Towards a Circular Nitrogen Bioeconomy: Integrating Nitrogen Bioconcentration, Separations, and High-Value Products for Nitrogen Recovery

Edward Apraku[a*], McKenna Farmer[b*], Chayse Lavallais[c], Danna A. Soriano[d], Justin Notestein[c], Keith Tyo[c], Jennifer Dunn[c], William A. Tarpeh[a,d]**, George F. Wells[b]**

[a]Department of Civil and Environmental Engineering, Stanford University, Stanford, CA, 94305, United States

[b]Department of Civil and Environmental Engineering, Northwestern University, Evanston, IL 60208, United States

[c]Department of Chemical and Biological Engineering, Northwestern University, Evanston, IL 60208, United States

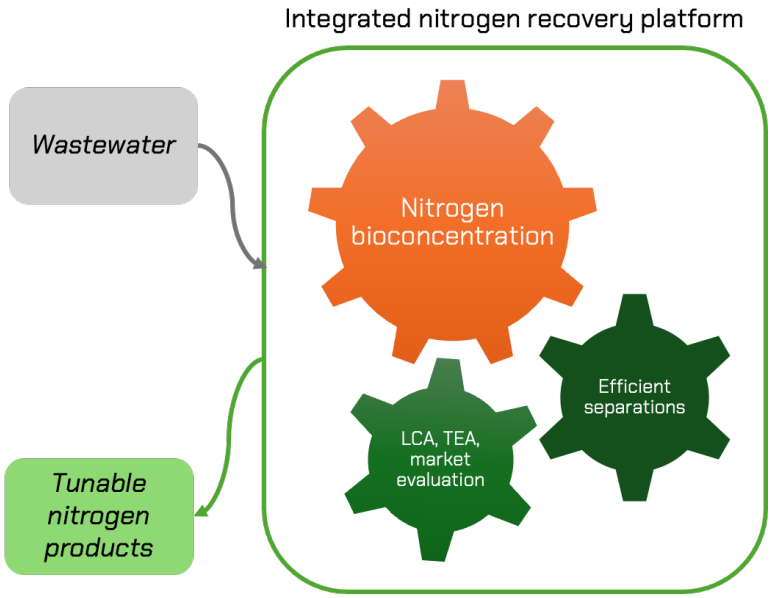
[d]Department of Chemical Engineering, Stanford University, Stanford, CA, 94305, United States

*Edward Apraku and McKenna Farmer contributed equally to this work

**Corresponding author, Email: george.wells@northwestern.edu Address: 2145 Sheridan Road, Evanston, IL 60208, United States

**Corresponding author, Email: wtarpeh@stanford.edu Address: 443 Via Ortega, Room 387, Stanford, CA, 94305, United States

Graphical Abstract



Abstract

Recovering nitrogen (N) from wastewater is a potential avenue to reduce reliance on energy-intensive synthetic nitrogen fixation via Haber-Bosch and subsequent treatment of N-laden wastewaters through nitrification-denitrification. However, many technical and economic factors hinder widespread application of N recovery, particularly low N concentrations in municipal wastewater, paucity of high-efficiency separations technologies compatible with biological treatment, and suitable products and markets for recovered N. In this perspective, we contextualize the challenges of N recovery today, propose integrated biological and physicochemical technologies to improve selective and tunable N recovery, and propose an expanded product portfolio for recovered N products beyond fertilizers. We highlight cyanophycin, an N-rich biopolymer produced by a diverse range of bacteria, as a potential target for N bioconcentration and downstream recovery from municipal wastewater. This perspective emphasizes the equal importance of integrated biological systems, physicochemical separations, and market assessment in advancing nitrogen recovery from wastewater.

Introduction

Nitrogen (N) transformation through the Haber-Bosch process propelled the Green Revolution through production of nitrogen fertilizers but created ongoing environmental challenges from fertilizer runoff and N-rich wastewaters. N-rich waste streams are produced in enormous quantities in both urban systems (municipal and industrial wastewater, food waste) and agriculture (manure, fertilizer runoff) settings (a combined 10^9-10^{16} L/year).[1] In current practice, N-rich wastewaters undergo energy intensive treatment that results in dissipation of N into N_2 or N_2O, a potent greenhouse gas (GHG) (**Figure 1**). Shifting the paradigm of wastewater treatment plants (WWTPs) from nitrogen removal to nitrogen recovery can reduce dependence on Haber-Bosch, generate N-rich products, and promote a circular nitrogen economy.

