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Research review paper

Technologies for biological removal and recovery of nitrogen from wastewater

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ABSTRACT

Water contamination is a growing environmental issue. Several harmful effects on human health and the environment are attributed to nitrogen contamination of water sources. Consequently, many countries have strict regulations on nitrogen compound concentrations in wastewater effluents. Wastewater treatment is carried out using energy- and cost-intensive biological processes, which convert nitrogen compounds into innocuous dinitrogen gas. On the other hand, nitrogen is also an essential nutrient. Artificial fertilizers are produced by fixing dinitrogen gas from the atmosphere, in an energy-intensive chemical process. Ideally, we should be able to spend less energy and chemicals to remove nitrogen from wastewater and instead recover a fraction of it for use in fertilizers and similar applications. In this review, we present an overview of various technologies of biological nitrogen removal including nitrification, denitrification, anaerobic ammonium oxidation (anammox), as well as bioelectrochemical systems and microalgal growth for nitrogen recovery. We highlighted the nitrogen removal efficiency of these systems at different temperatures and operating conditions. The advantages, practical challenges, and potential for nitrogen recovery of different treatment methods are discussed.

1. Introduction

Ground- and surface waters are contaminated by nitrogen via numerous routes (Fig. 1). Increasing application of fertilizers in agriculture was reported to contaminate surface and ground water sources with around 293,000 tonnes/yr of nitrogen in Canada (Ritter et al., 2002). Moreover, different types of waste, including industrial, animal, and domestic, result in contamination of water with nitrogen when those wastes get discharged in water sources without treatment. Municipal wastewater treatment plants (WWTPs) also contribute to nitrogen loading into the surface and ground waters with approximately 80,000 tonnes/yr (Ritter et al., 2002). Strict nitrogen discharge standards are enforced by many countries. For example, municipal WWTPs are allowed to discharge less than 5 mg L^{-1} ammonium and 15 mg L^{-1}

Abbreviations: AB, algal biofilm; ABMFC, algae biofilm microbial fuel cell; AHL, acyl homoserine lactone; ALE, adaptive laboratory evolution; AMO, ammonia monooxygenase; AnAOB, anaerobic ammonia oxidation bacteria; AOB, ammonium oxidizing bacteria; CANON, completely autotrophic nitrogen removal over nitrite; C/N, carbon to nitrogen ratio; 2C4NP, 2-chloro-4-nitrophenol; Comammox, complete ammonia oxidizers; COD, chemical oxygen demand; CSTR, continuous stirred tank reactor; DAMO, denitrifying anaerobic methane oxidation; DEA, diethylamine-functionalized polymer; DEAMOX, denitrifying ammonium oxidation; DO, dissolved oxygen; DSA, dimensionally stable anodes; EPS, extracellular polymeric substance; FA-MFC, flat-panel air-cathode MFC; GMM, genetically modified microorganism; HAO, hydroxylamine oxidoreductase; HF-MBfR, H_2-based hollow-fiber membrane biofilm reactor; HRAP, high rate algal pond; HRT, hydraulic retention time; LUC, land use change; MABR, membrane-aerated biofilm reactor; MBBR, moving bed biofilm reactor; MBfR, membrane-biofilm reactor; MEC, microbial electrolysis cells; MFCs, microbial fuel cells; m-WWTPs, municipal wastewater treatment plants; NADH, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; N/P, nitrogen to phosphorus molar ratio; NPs, nitrophenols; NOB, nitrite oxidizing bacteria; 3NT, 3-nitrotoluene; 4NT, 4-nitrotoluene; 2NTDO, 2-nitrotoluenene 2,3-dioxygenase; NTR, nitrate-to-nitrite transformation ratio; NXR, nitrite oxidoreductases; OLAND, oxygen-limited nitrification and denitrification; PAC, poly-aluminium chloride; PAOs, polyphosphate-accumulating organisms; PBRs, photobioreactors; PBS, poly(butylene succinate); PCL, poly(ε-caprolactone); PHAs, polyhydroxyalkanoates; PHBV, poly3-hydroxybutyrate-co-3-hydroxyvalerate; PLA, poly(lactic acid); PLLA, poly(l-lactic acid); PQS, *Pseudomonas* quinolone signal; PVA, polyvinyl alcohol; QS, quorum sensing; *rhl, acyl-homoserine-lactone synthase*; SHARON, single reactor high activity ammonia removal over nitrite; SNAD, simultaneous nitrification anammox and denitrification; SND, Simultaneous nitrification and denitrification; SRT, sludge retention time; VHb, *Vitreoscilla* Hemoglobin b

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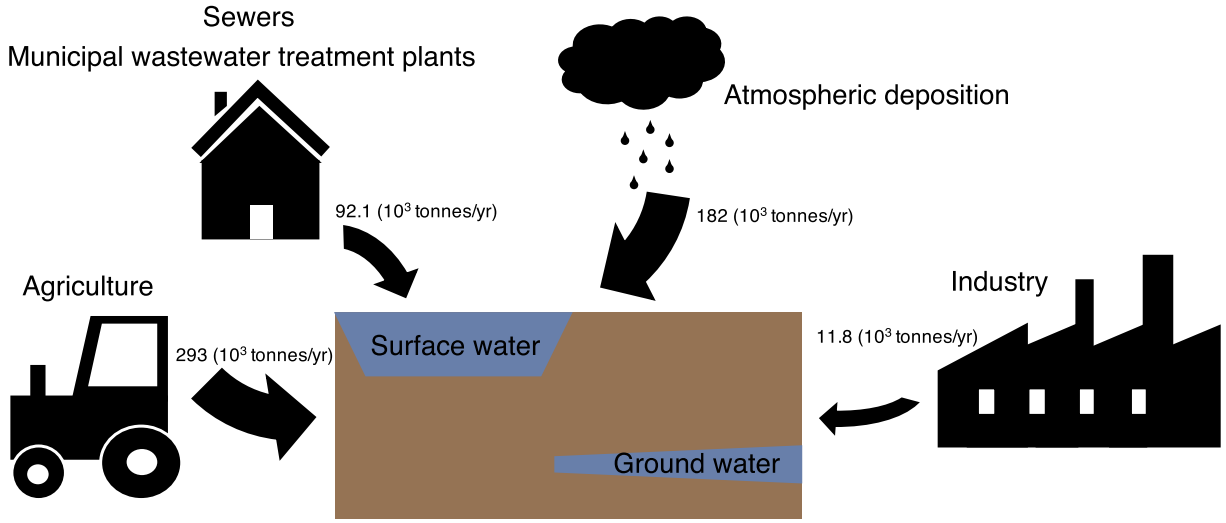

Fig. 1. Nitrogen loading (10^3 tonnes/yr) to the surface water and groundwater in Canada from various sources. The nitrogen loading rates values are provided from Ritter et al., 2002. It should be pointed out that not all atmospheric nitrogen deposition can be considered as contamination.

total nitrogen in China (GB18918-2002) (Du et al., 2015). Thus, nitrogen removal from wastewaters is extremely important to protect water resources, especially for regions facing water shortage. Eutrophication in freshwater ecosystems is one of the direct and harmful consequences of excessive nitrogen loading. The eutrophication phenomenon degrades freshwater ecosystems by developing algal blooms, spreading aquatic plants, oxygen depletion and hence loss of key species (Taziki et al., 2015). Furthermore, blue green algae blooms can produce natural toxins that pose risks to the human health (Ritter et al., 2002; Taziki et al., 2015).

Nitrogen exists in different oxidation states which makes the process of its removal from water complex and challenging. Adsorption or co-precipitation treatment is most often not feasible due to the stability and high solubility of nitrate, resulting in high energy and cost for treatment of nitrate-contaminated water (Rezvani et al., 2019). Most wastewater treatment systems have two levels of treatment: primary (physical settling of solids) and secondary (various forms of biological oxidation e.g. activated sludge or trickling filters). In regions where regulations mandate higher effluent quality, tertiary treatment is also used for nutrient removal and disinfection. Tertiary treatment as the final cleaning process removes inorganic compounds and improves the effluent quality before it is reused, recycled or discharged to the environment.

Biological approaches are known to effectively remove nitrogen compounds in wastewater (EPA, 1993). The activated sludge process, as the most common biological wastewater treatment method, was developed to enhance the effectiveness of nutrient removal. In a conventional nitrogen removal process, wastewater goes through the nitrification and then the denitrification process. Nitrification is the biological oxidation of ammonia or ammonium to nitrite followed by oxidation of nitrite to nitrate, however, denitrification reduces nitrate and ultimately produces N_2 through a series of intermediate gaseous nitrogen oxide products. When there is not sufficient organic carbon source in wastewater for denitrification, this negatively affects the nitrogen removal efficiency (Blackburne et al., 2008). Various technologies for nitrification and denitrification of wastewater have been reviewed previously (Ali and Okabe, 2015; Rodríguez Arredondo et al., 2015; Gonçalves et al., 2017; Ma et al., 2016; Taziki et al., 2015; Tomaszewski et al., 2017). In this review, we aim to compare the efficiency and energy cost of different methods to hopefully facilitate decisions on wastewater treatment policies based on local conditions. We first describe the fundamental biological nitrogen removal processes in WWTPs, such as nitrification, denitrification, and anaerobic ammonium oxidation (anammox). We then present a comparative overview on the advantages and challenges presented by different biological nitrogen removal processes, including their potential for obtaining high effluent quality as well as for chemical/energy recovery. We also discuss various physico-chemical factors that may affect the efficiency of the biological nitrogen removal methods.

2. Major biological enzymatic processes of nitrogen cycle that transform nitrogen into various oxidation states

Stein and Klotz (2016) suggested five nitrogen-transformation flows (coloured arrows in Fig. 2A) which are the following: ammonification, including reduction of dinitrogen or nitrogen fixation (red), assimilatory and dissimilatory reduction of nitrite to ammonium (DNRA) (blue); nitrification (green and orange) composed of ammonia oxidation to nitrite (nitritation), and oxidation of nitrite to nitrate (nitratation); denitrification (purple); anammox, as a form of coupled nitrification–denitrification (pink); and nitrite–nitrate interconversion (orange and cyan). The general processes of organic matter mineralization and assimilation by cellular life completes the movement of reactive nitrogen through the biosphere. Microorganisms that participate in the above-mentioned biological processes of nitrogen cycle include heterotrophic denitrifying bacteria, autotrophic nitrifying bacteria, anammox bacteria, and microalgae (Fig. 2B).

In primary wastewater treatment, the major forms of nitrogen present in the water are organic nitrogen and ammonium. During secondary treatment, these two major forms of nitrogen are rapidly converted to nitrate by nitrifying bacteria, including ammonium oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB) (Taziki et al., 2015). Newly identified complete ammonia oxidizers (Comammox) can perform ammonia oxidation to nitrate (Daims et al., 2015) (Fig. 2B). Nitrite is the most ephemeral form of nitrogen in the environment. In both wastewater treatment systems and surface waters, it occurs as the least prevalent form of inorganic nitrogen. Among the above-mentioned biological processes of nitrogen cycle, nitrification, denitrification, anammox, and nitrogen assimilation are discussed here.

3. Fundamental nitrogen removal processes

Biological wastewater treatment is operated based on the combined activity of microorganisms in microbial community. Thus, it is important to know about the nitrogen removal processes along with the microbial communities involved in the processes. Proteobacteria are a

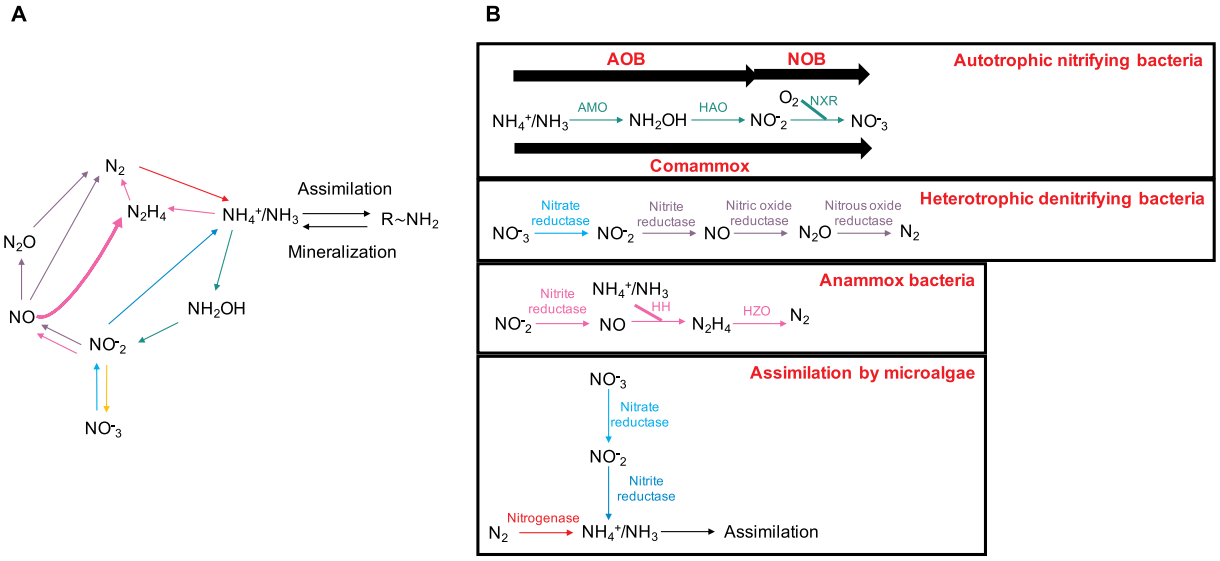

Fig. 2. Major biological processes of the nitrogen cycle (A). Arrows with different colours are the major processes in nitrogen cycle including, ammonification (red), assimilatory and dissimilatory reduction of nitrite to ammonium (DNRA) (blue), nitrification (green and orange), denitrification (purple), anammox (pink), nitrite–nitrate interconversion (orange and cyan). Organisms participated in the biological processes of nitrogen cycle (B). The coloured arrows and enzymes follow the rules in figure (A). Both ammonification and assimilatory reduction of nitrite to ammonium are performed by microalgae. It should be pointed out that archaea can also carry out nitrification and denitrification. AOB, ammonium oxidizing bacteria; NOB, nitrite oxidizing bacteria; AMO, ammonia monooxygenase; HAO; hydroxylamine oxidoreductase; HH, hydrazine hydrolase; HZO, hydrazine-oxidizing enzyme. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

dominant phylum in activated sludge as the most common technology for sewage treatment, followed by other groups such as Bacteroidetes, Chloroflexi, Actinobacteria, Planctomycetes, Firmicutes, etc (Ferrera and Sánchez, 2016). Here we discuss the nitrogen removal processes driven by specific microbes. Table 1 presents total nitrogen removal efficiency, chemical input, economic evaluation and main technical parameters of different nitrogen removal processes compared to the conventional process.

3.1. Nitrification

Nitrification consists of two sequential biological oxidation processes. The first step is NH_4^+ oxidation to NO_2^-, which is the limiting step and is carried out by the AOB. This reaction is catalysed by ammonia monooxygenase (AMO) and hydroxylamine oxidoreductase (HAO), along with hydroxylamine (NH_2OH) formation as the intermediate product. The NO_2^- produced in the first step is rapidly converted to NO_3^- in the second step, carried out by the NOB, in the presence of molecular oxygen. The conversion is catalysed by nitrite oxidoreductases (NXR) and nitrite-oxidizing systems, which are one-step oxidation enzymes found in *Nitrobacter* and in the genera of *Nitrococcus, Nitrospina and Nitrospira*, respectively (Fig. 2B).

