respectively [51]. The 100% inhibition of *NOB* activity was observed at pH less than 6.5 [52]. The specific growth rate of ammonium oxidizers and nitrite oxidizers decreased as the pH increases up 9-9.5 [53]. Effects of free ammonia, temperature, dissolved oxygen, and pH on the nitrification process are shown in Table 4.

A review of nitrification reveals that a number of environmental factors influence nitrogen removal in

Table 4. Effects of free ammonia, temperature, pH, and DO on the nitrification process.

Factor	Effect	References
Free NH_3-N (mg/L)		
10-150	Inhibition of ammonium oxidizers	[44]
30-50	Inhibition of *Nitrobacter* spp.	[45]
0.08-0.82	Inhibition of nitrite oxidizers	[44]
0.04-0.8	Inhibition of *Nitrospira* spp.	[45]
1-9	100% inhibition of *Nitrobacter* sp. anabolism, and 12-25% inhibition of *Nitrobacter* sp. catabolism	[46]
Temperature (T, º)		
< (8-10)	Inhibition of ammonium and nitrite oxidizers	[47]
=25	Ammonium oxidizers can out-compete nitrite oxidizers	[43]
=35	Maximum Growth rate of AOBs and NOBs	[54]
> (35-45)	Inhibition of ammonium and nitrite oxidizers	[48]
DO (mg/L)		
< 1.0	Low ammonium conversion and low NO_2^- and NO_3^- accumulation	[49]
=1.0	Stable and 100% nitrite accumulation	[50]
1.5	50% of ammonium conversion to nitrite	[50]
> 2.5	Full nitrification, NH_4^+ -N oxidation depended on applied ammonium load	[50]
2.0-5.0	Nitrite accumulation up to 60% of total ammonia conversion	[55]
pH		
< 6.5	Complete inhibition of the NOBs activity	[52]
7.9-8.2	Optimum range for ammonia oxidizers (*Nitrosomonas* spp.)	[53]
7.2-7.6	Optimum range for nitrite oxidizers (*Nitrobacter* spp.)	[53]
9-9.5	Inhibition of ammonium and nitrite oxidizers	[53]

biofilters. In practice, all of the environmental factors have different effects on the growth rate and enrichment of ammonium and nitrite oxidizers. However, the nitrification process may also be influenced by several inhibitory compounds, including heavy metals, salinity, and alkalinity, as well as sludge age [56-58]. This study therefore highlights the need for more research efforts to study the importance of inhibitory compounds in nitrogen removal in biofilters. For a long time, bacterial nitrifiers were believed to be the only significant mechanism of autotrophic nitrification. It has been proved that Archaea played an important role in aerobic nitrification. Archaeal ammonium-oxidizer *amoA* sequences have been identified in a laboratory-scale reactor [59]. Wang et al. [1] investigated the dynamic populations of Archaea in a lab-scale multimedia biofilter. The results suggested that Archaeal showed a much lower abundance (relative to bacteria). This study therefore highlights the need for more research efforts to study the importance of Archaeal in nitrogen removal in biofilters.

Denitrification Process

Denitrification is a sequential reduction process of NO_3^--N to N_2 (Table 3) [37]. The phylum proteobacteria, such as *Alcaligenes*, *Azospirillum, Magnetospirillum, Bradyrhizobium*, *Rhizobia*, and *Azoarcus* are assumed to be the key players in denitrification communities [60]. Different denitrification microbiotas played predominant roles during different processes and functioned with various activities in the presence of environmental changes [61-62]. Most denitrifying bacteria grow rapidly under anaerobic conditions, and they utilize organic compounds as electron donors in the denitrification process [63]. The six functional genes, including the membrane-boundnitrate reductase gene (*narG*), periplasmic nitrate reductase gene (*napA*), Cu-containing nitrite reductase gene (*nirK*), cytochromecd1- containing nitrite reductase gene (*nirS*), nitric oxide reductase gene (*norB*), and nitrous oxide reductase gene (*nosZ*), have been used as molecular markers to study preferable denitrification processes [37, 62]. Zhang et al. [17] reported that the remarkable decrease in NO_3^--N monitored in a biofilter manifests that denitrification is an important microbiological process, and denitrification was estimated to account for 90% of TN removal and 95% of NO_3^--N removal. The ratio of *norB*/*nosZ* served as the predominant driver for the transformation rates of NO_3^--N and NH_4^+-N, while the *norB*/*nosZ* ratio followed by the ratio of (*nirS* + *nirK*)/*nosZ* predominated a notable role in the accumulation of N_2O and NO in the biofilter [17].

