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Nitrogen removal with energy recovery through N_2O decomposition†

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A new process for the removal of nitrogen from wastewater is introduced. The process involves three steps: (1) partial nitrification of NH_4^+ to NO_2^-; (2) partial anoxic reduction of NO_2^- to N_2O; and (3) N_2O conversion to N_2 with energy recovery by either catalytic decomposition to N_2 and O_2 or use of N_2O to oxidize biogas CH_4. Steps 1 and 3 have been previously established at full-scale. Accordingly, bench-scale experiments focused on step 2. Two strategies were evaluated and found to be effective: in the first, Fe(II) was used to abiotically reduce NO_2^- to N_2O; in the second, COD stored as polyhydroxybutyrate (PHB) was used as the electron donor for partial heterotrophic reduction of NO_2^- to N_2O. For abiotic reduction with Fe(II), the efficiency of conversion of NO_2^- to N_2O was over 90% with 98% nitrogen removal from water. For partial heterotrophic denitrification, different selection conditions were imposed on acetate- and nitrite-fed communities initially derived from waste activated sludge. No N_2O was detected when acetate and nitrite were supplied continuously, but N_2O was produced when acetate and nitrite were added as pulses. N_2O conversion efficiency was dependent upon the method of addition of acetate and nitrite. When acetate and nitrite were added together (coupled feeding), the N_2O conversion efficiency was 9–12%, but when acetate and nitrite additions were decoupled, the N_2O conversion efficiency was 60–65%. Decoupled substrate addition selected for a microbial community that accumulated polyhydroxybutyrate (PHB) during an anaerobic period after acetate addition then consumed PHB and reduced NO_2^- during the subsequent anoxic period. The biological N removal efficiency from the water was 98% over more than 200 cycles. This indicates that decoupled operation can sustain significant long-term N_2O production. Compared to conventional nitrogen removal, the three-step process, referred to here as Coupled Aerobic–anoxic Nitrous Decomposition Operation (CANDO), is expected to decrease oxygen requirements, decrease biomass production, increase organic matter available for recovery as biogas methane, and enable energy recovery from nitrogen, but pilot-scale studies are needed.

Broader context

The release of reactive forms of nitrogen is a major environmental threat causing hypoxia and eutrophic zones in water bodies. Globally, rising energy costs and increasingly stringent discharge regulation are major drivers for efficient wastewater treatment processes that lower costs and increase recoverable energy from waste. While many processes recover energy from carbon waste as CH_4, none recovers energy from waste nitrogen. This work introduces a new wastewater treatment process that removes and recovers energy from nitrogen waste by exploiting the thermodynamic properties of N_2O for energy recovery. The proposed process, referred to here as *C*oupled *A*erobic–anoxic *N*itrous *D*ecomposition *O*peration (CANDO), involves three steps: (1) partial aerobic nitrification of NH_4^+ to NO_2^-, (2) partial anoxic denitrification of NO_2^- to N_2O, and (3) N_2O conversion to N_2 with energy recovery *via* catalytic decomposition of N_2O or use of N_2O as an oxidant in CH_4 combustion. If successfully scaled-up, this process has the potential to lower aeration and biosolid production (the two major operational costs), increase CH_4 recovery from "freed" organic matter, and introduces a new renewable energy source from CH_4 combustion with N_2O.

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Introduction

A major goal of biological wastewater treatment is removal of oxygen-depleting forms of carbon and nitrogen from water. These substances are routinely quantified in terms of the mass of oxygen required for complete oxidation. Organic compounds are collectively quantified as Chemical Oxygen Demand (COD); the mass of oxygen required for stoichiometric oxidation of the organics to CO_2. Reduced, oxygen-depleting forms of nitrogen

(ammonia, organic nitrogen, and nitrite) are likewise quantified as Nitrogenous Oxygen Demand (NOD); the mass of oxygen needed for their stoichiometric oxidation to nitrate. Theoretical Oxygen Demand (ThOD) is the sum of COD and NOD. Many processes efficiently remove ThOD, but these processes differ dramatically in production and consumption of energy and in production of biosolids. The use of aerobic processes to remove biodegradable COD (*i.e.*, BOD), for example, requires energy-intensive O_2 delivery and generates large quantities of biomass, but anaerobic processes remove COD as CH_4 for energy production and generate comparatively little biomass.