In comparison with the physicochemical processes for wastewater treatment, biological nitrogen removal via nitrification and denitrification is more cost-effective. However, several drawbacks remain, such as slow nitrification reaction, decreasing nitrification activity by ammonium and organic matter overload, necessity of oxygen control, and the demand for two reactors: an aerobic one for nitrification and an anaerobic one for denitrification. Also, large size reactor or long hydraulic retention time (HRT) are required to complete NH_4^+ removal due to low nitrification rate, resulting in high operational cost (Shoda, 2017). Several biological nitrogen removal process systems have been developed to reduce energy input into the process. These include simultaneous nitrification and denitrification, anammox, partial nitrification and denitrification, complete autotrophic nitrogen removal over nitrite (CANON), aerobic deammonification, oxygen-limited nitrification and denitrification (OLAND), as well as combination of these processes including membrane bioreactors and cell-immobilization (Ge et al., 2015; Shoda, 2017) (Table 2). Partial nitrification via nitrite offers several significant advantages in biological wastewater treatment compared to the conventional nitrification including: i) 40% reduction of chemical oxygen demand (COD) and 1.5–2 fold increase of nitrite reduction rates in the subsequent denitrification stage, ii) saving 25% oxygen consumption, 300% biomass reduction, and 20% CO_2 emission during denitrification. When partial nitrification is combined with anammox, ammonium partially oxidizes to nitrite aerobically, and remaining ammonium subsequently reacts with nitrite to form nitrogen gas anaerobically. This has several benefits, such as no requirement of external carbon source, 80% reduced sludge production, and less energy and 60% reduced oxygen requirement compared to the conventional nitrification/denitrification (Ge et al., 2015; Cao et al., 2017). The partial nitrification is based on the condition favouring AOB bacteria but preventing NOB bacteria. Therefore, there are several parameters preferred by AOB bacteria positively affected partial nitrification including i) pH (7.5 to 8.5) (Ge et al., 2015), ii) temperature (higher than 25.0 °C) (Paredes et al., 2007), iii) dissolved oxygen (DO) concentration (1.5 mg L^{-1}) (Ruiz et al., 2006), iv) real-time control of aeration and periodic anoxic and aerobic operation (Ge et al., 2014), v) sludge retention time (SRT) (5 d) (Galí et al., 2007), vi) C/N ratio ($0.3 < C/N < 6$) (Zafarzadeh et al., 2011; Mosquera-Corral et al., 2005), vii) NOB inhibitor (such as sulfide, hydroxylamine, salt, heavy metals, chlorate, cyanate, halide, azide, hydrazine, and organic chemicals) (Sinha and Annachhatre, 2007), and viii) ultrasonic treatment (frequency 40 kHz and density ultrasound 0.027 W mL^{-1} for 2 h (Zheng et al., 2013) (Fig. 3A).

The moving bed biofilm reactor (MBBR) is composed of an aeration tank with special plastic carriers for fixing biomass as the biofilm. The carriers are mixed in the tank by the aeration system and thereby leading a good contact between the biomass on the carriers and the substrate present in the influent wastewater. Recently, combination of nitrification and poly-aluminium chloride (PAC) adsorption of organic micropollutants were simultaneously performed in a nitrifying MBBR (Cimbritz et al., 2019). However, due to inhibitory effect of nitrite on polyphosphate-accumulating organisms (PAOs), the simultaneous

Table 1
Total nitrogen removal efficiency, chemical input, economic evaluation, main technical parameters of different nitrogen removal process compared to conventional process.

Operation technique	Total nitrogen removal efficiency	Chemical input	Economic evaluation	Main technical parameters	References
Partial nitrification via nitrite	1.5–2 fold increase of nitrite reduction rates in the subsequent denitrification stage	40% reduction of COD	25% reduced oxygen demand, 300% biomass reduction, 20% CO_2 emission during denitrification	pH, temperature, DO, real-time aeration control, sludge retention time, substrate concentration, alternating anoxic and aerobic operation, inhibitor, ultrasonic treatment	Ge et al. (2015)
Partial nitritation /anammox	≥85% of nitrogen removal	No need of external carbon source	60% reduced oxygen demand, 80% reduced sludge production, 24 Wh/p/day, compared to a 44 Wh/p/day consumption in conventional treatment	Carbon concentrating pretreatment, suppression of NOB especially under low temperatures (15–10 °C), intensification of anammox biofilm activity, reactor design, final polishing	Cao et al. (2017)
Simultaneous nitrification and denitrification (SND)	82% nitrogen removal	Requirement of external carbon source	Saving cost for anoxic tank, applicable only for low C/N ratio (< 5) wastewaters	Reactor design, oxygen availability for nitrification, effective carbon source utilization for denitrification	Guo et al. (2005)
Shortcut nitrification and denitrification	Nitrite denitrification rate is 1.5 to 2 times higher than nitrate denitrification rate	40% lower demand of electron donors in anoxic phase	25% reduced oxygen demand in aerobic phase with 60% saving energy, applicable for high ammonium concentrations or low C/N ratios wastewaters	DO, SRT, pH, temperature, substrate concentration and load, operational and aeration pattern, inhibitor	Peng and Zhu (2006)
Nitritation/anammox	81% nitrogen removal	No need of external carbon source	60% reduced oxygen demand, energy recovery by methane production, minimal surplus sludge production, consumption of inorganic carbon CO_2, no nitrous oxide emission, decrease in energy consumption from 2.66 to 1.50 kWh per kg N removed for reject water treatment	Poor effluent water quality, need of post-denitrification process	Du et al. (2015); Ma et al. (2016); Li et al. (2016a)
Simultaneous partial nitrification, anammox, and denitrification (SNAD)	99% nitrogen removal	Low concentrations of organic matter	Simultaneous removal of inorganic nitrogen and organic carbon, applicable for wastewater with complex composition and high ammonia concentration and low C/N ratio	Intermittent aeration, pH, DO	Zhang et al. (2017)
Denitrifying ammonium oxidation (DEAMOX)	94% nitrogen removal, simultaneous nitrate and ammonium removal	80% reduced demand of organic carbon	100% reduced aeration demand, 64.8% reduced sludge production, low nitrogen contained wastewater, low/high-strength nitrate and ammonium containing wastewater, reduced greenhouse gas (CO_2 and N_2O) emission	Co-existence of partial-denitrification and anammox bacteria	Du et al. (2017)
Partial-denitrification/anammox	More than 90% nitrogen removal	79% reduced demand of organic carbon	45% reduced oxygen demand, reduced biomass production, applicable for high-strength wastewater	Avoiding high organic matter in the effluent of partial denitrification reactor	Ma et al. (2016), Cao et al. (2016)
Denitrification by bioelectrochemical systems	100% nitrate removal	Required carbon source and buffering agent	100% reduced oxygen demand, producing power and current densities of 2.1 W/m3 and 26.6 A/m3	Generation of high concentrations of ammonium in anode and cathode	Naga Samrat et al. (2018)

COD- chemical oxygen demand; DO- dissolved oxygen concentration; SRT- sludge retention time; Wh/p/day- watt hours per person per day.

Table 2
Comparison of nitrogen removal efficiencies between different anammox methods.

Optimization method	Reactor type	Operation technique	Wastewater	Total nitrogen removal	Temperature	References
Combined anammox system	Sequencing batch reactors (SBR)	Anammox-partial denitrification	Synthetic wastewater	94.06%	30 °C 20–26 °C	Du et al. (2015)
	SBR	Anammox-denitrification	Synthetic wastewater	97.47%	30 °C	Du et al. (2014)
	Plug-flow granular sludge-based pilot-scale reactor	One-stage partial nitritation-anammox	A-stage of the WWTP of Dokhaven, Rotterdam	46%	19 °C	Lotti et al. (2015)
	Upflow anaerobic sludge blanket reactor (UASB) +SBR	Anammox-partial denitrification	Synthetic wastewater, domestic wastewater	97.8%	28 °C	Cao et al. (2016)
	Upflow reactor	Synergy of sludge fermentation, denitrification and anammox processes	Pre-treated domestic wastewater and synthetic wastewater	0.065 g N L^{-1} d^{-1}	16–29 °C	Wang et al. (2016a)
	Anaerobic upflow reactor	Granular bed anammox	Synthetic medium	60%	37 °C	Cho et al. (2010)
	Combining high rate anammox reactor with sequential biocatalyst addition (SBA)	SBA-anammox process	Pharmaceutical wastewater	9.4 g N L^{-1} d^{-1}	35 °C	Tang et al. (2011)
	Membrane biofilm reactor (MBfR)	Synergy of anammox and denitrifying anaerobic methane oxidation (DAMO) microorganisms	Synthetic wastewater	0.25 g N L^{-1} d^{-1}	NA	Shi et al. (2013)
	SBR	Simultaneous partial nitrification, anammox and denitrification (SNAD)	Synthetic wastewater	85-87%	35 °C	Lan et al. (2011)
	Lab-scale upflow membrane-aerated biofilm reactor (UMABR)	Granular nitritation-anammox	Synthetic wastewater	81%	25 °C	Li et al. (2016a)
	Full-scale SBR	Decentralized full-scale system	Municipal wastewater	50%	NA	Fernandes et al. (2013)
	Lab-scale sequencing batch biofilm reactors (SBBR)	Intelligent control system (ICS) and timer control system (TCS)	Synthetic wastewater	87.8%	25 °C	Jin et al. (2012)
	Lab-scale A2N-SBR	Anaerobic-anoxic/nitrification	Domestic wastewater	83%	22–25 °C	Wang et al. (2009)
	UASB	Anaerobic digester seedling sludge	Inorganic medium	42 g N L^{-1} d^{-1}	30-35 °C	Hu et al. (2010)
Low temperature	Aerobic/anoxic sequence batch reactor SBR	Removal of carbon and nutrients	Synthetic wastewater	71.15%	20 °C	Mansouri et al. (2014)
	UASB	Effect of seasonal temperatures on anammox	Synthetic wastewater	3.77 g N L^{-1} d^{-1}	2.5–15.8 °C seasonal variations	Guo et al. (2015)
	Continuous reactor	Gel carrier with entrapped anammox bacteria at lower temperature	Synthetic water	0.36 g N L^{-1} d^{-1}	6.3 °C	Isaka et al. (2008)
	Gas-lift reactor	Cold-adapted anammox species	Synthetic medium	0.02 g N L^{-1} d^{-1}	10 °C	Hendrickx et al. (2014)
	SBR	Direct treatment of municipal wastewater using anammox	Synthetic media and municipal wastewater from Dübendorf Switzerland	0.046 g N L^{-1} d^{-1}	12.5 °C	Laureni et al. (2015)
	SBR	Optimization of the nitrogen sludge loading rate (NSLR)	Synthetic sewage	0.029 g N L^{-1} d^{-1}	13.2 °C	Sánchez Guillén et al. (2016)
	Granular SBR	Completely autotrophic nitrogen removal over nitrite (CANON) process	Supernatant of an anaerobic sludge digester of the WWTP of Lugo Spain	0.2 g N L^{-1} d^{-1}	15 °C	Vázquez-Padín et al. (2011)
	Membrane bioreactor (MBR)	Anammox at lower temperatures	Synthetic nutrient medium	1.1 g N L^{-1} d^{-1}	15 °C	Awata et al. (2015)
	Upflow anaerobic biofilter (AF)	Anammox for treating low-strength wastewater	Synthetic wastewater	1.965 g N L^{-1} d^{-1}	15.3 °C	Taotao et al. (2015)
	UASB	High anammox activity and abundance	Low strength wastewater	2.28 g N L^{-1} d^{-1}	16 °C	Ma et al. (2013)
	SBR	Anammox at lower temperatures	Synthetic autotrophic medium	0.325 g N L^{-1} d^{-1}	18 °C	Dosta et al. (2008)
	Upflow anammox sludge bed (UAnSB) reactor	Anammox process at low temperature	Nitrite-amended pre-treated real urban wastewater	82 ± 4%	11 °C	Reino et al. (2018)
	Up-flow column reactor (UCR)	Porous polyester nonwoven fabric	Synthetic inorganic medium	0.18 g N L^{-1} d^{-1}	18 °C	Osaka et al. (2012)
	Gas-lift	Direct anaerobic treatment of municipal wastewaters	Synthetic influent	0.29 g N L^{-1} d^{-1}	20 °C	Hendrickx et al. (2012)

(*continued on next page*)

Table 2 (*continued*)

Optimization method	Reactor type	Operation technique	Wastewater	Total nitrogen removal	Temperature	References
	Anaerobic biological filtrated (ABF) reactor	Porous polyester nonwoven fabric carriers as a fixed bed for anammox bacteria	Synthetic water	8.1 g N L^{-1} d^{-1}	20–22 °C	Isaka et al. (2007b)
	Continuous reactor	Gel carrier with entrapped anammox bacteria	Synthetic water	2.8 g N L^{-1} d^{-1}	22 °C	Isaka et al. (2008)
	UCR	Gas–solid separator	Synthetic inorganic wastewater	17.5 g N L^{-1} d^{-1}	23 ± 2 °C	Yang et al. (2011)
	Single reactor high activity ammonia removal over nitrite (SHARON), SBR	Two-unit SHARON reactor coupled to anammox SBR	Supernatant of an anaerobic sludge digester of the WWTP of Lugo Spain	0.08 g N L^{-1} d^{-1}	20 °C	Vázquez-Padín et al. (2011)
	Moving bed biofilm reactor (MBBR)	Partial nitritation-anammox	Synthetic wastewater	0.007 g N L^{-1} d^{-1}	10 °C	Gilbert et al. (2015)
	MBBR	Partial nitritation-anammox	Synthetic influent	0.015 g N L^{-1} d^{-1}	10 °C	Gilbert et al. (2014)
	SBR	Combination of aerobic ammonium-oxidizing bacteria (AOB) and anammox	Synthetic pre-treated municipal wastewater	0.025 g N L^{-1} d^{-1}	12 °C	Hu et al. (2013)
	OLAND rotating biological contactor (RBC)	Oxygen-limited autotrophic nitrification/ denitrification (OLAND)	Synthetic wastewater	0.5 g N L^{-1} d^{-1}	15 °C	De Clippeleir et al. (2013)
	MBBR	Partial nitritation-anammox	Municipal wastewater	0.030 g N L^{-1} d^{-1}	15 °C	Laureni et al. (2016)
	MBBR	Integrated fixed-film activated sludge (IFAS) mode	Mainstream wastewater	0.010 g N L^{-1} d^{-1}	16 ± 1 °C	Trojanowicz et al. (2016)
	RBC	Anammox process accompanied with autotrophic nitrification and heterotrophic denitrification	Municipal landfill leachate and artificial $NaNO_2$ and NH_4Cl solutions	0.5 g N L^{-1} d^{-1}	17 °C	Cema et al. (2007)
	Air-lift sequencing batch reactor (SBAR)	Granular sludge-anammox based single stage	Synthetic medium	85-75 %	10-20 °C	Lotti et al. (2014)
Immobilization	Continuous stirred tank reactor (CSTR)	Polyvinyl alcohol (PVA)- immobilized system	Synthetic and partially nitrified swine wastewater	0.5 g N L^{-1} d^{-1}	33 °C	Magrí et al. (2012)
	CSTR	PVA, sodium alginate (SA)-immobilized system	Synthetic wastewater and modified reject water	8.2 g N L^{-1} d^{-1}	33 °C	Quan et al. (2011)
	CSTR	Polyethylene glycol (PEG)-immobilized system	Digester liquor of biogas plant	4 g N L^{-1} d^{-1}	30 °C	Furukawa et al. (2009)
	CSTR	PEG-immobilized system	Digester supernatant	3.8 g N L^{-1} d^{-1}	30 °C	Isaka et al. (2011)
	CSTR	PEG-immobilized system	Ammonia-rich wastewater	6 g N L^{-1} d^{-1}	36 °C	Isaka et al. (2008)
	CSTR	PEG-immobilized system	Synthetic water	3.7 g N L^{-1} d^{-1}	36 °C	Isaka et al. (2007a)
	CSTR	PEG-immobilized system	Digester supernatant for sludge	3.8 g N L^{-1} d^{-1}	30 °C	Kimura et al. (2013)
	CSTR	PVA-immobilized system	Synthetic wastewater	4.4 g N L^{-1} d^{-1}	30 °C	Ge et al. (2009)
	CSTR	PVA, SA-immobilized system	Synthetic wastewater	1.69 g N L^{-1} d^{-1}	33-35 °C	Qiao et al. (2013)
	SBR	PVA, SA-immobilized system	Synthetic medium	0.58 g N L^{-1} d^{-1}	35 °C	Zhu et al. (2014)
	Up-flow glass column reactors (UFCR)	PVA, SA-immobilized system	Culture medium	10.8 g N L^{-1} d^{-1}	37 °C	Ali et al. (2015)
	Lab-scale MBR	Bacillus methylotrophicus L7	Artificial wastewater	53%	25–30 °C	Yao et al. (2013)
	Pilot-scale SBR	Activated sludge + *A. tumefaciens* LAD9, *C. testosteroni* GAD3 and *A. xylosoxidans* GAD4	Municipal wastewater	80.5%	NA	Chen et al. (2015)