A number of studies have identified the key factors (i.e., temperature, pH, DO) governing denitrification in an attempt to achieve a satisfactory NO_3^--N removal. The denitrification microbial growth rate and the rate of biologically mediated reactions generally increase exponentially with increasing temperature. Denitrification activity would be limited below 20ºC or above 60-75ºC [64]. The pH of influent water has a strong influence on denitrifier activity. An optimum range of pH for denitrification rate is 7.0-7.5, and pH values below 4 and above 10 caused the almost complete inhibition of the denitrification rate [65]. The significant inhibition of denitrification activity was observed at pH of 6.5-7, and high nitrite accumulation was shown at pH 7.5-9 [66]. Another study carried out by Zhang et al. [17] showed NO_3^--N transformation rates ranging from 21.0 to 23.4 g/(m^3 h), whereas NO_2^--N and NH_4^+-N transformation rates stabilized less than 6.0 g/(m^3 h) as the DO level increased from 1.0 to 6.0 mg/L. Based on these observations, the growth rate and denitrification activity could vary due to the different responses of denitrification microorganisms to environmental factors.

Anammox Process

Under anaerobic conditions, NH_4^+-N and NO_2^--N could be transformed into N_2 through the anammox process (Table 3) [67]. *Candidatus* Brocadia, *Candidatus* Kuenenia, *Candidatus Jettenia* asiatica, and *Candidatus* Brocadia anammoxidans are assumed to be key players in the anammox process, and the anammox sequences have been identified [68]. The process of anammox is one of the most innovative technological advances in the removal of NH_4^+-N from wastewater. Because of its high efficiency in nitrogen removal, anammox has been widely researched. Zeng et al. [31] investigated nitrogen removal efficiency and microbial community in treating low-strength wastewater using an anammox biofilter at ambient temperature (15.3-23.2ºC). The results revealed that microbial community structures varied with the decrease of influent NH_4^+-N concentration, and the genus of functional anammox bacteria was *Candidatus Kuenenia stuttgartiensis*. Wang et al. [2] reported that the NH_4^+-N removal efficiencies ranged from 67.3% to 92.7% under hydraulic loading rate (1.0-3.0 m^3/ (m^2 d)) constraints. The result indicated that the abundance of anammox bacteria was 100-1,000 times greater than that of *amoA*, suggesting that anammox was the predominant removal pathway of NH_4^+-N in the biofilter.

The key environmental factors that influence anammox include NH_3-N, temperature, and pH. Fernández et al. [69] reported that 50% and 80% inhibition of anammox activity in short-term tests (0-140 days) were observed at 38 mg NH_3-N/L and 100 mg NH_3-N/L, respectively. Tang et al. [70] observed that high NH_3-N concentrations (57-187 mg/L) may be toxic to anammox microorganisms. However, Aktan et al. [71] reported that no inhibition of anammox activity was observed at NH_3-N concentrations below 150 mg/L, and the activity of anammox rapidly dropped to 10% when the NH_3-N concentration reached 190 mg/L (T = 34ºC and pH = 8). Another study carried out by Aktan et al. [71] found the removal efficiency and elimination capacity of laboratory-scale biofilters (8l reactor volume) significantly decreased as the inlet NH_3-N concentration increased to above 110 mg/m^3. Previous studies showed that the appropriate temperature for anammox was about 30-40°C [72-73]. It is still

a challenge for the anammox process to function well at low temperatures. The optimum range of pH for the process was 6.7-8.3 [12].

Dissimilatory/Assimilatory Nitrate Reduction to Ammonium

Dissimilatory nitrate reduction to ammonium (DNRA) and assimilatory nitrate reduction to ammonium (ANRA) are two notable processes involved with NO_3^- reduction and the increase (accumulation) of NH_4^+-N (Table 3) [37]. DNRA and ANRA could also occur, but the anaerobic environments in biofilter systems have high concentrations of NH_4^+-N and organic N, which repress this process or make it quantitatively insignificant [60]. In the absence of oxygen, NO_3^--N can be utilized by many microbes as a respiratory electron acceptor. NO_3^--N reduction is coupled to the anaerobic oxidation of organic carbon, producing either NH_4^+-N or N_2 gas during denitrification [37]. The NO_3^--N reduction coding gene *nas* is often used as a molecular marker for the ANRA process [37]. Wang et al. [7] investigated the absolute abundance and distribution pattern of *nas* gene in a biofilter. The results indicated that *nas* had a high direct negative contribution (-0.802) to NH_4^+-N removal rate and a remarkable direct negative contribution (-0.806) to NO_3^--N accumulation.