The situation with NOD is more complex. Conventionally, NOD is removed by nitrification, a two-step microbial process involving (1) oxidation of NH_4^+ to NO_2^- and (2) oxidation of NO_2^- to NO_3^-. Each step requires energy for O_2 delivery, and the NO_3^- produced can still fertilize a sensitive water body or pose human health risks. Removal of NO_3^- is typically accomplished through heterotrophic denitrification, a four-step process that can be fully intracellular within a single type of microorganism or partially intracellular, with different organisms mediating different steps. The steps of complete denitrification are: (1) NO_3^- reduction to NO_2^-, (2) NO_2^- reduction to NO, (3) NO reduction to N_2O, and (4) N_2O reduction to N_2. For heterotrophic denitrification, each step requires reducing power obtained from the oxidation of COD that could otherwise be recovered as CH_4. If reduction to N_2 is incomplete, N_2O, a potent greenhouse gas (310 times more powerful than CO_2) can be released to the atmosphere.[1,2] Such concerns have motivated research to quantify N_2O emissions from soil, seawater, and wastewater treatment systems, and to elucidate the underlying mechanisms.[2–4]

Over the past two decades, European researchers have vastly improved treatment options for nitrogen removal using ecological "short-circuits" that avoid NO_3^- production. Examples include SHARON,[5–8] OLAND,[7,9–11] and CANON[7,12–17] with Anammox. In these processes, NH_4^+ is only partially oxidized to NO_2^-, decreasing O_2 requirements, and NO_2^- is reduced to N_2 in three steps, rather than four, conserving COD for energy recovery as CH_4. Especially noteworthy was the discovery of anaerobic ammonium oxidation (Anammox) bacteria[18] and their successful deployment in full-scale wastewater treatment facilities.[12,19] Anammox bacteria obtain reducing equivalents for reduction of NO_2^- to N_2 from the oxidation of NH_4^+ rather than COD, with hydrazine (N_2H_4) as a critical intermediate. By avoiding the use of COD as the source of reducing equivalents, more COD is available for recovery as CH_4. Treatment of anaerobic digester centrate with Anammox has the potential to decrease energy consumption of a full-scale plant by >50% and increase CH_4 production by up to 25%.[13]

Many processes recover energy from waste COD as methane, but none recovers energy from NOD. In this article, we introduce a new nitrogen removal strategy that exploits the thermodynamic properties of N_2O for energy recovery. The proposed process, referred to here as *C*oupled *A*erobic–anoxic *N*itrous *D*ecomposition *O*peration (CANDO),[20,21] involves three steps: (1) partial aerobic nitrification of NH_4^+ to NO_2^-, (2) partial anoxic denitrification of NO_2^- to N_2O, and (3) N_2O conversion to N_2 with energy recovery *via* catalytic decomposition of N_2O or use of N_2O as an oxidant of CH_4.

N_2O has a positive enthalpy of formation, releasing 82 kJ $mole^{-1}$ when decomposed (eqn (1)). Thermal decomposition of N_2O occurs at approximately 850 °C, but the presence of a transition metal oxide catalyst can enable self-sustaining decomposition and net energy production at decomposition temperatures as low as 300 °C.[22–27] The energy released by decomposition of 1.0 mole of N_2O is approximately equivalent to the energy released by combustion of 0.1 mole of CH_4.

Eqn (1). Decomposition of N_2O.

$$N_2O \rightarrow \tfrac{1}{2}O_2 + N_2,\ \Delta\hat{H}^\circ_R = -82\ \text{kJ mol}^{-1} \quad (1)$$

N_2O can also act as a powerful oxidant in combustion reactions. It is commonly used to supercharge the engines of high performance vehicles (*i.e.* "Nitrox") and as an oxidant in hybrid rocket motors in the aerospace industry. When used to oxidize methane, N_2O increases the heat of reaction by −329 kJ mol^{-1} as compared to O_2 (eqn (2)).