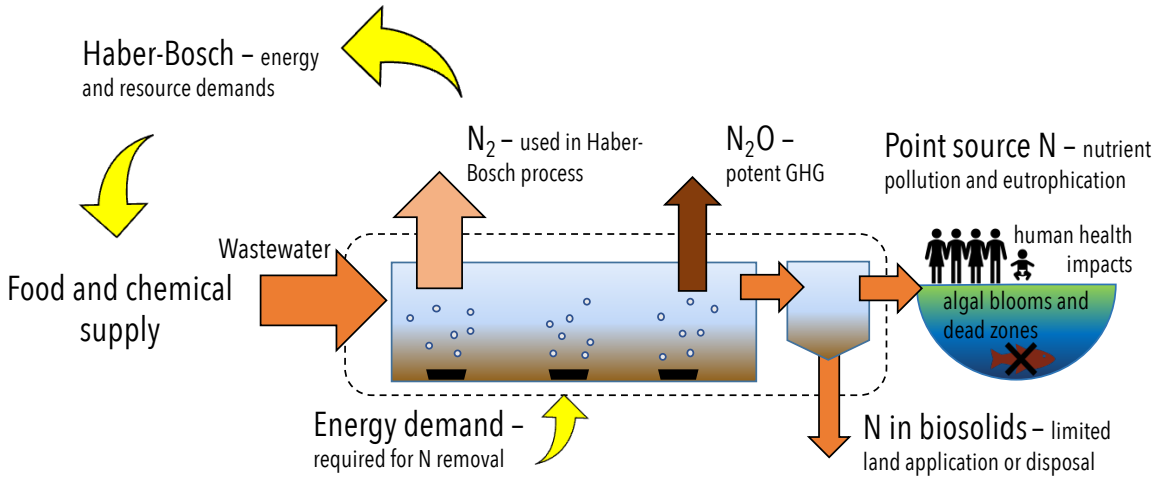


Figure 1. Linear model of N management from wastewaters.

Recent efforts have aimed to transition from removing N to upcycling N from wastewaters as a concentrated fertilizer that offsets fertilizer made by the Haber-Bosch process with its intensive energy use and CO_2 emissions.[2] However, the low price of commodity fertilizers[3] coupled to high cost and energy use of current fertilizer recovery approaches pose crucial barriers to economical N recovery.[4] Biological and physicochemical unit processes are widely used for N removal in wastewater treatment but are separated due to differences in operating conditions and the independent optimization of processes.[5] There is a comparative lack of research on integrated biological-physicochemical processes for N recovery, highlighting the need for low-energy, cost-effective approaches for sustainable N recovery from wastewater and conversion into valuable products. In this perspective, we identify the context and challenges of N recovery, propose integrated biological and physicochemical technologies to improve selective N recovery, and expand the product portfolio for N recovery outside of fertilizers.

N recovery from wastewater: context, challenges, and roadmap

N management practices at WWTPs are largely focused on N removal rather than recovery (Figure 1). Standard practice for N removal, biological nitrification-denitrification (NDN), converts influent ammonia into N_2 gas. NDN can facilitate N recovery through land application of biosolids; however, at least 80% of influent N is dissipated rather than recovered in biosolids.[6] Successful NDN opposes other

recovery goals (i.e. biogas production from anaerobic digestion) because reducing equivalents for denitrification come from organic C in influent wastewater or from an external source such as methanol. Furthermore, NDN produces N_2O as an unintended byproduct. N_2O has 300 times the global warming potential of CO_2, and wastewater treatment alone accounts for 5% of all N_2O emissions in the US.[7] NDN also requires aeration accounting for well over 50% of electricity use at WWTPs.[8] Newer biotechnologies such as anammox processes can significantly reduce electricity and C demands but still target N dissipation rather than recovery.