Complete Oxidation of Ammonia to Nitrate in One Organism (Comammox)

Nitrification is a extensively accepted characteristic of the nitrogen cycle [37]. A newly nitrogen removal process, comammox, was first discovered in a recirculation aquaculture system biofilter [36]. Van Kessel et al. [36] reported the enrichment and initial characterization of two *Nitrospira* species that encode all the enzymes necessary for NH_4^+-N oxidation via NO_2^--N to NO_3^--N in their genomes, and indeed completely oxidize ammonium to NO_3^--N to conserve energy. The findings of the comammox process will lead to a new recognition of the environmental abundance and distribution of ammonia-oxidizing microorganisms.

Simultaneous Partial Nitrification, Anammox, and Denitrification (SNAD)

SNAD comprises three main processes: partial nitrification (micro-aerobic), anammox (anoxic), and denitrification (anoxic) (Table 3). The anammox reaction is derived by a cluster of planctomycete bacteria, which always prefer to utilize NO_2^--N as electron acceptors [43]. Anammox prefers to be associated with partial nitrification, which can supply NO_2^--N substrate to the anammox process [74]QD Ru. The first step of the denitrification process (NO_3^--N→ NO_2^--N), catalyzed by *narG* and *napA* codase under aerobic conditions, provided NO_2^--N substrate for the anammox process. Due to the slow growth rate of anammox, long start-up time is needed for anammox to function well in biofilters [1]. Liang et al. [30] investigated a 2.65 L SNAD biofilter for treating synthetic wastewater with high NH_4^+-N and low organic carbon content. The influent ammonia, aeration rate, and temperature were 200 mg/L, 4.5L/min, and 25ºC. The nitrogen removal rate and TN removal efficiency were 3.26 kg/ (m^3 d), 65%-76%, respectively. *Nitrosomonas* and *Candidatus brocadia* were considered the predominant players in the biofilter. As noted by Wang et al. [62], SNAD were coupled at the molecular level (functional genes), and collaboratively contributed to NH_4^+-N removal. The coexistence of the SNAD processes was the primary mechanism response to the simultaneous and robust performance of NH_4^+-N and organic carbon treatment.

Single Reactor High Activity Ammonia Removal over Nitrite (SHARON)

The SHARON process is developed and applied for the treatment of high NH_4^+-N wastewater by means of nitritation (Table 3) [75]. Due to the distinct responses to an increase of temperature, the *AOB* grow faster than *NOB* at temperatures above 20-35ºC. The SHARON process especially utilizes the high temperature to enhance the specific growth rates of *AOB*, suggesting no sludge production. Thus temperature could be used as a controlling operational parameter to achieve a SHARON process in biofilters. Previous studies indicated that temperature, pH, and HRT are the three important process parameters for SHARON treating domestic sewage. Ammonium oxidizers have the corresponding maximum specific growth rate with a working temperature of 35ºC. A high pH (8.1-8.5) is preferable to outcompete nitrite oxidizers and obtain a lower effluent NH_4^+-N concentration. González-Martínez et al. [33] constructed a partial-SHARON biofilter and investigated the influence of the HRT on nitrification process of the biofilter. An HRT of 0.5 day could decrease the bacterial biodiversity in the biofilms constituted by *Nitrosomonas* and *Diaphorobacter*. HRTs of 0.4 and 0.5 days could enhance the formation of biofilms constituted by *Nitrosomonas* sp., *Nitrosospira* sp., and *Nitrosovibrio* sp.

Completely Autotrophic Nitrogen Removal over Nitrite (CANON)

In the CANON process, NH_4^+-N is first converted to NO_2^--N by aerobic *AOB*, after which the NO_2^--N and remaining NH_4^+-N are converted to N_2 by anaerobic *AOB* (Table 3) [76]. CANON has been a hot area of research in the wastewater treatment field nowadays. The CANON process provides several advantages, including less aeration demand, less sludge production, and no organic carbon consumption [32]. Liu et al. [77] studied the microbial diversity and population with the different influent NH_4^+-N in a CANON biofilter packed with volcanic filter. Biodiversity analysis showed that *Nitrosomonas*-related aerobic *AOB* and *Planctomycetales*-like anammox bacteria were dominant functional bacteria. Previous studies indicated that influent NH_4^+-N concentration

and temperature were two of the principal factors that constrained the CANON process to treat sewage. The decrease of DO, influent NH_4^+-N, and temperature had a negative effect on certain *AOB* and anammox bacteria, leading to either an acclimation of existing bacterial population to new conditions or a fluctuation of microbial community and population [77]. As noted by Liang et al. [78], high DO concentrations (around 5 mg/L) of the influent were required to achieve high-rate nitrogen removal, and the ratio of air inflow to water inflow should be maintained at 28-40 for stable operation.