Eqn (2). Comparison of the heat of reactions of CH_4 with N_2O (top) and CH_4 with O_2 (bottom).

$$CH_4 + 4N_2O \rightarrow CO_2 + 2H_2O_{(l)} + 4N_2,\ \Delta\hat{H}^\circ_R = -1219\ \text{kJ mol}^{-1} \quad (2A)$$

$$CH_4 + 2O_2 \rightarrow CO_2 + 2H_2O_{(l)},\ \Delta\hat{H}^\circ_R = -890\ \text{kJ mol}^{-1} \quad (2B)$$

Steps 1 and 3 in CANDO have been demonstrated at full-scale. Step 1 is achieved with the SHARON process: partial oxidation of NH_4^+ to NO_2^- (Step 1).[6] In our bench-scale studies, an enrichment of ammonia oxidizing bacteria (AOB) converted 80% of the incoming ammonia (2 g N L^{-1}) to NO_2^-, consistent with 80–85% conversion previously reported for a lab-scale SHARON process.[5] Full-scale SHARON processes have reported over 95% nitrogen removal efficiency.[28,29] The decomposition of N_2O and the use of N_2O for hydrocarbon combustion (Step 3) are also well-documented.[23,30,31] In earlier studies, we demonstrated that the decomposition reaction of eqn (1) occurs at N_2O flow rates comparable to those expected for a medium sized wastewater treatment facility (~20 MGD).[32,33] Accordingly, the focus of this study is step 2: partial reduction of NO_2^- to N_2O. Two strategies were investigated and documented below.

The first strategy builds on geochemical studies of NO_2^- reactivity with Fe(II). In carbonate buffered systems at pH = 6–8, Fe(II) precipitates with Fe(III) and carbonate to form carbonate "green rust" ($Fe^{II}_4Fe^{III}_2(OH)_{12}CO_3$). It also precipitates with carbonate alone to form siderite ($FeCO_3$). Fe(II) in the form of green rust or siderite reduces NO_2^- mainly to N_2O, as does Fe(II) absorbed to Fe (hydr)oxide precipitates.[34–42]

As shown in Fig. 1, reduction of NO_2^- to N_2O by carbonate green rust and siderite is thermodynamically favorable over a broad pH range, for conditions that are similar to those of partially oxidized anaerobic digester centrate. The key reactions are:

$$4FeCO_3\ (\text{siderite}) + 2NO_2^- + 5H_2O \rightarrow 4\gamma\text{-FeOOH} + 2H^+ + N_2O + 4HCO_3^-$$

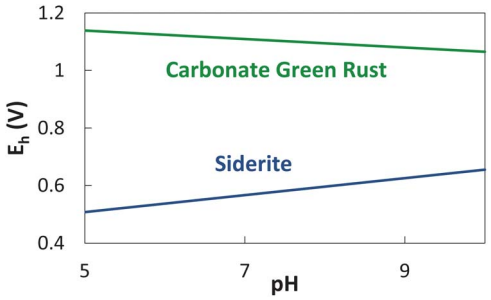

Fig. 1 Redox potential diagram (E_h *vs.* pH) for reduction of NO_2^- to N_2O coupled to the oxidation of Fe(II) in siderite and carbonate green rust. Assumed conditions are similar to partially oxidized anaerobic digester centrate: $[NO_2^-] = 35$ mM, $[N_2O] = 22$ mM, $[HCO_3^-] = 40$ mM. The sources of thermodynamic data were obtained from Rittmann and McCarty for aqueous solutes,[43] see ref. 44 for siderite and ref. 45 for carbonate green rust.

$$Fe^{II}_4Fe^{III}_2(OH)_{12}CO_3 \text{ (carbonate green rust)} + 2NO_2^- + H^+ \rightarrow 6\gamma\text{-FeOOH} + HCO_3^- + N_2O + 3H_2O$$

