Available online at www.sciencedirect.com

ScienceDirect

Current Opinion in Biotechnology

New directions in biological nitrogen removal and recovery from wastewater

Mari KH Winkler and Levi Straka

This review summarizes strategies for biological nitrogen removal (BNR) and recovery from wastewater. The most commonly used BNR technology nitrification/denitrification is also the most energy intensive, even though there are lower energy options, including nitritation/denitritation and more efficient partial nitritation/Anammox; the latter is well demonstrated for side-stream treatment and progressing toward mainstream applications. Nitrogen recovery can be done through cell assimilation with phototrophs, but bottlenecks with solids separation and space requirements limit applications to tertiary treatment. Whereas, microbial electrochemical cells are energy efficient at recovering nitrogen from side streams, but not capable of achieving low effluent levels. The combined strengths of these emerging approaches will improve wastewater nitrogen removal by reducing energy consumption, minimizing effluent nitrogen, and maximizing nitrogen recovery.

Address
Department of Civil and Environmental Engineering, University of Washington, 201 More Hall, Box 352700, Seattle, WA 98195-2700, USA

Corresponding author: Winkler, Mari KH (mwinkler@uw.edu)

Current Opinion in Biotechnology 2019, **57**:50–55

This review comes from a themed issue on **Environmental biotechnology**

Edited by **Lutgarde Raskin** and **Per Halkjær Nielsen**

For a complete overview see the Issue and the Editorial

Available online 29th January 2019

https://doi.org/10.1016/j.copbio.2018.12.007

Introduction

Nitrogen is used for the synthesis of proteins, nucleic acids, and other cell constituents, making it one of the most important nutrients in the biosphere [1]. It is, therefore, a large part of fertilizers and foods, which ultimately end up in wastewater. Left untreated, large fluxes of reactive nitrogen to receiving waters leads to a host of environmental problems, including: eutrophication, toxic algae blooms, groundwater contamination, and atmospherically active gases that contribute to global warming [2]. Anthropogenic activity has altered the nitrogen cycle far outside the natural Earth system, and it is important that we mitigate these deleterious effects [3].

The two main strategies for removal of nitrogen from wastewater are to convert it to dinitrogen gas or to concentrate and recover it as fertilizer. Nitrogen fertilization is indispensable for sustaining agricultural yields, but production of nitrogen fertilizers accounts for 1–2% of global energy consumption [4]. Offsetting this energy consumption by reusing nitrogen from wastewater is desirable; however, the nitrogen content in wastewater often gets diluted making it difficult and energy intensive to recover via physical/chemical processes [5]. The new paradigm in wastewater treatment is resource recovery and energy reduction, and, hence, this review will focus on recent innovations in biological wastewater treatment that enhance nitrogen recovery while also reducing energy costs and production of greenhouse gases.