Oxygen-Limited Autotrophic Nitrification-Denitrification (OLAND)

In the OLAND process, NH_4^+-N is autotrophically oxidized to N_2 with NO_2^--N as the electron acceptor under oxygen-limited conditions (Table 3) [79]. Compared with a traditional nitrification-denitrification process, OLAND requires lower oxygen consumption (63% lower), higher organic carbon degradation and removal efficiency, and lower operating cost [34]. De Clippeleir et al. [80] demonstrated the possibility of obtaining fully autotrophic TN removal in the OLAND biofilter through a coupling of aerobic *AOB* and anoxic ammonium oxidizing or anammox bacteria (*AnAOB*) activity. The abundance of aerobic *AOB*-AmoA, *AnAOB*-16SrRNA, and *Nitrospira*-16SrRNA was 2×10^2, 9×10^3, and 2×10^2 copies/ng DNA.

The review on nitrogen removal and transformation processes reveals that NH_4^+-N removal through nitrification and NO_3^--N removal through denitrification are considered satisfactory in biofilters. Anammox was a significant mechanism that accounted for robust NH_4^+-N removal in biofilters. The coupled multipath interactions of nitrification, denitrification, and anammox processes were the primary reason that accounted for the robust nitrogen removal performance in biofilters. Although the newly discovered nitrogen pathways have a high potential for nitrogen removal, more research efforts are needed to realize the full-scale application of biofilters. In addition, the less-studied ANRA process may be an existing but previously underestimated pathway in biofilters. The newly discovered comammox process will provide a new appreciation of the nitrogen cycle in biofilters.

Challenges and Perspectives

Biofilters have been intensively studied and used as a promising technology to achieve sustainable nitrogen removal in an aquatic environment. The coupling of microbial nitrogen transformation processes in biofilters were the primary reason accounting for high removal efficiency of nitrogen in an aquatic environment. The traditional biofilters for nitrogen removal have been the combined application of nitrification and denitrification processes. New biofilters based on partial nitrification coupled with the anammox process seem to be more favorable. The coupling of anammox bacteria, *AOB*, and *AOA* have been successfully achieved in a lab-scale biofilter. However, the presence of archaea has been reported in biofilters [7], but little detailed information of Archaea have been investigated. Thus, more investigation is needed to explore the applicability of the co-existence of archaea and anammox bacteria in biofilters.

The SNAD process in biofilters has been proved by molecular techniques and is a notable process for treating high NH_4^+-N and low-carbon concentration wastewaters. The SNAD process needs the simultaneous presence of aerobic and anaerobic conditions to function well. The limited oxygen supply is needed to inhibit *NOB* over the *AOB* and to further avoid inhibition of anammox bacteria. Therefore, more investigation is needed to explore optimal conditions for such notable co-existence.

Over the past few years, denitrification has been known as the only key mechanism in NO_3^--N removal. The DNRA and ANRA processes may be an underlying mechanism response for NO_3^--N removal [37, 81]. However, few investigations on DNRA and ANRA in biofilters have been reported. Thus, further research efforts are required to study the importance of DNRA or ANRA in the nitrogen balance. These efforts should include using stable isotope techniques (^{15}N) to accurately evaluate the contribution of DNRA or ANRA for nitrogen removal.

In biofilter systems, biomass, density, and thickness of biofilm affect the substrate (i.e., NH_4^+-N, NO_3^--N, and NO_2^--N) conversion rate, thus affecting the removal efficiency of nitrogen. Most current studies on biofilters are mainly concentrated on the practical applications and optimal operating parameters, which are largely based on one-dimensional monitoring and manipulation of traditional bulk physical-chemical parameters with limited scope for characterizing biofilter microbiology [15]. Furthermore, research of the density, porosity, and pore structure of biofilms should also be taken into consideration to further enhance nitrogen removal and to polish effluent. In addition, the quorum-sensing bacteria produce and release chemical signal molecules termed autoinducers, whose concentrations increase as a function of increasing cell density [82]. The quorum sensing in bacteria is supposed to function as the key role in microbial attachment. Thus, more research is required to study the function of quorum sensing on microbial attachment.

Conclusions

The literature reports the operational adaptability and feasibility of biofilters for nitrogen removal. The coupled multipath interactions of nitrification, denitrification, and anammox processes were the primary reason accounting for robust nitrogen removal performance. The newly discovered nitrogen pathways such as SNAD, SHARON, CANON, and OLAND have a high potential for nitrogen removal. In order to achieve satisfactory nitrogen removal, future studies should focus on exploring the co-existence of

archaea and anammox bacteria, optimizing the operational parameters of SNAD, exploring the contribution of DNRA or ANRA to nitrogen removal, and exploring the quorum-sensing function on microbial attachment.

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