The second strategy is based on a review of factors previously implicated in N_2O production by denitrifying heterotrophs: (1) low COD/N,[2,3,46–48] (2) high nitrite levels,[2,3,47,49–56] (3) transient feeding regimes (*i.e.* feast and famine),[3,47,48,51,52,54,57,58] (4) low pH (*i.e.* high concentration of free nitrous acid),[59] and (5) low dissolved oxygen.[2,3,53,55,58,60,61] In general, more extensive conversion to N_2O was associated with: (1) limited availability of COD;[2,3,46,47] (2) oxidation of endogenous COD in pulse fed systems;[48,51,52,56] or (3) inhibition of N_2O reduction at high NO_2^- levels.[2,3,47,50,51,53–55] Influent nitrogen was converted to N_2O with conversion efficiencies of >90%,[56] 77%,[51] and 32–64%.[48] The highest reported percent conversion (>90%[56]) occurred in batch studies in which the investigators supplied a single pulse of NO_2^-. Comparing results across studies is difficult because the conversion percentages were not replicated over many cycles, and the microorganisms used were obtained from parent reactors operated under different selection conditions (*i.e.* aerobic/anaerobic SBRs with denitrification from NO_3^- and steady state addition of carbon/nitrite feed). In the study reporting 32–64% conversion, adaptation occurred in some cultures, with a decrease in N_2O production after 10 cycles, presumably with a corresponding increase in N_2 production. One culture with a different operational history reportedly retained a high level of N_2O production, but no data were provided. We know of no bioreactor studies documenting sustained N_2O production at high levels in long-term operation.

To identify conditions favorable for sustained generation of N_2O, experiments were carried out with acetate as the electron donor, nitrite as the electron acceptor, and activated sludge as the source of microorganisms. Acetate was added at 703 mg L^{-1} (750 mg L^{-1} as COD) and nitrite-N was added at 500 mg L^{-1} to give a COD : N ratio of 1.5. In a preliminary experiment, N_2O production was negligible when acetate and nitrite were simultaneously fed to an enrichment initiated from activated sludge (data not shown). Two cyclical pulse feeding strategies were evaluated: in the first, addition of acetate and nitrite was "coupled", *i.e.*, both substrates were added simultaneously as a single pulse at the beginning of each cycle; in the second, acetate and nitrite were added as separated pulses.

The following sections provide a laboratory evaluation of the abiotic (reaction of NO_2^- with Fe(II)) and biotic (alternating acetate/NO_2^- pulsed-feeding) strategies, an assessment of the theoretical potential of CANDO for energy recovery, and possible treatment trains for further evaluation in the lab and field.

Materials and methods

Fe(II) reactor for abiotic N_2O production

Carbonate green rust was evaluated for reduction of NO_2^- to N_2O in a one-liter well-mixed vessel. Green rust (0.4 M) was prepared by combining solutions of $FeCl_2$ and $FeCl_3$, titrated to pH 8 with Na_2CO_3 prior to mixing, as described previously.[62] 20 mL of sodium nitrite stock solution (1.4 M) was pulsed at the beginning of the test to give an initial NO_2^- concentration of 28 mM N, ~400 mg L^{-1} N. Automatic addition of 0.1 M HCl and 0.1 M NaOH solutions maintained pH = 7. He carrier gas supplied at 250 mL min^{-1} was used to sweep gas phase products. The gas stream was automatically sampled every 5 minutes for analysis on a Varian 3400 Gas Chromatograph equipped with a 6-foot Porapak Q column (T = 80 °C), 6-foot 5 Å molecular sieve column, and thermal conductivity detector (T = 90 °C). A three-point external calibration curve was prepared with serial dilutions of a gas standard containing 500 ppm N_2O and 500 ppm N_2 (Scott Specialty Gases). NO_2^- was measured colorimetrically (Hach Company, TNT840 test vials).