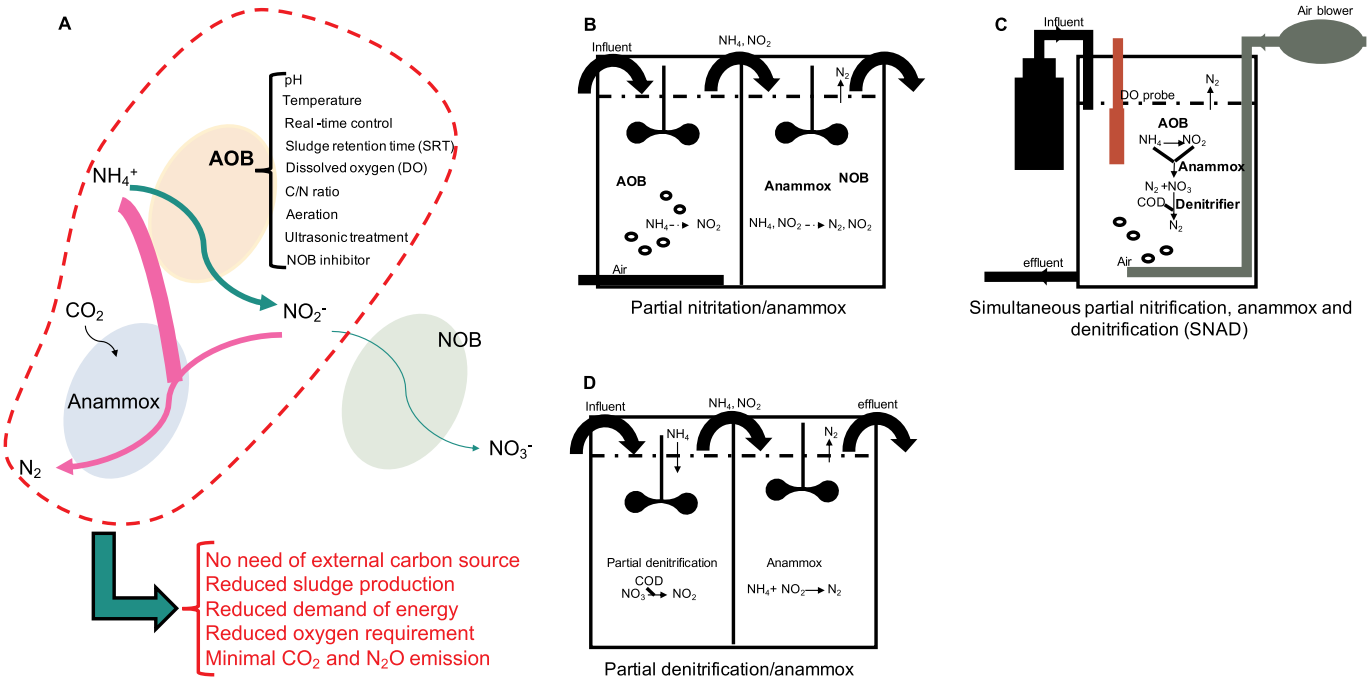

Fig. 3. Strategies for partial nitritation and anammox by ammonium oxidizing bacteria (AOB) and suppression of nitrite oxidizing bacteria (NOB). Schematic structure of partial nitritation/anammox (B), simultaneous partial nitrification, anammox and denitrification (SNAD) (C) and partial denitrification/anammox (D) reactors.

removal of nitrogen and phosphorus in partial nitrification systems has not been achieved and needs to be further investigated.

3.1.1. Microorganisms involved in nitrification

Nitrification, a two-step process of ammonia oxidation via nitrite to nitrate, is catalysed by chemolithoautotrophic microorganisms oxidizing either ammonia or nitrite. Two phylogenetically unrelated groups of AOB and NOB are Gram-negative autotrophic bacteria responsible for aerobic nitrification. They obtain energy and carbon from ammonia oxidation and CO_2, respectively and they use oxygen as the terminal electron acceptor (Shoda, 2017). AOB have multi-layered cell wall morphology and are motile by means of flagella. Five recognized genera of AOB include β-subclass of proteobacteria such as *Nitrosomonas*, *Nitrosospira*, *Nitrosovibrio* and *Nitrosolobus*, and only *Nitrosococcus* from the γ-subclass proteobacteria (Ge et al., 2015). A total 25 AOB species have been collected from various environments, certain of which can grow under both aerobic and anaerobic conditions (Ge et al., 2015). Interestingly, nitrification products of AOB species vary based on the availability of DO. For example, *Nitrosomonas eutropha* aerobic oxidation product at the DO higher than 0.8 mg L^{-1} is only nitrite while some other products such as nitrogen gas, nitrite and nitric oxides are produced at DO below 0.8 mg L^{-1} (Ge et al., 2015). NOB are more widespread than AOB and they are classified into four phylogenetically distinct groups (Ge et al., 2015). Genera of *Nitrococcus* and *Nitrobacter* belong to the α- and γ-subclass, respectively. Meanwhile, the *Nitrospira* genus with the first-deciphered complete genome of *Candidatus Nitrospira defluvii*, is classified in the δ-subclass (Ge et al., 2015). Recent characterization of a complete nitrifying bacterium *Nitrospira*, that carries out a process called Comammox, fundamentally changed the picture of microbial nitrification (Daims et al., 2015). Pathways for ammonia and nitrite oxidation are concomitantly expressed in this organism during growth, leading to ammonia oxidation to nitrate.

Overall, among AOB and NOB microorganisms previously detected as the main nitrifiers in activated sludge and engineered system samples, *Nitrosomonas* sp. was present in all the systems described, as well as the NOB *Nitrospira* (Ferrera and Sánchez, 2016). However, a single AOB/NOB species was found as the dominant in some of these systems, while different species existed in other systems. Furthermore, nitrifying granules with high sedimentation property maintain β-proteobacterial ammonia oxidizing bacteria and the Nitrospira-like nitrite-oxidizing bacteria as the dominant bacteria in a reaction tank. As high-rate nitrification of 1.5 kgN m^{-3} day^{-1} is achieved using real electronics industry wastewater, which is 2.5–5 times faster than traditional activated sludge methods (Hasebe et al., 2017).

3.1.2. Reactor technologies in nitrification

3.1.2.1. Partial nitritation and anammox. 200,000 m^3/day step-feed activated sludge process of the Changi WRP, Singapore, and the deammonification process in Strass WWTP, Austria, are two examples full-scale application of partial nitritation and anammox suspended sludge processes (Cao et al., 2013a; Cao et al., 2016; Wett et al., 2013). Partial nitritation and anammox can be operated in a single-stage process in one reactor which is cost-effective with less emission of greenhouse gas N_2O compared with two-stage process in two reactors (Fig. 3B) (Ge et al., 2015; Kampschreur et al., 2009). In spite of remarkable progress in feasibility of mainstream partial nitritation and anammox process at low temperatures (Lotti et al., 2014), "warm anammox" operating between 20-30 °C is still promising compared with the cold anammox (Sánchez Guillén et al., 2015; Li et al., 2016a). Suppression of NOB bacteria can be achieved in partial nitritation and anammox by controlling competition of AOB and NOB for oxygen in oxygen-limited condition (Pérez et al., 2014).

AOB bacteria are the main producer of N_2O during nitrification. However, there is no contribution by anammox to N_2O formation. The aeration pattern was found to be the principal factor influencing the emission and formation of N_2O in reactors (Ma et al., 2017; Ni et al., 2013; Castro-Barros et al., 2015). Periodic aeration with increasing cycle frequency, transient aeration patterns from low (or anoxic) to high aeration, and continuous aeration are some of the effective aeration patterns for reducing N_2O production in reactor setups (Ni et al., 2013; Castro-Barros et al., 2015).

3.1.2.2. NOB suppression in biofilm reactors. The membrane-biofilm

reactor (MBfR), also known as the membrane-aerated biofilm reactor (MABR), is an attractive nitrogen removal technology in wastewater treatment based on gas-transferring membranes. Gaseous substrates acting as the electron donor or acceptor (such as oxygen, hydrogen, and methane) are diffused through the hydrophobic and gas-permeable membranes to a biofilm which is forming on the membrane surface (Nerenberg, 2016).

Layered biofilm structure and oxygen-controlled system in MABRs repress NOB activity, while providing favorable growth conditions for AOB and/or anammox bacteria. Therefore, MABR setup can be particularly beneficial for allowing other bacteria to out-compete NOB. This setup is much more efficient in this respect compared to e.g. adjustment of DO in liquid phase of suspended sludge systems (Picioreanu et al., 2016). In aerobic conditions, AOB bacteria grow in the outer surface of biofilms, NOB can be found several μm deeper, and anaerobic ammonia oxidation bacteria (AnAOB) are found in the anoxic interior. This arrangement requires substrate diffusion among these different layers for the full conversion (ammonium oxidation using nitrite, as electron acceptor, to produce nitrogen gas) (Cao et al., 2017). Another approach for suppression of NOB in MBBR consists of supplying a small amount of oxygen (Gilbert et al., 2015). Furthermore, biofilm thickness is a critical property influencing the community structure and function. Aerobic nitrifiers were abundant in 50 μm biofilms whereas anaerobic ammonium oxidizers abundantly localized in 400 μm biofilms. Nitrifying biofilms differing in thickness are composed of different nitrogen-transforming bacteria and vary in their nitrogen transformation rates (Suarez et al., 2018; Suarez et al., 2019).

3.2. Denitrification

Denitrification is the process of complete removal of nitrate to harmless nitrogen gas as the end product, with relatively low generation of waste brine (Rezvani et al., 2019) (Fig. 2A). Denitrification treats various contaminants at the same time, leading to a reduced waste disposal cost. For denitrification, several primary factors are essential: requirement for strict anoxic conditions, carbon sources, and post-treatment. Additional organic carbon sources work as the electron donor and are required for cell growth and heterotrophic denitrification (Modin et al., 2007; Miao and Liu, 2018). Glucose, alcohols such as methanol and ethanol, succinate and acetate are the most common carbon sources supplemented to the denitrification systems (Ji et al., 2015; Miao and Liu, 2018). Due to the high biodegradability a wide range of biopolymers including poly(ε-caprolactone) (PCL), poly(butylene succinate) (PBS); polyhydroxyalkanoates (PHAs), poly (l-lactic acid) (PLLA), polyvinyl alcohol (PVA), poly3-hydroxybutyrate-co-3-hydroxyvalerate (PHBV)/PLA, starch/PCL, and starch/PVA are examined for nitrate removal (Xu et al., 2018). It was also found that the aerobic methane oxidation by methanotrophic bacteria can serve organic compounds acting as the electron donors for heterotrophic denitrification (Modin et al., 2008; Modin et al., 2010).

In spite of several advantages offered by denitrification, these substrates result in turbidity as the consequence of excessive biomass and remnant carbon source, thereby necessitating further treatment.

There are several issues impeding large scale application of biodenitrification. There is a risk of gaseous nitrous oxide production, which is a greenhouse effect-causing gas, more potent than the CO_2 (Wang and Wang, 2013). The requirement for continuous carbon source supply is a considerable burden, combined with the need for precise dosing to avoid deterioration of effluent water quality by excessive biomass of bacterial cells and remnant carbon source (Boley et al., 2000). Furthermore, presence of oxygen during denitrification negatively affects nitrogen removal efficiency and increases nitrite concentration in treated water. This negative effect of oxygen varies according to the type of electron donor used as the carbon source. For example, denitrification is less affected by DO when using alcohols, such as ethanol and methanol, compared with sucrose as the carbon source (Gómez et al., 2002). This effect of alcohols is due to formation of smaller size of biofilms with higher density of bacteria, causing higher denitrification rate versus nitrate reduction rate. These carbon sources are more appropriate for the biological denitrification of water contaminated with nitrate and containing DO. Other challenges with denitrification include slow reaction rate due to high start-up time and HRT, need for pH adjustment and drop of productivity at cold temperatures (Rezvani et al., 2019). Some of these problems such as the slow reaction rate may be circumvented by increasing nitrate loading up to 130 mg L^{-1}, however, nitrate loading above this value adversely affects nitrate removal (Rezvani et al., 2019). One known exception is *Thiobacillus denitrificans*-dominated biofilms, which completely remove nitrate even at a nitrate loading rate of 600 mg L^{-1} h^{-1} and an HRT of 10 min (Zou et al., 2016). While biological denitrification effectively converts nitrate to nitrogen gas, it is not amenable to nitrogen recovery, which can be considered as a drawback of this process.

3.2.1. Microorganisms involved in denitrification

Both autotrophic and heterotrophic bacteria can reduce nitrate. A limited number of autotrophic denitrifiers have been discovered. Compared to the heterotrophic denitrifiers, they exhibit slow growth with low biomass production and inefficient assimilation (Rezvani et al., 2019). Autotrophic denitrifiers oxidize inorganic matters and the released electrons are delivered to the nitrate as the terminal electron acceptor. There are two types of autotrophic denitrification: hydrogen-based (*Micrococcus denitrificans* and *Paracoccus denitrificans*) and sulfur-based autotrophic denitrification (*Thiobacillus denitrificans* and *T. thioparus*), oxidizing H_2 and sulfur compounds (such as elemental sulfur or thiosulfate), as the electron donors, respectively (Rezvani et al., 2019; Zou et al., 2016). Di Capua et al. (2019) investigated twelve electron donors for autotrophic denitrification including hydrogen gas, chemically synthesized and biogenic elemental sulfur, sulfide, thiosulfate, sulfite, pyrite, thiocyanate, zero-valent iron, ferrous iron, arsenite and manganese. Denitrification kinetics was found to be strongly affected by the type of electron donor. Regardless of safety issues, fast kinetics, low biomass yield, ecosustainability and reasonable cost of H_2, make it the most promising electron donor. Meanwhile, reduced inorganic sulfur compounds are alternative to H_2, limiting effluent sulfate concentration and treating a wide range of nitrate-contaminated waters. Tian et al. (2020) recently confirmed exclusive autotrophic iron-dependent denitrification seeded with activated sludge. A novel sulfur-based denitrification process coupled with iron(II) carbonate ore (SICAD system) synergistically enhanced denitrification rate (up to 720.35 g N/m^3 d) with reduced accumulation of intermediates (NO_2^- and N_2O) and production of sulfate (Zhu et al., 2019). Sulfur oxidizing bacteria including *Acidithiobacillus thiooxidans* and *Thiobacillus denitrificans* use iron sulfides as the efficient electron donors for autotrophic denitrification treating nitrate in wastewater (Yang et al., 2017). OH^- ions generated by dissolved S^{2-} hydrolysis buffer H^+ ions produced by autotrophic denitrification of iron sulphides, thereby eliminating the need for alkaline reagents for neutralization. Sulfur autotrophic denitrification also leads to SO_4^{2-} accumulation. This issue is less serious in iron sulfide autotrophic denitrification, probably due to the incomplete S^{2-} oxidation to SO_4^{2-}.

In wastewater treatment systems, *Thiobacillus* sp. comprises the majority of autotrophic denitrifiers (Miao and Liu, 2018). Interestingly, cooperation of predominated hydrogenotrophic and heterotrophic denitrifiers demonstrated a shift from autotrophic to heterotrophic denitrification in H_2-based hollow-fiber membrane biofilm reactor (HF-MBfR) (Park et al., 2016).