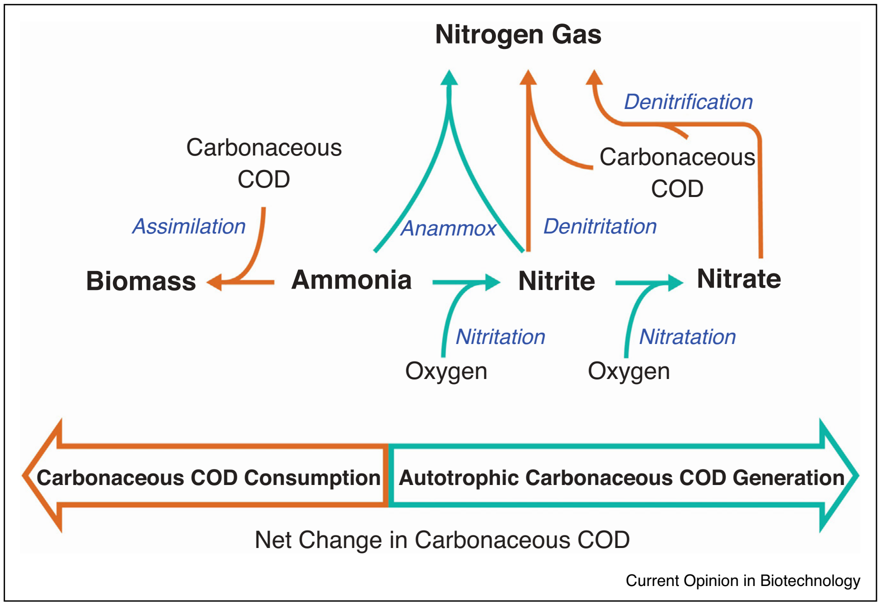
Nitrification/denitrification

Most wastewater nitrogen is present as ammonia (referring to total ammonia, or NH_4^+ and NH_3), and conventional biological nitrogen removal (BNR) is carried out by (i) aerobic nitrification, where ammonium oxidizing bacteria (AOB) convert ammonia to nitrite (nitritation) and nitrite oxidizing bacteria (NOB) convert nitrite to nitrate (nitratation), in addition to (ii) denitrification, where denitrifiers subsequently convert nitrate to dinitrogen gas with organic carbon as an electron donor [5]. Although effective, both steps produce N_2O (a greenhouse gas). In addition, nitrification requires energy intensive aeration (BNR increases the energy for aeration, pumping, and solids processing by 30–50% [5]), and denitrification requires organic carbon, which could instead be recovered as energy and for wastewaters with low COD/N is a costly addition ($1.14 per kg residual nitrate removed using methanol) [6,7]. Process optimization with real-time monitoring and control can minimize carbon and oxygen requirements [8,9], however nitritation coupled with denitritation or anaerobic ammonium oxidation (Anammox) are promising alternatives (Figure 1).

Nitritation/denitritation

Denitrifiers can use nitrite or nitrate as their electron acceptor, and while full nitrification requires 4.57 mgO_2/mgN, nitritation only requires 3.43 mgO_2/mgN, therefore, suppressing nitratation can save ~25% of aeration costs. Furthermore, denitritation rates occur 1.5 to 2 times faster than denitrification, the organic carbon requirement is up to 40% less, and the sludge production is theoretically reduced by ~33% for nitrification and ~55% for denitrification [10]. The key to successful nitritation/denitritation (or

Figure 1



Microbial nitrogen transformations for removing ammonia from wastewater, including: nitrification/denitrification, nitritation/denitritation, partial nitritation/Anammox, and assimilation. Green arrows indicate autotrophic processes, where the nitrogen species is the electron donor and brown arrows indicate the need for a non-nitrogen electron donor (organic carbon, or water for photosynthesis). From left to right roughly indicates the net increase or decrease in total chemical oxygen demand (COD) from organic carbon.

'short-cut' BNR) is enriching AOB while inhibiting NOB. Different strategies have been developed to control NOB while still enriching AOB, including: alternating anoxic and oxic conditions, aggressive SRT control, step feeding, and intermittent aeration [11,12]. Intermittent aeration is widely applied in full-scale treatment plants showing good nitrogen removal and substantial energy savings compared to conventional BNR [13]. NOB activity experiences a time lag following a transition from anoxic to oxic conditions [14], and intermittent aeration also restricts dissolved oxygen (DO), which has proven effective at limiting NOB activity and is in line with literature that reports generally higher oxygen affinity of AOB to NOB [15]. A downside to short-cut BNR is that N_2O production is triggered at low DO, which offsets some of the greenhouse gas savings from less energy consumption [7].

Denitrifying polyphosphate accumulating organisms (dPAO) can simultaneously remove phosphorus and nitrogen [16]. The dPAO are enriched under alternating anaerobic/anoxic conditions, storing organic carbon in the anaerobic phase that is subsequently utilized in the anoxic phase as the electron donor to reduce nitrate or nitrite to dinitrogen gas. Compared to ordinary denitrifiers, this is advantageous to nitrogen removal because dPAO denitrify in the absence of an external electron donor. One way in which dPAO have been implemented at lab, pilot, and full-scale, is through aerobic granular sludge technology [17•]. The culture is anaerobically fed where dPAO release phosphorus and accumulate organic carbon. During aerobic conditions, AOB produce nitrite on the granule periphery, which can diffuse into the oxygen protected core where dPAO denitrify the nitrite and accumulate phosphorus utilizing their stored carbon.

Side-Stream partial nitritation/Anammox

A further energy-saving improvement to nitritation/denitritation is teaming partial nitritation with Anammox (PNA). Anammox bacteria oxidize ammonium directly to dinitrogen gas using nitrite (produced by AOB during nitritation) as the electron acceptor. In current applications, about half of the ammonia is oxidized by AOB to nitrite, and the remaining half is anaerobically oxidized by Anammox. Key advantages of PNA over conventional BNR are: 1) no organic carbon needed; fully autotrophic nitrogen removal, 2) about 60% less energy for aeration, 3) about 75% less sludge production, and 4) lower emissions of CO_2 and potentially N_2O since both gases are not produced in Anammox metabolism [18,19] noting that AOB are reported to produce more N_2O under oxygen limited conditions [7]. More than 200 full-scale wastewater treatment plants successfully use PNA to treat warm (>25 °C) and ammonium laden (500–1500 mgN/L) anaerobic-digester-centrate-side streams with low COD/N ratios (<1 g COD/g N) [20••,21].