Advancing beyond NDN will facilitate greater N recovery, but an unavoidable challenge to N recovery will still remain: the relatively dilute concentrations of N in municipal wastewater, typically between ~20-40 mg N/L.[8] Electrochemical and membrane separations can achieve efficient and selective N recovery with high strength, low volume streams such as anaerobic digester centrate and source-separated urine, with typical concentrations above 1000 mg N/L.[9] New biotechnologies show great promise to bridge the gap between existing systems and emerging N recovery technologies through the Partition-Release-Recovery (PRR) approach: sequestering dilute mainstream N into concentrated sidestreams that can be transformed into a variety of endproducts.[10] Biological approaches to N concentration offer advantages over physicochemical concentration methods, including high affinity to low substrate concentrations and suitability in ambient temperature and pressure. The PRR approach is already employed in P and C recovery biotechnologies, particularly in enhanced biological phosphorus removal (EBPR) and high rate activated sludge (HRAS), which can serve as roadmaps for N recovery.

P recovery from municipal wastewater is enabled by phosphorus accumulating organisms (PAOs), which convert soluble phosphate to intracellular polyphosphate under cyclic redox conditions (partition). The P-rich biomass is then diverted to the sidestream. Under anaerobic conditions, soluble P can be released from PAOs back into solution (release), and the P-rich liquid solution can be used as a feedstock for struvite ($MgNH_4PO_4 \cdot 6H_2O$) production (recovery) with end products including fertilizer and flame retardant.[8,11] P partitioning by PAOs is standard practice for removing P from wastewater. Furthermore, P release under controlled conditions mitigates nuisance struvite build-up in pipes and equipment. For C recovery, the

HRAS process follows a similar PRR framework; fast-growing microbes incorporate a majority of influent carbon into biomass (partition) which is directed to anaerobic digesters for hydrolysis (release) and conversion to methane for energy and heat generation (recovery).[12] C recovery via biogas generation works in tandem with sludge stabilization, the process of reducing sludge volume and minimizing odor and pathogens.

These examples of PRR pathways for P and C recovery are mature technologies and are far more developed than N recovery, in part due to their compatibility and co-benefits with other treatment and operations goals. Given that wastewater practitioners prioritize cost, effluent concentration, and removal efficiencies in their technology decisions[13], N bioconcentration technologies should be developed with these priorities at the forefront.

In Pursuit of Nitrogen Accumulating Organisms

N bioconcentration may incidentally occur in existing processes but is not the primary goal. HRAS can facilitate partial N recovery through assimilation or adsorption to biomass. However, N capture in HRAS is associated with longer solids retention time (SRT), which opposes C recovery goals; HRAS may capture up to 50% of influent N at SRT between 1.5-3 days, but methane production from HRAS biomass is often optimized for SRT below 1 day.[14] Furthermore, HRAS systems typically require additional processes to manage downstream N and P to meet effluent discharge limits. Algae and mixed algae-bacteria systems can also assimilate N, but similar to HRAS, maximizing N recovery in algal systems is at odds with other resource recovery goals, namely maximizing total algal biomass yield.[15] Furthermore, algal systems face significant challenges in scale-up for optimal light exposure, efficient biomass separations, and product extraction[16].

An emerging method for N bioconcentration is intracellular biopolymer accumulation in activated sludge, analogous to P accumulation by PAOs. A leading but underexplored candidate biopolymer is cyanophycin, which consists of an aspartate backbone and arginine or lysine residues. Cyanophycin has a relatively high N content (approximately 10:5 C/N ratio on a molar basis) and is selectively soluble as a

function of pH.[17] A diverse range of phototrophs, mixotrophs, and heterotrophs have cyanophycin accumulation and degradation metabolisms.[18,19] Surprisingly, cyanophycin metabolism genes have recently been found in important genomes or metagenome assembled genomes of a broad range of microbes commonly present in existing biological nutrient removal processes, including PAOs *Ca.* Accumulibacter, *Tetrasphaera*, and *Dechloromonas*[20,21] as well as nitrifiers *Nitrosomonas* and *Nitrosospira*[19], and gene expression of cyanophycin synthetase has also been demonstrated in *Ca.* Accumulibacter and *Tetrasphaera.*[20]

Moreover, cyanophycin has been measured at 124 mg/g mixed liquor suspended solids in a mixed algal-bacterial system designed for cyanophycin production (achieving ~50% total N removal and ~90% total P removal)[22], and over 100 mg/g dry weight in full-scale activated sludge facilities designed for conventional N and P removal (achieving <10 mg/L total N and <0.5 mg/L total P).[23] Both of these systems have cyanophycin yields comparable to 10-20% cell dry weight from a cyanobacteria *Synechocystis* sp. PCC6803.[24] These early studies highlight the unexpected diversity and prevalence of taxa putatively capable of cyanophycin metabolisms, and suggest that N bioconcentration (partitioning) via cyanophycin can occur in tandem with other treatment goals. While additional research is needed, these studies also point to cyanophycin as a possible basis for a long missing "nitrogen accumulating organism" that may provide the foundation for N recovery in a PRR framework—potentially using existing process infrastructure. Cyanophycin accumulation and production in existing systems should be investigated in tandem with efficient separations processes to rapidly advance this nascent method for N bioconcentration and recovery from wastewater.