Bioreactor operation and strategies for N_2O production

A two-liter continuous flow bioreactor was operated for heterotrophic denitrification under the following conditions: HRT = 12 days, mixing speed of 100 rpm, T = 22 °C, and pH = 6.5. The reactor was initially seeded with 100 milliliters of activated sludge from the Palo Alto Regional Water Quality Control Plant in Palo Alto, CA and operated as a continuously fed reactor for 4 months, achieving steady-state, with acetate as the electron donor (750 mg COD L^{-1}) and nitrite as the electron acceptor (500 mg N L^{-1}). The medium contained 0.1 g L^{-1} $MgSO_4$, 0.1 g L^{-1} KH_2PO_4, 0.3 g L^{-1} $CaCl_2 \cdot 2H_2O$, and 1.0 g L^{-1} $NaHCO_3$. Each liter of mineral medium contained 1 mL of Fe stock solution and 1 mL of trace element solution. The Fe stock solution contained 0.05 M $FeSO_4$ and 0.026 M EDTA. The trace element solution contained 100 mg L^{-1} $Na_2MoO_4 \cdot 2H_2O$, 200 mg L^{-1} $MnCl_2 \cdot 4H_2O$, 100 mg L^{-1} $ZnSO_4 \cdot 7H_2O$, 2 mg L^{-1} $CoCl_2 \cdot 6H_2O$, and 20 mg L^{-1} $CuSO_4 \cdot 5H_2O$.

No N_2O was detected in gas emissions from a well-mixed steady state reactor operated with a continuous feed of acetate and nitrite. Accordingly, two transient feed strategies were investigated. Mineral medium was fed continuously, but acetate and nitrite were added as pulses. In the first strategy, 20 mL of stock sodium acetate solution (1.1 M) and 40 mL of stock sodium nitrite solution (1.80 M) were added as a single daily pulse, resulting in an initial acetate concentration of 11 mM (~700 mg COD L^{-1}) and an initial NO_2^- concentration of

36 mM (~500 mg L^{-1}-N); in the second strategy, acetate pulses and nitrite pulses were separated in time to create alternating anaerobic and anoxic (nitrite-reducing) periods. Anaerobic periods (one day in duration) were initiated by addition of a 20 mL pulse of sodium acetate stock (1.1 M), resulting in an initial acetate concentration of 11 mM. Anoxic periods (also one day in duration) were initiated by addition of a 20 mL pulse of sodium nitrite (1.80 M), resulting in an initial concentration of NO_2^--N of 18 mM (~250 mg L^{-1}). To distinguish nitrite removal due to dilution and washout from nitrite consumption due to microbial activity, 20 mL of bromide stock (1 M) were added as a conservative tracer, along with the nitrite, giving an initial Br^- concentration of 10 mM.

Bioreactor monitoring

Helium carrier gas supplied at 250 mL min^{-1} was used to sweep dissolved N_2O and N_2 from the liquid phase of the bioreactor. Dissolved N_2O did not exceed 1% of saturation (~22 mM). The gas stream was automatically sampled every 5 minutes for analysis on a Varian 3400 Gas Chromatograph equipped with a 6-foot Porapak Q column ($T = 80$ °C), 6-foot 5 Å molecular sieve column, and thermal conductivity detector ($T = 90$ °C). For calibration, a three-point external calibration curve was prepared by serial dilutions of a gas standard containing 500 ppm N_2O and 500 ppm N_2 (Scott Specialty Gases).

NO_2^- was measured colorimetrically (Hach Company, TNT840 test vials). Acetate was assayed using a Dionex DX-500 ion chromatograph using a heptafluorobutyric acid eluant and equipped with a GP50 gradient pump, CD25 conductivity detector, AS40 Automated Sampler, and As6 ion-exchange column.

Total and volatile suspended solids[63] were 1820 mg L^{-1} and 780 mg L^{-1}, and remained stable. Percentage of polyhydroxybutyrate (PHB) in the dry cell mass was determined by Nile Red fluorescence (BD Biosciences, BD LSR II flow cytometer equipped with a 532 nm laser) and calibrated by gas chromatography (Agilent 6890N gas chromatograph equipped with an HP-5 column and FID detector), as described previously.[64]

Methods used for imaging of PHB granules, quantitative-PCR (qPCR) calibration, qPCR of 16S rDNA and *phaC*, and pyrosequencing of 16S rDNA and microbial community analysis are described in the ESI.†

Results

Abiotic production of N_2O

As shown in Fig. 2, the Fe(II) in carbonate green rust rapidly reduced nitrite to a mostly N_2O end product. Over 90% of the NO_2^- was reduced to N_2O within 2.5 hours, with 98% of the reduced nitrogen accounted for as N_2O and N_2. No NO_3^- or NH_4^+ were detected.