The PNA process can be designed as a one or two-stage process. In a two-stage process, half of the ammonia is oxidized to nitrite by AOB and the resulting ammonium and nitrite rich stream is fed to an Anammox reactor. The first stage is a nitritation reactor that exploits the higher growth rate of AOB compared to NOB at higher temperatures present in centrate [21,22]. In the subsequent Anammox reactor, AOB and NOB cannot grow due to absence of oxygen. In single-stage PNA, nitritation and Anammox are combined in one reactor and conditions are differentiated with biofilm gradients such as in: moving bed biofilm reactor, granular sludge, and rotating biological contactors [21]. The outer layer of the biofilm harbors AOB that generate nitrite, which can be used by Anammox in the oxygen protected inner layer.

Side stream PNA is a low-energy approach for reducing nitrogen recycled to the front of the treatment plant. However, due to high ammonia (and phosphate) concentrations this side stream is most suited for nitrogen recovery by physical/chemical approaches or microbial electrochemical cells.

Mainstream partial nitritation/Anammox

There is great interest in applying PNA to mainstream treatment because of the successes in side-stream treatment and the dramatic savings in carbon and aeration energy. However, some key differences make mainstream treatment challenging: 1) the COD/N ratio is higher leading to an excess of heterotrophic growth hence out selecting the slower growing Anammox, 2) the ammonia load is much lower; restricting Anammox and AOB growth, 3) the temperatures are lower, which disproportionately favors NOB relative to Anammox and AOB, and 4) effluent ammonia from mainstream needs to be much lower than from side stream [20••]. In theory, there are ways to design around these challenges: 1) carbon can be removed ahead of PNA treatment, 2) excess biomass can be retained with a membrane bioreactor or biofilm/granule system to compensate for lower Anammox activity associated with temperature and ammonia concentration [23], 3) NOB can be suppressed with mechanisms not involving temperature (f low DO, step feeding, and intermittent aeration [12]), and 4) tight process control can be employed for low effluent concentrations [24]. At present, however, successful lab demonstrations of PNA have, compromised on one or more of these challenges [20••].

There are two successful full-scale mainstream Anammox plants in operation: Strass (Austria), and Changi (Singapore). Strass supplements their mainstream sludge with Anammox granules from side-stream treatment, and applies intermittent aeration to control NOB [25••]. They do not report the relative contribution of Anammox to nitrogen removal, but report efficient granule retention in the mainstream [25••]. Alternatively, Changi uses flocculent activated sludge with step feeding and alternating aerobic and anoxic conditions to suppress NOB. Because the plant is located in a tropical environment, the wastewater temperature stays around 30°C and sludge is enriched with Anammox [26••]. While Anammox activity is demonstrated, they estimate it only accounts for 37.5% of nitrogen removal (denitrification accounts for 27.1%) [27]. These plants are testaments to the potential of mainstream PNA, but are unique situations, and further understanding is needed before widespread adaption is possible.

Recently, organisms capable of complete ammonia oxidation (ammonia to nitrate; comammox) have been discovered [28]. These organisms show an extraordinarily high affinity for ammonia [29], and, therefore, may be problematic in PNA systems if the comammox are producing nitrate. However, early results suggest comammox are not as efficient at nitrite oxidation, and, therefore may simply function as an ammonia oxidizer [29]. Additionally, it has been suggested comammox can perform dissimilatory nitrate reduction to nitrite, which could be beneficial to Anammox systems, but this is speculative [29].

Emerging research strategies for mainstream Anammox include coupling them with either ammonium oxidizing Archaea (AOA) or nitrate-dependent anaerobic methane oxidizing (N-damo) archaea. AOA oxidize ammonia to nitrite like AOB, but they possess a higher affinity for ammonia and oxygen [30], and, therefore, could be better suited for mainstream PNA with low effluent ammonia. N-damo can reduce nitrate to nitrite with methane as an electron donor, which might offer a more reliable nitrite supply to Anammox than AOB, and eliminates the requirement for NOB suppression [31]. Methane can be utilized from the grid or utilized from a digester. If used from a digester, methane will not be available for energy recovery, but aeration energy will still be lowered compared to conventional BNR if combined with Anammox. Handling and stripping of methane (losing dissolved methane to gas), however, are potential bottlenecks for a successful application [32]. Besides nitrite, Anammox has also been shown to use small organic molecules, sulfate manganese, and iron (III) as electron acceptors for ammonia oxidation [33]. This opens a myriad of alternate metabolisms that could be exploited in niche applications.