Exploiting Synergies between Biological and Physicochemical/Electrochemical Techniques to Advance N Recovery

Increased interest in N recovery from waste streams has advanced physicochemical techniques that separate N at various stages of wastewater treatment. A novel PRR approach to N recovery will require product separation after bioconcentration and can integrate existing physicochemical techniques.

Established physicochemical techniques for N recovery include air stripping, membrane filtration, adsorption, and struvite precipitation.[25] While promising, these approaches alone can be energy-intensive, costly, and often require pretreatment or highly concentrated waste streams.[4] However, combining biological and physicochemical techniques offers attractive options to circumvent the N recovery challenges faced by both techniques. This integrated biological and physicochemical approach has proven useful for emerging technologies for the goal of N removal. For example, the low ammonium concentration and slow growth of anammox bacteria limit mainstream application of anammox for N removal.[26] However, when incorporated with zeolite-coated ion exchange (IX) membranes, the growth of anammox bacteria improves due to cation exchange and ammonium adsorption, resulting in an enhanced average total nitrogen removal of 73%.[27] Similarly, several approaches to combining biological and polymeric membrane filtration materials have shown promise to advance energy-efficient N removal, increase oxygen utilization efficiency, and overcome competing growth with heterotrophs in environments with abundant biodegradable C. Of these technologies, the membrane aerated biofilm reactor (MABR) stands out due to its high effluent quality, carbon processing efficiency, and high nitrification rate (1-3 g N/m^2-d).[28] Further exploration into bio-based polymeric materials can improve selectivity for target ions, decrease dependence on fossil fuels, and advance a bioeconomy by utilizing biomass.[29]

We suggest that a similar approach to exploiting synergies between biological (e.g. cyanophcyin accumulation) and physicochemical technologies is needed to advance the goal of N recovery, rather than N removal. In the case of cyanophycin, selective large-scale recovery from microbial biomass after bioconcentration and sidestream diversion may be limited by laborious pH cycling that requires multiple chemical inputs.[30] Improving the intrinsic N-selectivity of physicochemical techniques can also work in tandem with P removal to advance nutrient recovery technologies. For example, simultaneous recovery of NH_4^+ and PO_4^{3-} from wastewater via struvite precipitation often follows diversion of PAO rich biomass to the sidestream in biological phosphorus removal processes[31], but faces limitations in terms of N recovery from dilute wastewaters. Struvite precipitation separates equimolar NH_4^+ and PO_4^{3-}, but the molar ratio of N:P often exceeds 5 in municipal wastewater, leading to reduced N removal efficiency.[32] Integrating PAOs

147 or cyanophycin with ion exchange materials could enhance the efficiency for nutrient recovery by
148 leveraging the specific binding affinities of these biomolecules for phosphorus and nitrogen compounds.

149 Alternatively, electrochemical techniques can utilize renewable energy to valorize solutes through
150 electrocatalysis, stoichiometric electrochemical conversions, and electrochemical separations.[1] Broader
151 electrochemical and electrokinetic techniques have successfully removed both ammonia and ammonium
152 from fertilizer effluent, municipal wastewater, and food waste digestate with upwards of 93% recovery
153 efficiency.[33] However, due to the instability of reactive inorganic nitrogen intermediates, further exploration
154 into electrocatalytic separations is required to expand the N portfolio. Microbial electrochemical systems
155 (MECs) and biocatalysts are technologies that integrate biological and electrochemical techniques into a
156 single unit. In the case of MECs, reducing equivalents from influent organics are harnessed to produce H_2
157 and CH_4.[34,35] More broadly, microbial enzymes can be leveraged to facilitate redox reactions, enabling the
158 production of a wide-range of products (e.g., pharmaceuticals, fine chemicals, polymers).[36] Future efforts
159 to integrate a PRR approach with cyanophycin can incorporate electrochemical techniques to improve N
160 separations. For example, the cyclical acid and base addition that may limit large-scale cyanophycin
161 production can be circumvented with electrochemical water splitting, which generates acid via water
162 oxidation and base via water reduction.