Faster denitrification reactions performed by heterotrophs require smaller reactor volumes, thereby reducing the cost. *Pseudomonas* and *Bacillus* are the most common heterotrophic denitrifiers (Rezvani et al., 2019). Heterotrophic bacteria utilize carbon from the complex organic compounds, prefer low to zero DO, and use nitrate as the terminal electron acceptor. Under both aerobic and anoxic environments, nitrate

can be removed by heterotrophic bacteria from wastewater. Under anoxic conditions, nitrate is used as the terminal electron acceptor for cell respiration instead of oxygen. Thus, nitrate gets reduced simultaneously with oxidation of organic matters. Some bacteria, such as *Thiosphaera pantotropha, Alcaligenes faecalis*, and *Bacillus* sp. possess the capacity of aerobic denitrification, in addition to heterotrophic nitrification (Chen et al., 2015). DO concentration, carbon to nitrogen ratio (C/N) as well as temperature and pH are known to influence aerobic denitrification rates (Ji et al., 2015). There are several advantages of aerobic denitrification using these organisms, such as high growth rates, aerobic removal of ammonium and nitrate by simultaneous nitrification and denitrification, minimized acclimation problems, and reduced requirement for buffering (alkalinity produced by denitrification partially compensates the alkalinity required for nitrification) (Chen et al., 2015). The nitrate and oxygen co-respiration in these conditions are believed to result from microbial adaption to harsh environment with high dosage of nitrate to degrade the toxic nitrogen (Ji et al., 2015).

In wastewater treatment systems, members of the genera *Thauera, Paracoccus, Hyphomicrobium, Comamonas, Azoarcus, Denitratisoma, Dechloromonas*, and family Comamonadaceae are the major denitrifiers contributing into the nitrogen removal (Jiang et al., 2012; Baumann et al., 1996; Carvalho et al., 2007; Cowan et al., 2005; Neef et al., 1996; Martineau et al., 2013; Gumaelius et al., 2001; Khan et al., 2002). Interestingly, microbial species composition in wastewater affects nitrite accumulation in denitrification process. It can be due to the differential pattern of denitrification pathway in various bacteria. The denitrifying bacteria contributing in nitrate removal are shown in Fig. 4, which are functioning in nitrite accumulation and or complete denitrification. Three different patterns of denitrification process are found in wastewater treatment plants including i) reducing nitrate only to nitrite, ii) reducing both nitrate and nitrite with no nitrite accumulation which the rate of reducing nitrite is higher than that of nitrate in these bacteria, iii) reducing nitrate and nitrite, along with a transient nitrite accumulation which rate of nitrite reducing is lower than that of nitrate in these bacteria (Ma et al., 2016; Martienssen and Schops, 1997). To demonstrate the effect of denitrifying community composition on nitrite accumulation, Cao et al. (2013a) investigated three different seeding sludges (SA, SA–A–O, SA–A). SA was collected from the anoxic zone in lab-scale anoxic and aerobic reactor treating domestic wastewater with a long SRT; SA–A–O was collected from an anaerobic/anoxic and aerobic reactor with high denitrifying phosphorus removal efficiencies;

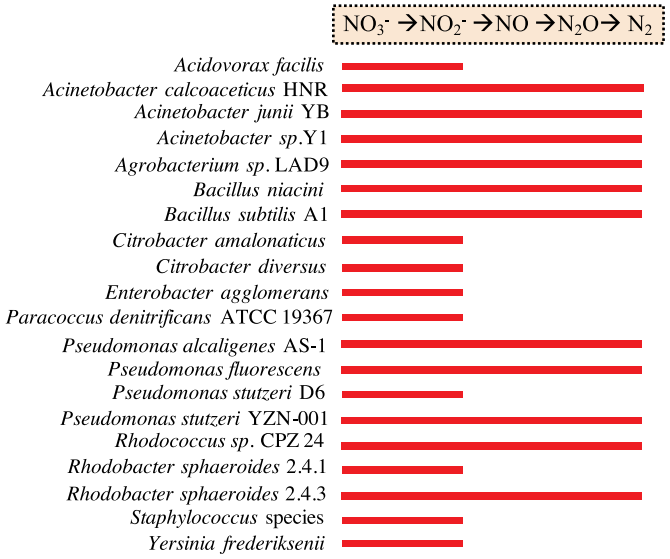

Fig. 4. Nitrite accumulation and complete denitrification by denitrifying bacteria.

and SA–A was collected from an anaerobic sludge fermentation coupling a anoxic denitrification reactor with a carbon source produced by sludge fermentation. In denitrification process, SA and SA–A–O sludges showed the transient accumulation of nitrite, however SA–A showed high nitrite accumulation with a nitrate-to-nitrite transformation ratio (NTR) of 80% before the nitrate reduced completely. Therefore, it can be assumed that the denitrifiers, reducing nitrate only to nitrite, are dominated microorganism communities (Cao et al., 2013b). Furthermore, nitrite accumulation due to inconsistent nitrite reductase and nitrate reductase activities is also induced in several conditions including carbon-limited condition, type of carbon sources such as glucose, pH and nitrate concentration, oxygen concentration and toxic compounds such as pesticide (Gong et al., 2013; Yang et al., 2012; Ge et al., 2012; Glass and Silverstein, 1998; Cao et al., 2013c; Sáez et al., 2003).

Bioaugmentation is a biological approach of adding specific microorganisms into a microbial community to enhance the capacity of microbial community for degradation of specific contaminants. Altogether, bioaugmentation with the strains that possess complete denitrification capacity would be beneficial to achieve a complete denitrification system for wastewater treatment.

3.2.2. Reactor technologies in denitrification

3.2.2.1. Simultaneous nitrification and denitrification (SND). SND particularly for treating wastewaters with low C/N ratio (< 5) is the occurrence of both nitrification and denitrification simultaneously in the same reactor and saving cost for anoxic tank (Guo et al., 2005). SND can be operated through the physical and biological mechanisms. Physical mechanism is based on gradient of DO due to limitation of oxygen diffusion through the flocs or biofilms. The nitrifiers and denitrifiers localize in higher (more than 1–2 mg L^{-1}) and lower concentration of DO (less than 0.5 mg L^{-1}), respectively (Zhu et al., 2008). However, the biological mechanism of SND is based on the activity of heterotrophic nitrifiers and aerobic denitrifiers with the capacity of denitrification even at oxygen-saturated condition (Chen et al., 2015).

3.2.2.2. Shortcut nitrification and denitrification. Shortcut nitrification and denitrification also called as partial nitrification-denitrification, is feasible technology for treatment of wastewaters with high ammonium concentrations or low C/N ratios. In this case, nitrite is produced by nitrification as an intermediate product instead of nitrate and subsequently reduced to N_2 by nitrite denitrification (Zhu et al., 2008). There are several advantages by shortcut nitrification and denitrification compared with the conventional nitrification and denitrification via nitrate; i) 25% lower consumption of oxygen in aerobic phase with 60% saving energy within whole process, ii) 40% lower demand of electron donors in anoxic phase, iii) nitrite denitrification rate is 1.5 to 2 times higher than nitrate denitrification rate (Peng and Zhu, 2006).

3.2.3. Synthetic biology in denitrification

Quorum sensing (QS) including *rhl and las* -two acyl homoserine lactone (AHL)-mediated QS systems- and *Pseudomonas* quinolone signal (PQS) systems, is a cell–cell communication mechanism of *Pseudomonas* playing crucial role in wastewater treatment (Kalia et al., 2018; Yong et al., 2015). The denitrification activity of *Pseudomonas* aeruginosa PAO1 was positively affected by QS mutants (*Δ(Acyl-homoserine-lactone synthase I)rhlI* and *ΔrhlR*), indicating involvement of QS in denitrification process (Yoon et al., 2002; Toyofuku et al., 2007). Regulatory mechanism of denitrification by QS systems proposed that the denitrification processes can be improved by engineering of QS systems. Furthermore, regulatory role of PQS signalling molecule in denitrification suggests potential regulation of denitrification process by manipulation of PQS signalling molecule biosynthesis in various denitrifying bacteria and microbial consortia (Toyofuku et al., 2008).

3.3. Anammox

Anaerobic digestion, transforming organic materials into mostly CO_2 and methane (CH_4) is one of the technologies for commercial production of energy from waste (Appels et al., 2008). The transformation of organic matter into biogas (60–70 vol% of CH_4) by anaerobic digestion reduces final sludge solids, destroys most of the sludge pathogens and decreases odour problems associated with the residual putrescible matter (Appels et al., 2008). Hydrolysis, acidogenesis, acetogenesis and methanogenesis are the subsequent steps in anaerobic digestion of organic materials. Organic matter as the main energy resource is used by the anaerobic methanogenic bacteria during methanogenesis step of anaerobic digestion process. However, it is also used by the heterotrophic denitrifiers present in wastewater microbial community thereby lowering energy production by methanogenic bacteria. However, nitrogen removal by autotrophic microorganisms would be preferable, since it leaves the organic matter for methanogenic bacteria during wastewater treatment. Autotrophic denitrification (Ma et al., 2016), photoautotrophic systems (Ma et al., 2016) and anammox processes (Kartal et al., 2010) are the autotrophic nitrogen removal approaches. Among them, anammox process seems to be the most promising for energy-neutral or energy-generating sewage treatment (Kartal et al., 2010). In the anammox process, nitrite and ammonium are utilized to form nitrogen gas through NO and N_2H_4 intermediates (Fig. 2B). In 2002, the first full-scale anammox reactor was set up for treatment of reject water at Dokhaven, Rotterdam, Netherlands. In 2015, there were reportedly 114 full-scale anammox installations around the world with a capacity to treat 134 tons of N per day (Ali and Okabe, 2015). Along with increasing number of installations, capacity volume of the anammox plants is also increasing. These full-scale treatment plants are mainly treating reject water streams, however, in terms of nitrogen loading rates, their main targets are glutamate and amino acids industries and slaughterhouses (Ali and Okabe, 2015).

Anammox bacteria oxidize ammonium using nitrite as the electron acceptor. Nitrite can be obtained from nitritation (oxidizing ammonium to nitrite) and partial denitrification (reducing nitrate to nitrite) (Ali and Okabe, 2015). Thus, nitritation/anammox and partial denitrification/anammox can remove biological nitrogen from wastewater (Table 2). Since both anammox and nitritation are autotrophic processes, they are advantageous compared with nitrification/denitrification, since they do not utilize the carbon sources present in the wastewater. By comparison, nitrification/denitrification can use up to 100% of organic matter present in treated wastewater. With nitritation/anammox, methane can be produced using organic matter, thereby enhancing energy recovery from wastewater (Kartal et al., 2010). Furthermore, energy consumption can be reduced due to about 60% less oxygen demand in nitritation/anammox processes, and only approximately 50% of the ammonium must be oxidized to nitrite instead of nitrate. In addition, surplus sludge production is minimal with nitritation/anammox, due to lower cell production but higher rate of nitrogen removal (Du et al., 2015; Kartal et al., 2010). The chemoautotrophic anammox utilizes inorganic carbon source of CO_2 as it can positively affect the anammox growth and activity. Therefore, nitritation/anammox processes could also decrease the emission of greenhouse gases due to consumption of inorganic carbon CO_2 as well as lower N_2O emission (Du et al., 2015).

Besides the above-mentioned advantages of anammox process, there is one crucial shortcoming: usually excessive amounts of nitrate are introduced into the effluent. Consequently, a post-denitrification process is required to meet the discharge standard, which is in demand of external carbon source (Modin et al., 2007) (Fig. 5A). This comes at a cost: energy consumption, surplus activated sludge and CO_2 emission.

High C/N ratio, low temperature, and poor effluent water quality are the main challenges for application of anammox process in treating mainstream wastewater. When organic matter is present, heterotrophic denitrifying bacteria are able to compete with anammox bacteria resulting in lower ammonium removal efficiency. In addition, certain organic compounds, such as methanol, inactivate anammox activity completely or partially (Ali and Okabe, 2015). Low concentrations of organic matter do not significantly affect anammox activity, so nitrogen removal in the presence of low concentrations of organic matter can be promoted via heterotrophic denitrification. This process is called simultaneous nitrification, anammox and denitrification (SNAD) (Fig. 5A) (Lan et al., 2011), and can be used for treatment of mainstream wastewater. Optimal temperature for anammox process is 37 °C (Fig. 5B, Table 2), however, at 45 °C anammox irreversibly loses activity due to the biomass lysis. Anammox bacteria can adapt to lower temperatures and they are able to grow and maintain their activity at 10-20 °C in wastewater. Anammox activity can be detected at temperatures as low as 4 °C in laboratory conditions (Oshiki et al., 2011). It was shown when temperature decreased to 15 °C, the maximum reactor capacity and system stability negatively affected due to nitrite accumulation. However, anammox was adapted to low temperature and no changes in sludge physical properties and/or bacterial populations were found during the operation (Dosta et al., 2008). In contradiction with previous finding, prolonged cultivation of anammox bacteria at 20 °C results in deterioration of biomass-specific activity due to the required long SRT in the system. The SRT controls the bacteria concentration within the treatment and higher SRT can contribute to a higher bacterial concentration in the reactor. Long SRT causes non-active and non-anammox bacterial cells such as heterotrophs growing on minimal organic carbon present in the influent and becoming dominant in the reactor, thereby decreasing the biomass-specific activity (Hoekstra et al., 2018).

Average NH_4^+ and NO_3^- concentrations of 100 and 50 mg L^{-1} are found in the effluent of full-scale anammox installations, respectively (Ali and Okabe, 2015). Hence, the effluent water quality needs to be improved to meet the effluent discharge standard by further post-treatment (increasing the cost and energy consumption).

3.3.1. Microorganisms involved in anammox

Anammox is a recently discovered process in the nitrogen cycle. The representative anammox bacteria are Candidatus "*Brocadia anammoxidans*" and Candidatus "*Kuenenia stuttgartiensis*" (Shoda, 2017). Anammox bacteria belonging to phylum Planctomycetes (Strous et al., 1999) and are ubiquitously found in anoxic environments such as marine, freshwater and terrestrial ecosystems, where the nitrogen loss mainly occurs due to anammox processes (Ali and Okabe, 2015). About nineteen species and broadly six genera of anammox bacteria have been characterized. No pure culture was found for anammox species, however, some of the anammox cultures have been enriched as the mono-species in laboratory conditions (Oshiki et al., 2011).

3.3.2. Reactor technologies in anammox

3.3.2.1. Simultaneous partial nitrification, anammox, and denitrification (SNAD). As there is no demand of organic carbon for the anaerobic conversion of ammonia to N_2 by anammox, it is an attractive technology for the treatment of nitrogen rich wastewaters. However, combination of partial nitrification with anammox through the SHARON (single reactor high activity ammonia removal over nitrite)-anammox (two-reactor system) and CANON (completely autotrophic nitrogen removal over nitrite in single reactor system) produces the electron acceptor nitrite required for anammox process. However, these two processes are not able to eliminate organic carbon along with ammonium. Furthermore, nitrate production in the system making the necessity of further treatment. Therefore, SNAD process (simultaneous partial nitrification, anammox, and denitrification) has been developed for simultaneous removal of inorganic nitrogen and organic carbon. It conducts three chemical reactions by three bacterial communities aerobic AOB, anammox, and denitrifying bacteria in a single reactor under oxygen limiting conditions (Fig. 3C) (Chen et al., 2009) (Fig. 5A).