Besides Anammox, another autotrophic ammonium removal process is the sulfate reduction, autotrophic denitrification, nitrification integrated (SANI). The SANI process was demonstrated in Hong Kong where toilets are flushed with salt water leading to high sulfate concentrations (500 mgS/L) in the wastewater. Organic carbon is oxidized with sulfate as the electron acceptor (instead of utilizing energy intensive aeration), and thus formed sulfide is used to reduce nitrate (from a nitrification reactor) to dinitrogen gas [34].

Nitrogen recovery

Recovering a pure or highly concentrated ammonia stream can be completed with physical/chemical methods: air stripping, steam stripping, hollow fiber membrane contactors, and struvite precipitation [35•]. These methods are most cost effective at high initial ammonia concentrations and hence only useful on side-stream effluents or source-separated urine [36]. In the mainstream where nitrogen is very dilute, chemical recovery becomes economically unfeasible. Instead, nitrogen captured in stabilized biosolids can be directly applied to fields offsetting chemical fertilizers [5]. In the United States approximately half of all biosolids are recycled to land (US EPA; URL: https://www.epa.gov/biosolids). Unfortunately, for social/economic reasons, some biosolids cannot be used, and one reason is that the quality of biosolids is not high enough for land application (due to pathogens or pollutants), so they are instead landfilled or incinerated. These biosolids need to be further treated or 'stabilized' for reuse. One promising option is biodrying, which is a rapid composting/drying process that stabilizes and kills pathogens in biosolids while generating ammonia-rich air that can be scrubbed and recovered [37•]. In a full-scale biodrying installment in Zutphen, The Netherlands treating 150 kton/year of waste activated sludge, 7.3 kton/year ammonium sulfate was recovered and biosolids were produced that complied with the Dutch quality standards for land application and had a caloric value between 7700–10 400 kJ/kg making them a good source of energy if land application is not an option [37•].

Capturing soluble nitrogen in biosolids involves growing organisms that assimilate nitrogen. Heterotrophic organisms consume approximately 20 gCOD/gN, but municipal wastewater contains around 11 gCOD/gN [5]. As a result, only a 10–20% of influent wastewater nitrogen is sequestered in heterotrophic biomass. Phototrophs are an attractive alternative to capture nitrogen from low COD/N wastewater because they can obtain additional energy from light, and, therefore, assimilate nitrogen with no or less organic carbon.

Phototrophic systems

Phototrophic systems take advantage of energy from sunlight to reduce the COD/N uptake of the BNR system. Additionally, many phototrophs can grow heterotrophically in the dark (at night). The two primary classes of phototrophic organisms considered in wastewater applications are phototrophic purple bacteria (PPB) for their flexible metabolism, and single-celled algae and cyanobacteria (collectively microalgae) for their ability to perform oxygenic photosynthesis. PPB can grow photo-autotrophically, photo-heterotrophically (on light frequencies down to near infrared), and chemo-heterotrophically [38,39]. PPB also grow well when fed with high strength wastewaters, and have, therefore, been used to treat a variety of agricultural waste streams [40]. While less commonly applied to domestic wastewater, PPB have been shown to assimilate approximately 16 gCOD/gN and are capable of reducing COD and nitrogen in treated water to discharge limits [41•,42]. While better than heterotrophs, this is not sufficient for complete nitrogen removal in typical municipal wastewater and would require supplemental carbon addition or an additional treatment step. Another limitation is that PPB are restricted to organic acids, alcohols, and some sugars, and would likely need additional pretreatment (such as pre-fermentation) for complete COD removal [35•,41•]. The application of PPB, is, therefore, limited to specific situations.

Microalgae also have a variety of metabolisms, and, unlike PPB, can grow photo-autotrophically with only water as the electron donor (oxygenic photosynthesis) and assimilate nitrogen without organic carbon [1]. Cultivating microalgae on wastewater has been researched for a long time (reviewed in Refs. [43,44]), and a key challenge is solids separation. When growing suspended cultures, the use of light inherently requires low solids concentration to supply adequate light to the cell suspension. Additionally, most microalgae settle poorly making solids separation difficult [45–47]. Emerging strategies include membrane photobioreactors, photogranular processes, and immobilizations, which could herald more compact reactor footprints and less energy intensive solids separation compared to conventional ponds or photobioreactors [48,49].