Microbial production of N_2O

Both the coupled and the decoupled strategies were evaluated for over 100 cycles. The fraction of N converted to N_2O was stable with 9–12% conversion for the coupled strategy and 60–65% conversion for the decoupled strategy. The two strategies also resulted in different patterns of denitrification and different community structures.

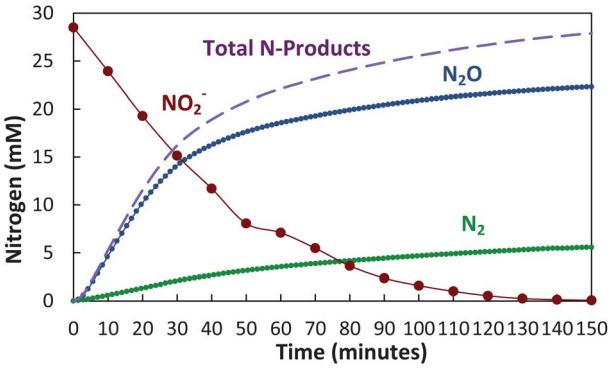

Fig. 2 Reduction of nitrite by carbonate green rust ($Fe^{II}_4Fe^{III}_2(OH)_{12}CO_3$) with over 90% conversion of NO_2^- to N_2O.

Fig. 3 shows the denitrification pattern of the coupled strategy. Initially, acetate and nitrite were present at high levels. N_2O production exceeded N_2O reduction, and N_2O levels increased rapidly. High free nitrous acid (HNO_2) levels may have inhibited N_2O reductase, previously reported at levels >0.004 mg HNO_2-N L^{-1}.[47] After ~1 hour, however, N_2O levels peaked then decreased as the N_2O reduction rates exceeded N_2O production rates. During the second half of the cycle, the fraction of N_2O product decreased from ~0.6 to ~0.4. After 12 hours, acetate levels decreased to zero, and nitrite removal rates approximated the washout rate of the bromide tracer, indicating little nitrite conversion to N_2O production. However, the N_2O to N_2 fraction increased in the absence of acetate, suggesting an endogenous source of electrons.

The decoupled strategy explored the possibility that a storage polymer could serve as the source of reducing equivalents for NO_2^- reduction to N_2O. Separate pulses of acetate and nitrite were delivered daily (Fig. 4) to create alternating anaerobic and anoxic (partial denitrifying) periods (Fig. 4 and 5). These cycles (>200) resulted in a repeating pattern of acetate consumption and nitrite production, with approximately 60–65% of the NO_2^-

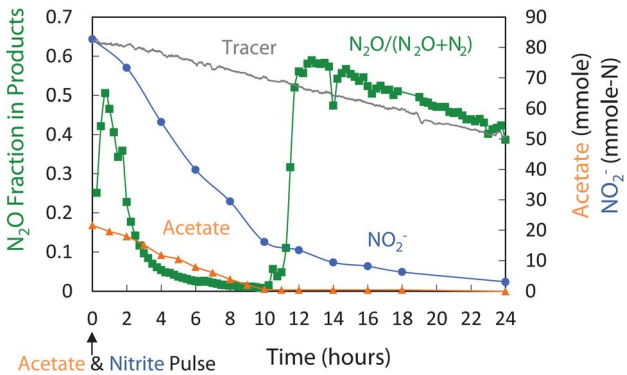

Fig. 3 Coupled acetate–nitrite addition (cycle 107): changes in acetate, NO_2^-, and N_2O production. Of the NO_2^- consumed, 12% was reduced to N_2O, the rest to N_2.