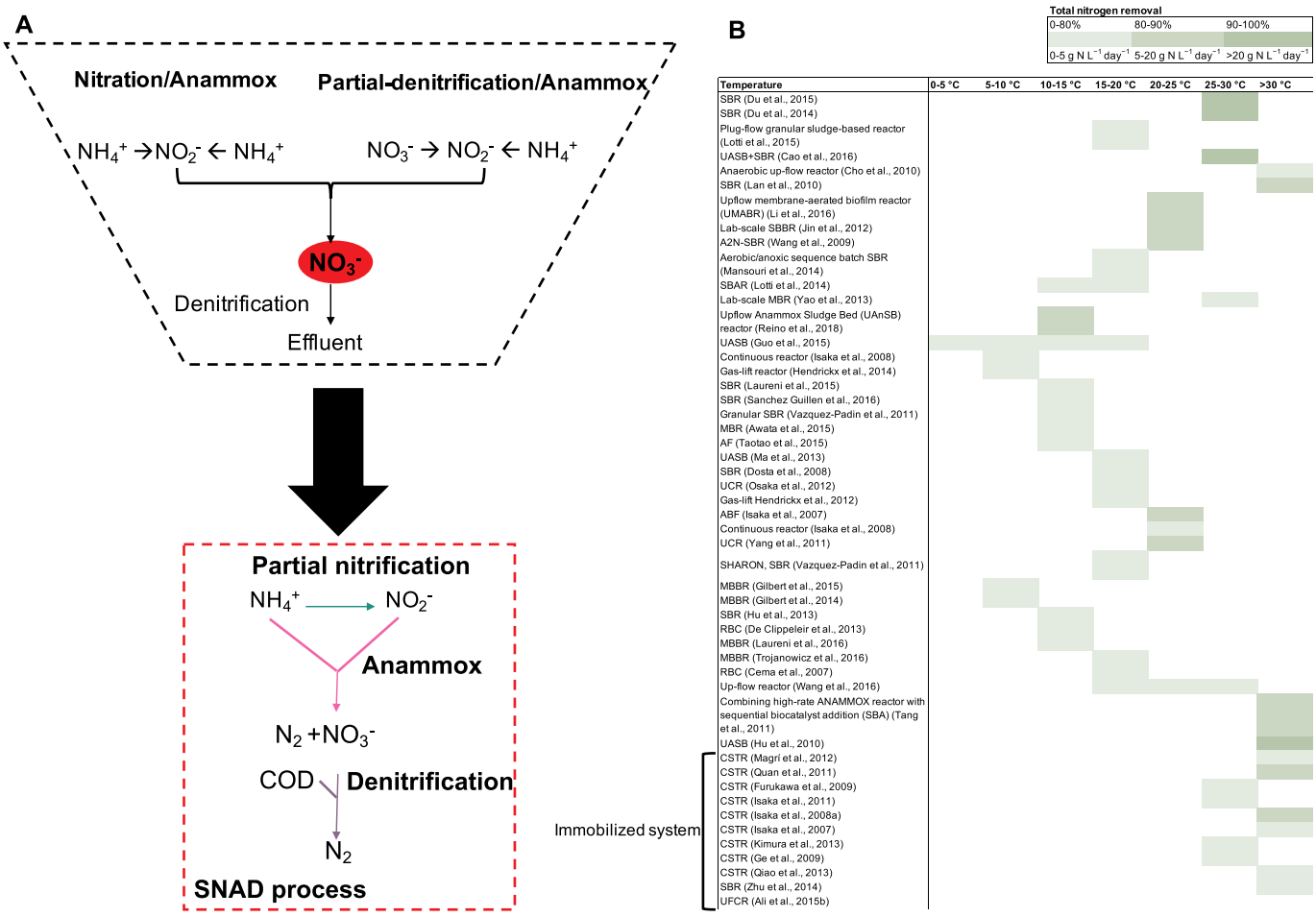

Fig. 5. Anammox process with the need of post denitrification treatment leading SNAD (simultaneous nitrification, anammox and denitrification) process (A). In SNAD process, ammonia is partially oxidized to form nitrite, and then anammox and denitrification together remove ammonia and nitrate, respectively. Comparison of the nitrogen removal efficiencies at various temperatures (B), the graph was drawn based on the data shown in Table 2.

Nitrate production in mainstream anammox process and variation in nitrite to ammonium ratio result in poor quality of anammox effluent with total nitrogen concentration of above 10 mg N L^{-1} (Xie et al., 2018). The counter-diffusion delivery in MABR allows a higher efficiency of gas transfer and substrate consumption compared to the conventional methods (Xie et al., 2018). The gaseous methane delivery through the membrane causes growth of denitrifying anaerobic methane oxidation (DAMO) organisms along with anammox bacteria. Thus, nitrate produced by anammox reaction is consumed by DAMO organisms. If the feed has non-optimal nitrite to ammonium ratio for the anammox reaction, the additional nitrite is also removed by DAMO organisms. Therefore, combination of anammox and denitrifying anaerobic methane oxidation (DAMO) has been established in MBfR to improve the mainstream anammox process. High nitrogen removal rate and satisfactory effluent quality are achieved by this method in mainstream wastewater treatment (Xie et al., 2018).

3.3.2.2. Denitrifying ammonium oxidation (DEAMOX). DEAMOX is the novel process of anammox coupling with partial denitrification (nitrate generates nitrite) simultaneously treats ammonia and nitrate containing wastewaters. It obtains a stable performance (total nitrogen removal of 93.6%) despite the seasonal changes of temperature (29.2 °C–12.7 °C). It is due to enhanced anammox activity by high accumulation of nitrite through the partial-denitrification process (Du et al., 2017). Interestingly, the partial-denitrification process creates broader range of capacity for application of anammox technology (Fig. 3D). This economically recommended technology has advantages compared to the conventional biological nutrient removal such as i) 100% reduced demand of aeration, ii) simultaneous nitrate and ammonium removal with 80% reduced demand of organic carbon, iii) 64.8% reduced sludge production, iv) low nitrogen contained wastewater, low/high-strength nitrate and ammonium containing wastewater, and v) significant reduction in greenhouse gas (CO_2 and N_2O) emission (Cao et al., 2019a).

3.3.3. Synthetic biology in anammox

AHLs-regulated metabolic pathways and AHLs-mediated QS mechanism in wastewater treatment were explored in anammox consortia (Sun et al., 2018; Tang et al., 2018). Anammox fed with influent comprising high ammonium concentration enriched more QS organisms producing more AHLs, which likely benefited anammox activity. Furthermore, more hydrophobic amino acid and protein were produced in extracellular polymeric substances (EPS). Thus, active QS and EPS synthesis in anammox might cause high nitrogen removal capacity and dense biofilm (Sun et al., 2018). AHLs-mediated regulation of anammox activity, growth rate, and EPS production implied that genetically engineered bacteria carrying signal gene for AHLs synthesis could improve nitrogen removal rate or biomass aggregation and thereby overcoming limitations of long start-up required for wastewater treatment in anammox reactor (Tang et al., 2018).

3.4. Nitrogen removal from industrial wastewater

Industrial wastewaters containing over 500 mg L^{-1} ammonium are

produced from oil refining, organic chemicals, glass manufacturing, feed production, chemical fertilizer, iron alloy, meat processing, animal husbandry, pharmaceutical industry, and other industries (Tabassum et al., 2018). Industrial wastewater treatment was developed using various anammox processes, as one-stage nitritation/anammox system is feasible to treat numerous types of ammonium-enriched industrial wastewaters (Zhang et al., 2015; Miao et al., 2014; Wang et al., 2016b; Shen et al., 2012; de Graaff et al., 2011; Tang et al., 2011; Molinuevo et al., 2009; Daverey et al., 2013). Complex components (heavy metals, antibiotics and salinity) present in ammonium-enriched industrial wastewaters affect anammox activity, autotrophic nitrogen removal performance, microbial community structure transformation, and system stability (Li et al., 2018). Therefore, studying impact of these components on anammox is of practical significance to provide insights on anammox technology for treatment of different industrial wastewaters.

High concentration of nitrate is also discharged from certain industries including stainless steel pickling rinse wastewater (450 mg L^{-1}), fertilizer industry (950 mg L^{-1}), liquid-liquid extraction process of uranium nitrate raffinate (up to 77,000 mg L^{-1}) (Fernández-Nava et al., 2008; Zala et al., 2004; Biradar et al., 2008). Partial denitrification coupled with anammox process (PD-A) is a new approach for nitrate-enriched industrial wastewater treatment (Cao and Zhou, 2019). Toxic substances including metals, fluoride, sulfate, chloride, toxic organics present in nitrate-enriched industrial wastewaters could affect nitrate and nitrite reductase activities, nitrite production for anammox process and likely anammox activity (Jin et al., 2012). Granular sludge with compact physicochemical structure as well as protection mechanisms offered by EPS enhance tolerance of microorganisms to toxic compounds (Cao and Zhou, 2019). Furthermore, granules are able to absorb toxic substances. Therefore, granule-based systems should be favoured to mitigate negative effects of toxic components in nitrate-enriched industrial wastewater.

3.4.1. Removal of nitro group-containing chemicals from industrial wastewater

Industrial wastewater also contains toxic nitro group-containing chemicals such as nitrophenols (NPs) composed of benzene rings and nitro (-NO_2) and hydroxyl (-OH) groups. NPs are widely used as a raw material and intermediate in production of pharmaceuticals, pigments, wood, preservatives, dyes, pesticides, explosives and rubbers, while listed as a priority pollutant (Park and Bae, 2018; Keith and Telliard, 1979). Common methods for NPs wastewater treatment are adsorption, extraction and oxidation. These are complicated and costly processes and generate secondary pollution. However, NPs can be consumed as the sole carbon sources by some bacteria, which is a basis for an effective technology of their removal from the environment (Arora et al., 2014; Xiong et al., 2019). Microbial degradation of NPs is considered to be efficient when the concentration of NPs is under 200 mg L^{-1}. Recently, Mei et al. (2019) constructed an integrated membrane-aerated bioreactor system with anoxic and aerated zones. Using this reactor, efficient simultaneous removal of NPs (95.86%) and nitrogen (94.81%) was demonstrated on influent water with NPs concentration of 500 mg L^{-1}. The presence of different types of NP molecules in aqueous solution limits bacterial remediation (Xiong et al., 2019).

3.4.2. Synthetic biology in removal of nitro group-containing chemicals from industrial wastewater

Genetically modified microorganisms (GMM) show potential for degrading pollutants in wastewater, but bio-environmental concerns restrict their applications. For example, *Bacillus cereus* strain isolated from pulp and paper wastewater effluent showed higher degradation of NPs when it expressed a heterologous *vgb* gene encoding hemoglobin-like protein, *Vitreoscilla* Hemoglobin b (VHb) from *Vitrocilla stercoraria* (Vélez-Lee et al., 2016). As an alternative to such targeted genetic engineering, genetic mutations obtained during adaptive laboratory evolution (ALE) can be used to facilitate NPs removal, thereby circumventing the necessity of ecological safety evaluation for introduction of GMM into contaminated sites (Elena and Lenski, 2003; Chaudhary and Kim, 2019). *Pseudomonas* sp. strain WBC-3 is an example of NP-degrading bacteria that evolved to utilize 2-chloro-4-nitrophenol (2C4NP) as a growth substrate in an ALE experiment. The key mutation obtained by ALE was detected in a transcriptional regulator PnpR, which activated expression of *pnpA* and *pnpB* operons. These genes encode enzymes for the initial reactions in NP and 2C4NP catabolism. In the mutated ALE strain, they were strongly expressed in the absence of inducer and even higher induction was obtained by inducer supplementation (Deng et al., 2019). Similarly, *Acidovorax* sp. strain JS42 was able to grow either on 3-nitrotoluene (3NT) or on 4-nitrotoluene (4NT) after and ALE experiement, due to variations in 2-nitrotoluenene 2,3-dioxygenase (2NTDO) and evolved activities of 2NTDO against 3NT and 4NT (Mahan et al., 2015). Therefore, the ALE-based approaches can be suggested to optimize the capacity for biodegradation of toxic nitro group-containing components.

3.5. Bioelectrochemical systems

Specific interests are found in bioelectrochemical reactors for nitrogen removal from wastewaters, along with energy and/or chemicals production. Significant progress has been made to improve bioelectrochemical system technology for large-scale treatment of various wastewaters (Fig. 6B, Table 3). Bioelectrochemical systems utilize electrochemically active microorganisms (bioanode/biocathode) with extracellular electron transfer capacity. Electrons driven by oxidation of pollutants are transferred into the anode, resulting in pollutants removal such as organic matter decomposition. According to the type of cathodic reaction, there are two types of bioelectrochemical systems: microbial fuel cells (MFCs), which generated electrical power because the anodic oxidation is coupled to a cathode reducing an electron acceptor with high reduction potential (such as O_2, Fe^{3+}, and Cu^{2+}) and microbial electrolysis cells (MEC), which require electrical power because the anodic oxidation is coupled to a cathode operated at low potential, for example for the reduction of protons to hydrogen gas (Rodríguez Arredondo et al., 2015).

3.5.1. Denitrification by bioelectrochemical systems

Biological nitrogen removal using bioelectrochemical systems is based on denitrification at the cathode (Rodríguez Arredondo et al., 2015; Wu and Modin, 2013). Denitrification can be performed in the cathode compartment of BES either by the production of H_2, which is consumed by denitrifying autotrophs (Fig. 6A) or by denitrifiers directly accepting electrons from the cathode surface. An MFC functioning with acetate as electron donor at the anode and nitrate as electron acceptor at the cathode was demonstrated by Clauwaert et al. (2007). Another study showed that denitrification is enhanced with a constant electric field in a continuous stirred tank reactor (CSTR), when the cathode potential is maintained constant around the standard potential value of the nitrate/nitrite redox couple (Parvanova-Mancheva and Beschkov, 2009). The denitrification rate is influenced by applied potential and current. When DO level is low, the positive effect of current on nitrate removal is elevated (Sakakibara and Kuroda, 1993; Tanaka and Kuroda, 2000).

There are several advantages offered by bioelectrochemical methods of wastewater treatment, including removal of specific contaminants, reduced environmental footprint, stable operation at ordinary environmental temperatures, processing speed, and cost-effectiveness due to cheap and self-propagating microorganisms. This type of treatment also uses wastewater as a renewable source of energy (Kato, 2015; Rajmohan et al., 2016). Furthermore, bioelectrochemical systems can easily be coupled with other techniques or may be used for post-treatment of effluents of other techniques. For example, the anodic oxidation of organic matter produces electricity, while denitrification

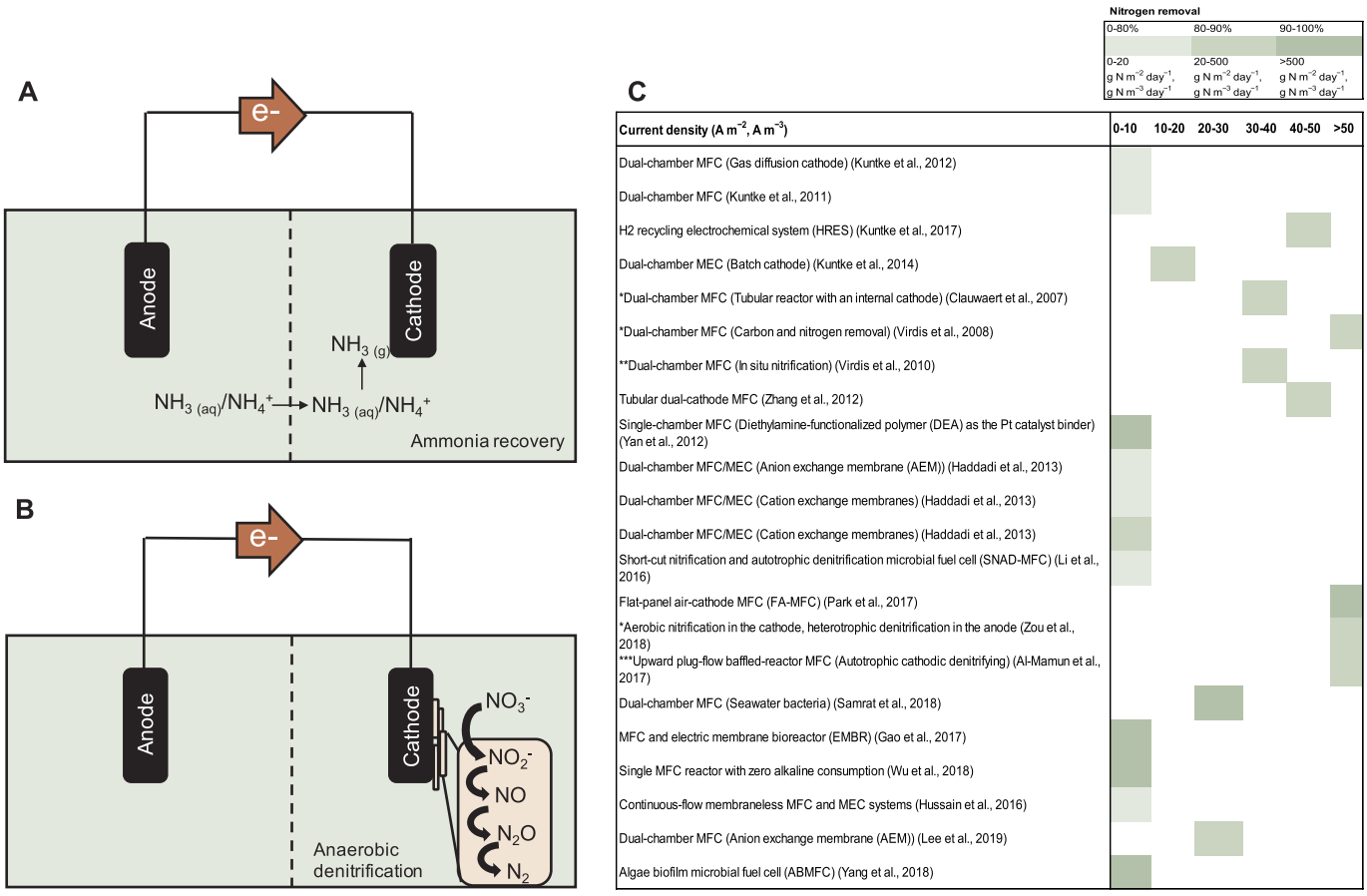

Fig. 6. Bioelectrochemical system for biological nitrogen removal from wastewater (A). Comparison of the nitrogen removal efficiencies of different bioelectrochemical setups (B), the graph was drawn based on the data shown in Table 3. *Measurement is based on net cathodic compartment (NCC), **Total compartment volume (TCV), ***Net cathode volume (NCV).