The most practical use for microalgae in wastewater treatment may be as a tertiary step to decrease nitrogen to low discharge levels without organic carbon addition [50••]. Studies have shown that microalgae can reduce nitrogen to very low levels in constant and diurnal light [50••,51,52]. As tertiary treatment, the impairments of high capitol and operational costs would be minimized compared with a full scale microalgal treatment system. Further understanding the effects of fluctuating nutrient levels, naturally assembling communities, and ideal reactor configurations still needs to be elucidated, but tertiary microalgal treatment is a promising prospect.

Microbial electrochemical cells

Applying microbial electrochemical cells (MXCs) to wastewater treatment provide a unique opportunity for recovering energy, valuable products, and ammonia [53,54]. In MXCs, the oxidation and reduction reactions are separated by a membrane. At the cathode (where the reduction reaction occurs) the pH increases causing NH_4^+ to speciate to NH_3. This creates an NH_4^+ gradient across the membrane that pulls ammonia into the cathode chamber (if a cation exchange membrane is used), and because of the speciation to NH_3, ammonia is more easily separated [55]. Although many cathode reactions are possible, the two demonstrated for ammonia recovery are (i) oxygen reduction, which generates electricity and (ii) hydrogen evolution, which requires an applied voltage [54]. Ammonia recovery is tightly coupled to current, and, therefore, systems with applied energy show higher ammonia removal [54,56]. In the first scaled-up system (0.5 m^2), 31 ± 59% recovery was achieved over a 6 month period treating urine having gone through struvite precipitation with an applied voltage of 0.5 V [57••]. This resulted in an energy consumption of 4.9 ± 1.0 MJ kgN^{-1} [57••], which is far below the energy cost of fixation using Haber-Bosch (approximately 45 MJ kgN^{-1} [58]). This is unlikely to achieve the low effluent ammonia required for mainstream treatment; however, it shows great promise for nitrogen recovery from a side stream.

Conclusions

All biological nitrogen removal strategies have advantages and disadvantages. They are also not mutually exclusive, where processes more efficient at high nitrogen loads can be applied in sequence with processes more efficient at low nitrogen. A single stage nitrogen removal system will typically save capitol cost, but it may not be as robust or energy efficient as multiple stages. Looking forward, nitrogen removal needs to move away from the prohibitively energy intensive nitrification/denitrification and into next generation processes. We specifically highlight a system with mainstream partial nitritation/Anammox for energy efficient bulk removal, tertiary algal treatment for low effluent concentrations, MXC side stream treatment for energy efficient recovery, and biodrying for additional recovery and land application.

Conflict of interest statement

Nothing declared.

Acknowledgements

This work was supported by the Defense Advanced Research Projects Agency [Contract Number: HR0011-17-2-0064]. The authors would like to thank David Stensel and David Stahl for their critical reviews of the manuscript, and Bénédicte Rossi for assistance in creating the figures.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Madigan MT, Martinko JM, Stahl DA, Clark DP: *Brock Biology of Microorganisms*. Pearson Education, Inc.; 2012.

2. EPA US: *Reactive Nitrogen in the United States: An Analysis of Input, Flows, Consequences and Management Options*. 2011. doi: EPA-SAB-11-013.

3. Rockström J, Steffen W, Noone K, Persson A, Chapin FSI, Lambin EF, Lenton TM, Scheffer M, Folke C, Schellnhuber HJ *et al.*: **A safe operating space for humanity**. *Nature* 2009, **461**:472-475.

4. Swaminathan B, Sukalac KE: **Technology transfer and mitigation of climtae change: the fertilizer industry perspective**. In *Proceedings from IPCC Expert Meeting on Industrial Technology Development, Transfer And Diffusion*. 2005:140-154.

5. Metcalf, Eddy I: *Wastewater Engineering Treatment and Reuse*. The McGraw-Hill Companies, Inc.; 2003.

6. Sun S-P, Nacher CP, Merkey B, Zhou Q, Xia S-Q, Yang D-H, Sun J-H, Smets BF: **Effective biological nitrogen removal treatment processes for domestic wastewaters with low C/N ratios: a review**. *Environ Eng Sci* 2010, **27**:111-126.

7. Massara TM, Malamis S, Guisasola A, Baeza JA, Noutsopoulos C, Katsou E: **A review on nitrous oxide (N2O) emissions during biological nutrient removal from municipal wastewater and sludge reject water**. *Sci Total Environ* 2017, **596–597**:106-123.