163

164 **Expanding the Nitrogen Product Portfolio**

165 Recovered nitrogen has a variety of potential end uses (**Table 1**), many of which are significantly
166 higher value than nitrogenous fertilizers. For example, recovered nitrogen in the form of ammonia can be
167 used to produce nitrogen-containing chemical compounds such as alkyl amines, which can be further
168 transformed to produce rubber, pesticides, or as a solvent for carbon capture[37]. Ammonia can also be used
169 to produce nitric acid, nitroglycerin, and specialty organic compounds[37]. Cyanophycin contains the amino
170 acids arginine, lysine, and aspartic acid, which are found in traditional protein sources for animal feed
171 formulations such as soybean meal and fish meal.[38] Replacing those protein sources with cyanophycin could
172 provide additional environmental benefits such as reduced N_2O emissions from crop production, reduced

agricultural land requirements for crops, and improved efficiencies for nitrogen feed to food chains. Alternatively, these amino acids can be extracted from the cyanophycin and directly used in multiple industries such as food, cosmetics, and pharmaceuticals.[39,40] These amino acids could also be further processed to produce various high-value chemical products such as acrylonitrile, acrylamide, and 1,4-diaminobutane[39]. Having several potential end products can allow WWTPs to optimize nitrogen recovery and processing to meet dynamic market demands.

Table 1. Potential target products for N recovery, markets, and prices

Product	**Market $B. (yr)**	**Compound Average Growth Rate % (yrs)**	**Market Price ($/metric ton)**	**Use(s)**
Fertilizer [41]	57.1 (2021)	5.4 (2020-2030)	360-570	Agriculture
Nitric Acid [42]	29.8 (2022)	2.4 (2023-2030)	300-400	Dye, explosives
Amines[43]	15.6 (2021)	4.8 (2022-2030)	2900-3500	Surfactants, pesticides, carbon capture
Animal Feed [38,44]	570.7 (2022)	4.6 (2023-2030)	150-370	Livestock, aquaculture
Amino Acids [45]	27.2 (2023)	8.5 (2024-2030)	1400-100,000+	Food flavoring, pharmaceuticals, cosmetics
Acrylonitrile [46]	11.2 (2022)	2.2 (2023-2030)	1900-2400	Fibers, ABS
Acrylamide[47]	3.6 (2023)	3.5 (2024-2032)	1300-2200	Wound care, agriculture (slow release fertilizers)
Nylon[48,49]	34.4 (2023)	6.5 (2024-2030)	2000-3200	Auto-mobile, engineering plastics
1,4-diaminobutane[50]	0.1 (2018)	4.1 (2018-2025)	-	Nylon 4,6

Conclusion

Advancing wastewater N recovery will require research and development progress on multiple fronts, including innovation in N bioconcentration strategies, efficient and tunable N separations, and N

product development. We propose that established C and P recovery methods in a PRR framework can serve as inspiration for N recovery, and we chart opportunities for tandem recovery with aqueous N species. We highlight cyanophycin as a novel target biopolymer for N bioconcentration, N-rich sidestream diversion, and recovery in this PRR framework. We anticipate that integrated research in biological processes and physicochemical separations will open opportunities for targets beyond cyanophycin that are tunable to desired product qualities. Moreover, we postulate that expanding the product portfolio for N recovery is needed to allow WWTPs to integrate technologies that enable them to produce various high-value products and update their product slates based on changes in the market. This economic viability could be achieved by WWTPs collaborating with local stakeholders such as farmers or chemical facilities and developing mutually beneficial partnerships. These partnerships will be crucial as novel biological pathways are identified and incorporated to expand the N portfolio. Combining established physicochemical and emerging electrochemical techniques can further improve the selectivity and recovery of N-rich bioproducts (like cyanophycin) as well as inorganic nitrogen species. Ultimately, N recovery can offset fossil energy and CO_2 emissions associated with the Haber-Bosch process while promoting a circular water economy that reimagines wastewater pollutants as chemical commodities.

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