occurs at the cathode. In addition, an external nitrification reactor enables the removal of ammonium from wastewater (Virdis et al., 2008). Even simultaneous nitrification and denitrification can be achieved by optimizing the oxygen supply in the aerated cathode chamber of an MFC (Virdis et al., 2010). Furthermore, a tubular dual-cathode MFC with an anoxic inner cathode for denitrification and an aerobic outer cathode for the nitrification process was designed using both anion and cation exchange membranes (Zhang and He, 2012). Interestingly an algal biofilm (AB) integrated with an MFC was established as an algae biofilm microbial fuel cell (ABMFC). It was shown to facilitate nitrogen and phosphorous removal much better than an AB and MFC alone. In case of bioenergy generation, ABMFC produced 18% higher power density than the MFC alone (Yang et al., 2018). Another example of combining MFCs with other technology comes from the cheese industry wastewater treatment. This type of wastewater is treated with a membrane bioelectrochemical reactor coupled with an MFC, in which contaminants such as COD, suspended solids and nitrite are removed by MFC. Since ratio of energy recovery and consumption is more than one thereby energy gets recovered. Moreover, a high-quality effluent is obtained by post-treatment in multiple bioelectrochemical reactors (Li et al., 2014).

3.5.2. Microorganisms involved in bioelectrochemical systems

Denitrification by autotropic microorganisms requires CO_2 as the inorganic carbon source and hydrogen as the electron donor (Ghafari et al., 2008). However, faster denitrification reaction rate by heterotrophs requires smaller reactor volumes, thereby reducing the cost and time. Firmicutes (Bacilli) are the dominant component of cathodic denitrifying biofilms in anaerobic denitrification and under low DO condition. Increasing DO results in a change of the predominant phylum to Proteobacteria, predominantly α, β, and γ, classes (Zhao et al., 2017). Several proteobacteria such as *Geobacter*, *Shewanella*, *Acidithiobacillus*, as well as *Bacteroidetes*, *Chloroflexi,* and Gram-positive bacteria *Firmicutes*, and Archaea have extracellular electron transfer ability which can support applications in bioelectrochemical systems for wastewater treatment (Kato, 2015). Enrichment of the electrochemically active bacterial community on the electrode can be positively affected by the electrode pre-treatment, optimization of potential, current, external resistance, chemical additions, bioaugmentation and temperatures (Butti et al., 2016). Our understanding of the microbial extracellular electron transfer is not complete, and further studies are needed to provide engineering strategies for improving bioelectrochemical reactors.

3.5.3. Synthetic biology in bioelectrochemical systems

Phenazines, heterocyclic nitrogen-containing and redox active secondary metabolites, are produced by *Pseudomonas* (Mavrodi et al., 2006). Regardless of whether phenazines are produced by *Pseudomonas* or by chemical synthesis, they play a crucial role as electron shuttles for extracellular electron transfer between bacteria and electrodes and thereby enable efficient electricity production in MFC (Jayapriya and Ramamurthy, 2012; Pham et al., 2008; Rabaey et al., 2005). Phenazine biosynthesis by *Pseudomonas* species is regulated by QS systems (Yong et al., 2011). Engineered QS systems in *P. aeruginosa* (overexpressing *rhlI* and *rhlR* genes) resulted in higher phenazine biosynthesis, efficient extracellular electron transfer and thereby enhanced electricity

Table 3
Bioelechtochemical systems performance for removal and recovery of nitrogen.

Reactor type	System	Type of wastewater	Removal mechanism	Removal efficiency/ rate	Current density A m^{-2}	References
Single-chamber MFC	Air-cathode	Swine wastewater	Ammonia removal	60%	NA	Kim et al. (2008)
Dual-chamber MFC	Anode with ferricyanide catholyte	Swine wastewater	Ammonia removal	69%	NA	Kim et al. (2008)
Dual-chamber MFC	Gas diffusion cathode	Urine	Ammonia removal	3.3 g N m^{-2} d^{-1}	0.5 A m^{-2}	Kuntke et al. (2012)
Dual-chamber MFC		Urine	Ammonia removal	9.57 g N m^{-2} d^{-1}	2.6 A m^{-2}	Kuntke (2013)
Three compartment electrochemical system	H_2 recycling electrochemical system (HRES)	Synthetic urine	Ammonia removal	335 gN m^{-2} d^{-1}	50 A m^{-2}	Kuntke et al. (2017)
Dual-chamber MEC	Batch cathode	Urine	Ammonia removal	162.18 g N m^{-2} d^{-1}	14.7 A m^{-2}	Kuntke et al. (2014)
Dual-chamber MFC	Tubular reactor with an internal cathode	Synthetic	Nitrate reduction	146 g N m^{-3} NCC d^{-1}	35 A m^{-3}	Clauwaert et al. (2007)
Dual-chamber MFC	Carbon and nitrogen removal	Synthetic	Carbon removal, nitrification, denitrification	67.4% TN, 410 g NO_3^- m^{-3} NCC d^{-1}	133 A m^{-3} INCLUDEPICTURE "https://www.rsc.org/images/entities/char_2009.gif" * MERGEFORMATINET NCC	Virdis et al. (2008)
Dual-chamber MFC	*In situ* nitrification	Synthetic	Simultaneous nitrification and denitrification	94.1% TN, 104 g N m^{-3} TCV d^{-1}	39.7 A m^{-3} TCV	Virdis et al. (2010)
Dual-cathode MFC	Tubular dual-cathode MFC	Synthetic	Nitrification and denitrification	67–90% TN, 140 g TN m^{-3} d^{-1}	43 Am^{-3}	Zhang and He (2012)
Single-chamber MFC	Diethylamine-functionalized polymer (DEA) as the Pt catalyst binder	Synthetic	Simultaneous nitrification and denitrification	96.8%	3.6 A m^{-2}	Yan et al. (2012)
Dual-chamber MFC/MEC	Anion exchange membrane (AEM)	Synthetic	Ammonia removal	2.94 g N m^{-2} d^{-1}	3.6 A m^{-2}	Haddadi et al. (2013)
Dual-chamber MFC/MEC	Cation exchange membrane	Synthetic	Ammonia removal	8.5 g N m^{-2} d^{-1}	3.6 A m^{-2}	Haddadi et al. (2013)
Dual-chamber MFC/MEC	Cation exchange membrane	Synthetic	Urea removal	37.8 g N m^{-2} d^{-1}	5 A m^{-2}	Haddadi et al. (2013)
Biofilm-electrode reactor		Synthetic	Nitrate removal	0.17 mg NO_3^-N cm^{-2} biofilm surface area d^{-1}	NA	Park et al. (2005)
Integrated SNAD-MFC	Short-cut nitrification and autotrophic denitrification (SNAD)-MFC	University of Connecticut Wastewater Treatment Facility UConn-WTF	Ammonia removal	99.9%, 12.5 g N m^{-3}	0.158 mA/cm^2	Li et al. (2016b)
Flat-panel air-cathode MFC FA-MFC	Two separator electrode assembly (SEA)	Domestic wastewater	Organic and nitrogen compounds removal	620 g N m^{-3} d^{-1}	401 ± 13 A m^{-3}	Park et al. (2017)
MFC	Aerobic nitrification in the cathode, heterotrophic denitrification in the anode	Recirculating aquaculture system (RAS) water	Nitrate, ammonia removal	51 g N m^{-3} NCC d^{-1}	74.00 A m^{-3}	Zou et al. (2018)
Upward plug-flow baffled-reactor MFC	Autotrophic cathodic denitrifying	Synthetic	Nitrate removal	148.3 ± 1.4 g N m^{-3} NCV d^{-1}	76.5 ± 0.5 A m^{-3} NCV	Al-Mamun et al. (2017)
Dual-chamber MFC	Seawater bacteria	Synthetic	Nitrate removal	100%	26.6 A m^{-3}	Naga Samrat et al. (2018)
MFC and electric membrane bioreactor (EMBR)	Proton exchange membrane (PEM)-free MFC	Synthetic	Phosphorus, ammonia removal	> 93%	0.0137 A m^{-2}	Gao et al. (2017)
Dual-chamber MFC	Single MFC reactor with zero alkaline consumption	Tannery wastewater	Carbon and nitrogen removal	97.9%	0.83 A m^{-2}	Wu et al. (2018)
MFC and MEC	Continuous-flow membraneless MFC and MEC systems	Synthetic, municipal wastewater	Carbon and nitrogen removal	0.38 g N m^{-3} d^{-1}	0.34 A m^{-3}	Hussain et al. (2016)
Dual-chamber MFC	Anion exchange membrane AEM	Synthetic	Nitrate removal	48 g NO_3^- m^{-3} d^{-1}	23.7 A m^{-3}	Lee et al. (2018)
Algae biofilm microbial fuel cell (ABMFC)	Integrating an algal biofilm (AB) with a MFC	Domestic wastewater	Carbon and nitrogen removal	96%	0.17 A m^{-2}	Yang et al. (2018)

NCC: net cathodic compartment; TN: total nitrogen. d TCV: total compartment volume; NCV: net cathode volume.

production in MFCs. These results imply that engineering the QS system is a promising strategy for improving the efficiency of bioelectrochemical systems.

4. Nitrogen recovery

Nitrogen is one of the key nutrients for survival of living organisms. It is an important constituent of several biomolecules such as proteins and DNA. Large proportion of nitrogen exist in N_2 gas form in the atmosphere. However, it cannot be utilized by most of living organisms except some bacteria. Instead, it should be converted to reactive forms such as nitrate, nitrite, ammonium, and ammonia to be used as a nutrient by living organisms. To supply food to a growing human population, large amounts artificial fertilizers are applied to agricultural land. The nitrogen content of artificial fertilizers is obtained by converting N_2 using the Haber-Bosch process, which is very energy-intensive. The fixation of N_2 and application of fertilizers have led to an excess of reactor nitrogen in the environment, which has caused pollution. Recovery of reactive nitrogen from wastewater for use in food production could thus contribute both to lower energy consumption and less pollution (Matassa et al., 2015). Two biological techniques that potentially could be used for nitrogen recovery include bioelectrochemical systems and photosynthetic microorganisms. Bioelectrochemical systems have a wide range of applications including nitrogen removal and recovery from wastewaters while producing electricity. Photosynthetic microorganisms include eukaryotic microalgae and prokaryotic cyanobacteria, which can assimilate nitrogen and phosphorus present in wastewaters and recycle them in the form of microalgal biomass for producing fertilizers. Nitrogen can be alternatively removed or recovered by chemical methods, such as stripping process and struvite precipitation. Table 4 presents nitrogen recovery efficiency, chemical input, economic evaluation and main technical parameters of different nitrogen recovery processes.

4.1. Ammonia/ammonium recovery by bioelectrochemical systems

Biological nitrogen recovery using bioelectrochemical systems is based on ammonia/ammonium recovery (Rodríguez Arredondo et al., 2015; Wu and Modin, 2013). In the first mechanism, the biological oxidation of organic matter in wastewater at the anodic compartment contributes to energy recovery. At the same time, the ammonia/ammonium in the wastewater is transported through the cation ion exchange membrane to the cathode compartment with elevated pH catholyte, leading to ammonia recovery by stripping (Fig. 6A). The electric field induces the ammonia/ammonium migration across the ion exchange membrane. This process ultimately allows for chemical recovery of ammonia/ammonium from wastewater in a bioelectrochemical system. Ammonium recovery is also performed from urine with a pre-treatment step for phosphate recovery via struvite precipitation (Rodríguez Arredondo et al., 2015). Ammonium was recovered from urine at rate of 9.7 gN m^{-2} d^{-1} with an energy yield of -10 kJ gN^{-1} at a current density of 2.6 A m^{-2} (Kuntke, 2013).

Bioelectrochemical systems can also support ammonium oxidation at the cathode. Modin et al. (2011) also utilized the alkalinity produced in the cathodic compartment of an MFC to support nitrification of reject water. Yan et al. (2012) used a cathode pre-enriched with a nitrifying biofilm in a single-chamber MFC. The nitrifiers scavenged oxygen and oxidized ammonium at the gas-diffusion electrode.

In spite of many advantages of reactors, there are several issues with this technology. Electrolytes in bioelectrochemical systems have large number of cations and anions other than H^+ and OH^-. The transport of these cations and anions through the ion exchange membranes determines the pH gradient between the anode and cathode as well as the membrane potential. Although transport of NH_4^+ is beneficial for N recovery in some systems, pH gradients as well as concentration gradients of other cations than H^+ are responsible for potential loss in cation exchange membranes, which significantly affects the performance of bioelectrochemical reactors (Prosnansky et al., 2002; Sleutels et al., 2017).

Performance of bioelectrochemical reactors has significantly improved recently, as the current density of 400 A m^{-3} has been achieved in flat-panel air-cathode MFC (FA-MFC) with a promise for energy sustainable wastewater treatment (Park et al., 2017). To achieve practical application of bioelectrochemical systems, it is expected that the full-scale bioelectrochemical reactors should exhibit a volumetric current density of 1000 A m^{-3} reactor volume (Rozendal et al., 2008). The challenge is still scaling-up of bioelectrochemical reactor volume from lab scale reactors to larger volume with less internal resistance.

4.2. Nitrogen recovery by microalgae and cyanobacteria

Unicellular species of microscopic photosynthetic microorganisms, microalgae and cyanobacteria, are present in freshwater and marine systems. Their cells can exist individually or in chains or groups and are characterized by relatively fast growth and adaptability to harsh conditions. Microalgae and cyanobacteria have been recently considered as an alternative system for biological wastewater treatment with several applications (Delgadillo-Mirquez et al., 2016; Sood et al., 2015). In wastewater treatment, they photosynthetically generate O_2, which is consumed by bacterial populations to decompose organic wastes to simple inorganic nutrients. Furthermore, microalgae and cyanobacteria remove inorganic nutrients in tertiary treatment before discharge to receiving waters (Taziki et al., 2015; Pouliot et al., 1989). Large amounts of nitrogen and phosphorus are required for fast growth of microalgae and cyanobacteria, which can be effectively provided by uptake of nitrogen and phosphorus from wastewaters. Their potential for nitrogen recovery is the specific advantage of these microorganisms. Nitrate and ammonia assimilated by microalgae and cyanobacteria are converted to biomass rather than being released to the atmosphere as the N_2 gas by the dissimilatory nitrate reduction (Taziki et al., 2015). The nitrogen removal capacity of microalgae and cyanobacteria relies on ammonification and assimilatory reduction of nitrite to ammonium (Fig. 2B). Nitrogen is fixed by cyanobacteria converting atmospheric molecular nitrogen into ammonia through the following reaction; $N_2 + 8H^+ + 8e^- + 16ATP \rightarrow 2NH_3 + H_2 + 16ADP + 16Pi$. Later, it can be incorporated into the amino acids and proteins or excreted to the environment (Barsanti and Gualtieri, 2006).