8. Zanetti L, Frison N, Nota E, Tomizioli M, Bolzonella D, Fatone F: **Progress in real-time control applied to biological nitrogen removal from wastewater A short-review**. *DES* 2012, **286**:1-7.

9. Simion G, Antonio J, Guerrero J, Guisasola A, Mircea V, Paul S: **Development and economic assessment of different WWTP control strategies for optimal simultaneous removal of carbon, nitrogen and phosphorus**. *Comp Chem Eng* 2013, **53**:164-177.

10. Peng Y, Zhu G: **Biological nitrogen removal with nitrification and denitrification via nitrite pathway**. *Appl Microbiol Biotechnol* 2006, **73**:15-26.

11. Gu J, Yang Q, Liu Y: **A novel strategy towards sustainable and stable nitritation-denitritation in an A-B process for mainstream municipal wastewater treatment**. *Chemosphere* 2018, **193**:921-927.

12. Erdirencelebi D, Koyuncu S: **Operational strategies and environmental conditions inducing aerobic denitritation in short-cut biological nitrogen removal at side-line treatment**. *J Environ Sci Heal Part A* 2017, **52**:607-615.

13. Sun Y, Guan Y, Pan M, Zhan X, Hu Z, Wu G: **Enhanced biological nitrogen removal and N2O emission characteristics of the intermittent aeration activated sludge process**. *Rev Environ Sci Biotechnol* 2017, **16**:761-780.

14. Kornaros M, Dokianakis SN, Lyberatos G: **Partial nitrification/denitrification can be attributed to the slow response of nitrite oxidizing bacteria to periodic anoxic disturbances**. *Environ Sci Technol* 2010, **44**:7245-7253.

15. Blackburne R, Yuan Z, Keller J: **Partial nitrification to nitrite using low dissolved oxygen concentration as the main selection factor**. *Biodegradation* 2008, **19**:303-312.

16. Kim JM, Lee HJ, Lee DS, Jeon CO: **Characterization of the denitrification-associated phosphorus uptake properties of "Candidatus Accumulibacter phosphatis" clades in sludge subjected to enhanced biological phosphorus removal**. *Appl Environ Microbiol* 2013, **79**:1969-1979.

17. • Pronk M, Kreuk MK, De, Bruin B, De, Kamminga P, Kleerebezem R, Loosdrecht MCM, Van: **Full scale performance of the aerobic granular sludge process for sewage treatment**. *Water Res* 2015, **84**:207-217.

Full-scale demonstration of aerobic granular sludge exhibiting dPAO activity.

18. Kartal B, Kuenen JG, van Loosdrecht MCM: **Sewage treatment with Anammox**. *Science (80-)* 2010, **328**:702-703.

19. Ma B, Wang S, Cao S, Miao Y, Jia F, Du R, Peng Y: **Biological nitrogen removal from sewage via Anammox: recent advances**. *Bioresour Technol* 2016, **200**:981-990.

20. •• Cao Y, van Loosdrecht MCM, Daigger GT: **Mainstream partial nitritation–anammox in municipal wastewater treatment: status, bottlenecks, and further studies**. *Appl Microbiol Biotechnol* 2017, **101**:1365-1383.

Review of Anammox use for mainstream wastewater treatment

21. Lackner S, Gilbert EM, Vlaeminck SE, Joss A, Horn H, van Loosdrecht MCM: **Full-scale partial nitritation/Anammox experiences - an application survey**. *Water Res* 2014, **55**:292-303.

22. Hellinga C, Schellen A, Mulder J, van Loosdrecht M, Heijnen J: **The SHARON process: an innovative method for nitrogen removal from ammonium-rich waste water**. *Water Sci Technol* 1998, **37**:135-142.

23. Laureni M, Falås P, Robin O, Wick A, Weissbrodt DG, Nielsen JL, Ternes TA, Morgenroth E, Joss A: **Mainstream partial nitritation and anammox: long-term process stability and effluent quality at low temperatures**. *Water Res* 2016, **101**:628-639.

24. Jiang H, Liu G, Ma Y, Xu X, Chen J, Yang Y, Liu X, Wang H: **A pilot-scale study on start-up and stable operation of mainstream partial nitrification-anammox biofilter process based on online pH-DO linkage control**. *Chem Eng J* 2018, **350**:1035-1042.

25. •• Wett B, Podmirseg SM, Gomez-Brandon M, Hell M, Nyhuis G, Bott C, Murthy S: **Expanding DEMON sidestream deammonification technology towards mainstream application**. *Water Environ Res* 2015, **87**:2084-2089.
Description of Strass full-scale mainstream Anammox plant.

26. •• Cao Y, Kwok BH, Yong WH, Chua SC, Wah YL, Ghani YA: **Mainstream partial nitritation–anammox nitrogen removal in the largest full-scale activated sludge process in singapore: process analysis**. *WEF/IWA Nutrient Removal and Recovery 2013: Trends in Resource Recovery and Use* 2013. 23.
Description of Chagi WRP full-scale mainstream Anammox plant.

27. Yeshi C, Kwok BH, Zhou Y, Liu Y, He J, Chua SC, Wah YL, Ghani Y: **Mainstream partial nitritation/Anammox nitrogen removal process in the largest water reclamation plant in Singapore**. *J Beijing Univ Technol* 2015, **41**:1441-1454.

28. Daims H, Elena V, Palatinszky M, Vierheilig J, Bulaev A, Kirkegaard RH, von Bergen M, Rattei T, Bendinger B, Nielsen PH *et al.*: **Complete nitrification by Nitrospira bacteria**. *Nature* 2015, **528**:504-509.

29. Dimitri Kits K, Sedlacek CJ, Lebedeva EV, Han P, Bulaev A, Pjevac P, Daebeler A, Romano S, Albertsen M, Stein LY *et al.*: **Kinetic analysis of a complete nitrifier reveals an oligotrophic lifestyle**. *Nature* 2017, **549**:269-272.

30. Limpiyakorn T, Fürhacker M, Haberl R, Chodanon T, Srithep P, Sonthiphand P: **AmoA-encoding archaea in wastewater treatment plants: a review**. *Appl Microbiol Biotechnol* 2013, **97**:1425-1439.

31. van Kessel MAHJ, Stultiens K, Slegers MFW, Cruz SG, Jetten MSM, Kartal B, Op den Camp H: **Current perspectives on the application of N-damo and anammox in wastewater treatment**. *Curr Opin Biotechnol* 2018, **50**:222-227.

32. Winkler MH, Ettwig KF, Vannecke TPW, Stultiens K, Bogdan A, Kartal B, Volcke EIP: **Modelling simultaneous anaerobic methane and ammonium removal in a granular sludge reactor**. *Water Res* 2015, **73**:323-331.

33. Li X, Huang Y, Liu H, Wu C, Bi W, Yuan Y, Liu X: **Simultaneous Fe (III) reduction and ammonia oxidation process in Anammox sludge**. *J Environ Sci* 2017, **64**:42-50.

34. Wang J, Lu H, Chen G, Lau GN, Tsang WL, Loosdrecht MCM, Van: **A novel sulfate reduction, autotrophic denitrification, nitrification integrated (SANI) process for saline wastewater treatment**. *Water Res* 2009, **43**:2363-2372.

35. • Lema JM, Suarez S: **This work reviews strategies for wastewater treatment and resource (like nitrogen) recovery.**. *Innovative Wastewater Treatment & Resource Recovery Technologies*. IWA Publishing; 2017.

36. Batstone DJ, Hülsen T, Mehta CM, Keller J: **Platforms for energy and nutrient recovery from domestic wastewater: a review**. *Chemosphere* 2015, **140**:2-11.

37. • Winkler M-K, Bennenbroek M, Horstink F, van Loosdrecht M, Van de Pol G-J: **The biodrying concept: an innovative technology creating energy from sewage sludge**. *Bioresour Technol* 2013, **147**:124-129.
This work describes a full-scale low energy sludge stabilization/drying method using a combination of composting and forced aeration.

38. Ghosh S, Dairkee UK, Chowdhury R, Bhattacharya P: **Hydrogen from food processing wastes via photofermentation using purple non-sulfur bacteria (PNSB) – a review**. *Energy Convers Manag* 2017, **141**:299-314.

39. Hülsen T, Batstone DJ, Keller J: **Phototrophic bacteria for nutrient recovery from domestic wastewater**. *Water Res* 2014, **50**:18-26.

40. Wen S, Liu H, He H, Luo L, Li X, Zeng G, Zhou Z, Lou W, Yang C: **Treatment of anaerobically digested swine wastewater by Rhodobacter blasticus and Rhodobacter capsulatus**. *Bioresour Technol* 2016, **222**:33-38.