NH_4^+, NO_3^-, and NO_2^- are fixed nitrogen forms which can be assimilated and transported into the microalgae. NO_3^- is the most oxidized and stable inorganic form of nitrogen in aquatic environments. It needs to be reduced into NH_4^+ before assimilation. It goes into a two-step process catalysed by the cytosolic nitrate reductase and chloroplastic nitrite reductase through the following reactions, $NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O$, $NO_2^- + 8H^+ + 6e^- \rightarrow NH_4^+ + 2H_2O$ (Barsanti and Gualtieri, 2006) (Fig. 7A). NH_4^+ (resulting from NO_3^- and NO_2^- reduction) is incorporated into the amino acids via glutamate dehydrogenase (at high levels of ammonium concentrations) or glutamine synthetase/glutamate synthase cycle (at low levels of ammonium concentrations) (Barsanti and Gualtieri, 2006; Taziki et al., 2015).

Higher production of biomass and absence of structural carbon such as cellulose in cyanobacteria and microalgae (C/N 5-20) make them more efficient in water reclamation and nitrogen recovery compared with complex plants (C/N ratio 18-120) (Taziki et al., 2015). Phylogenetic position of nitrate reductase and nitrite reductase in microalgae in comparison with other organisms was demonstrated in phylogenetic trees constructed via the Neighbour-Joining method and established the reliability of each node through bootstrap methods, using MEGA 4 (Fig. 7B, C). Furthermore, the chloroplast localization of nitrate reductases and nitrite reductases was investigated using Target P and Chloro P and shown by green arrow in Fig. 7B and C. As it was shown, nitrate reductase in some but not all of higher plants is located in chloroplast while this enzyme is localized in chloroplast of microalgae

Table 4
Nitrogen recovery efficiency, chemical input, economic evaluation, main technical parameters of different nitrogen recovery process.

Operation technique	Recovery efficiency	Chemical input	Economic evaluation	Main technical parameters
Ammonia/ammonium recovery by MFCs	Energy recovery by biological oxidation of wastewater organic matter at the anodic compartment	No demand for additional substances to adjust pH for ammonia stripping at the cathodic compartment	Reduced COD consumption to 0.57 kg by MFC for 1 kg N recovery compared to 2.86 kg COD for 1 kg N removal, no aeration demand	Biocatalyst microorganism, electrode materials, reaction control, resistance for electron transfer, large-scale reactor, long-term durability, cost of electrode materials, cost of fabrication and cost of operation
Microalgae and cyanobacteria	Nitrogen and phosphorus recovery	No chemical input	Reduced energy demand to 1.5-8 Wh per m^3 compared to 500 Wh per m^3 by activated sludge process, reduced greenhouse gas (CO_2) emission	Requires vast land area for installing ponds, optimization of wastewater characteristics (nutrients and toxic compounds), environmental factors (pH, temperature, light, O_2, and CO_2)
Chemical stripping process	Only NH_4^+ recovered, no impact on phosphorous and COD removal from wastewater	Additional substances to adjust pH	Expensive due to the complex process, cost of chemicals and reconditioning of the ammonium sulfate solution to a fertilizer product	pH, temperature and mass transfer area
Chemical struvite precipitation	NH_4^+ and phosphorus recovery	Required additional magnesium salts and phosphate	Expensive due to required chemicals, dewatering and drying of the precipitate	Molar ratio of $Mg:NH_4:P$, pH

COD- chemical oxygen demand.

Chlorella sorokiniana and *Dunaliella salina*. Immuno-specific electron microscopy observation has indicated that the nitrate reductase is localized in pyrenoids of *Monoraphidium braunii, Chlamydomonas reinhardii, Chlorella fusca, Dunaliella salina*, and *Scenedesmus obliquus* (Lopez-Ruiz et al., 1985). Pyrenoids are sub-cellular micro-compartments in microalgal chloroplasts, with a possible role in photosynthesis. Both photosynthesis-derived nicotinamide adenine dinucleotide (NADH) and nicotinamide adenine dinucleotide phosphate (NADPH) can be used as the electron donors for nitrate reduction in *Chlorella* sp. Therefore, it can be hypothesized that the energy-intensive process of nitrate reduction may be the reason for the chloroplast localization of nitrate reductase in microalgae (Taziki et al., 2015).

Nitrogen and phosphorus taken up by microalgal biomass can be used for producing fertilizers, bioenergy, food, animal feed and pharmaceuticals. Wastewater treatment by algae also includes assimilation of organic pollutants into cellular constituents such as lipids and carbohydrates. Furthermore, it is more environmentally friendly compared to the conventional wastewater treatments with introduction of activated sludge, a biological floc. Biological treatments using microalgae and cyanobacteria can offer a solution for the limitations of the current tertiary treatment methods. Secondary and tertiary treatments use more than half of the energy cost devoted to the municipal wastewater treatment. This cost is mostly related to the oxygen transfer into the secondary treatment and chemical demands in the tertiary treatment.

Chlorella sp. is frequently investigated for its nitrogen removal capacity in combination with other microalgae or bacteria (Table 5). Both native and non-native consortia can be used for wastewater treatment. The nitrogen removal efficiencies shown in Table 5 represent the combined effects of such consortia. Few reports exist on the individual capacity of microalgal species for nitrogen recovery, namely it was found that *Dunaliella tertiolecta*, *Neochloris oleobundans*, and *Chlorella vulgais* could individually uptake 155, 150, and 103 mg L^{-1} d^{-1}, respectively (Li et al., 2008; Hulatt et al., 2012).

4.2.1. Microalgae growth

Microalgae are cultivated through the suspended- and immobilized-cell systems for wastewater treatment purposes (Table 5). Nutrients supply including inorganic carbon as well as nitrogen (NO_3^-, NH_4^+, and urea) and phosphorus are required for the photosynthesis and production of proteins and nucleic acids during microalgal growth (Grobbelaar, 2004). Microalgal biomass production but not nitrogen and phosphorus removal is critically affected by nitrogen to phosphorus molar ratio (N/P) in wastewater. The ratio lower than 5 and higher than 30 causes nitrogen and phosphorus deficiency for microalgal growth, respectively (Choi and Lee, 2015). Different wastewaters, including the piggery industry and domestic wastewaters, as well as dairy manure and municipal sewage anaerobically-digested wastewaters with the appropriate N/P molar ratios (9, 11, 14 and 15, respectively) are suitable for microalgal growth (Gonçalves et al., 2017). Interestingly, the nitrogen fixation capacity of cyanobacteria make them able to survive at low concentration of nitrogen (Sood et al., 2015).

4.2.2. Factors affecting nitrogen removal by microalgae and cyanobacteria

For an optimal growth and nitrogen removal by microalgae, several principle parameters should be carefully controlled. The solubility of CO_2, required for microalgae photosynthesis, is negatively affected by the medium pH and temperature. NH_4^+ volatilization also occurs at increased pH and temperature, which adversely affects NH_4^+ removal process by microalgae (Gonçalves et al., 2017). CO_2 enrichment and buffering can prevent the harmful effect of pH increase on growth and nitrogen removal (Taziki et al., 2015). The microalgae optimal growth is influenced by temperature. Optimal temperature for microalgae growth differs among various species from below 10 °C to moderate temperatures (10–20 °C) and even some of them above 30 °C. Although, increasing temperature usually increase metabolic activity when microalgal growth is reduced at lower temperatures (Xin et al., 2011; Robarts and Zohary, 1987).

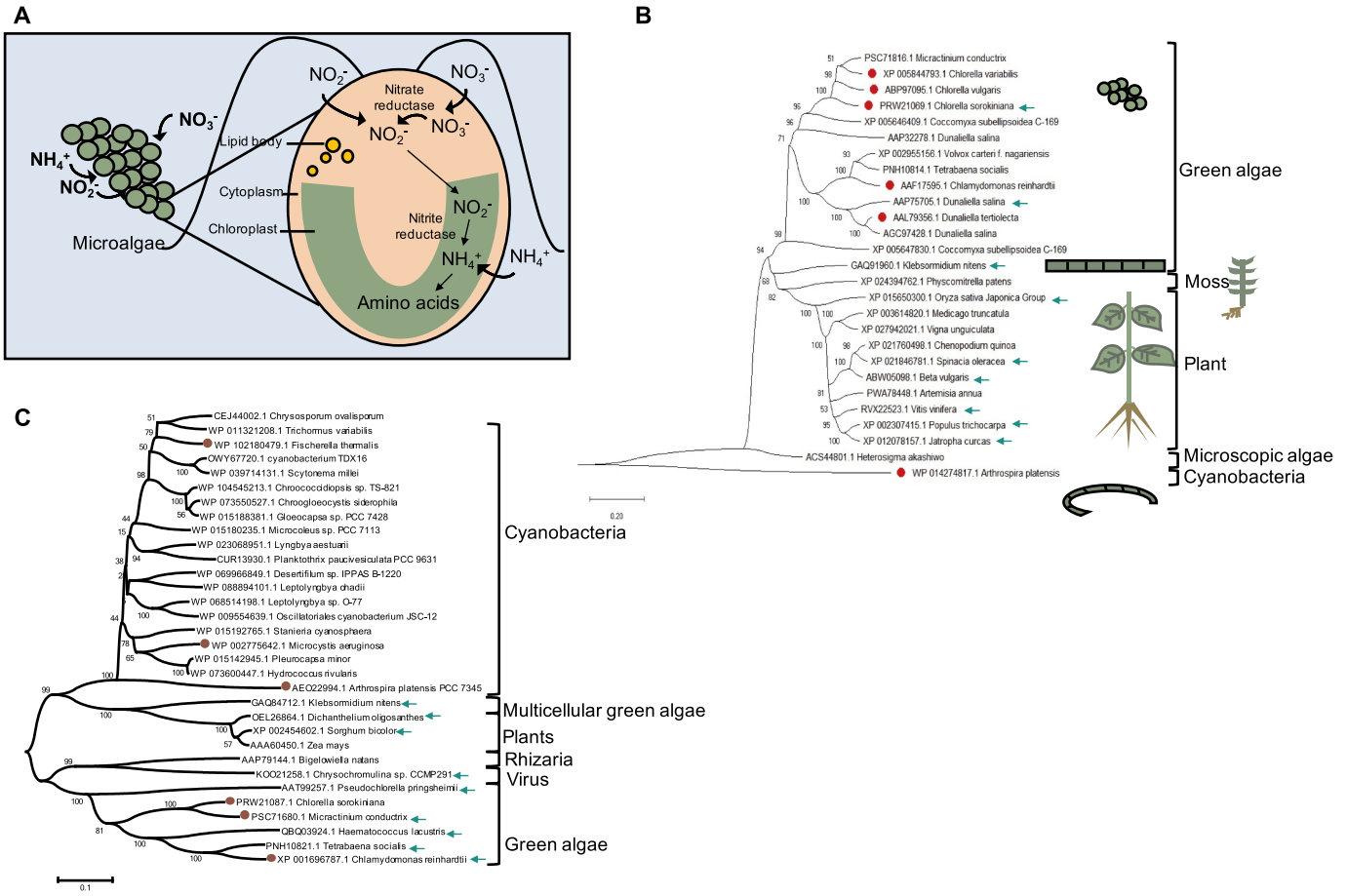

Fig. 7. Biological nitrogen removal mechanism by microalgae (A). Phylogenetic tree of nitrate reductase (B) and nitrite reductase (C) proteins in microalgae in comparison with other organisms. Microorganisms used for wastewater treatments are marked with red circle.

In terms of nitrogen recovery, a temperature-dependent specific affinity is found for nitrate in microalgae (Q10 ≈ 3, Q10 is the proportional change with 10°C increase in temperature), with no clear temperature-dependence for ammonium assimilation. Temperature-dependent nitrogen preference shows reduced affinity of microalgae for nitrate utilization at reduced temperatures whereas ammonium uptake and affinity is not affected suggesting ammonium as the more essential nutrient for microalgae (Reay et al., 1999).

The capacity of cyanobacteria for nutrient removal is also influenced by environmental factors such as light, temperature, pH, etc (Sood et al., 2015). However, a cyanobacteria Phormidium strain isolated from polar environment and *Phormidium bohneri* can efficiently remove nutrients at temperatures below 10 °C and high temperatures around 30 °C, respectively (Tang et al., 1997; Talbot and De la Noüe, 1993). These strains are sedimented for easy harvesting and they can be suggested as the suitable strains for wastewater treatment at low and high temperatures, respectively.

Light, both in quality (wavelength) and quantity can affect the microalgae growth and nitrogen removal efficiency. For example, red light is suggested as the optimum wavelength for *C. vulgaris* reproduction and wastewater total nitrogen removal efficiency. Whereas the stimulation of nitrate uptake and activation of nitrate reductase require blue light (Azuara and Aparicio, 1984; Calero et al., 1980; Yan et al., 2013). Wastewater treatment at dark condition is the best in terms of economy and efficiency. Thus, wastewater treatment using combined culture of *Rhodobacter sphaeroides* and *Chlorella sorokiniana* is suggested to efficiently remove nutrients under aerobic dark heterotrophic condition (Ogbonna et al., 2000).

4.2.3. *Mixed heterotrophic bacteria-microalgae culture*

An interesting potential for nitrogen recovery is found in polycultures of microalgae or microalgae and bacteria. These polycultures have a broad range of metabolic activities which allow them to adapt to various conditions. These valuable consortia can grow in different environments with various nutrient loads. The cooperative metabolic interactions of microorganisms in consortium can lead to nutrient removal from wastewater (Renuka et al., 2013). In such a beneficial consortium, the oxygen generated by microalgae photosynthesis is used by heterotrophic aerobic bacteria to biodegrade organic pollutants from wastewater. Heterotrophic bacteria discharge CO_2, which can be used by microalgae in the presence of light. Autotrophic, heterotrophic, mixotrophic, and photoheterotrophic are different metabolic types of microalgae, which can be shifted in response to changes in environmental conditions (Delgadillo-Mirquez et al., 2016). Under certain conditions, they may also compete with heterotrophic bacteria. High rate algal pond (HRAP) technology is used for mixed bacteria-microalgae culture to treat municipal, industrial and agricultural wastewaters. HRAP is essentially a shallow race-track reactor (0.3-0.4 m depth), equipped with mechanical mixing for the simultaneous growth of algae and bacteria. HRAP is fed with the primary/secondary wastewater, which is mixed with the algal and bacterial culture inside the bioreactor. In this system, the removal of pollutants is caused by algal assimilation, bacterial biological processes (nitrification/denitrification) and stripping phenomena such as ammonia volatilization and phosphorus precipitation (facilitated by high pH levels induced by photosynthetic microalgal growth). Microalgal-bacterial consortia are more beneficial than only microalgal consortia, as the microalgal-bacterial consortia can replace both secondary and tertiary treatment of

Table 5
Application of microalgal and microalgal-bacterial consortia in nitrogen removal from different wastewaters and respective removal efficiencies.

Microorganisms	Wastewater	Operation technique	Nitrogen removal rate %	References
Centrate wastewater native algal-bacterial consortium	Centrates wastewater, primary-treated domestic wastewater	Immobilized system with higher nutrient removal rates	60 70	Posadas et al. (2013)
Centrate wastewater native algal-bacterial consortium	Synthetic domestic wastewater	Closed suspended system and mixotrophic metabolism	99.8	Alcántara et al. (2015b)
Chlorella sorokiniana and activated sludge native bacteria	Potato-processing wastewater	Open suspended system and biogas production	≥ 95	Hernández et al. (2013)
Chlorella sorokiniana and activated sludge native bacteria	Primary-treated piggery wastewater	Open suspended system and biogas production	82.7	Hernández et al. (2013)
Chlorella sorokiniana and activated sludge native bacteria	Primary-treated piggery wastewater	Immobilized system biofilm reactor with simultaneous denitrification/nitrification	94–100	De Godos et al. (2009)
Chlorella sp., *Pediastrum* sp., *Phormidium* sp., *Scenedesmus* sp. and activated sludge native bacteria	Primary-treated municipal wastewater	Closed suspended system with microalgal bacterial flocs MaB-flocs	61.2	Van Den Hende et al. (2011)
Chlorella spp. and *Azospirillum brasilense*	Municipal wastewater	Co-immobilized system using alginate beads	15 NO3-N 100 NH4-N	De-Bashan et al. (2004)
Chlorella vulgaris and *Azospirillum brasilense*	Synthetic wastewater	Co-immobilized system using alginate beads	93 91 100	De-Bashan et al. (2002)
Chlorella vulgaris and *Bacillus licheniformis*	Synthetic medium	Closed suspended system with pH control	78	Liang et al. (2013)
Chlorella vulgaris and activated sludge native bacteria	Synthetic wastewater	Closed suspended system considering food to microorganisms ratio F/M and hydraulic retention time HRT	33.43–65.96	Medina and Neis (2007)
Chlorella vulgaris and primary-treated municipal wastewater native bacteria	Primary-treated municipal wastewater	Closed suspended system	30.9–100	He et al. (2013)
Microalgal consortium from a high rate algal pond treating diluted vinasse and activated sludge native bacteria	Synthetic wastewater	Anoxic–aerobic closed suspended system with nitrous oxide emission	75–96	Alcántara et al. (2015a)
Microalgal consortium from a high rate algal pond treating domestic wastewater and activated sludge native bacteria	Domestic wastewater	Open suspended system with and without CO_2 addition	91.8–96.9	Park and Craggs (2011)
Municipal wastewater native microalgae and activated sludge native bacteria	Primary-treated municipal wastewater	Open suspended system and effect of sludge inoculation ratios	93.7–95.8	Su et al. (2012b)
Piggery wastewater native algal-bacterial consortium	Fresh piggery wastewater	Open suspended system	58.2–94.8	González-Fernández et al. (2011)
Primary-treated wastewater native algal-bacterial consortium	Primary-treated municipal wastewater	Closed suspended system	100	Su et al. (2011)
Scenedesmus sp. and anaerobic sludge native bacteria	Starch wastewater	Closed suspended system	88.7	Ren et al. (2015)
Microalgal consortium composed by *Ulothrix zonata*, *Ulothrix aequalis*, *Rhizoclonium hieroglyphicum* and *Oedogonium* sp.	Digested dairy manure wastewater	Immobilized system biofilm reactor and dry matter/crude protein yields	62	Wilkie and Mulbry (2002)
Chlamydomonas reinhardtii, Scenedesmus rubescens and *Chlorella vulgaris*	Primary-treated municipal wastewater	Closed suspended system considering biotic and abiotic factors	41.2–100 TN	Su et al. (2012a)
Chlorella sp. and *Scenedesmus* sp.	Primary-treated municipal wastewater at different concentrations	Closed suspended system under the climatic conditions specific to Lithuania	88.6–96.4	Koreiviene et al. (2014)
Chlorella sp., *Scenedesmus* spp. and *Chlorella zofingiensis*	Dairy wastewater	Closed suspended system for biodiesel production	87.0–91.0	Qin et al. (2016)
Chlorella vulgaris and *Planktothrix isothrix*	Municipal wastewater	Closed suspended system and co-culture effect	43.9–81.5	Silva-Benavides and Torzillo (2012)
Microalgal consortium composed by the families Chlorophyta, Cyanobacteria, Euglenozoa and Ochrophyta	Primary-treated municipal wastewater	Closed suspended system	100	Samorì et al. (2013)
Carpet mill industry wastewater native microalgal consortium	Carpet mill industry wastewater	Closed suspended system for biodiesel production	99.7–99.8	Chinnasamy et al. (2010)
Phormidium sp., *Limnothrix* sp., *Anabaena* sp., *Westiellopsis* sp., *Fischerella* sp. and *Spirogyra* sp.	Primary-treated sewage water	Closed suspended system with native filamentous microalgal strains, native unicellular microalgal strains, selected microalgae from germplasm	83.3 100	Renuka et al. (2013)
Rhodobacter sphaeroides and *Chlorella sorokiniana*	Synthetic high strength organic wastewater	Closed suspended system	100 100	Ogbonna et al. (2000)
Synechocystis salina and *Chlorella vulgaris*	Synthetic medium	Closed suspended system with polyculture	84.5	Gonçalves et al. (2016)
Chlorella vulgaris	Treated sewage	Algal biofilm airlift photobioreactor ABA-PBR with solid carriers	61.6	Tao et al. (2017)
Betaproteobacteria, Gammaproteobacteria, Flavobacterium, Chlorophyta, Trebouxiophyceae, Bacillariophyceae, Cyanobacteria	Synthetic domestic wastewater	Photobioreactor with algal–bacterial granules	59.8-70.5 TN 99% NH4-N	Zhang et al. (2018)
Chlorella vulgaris-Bacillus licheniformis *Microcystis aeruginosa-Bacillus licheniformis*	Synthetic wastewater	Algae-bacteria symbiotic system	88.95 TN 21.56 TN	Ji et al. (2018)

TN - total nitrogen mg N L^{-1}.

wastewater whilst microalgal consortia can be only applied in tertiary treatment of wastewater (Gonçalves et al., 2017). HRAP with algae-dominated consortium shows high removal efficiency for COD, total nitrogen and phosphorus from untreated municipal wastewater (Kim et al., 2014). Furthermore, a hydrodynamic model of HRAP with a ratio length/width (L/W) higher than 10 shows better performance with respect to the velocity uniformity and reduced shear stresses (Hadiyanto et al., 2013). However, increasing depth significantly increases overall areal productivity (Sutherland et al., 2014).

Despite their utility, the HRAP systems present some hurdles. In these systems, there is lack of balance in oxygen consumption and production by bacteria and algae, caused by difficulties in light penetration and mixing. However, closed photobioreactors (PBRs) and closed tanks have been proposed to overcome the obstacles of light penetration, mixing and presence of predators (He et al., 2013; Alcántara et al., 2015a). The demand of an additional step to harvest microalgae biomass from treated effluent is another issue with HRAPs, which is addressed by immobilized growth system. There are several immobilization techniques to decrease time and costs for harvesting microalgae as following; i) a centrate (the liquid removed from thickened sludge) microalgal-bacterial consortium in biofilm reactor (Posadas et al., 2013; De Godos et al., 2009), ii) growth immobilization in solid carriers such as beads (De-Bashan et al., 2004), iii) usage of microorganisms with flocculation characteristics as artificial consortia (Van Den Hende et al., 2011). To further improve the capacity of these systems in nutrient removal, some studies investigated the complex interactions between physico-chemical factors such as light, photoperiod, temperature, nutrients concentration, pH, microalgal: bacterial ratio and biological factors (Gonçalves et al., 2017). Examples of relevant biological factors include pathogens, viral attack, protozoa predation and competition with bacteria over the available nutrients (Gonçalves et al., 2017). Large seasonal variations in photoperiod and temperature affect wastewater treatment efficiency and biomass productivity in HRAP (Delgadillo-Mirquez et al., 2016). The photoperiod duration has been shown to affect algal-bacterial population dynamics in a photo-bioreactor for municipal wastewater treatment (Lee et al., 2015).

4.3. Nitrogen recovery by chemical processes

4.3.1. Stripping process

The stripping technology is a chemical ammonium removal process promoting conversion of NH_4^+ to NH_3 by forcing air or other gas into the wastewater to get NH_3 into the gas phase. The ammonia stripping process includes four major steps including: i) NH_4^+ conversion to ammonia gas (NH_3), ii) NH_3 diffusion to the air-water interface, iii) NH_3 release to the air at the interface, and iv) NH_3 diffuses from the air-water interface into the air above. The process is affected by pH, temperature and mass transfer area. In common applications, NH_4^+ in wastewater can be released from the aqueous phase by air, steam or biogas (Limoli et al., 2016).

Both continuous and batch mode can be used for operation of strippers. To avoid NH_3 emission into the air causing the greenhouse effect, NH_3 is typically absorbed by phosphorus acid (Shen et al., 2017). Sulfuric acid is also used for the absorption of the stripped ammonia as ammonium sulphate. According to the initial ammonia concentration in wastewater, it is introduced at stoichiometric concentration. The most common use for nitrogen recovered in this process is the conversion to fertilizer with 40%–60% ammonium sulfate solution and low organic contamination. This can be produced directly from the ammonia stripping process effluent after pH neutralization (Laureni et al., 2013). Thus, the ammonia stripping process can be suggested as a beneficial technology to support agriculture in areas where this is required. However, only NH_4^+ can be removed by this method, with no impact on phosphorous and COD removal from wastewater (Cao et al., 2019b).

4.3.2. Struvite precipitation

Struvite precipitation is considered as the one of the preferred chemical technologies in NH_4^+ and phosphorus removal from wastewater, with significant potential for nitrogen recovery (Cao et al., 2019b; Huang et al., 2016; Song et al., 2018). It is a highly effective, simple and environmentally friendly method, with nitrogen recoverable as the fertilizer (Barbosa et al., 2016). Struvite is a valuable fertilizer in the form of white crystalline solid which is poorly soluble in water. It is formed through the following simplified reaction; $Mg^{2+} + NH_4+ + PO_4^{3-} + 6H_2O \rightarrow MgNH_4PO_46H_2O$. This reaction can be mainly affected by two factors including molar ratio of Mg:NH_4:P and pH (optimum pH 9.0–10.0) (Cao et al., 2019b). Struvite precipitation with an Mg:NH4:PO4 ratio of 1:1:1 could remove 95% of NH_4^+ from anaerobically treated effluents in 30 s (Escudero et al., 2015). At higher pH, the solubility of struvite reduces resulting in struvite crystals formation.

Several studies investigated struvite formation and phosphorus and ammonium recovery from urine, livestock manure, anaerobically-treated effluents, industrial wastewater and landfill leachate (Etter et al., 2011; Huang et al., 2011; Song et al., 2011; Crutchik and Garrido, 2011; Di Iaconi et al., 2010). One considerable difficulty with struvite precipitation is the large amount of ammonium and phosphorus found in anaerobically-treated effluents from livestock manures. These require large amounts of magnesium salts for effective struvite precipitation. Unfortunately, due to high cost of the reagents and necessity of pH control, very few industrial-scale struvite crystallization plants are in operation (Escudero et al., 2015). However, magnesium oxide (MgO) is recently suggested as a proper Mg source for struvite crystallization. It is cheap, existing in large quantities, and also it has high alkalinity and adsorption capacity for removal of organic and polymeric substance, such as polysaccharide, polyphenols, and organic acid (Cao et al., 2019b; Chimenos et al., 2003; Cai et al., 2017). Furthermore, combining ammonia stripping with struvite precipitation could feasibly remove ammonia nitrogen, phosphate, and COD from digested swine wastewater with high efficiency, low cost, and environmental friendliness (Cao et al., 2019b). Furthermore, no pH adjustment is needed using $Na_3PO_4{\cdot}12H_2O$ as the PO_4^{3-} source which is an effective reagent for ammonium removal and struvite precipitation due to reaching to high pH in the medium.

5. Perspectives

Partial nitritation and anammox for mainstream wastewater treatment is an attractive concept because it would reduce energy requirements for aeration and allow a larger fraction of organic matter to be valorized as biogas. However, the concept is challenging because of the low growth rate of anammox bacteria at low temperatures, full-scale operation is remaining a challenge under winter conditions. The long-term stability of the process has also been pointed out as an aspect requiring further research. Stable nitrite production plays a key role for long-term operation of coupled partial nitritation and anammox. Therefore, effective strategies such as organic carbon and reaction time, etc should be further investigated to maintain nitrite production for different wastewater compositions and operational conditions.

Another concern with anammox processes is the nitrate present in effluent. Both the anammox bacteria themselves and failure to suppress NOB activity will lead to nitrate production, which means the effluent may require further treatment. This challenge could be addressed by a SNAD process simultaneously accomplishing partial nitrification, anammox, and denitrification. However, these conditions present serious difficulties for conventional nitrification and denitrification methods.

To address the issue with toxic substances present in nitrate-enriched industrial wastewaters, it is required to perform a comprehensive analysis of chemical composition and then adapt the treatment process accordingly. Granules or biofilms based systems are promising approaches when it comes to mitigating negative effects of toxic components in nitrate-enriched industrial wastewater.

In case of nitrogen recovery, bioelectrochemical systems can make a shortcut in ammonium removal from wastewater through the direct recovery in the form of ammonia rather than reduction to nitrogen gas. Nitrogen recovery by bioelectrochemical systems is advantageous, as there is no demand for additional substances to increase pH for ammonia stripping at the cathodic compartment. Furthermore, no aeration is required for ammonium oxidation and nitrogen removal which makes it an energy-efficient ammonia recovery system compared with other nitrogen removal technologies.

The MFC capital cost is around 30 times higher than that of conventional treatment of activated sludge for domestic wastewater. For the commercialization of MFC-based wastewater treatment systems, further assessment of several factors such as biocatalyst microorganism, electrode materials, reaction control, resistance for electron transfer, large-scale reactor, long-term durability, and the relatively high cost of electrode materials, cost of fabrication and cost of operation are required to be considered.

From the synthetic biology point of view, ALE-based approaches leading to evolved NP-degrading bacteria can facilitate biodegradation of toxic nitro group-containing components circumventing necessity of safety evaluation for introduction of GMM into contaminated sites. Moreover, QS system plays a crucial role in wastewater treatment and further engineering of the QS system can be promising strategy for regulating denitrification process, improving activity and performance of anammox, and efficient bioelectrochemical systems.

One kilogram phosphorus and nitrogen removal costs about 3.0 and 4.4 USD using biological conventional wastewater treatment, respectively. Meanwhile, utilization of wastewater for microalgae cultivation decreases almost 100% demand of freshwater and nutrient leading remarkable reduction in their production cost. The utilization of microalgae for wastewater treatment has high efficiency for treatment of various wastewaters, nutrient recovery in the form of valuable biomass for biofertilizers, biopesticides, animal feed, and pharmaceuticals as well as for energy recovery as feedstock to produce biofuels such as biodiesel and bioethanol and reducing greenhouse gas (CO_2) emission. In terms of energy demand, it competes with activated sludge processes with energy demand of ~500 Wh per m^3 of wastewater. Whereas only energy ranges from 1.5 to 8.0 Wh per m^3 is needed for mixing which is the most energy demanding factor in conventional HRAPs. However, several challenges for microalgae-based technology should be addressed for the large-scale operation. This technology requires vast land area for installing ponds. Therefore, possible environmental impacts of land use change (LUC) and changes carbon stocks in soil should be investigated. Furthermore, characteristics of wastewater such as nutrients and toxic compounds and environmental factors including pH, temperature, light, O_2, and CO_2 should be optimized for microalgae growth and thereby efficient wastewater treatment.

Last but not least, to obtain more effective consortia for treating different pollutants such as nitrogen sources, selection and bioaugmentation of effective microorganisms from broad possible combinations need to be evaluated. Novel computational tools such as genome-scale metabolic models (GEMs) of relevant microbial species can be employed to predict the efficient microbial species as well as optimum ratio of those species in consortia achieving the maximum nitrogen removal in wastewater.

Additionally, interactions between organisms in the consortia need to be investigated through metabolite profiling to make beneficial communications among microorganisms for optimal pollutant removal. “Multi-omics” such as metagenomic/metatranscriptomic/metaproteomic approaches and microbiome analysis of wastewater treatment processes are needed to enhance our understanding of genes involved in wastewater treatment processes in specific microbial communities. This information can be useful for improvement of treatment systems based on different nitrogen removal technologies.

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