41. • Hülsen T, Barry EM, Lu Y, Puyol D, Keller J, Batstone DJ: **Domestic wastewater treatment with purple phototrophic bacteria using a novel continuous photo anaerobic membrane bioreactor**. *Water Res* 2016, **100**:486-495.
This work demonstrates nitrogen assimilation with purple-nonsulfur bacteria from wastewater.

42. Lu H, Han T, Zhang G, Ma S, Zhang Y, Li B, Cao W: **Natural light-micro aerobic condition for PSB wastewater treatment: a flexible, simple, and effective resource recovery wastewater treatment process**. *Environ Technol (United Kingdom)* 2018, **39**:74-82.

43. Gonçalves AL, Pires JCM, Simões M: **A review on the use of microalgal consortia for wastewater treatment**. *Algal Res* 2017, **24**:403-415.

44. Cuellar-Bermudez SP, Aleman-Nava GS, Chandra R, Garcia-Perez JS, Contreras-Angulo JR, Markou G, Muylaert K, Rittmann BE, Parra-Saldivar R: **Nutrients utilization and contaminants removal. A review of two approaches of algae and cyanobacteria in wastewater**. *Algal Res* 2017, **24**:438-449.

45. Cai T, Park SY, Li Y: **Nutrient recovery from wastewater streams by microalgae: status and prospects**. *Renew Sustain Energy Rev* 2013, **19**:360-369.

46. Barros AI, Gonçalves AL, Simões M, Pires JCM: **Harvesting techniques applied to microalgae: a review**. *Renew Sustain Energy Rev* 2015, **41**:1489-1500.

47. Uduman N, Qi Y, Danquah MK, Forde GM, Hoadley A: **Dewatering of microalgal cultures: a major bottleneck to algae-based fuels**. *J Renew Sustain Energy* 2010, **2**:1-15.

48. Abouhend AS, McNair A, Kuo-Dahab WC, Watt C, Butler CS, Milferstedt K, Hamelin J, Seo J, Gikonyo GJ, El-Moselhy KM *et al.*: **The oxygenic photogranule process for aeration-free wastewater treatment**. *Environ Sci Technol* 2018, **52**:3503-3511.

49. Luo Y, Le-Clech P, Henderson RK: **Simultaneous microalgae cultivation and wastewater treatment in submerged membrane photobioreactors: a review**. *Algal Res* 2017, **24**:425-437.

50. •• Sturm BSM, Lamer SL: **An energy evaluation of coupling nutrient removal from wastewater with algal biomass production**. *Appl Energy* 2011, **88**:3499-3506.
This work demonstrates nitrogen recovery in full-scale algae system treating secondary effluent.

51. Whitton R, Le Mével A, Pidou M, Ometto F, Villa R, Jefferson B: **Influence of microalgal N and P composition on wastewater nutrient remediation**. *Water Res* 2016, **91**:371-378.

52. Gardner-Dale DA, Bradley IM, Guest JS: **Influence of solids residence time and carbon storage on nitrogen and phosphorus recovery by microalgae across diel cycles**. *Water Res* 2017, **121**:231-239.

53. Logan BE, Rabaey K: **Convesion of waste into bioelectricity and chemical by using microbial electrochemical technologies**. *Science* 2013, **337**:686-690.

54. Kuntke P, Sleutels THJA, Rodríguez Arredondo M, Georg S, Barbosa SG, ter Heijne A, Hamelers HVM, Buisman CJN: **(Bio) electrochemical ammonia recovery: progress and perspectives**. *Appl Microbiol Biotechnol* 2018, **102**:3865-3878.

55. Jung RK, Zuo Y, Regan JM, Logan BE: **Analysis of ammonia loss mechanisms in microbial fuel cells treating animal wastewater**. *Biotechnol Bioeng* 2008, **99**:1120-1127.

56. Arredondo MR, Kuntke P, ter Heijne A, Hamelers HVM, Buisman CJN: **Load ratio determines the ammonia recovery and energy input of an electrochemical system**. *Water Res* 2017, **111**:330-337.

57. •• Zamora P, Georgieva T, Ter Heijne A, Sleutels THJA, Jeremiasse AW, Saakes M, Buisman CJN, Kuntke P: **Ammonia recovery from urine in a scaled-up microbial electrolysis cell**. *J Power Sources* 2017, **356**:491-499.
This work demonstrated ammonia recovery in a scaled-up MXC system treating urine.

58. Maurer M, Schwegler P, Larsen TA: **Nutrients in urine: energetic aspects of removal and recovery**. *Water Sci Technol* 2003, **48**:37-46.