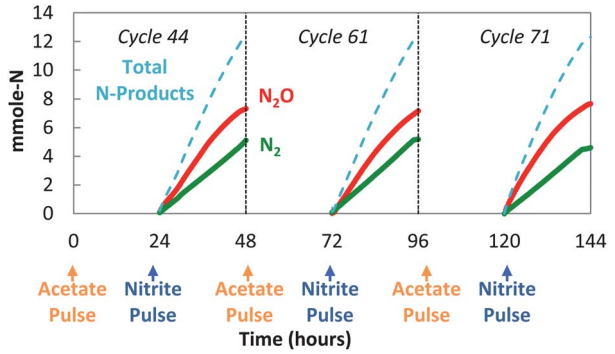

Fig. 4 Decoupled acetate/nitrite addition (cycles 44, 61, and 71): sustained production of N_2O.

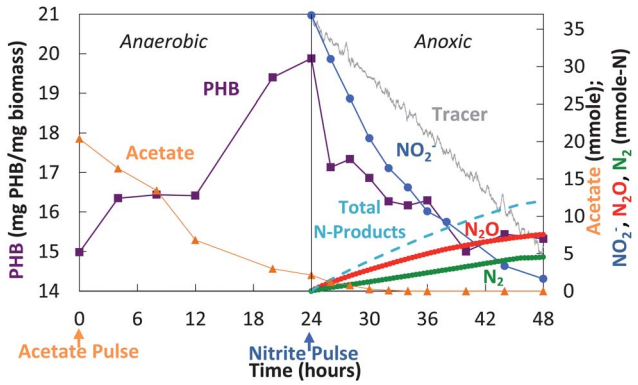

Fig. 5 Decoupled acetate/nitrite addition (cycle 61): changes in acetate, NO_2^-, N_2O, N_2, and PHB.

reduced to N_2O. Acetate pulsed at the beginning of the anaerobic phase was incorporated into biomass as PHB (Fig. 5 and 6). NO_2^- was then added. Reduction of NO_2^- to N_2O coincided with PHB consumption. The nitrite mass reduced was estimated from the area between the bromide tracer and nitrite curves. Sixty two percent of the NO_2^- was converted to N_2O, with 98% of the reduced nitrogen accounted for as N_2O and N_2 (mass balance for cycle 61: 12.3 mmol NO_2^--N consumed, 7.5 mmol N_2O-N produced, 4.5 mmol N_2-N produced). The average specific rate of N_2O production was 200 μmol N_2O per g VSS per h.

The reactor has since been converted to an SBR operation treating real anaerobic digester filtrate from the Sunnyvale Water Pollution Control Plant. Acetate is used as the electron donor and nitrite, from the partially oxidized filtrate, as the electron acceptor. Preliminary results reveal an 82–85% NO_2^- to N_2O conversion fraction over a few cycles.

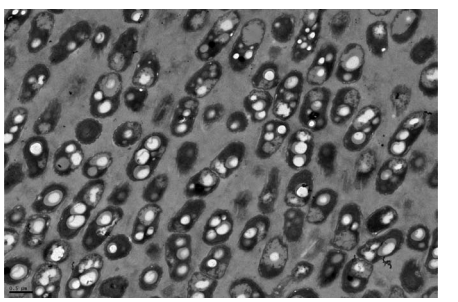

Fig. 6 Decoupled acetate/nitrite addition (cycle 61): PHB inclusion granules at the end of the anaerobic period.

Table 1 Q-PCR results for bacterial and archaeal 16S rDNA and *phaC* gene between the coupled and decoupled feeding strategies

Target gene	Coupled (cycle 107)	Decoupled (cycle 61)
16S rDNA Bacteria (gene copies per L)	4.9×10^{11} ($\pm 1.4 \times 10^{10}$)[a]	2.7×10^{11} ($\pm 1.9 \times 10^{10}$)
16S rDNA Archaea (gene copies per L)	3.1×10^{8} ($\pm 1.1 \times 10^{7}$)	3.1×10^{8} ($\pm 2.4 \times 10^{7}$)
phaC (gene copies per L)	1.1×10^{9} ($\pm 4.3 \times 10^{7}$)	1.3×10^{10} ($\pm 2.6 \times 10^{7}$)
phaC/16S rDNA Bacteria (%)	0.22 (±0.02)	4.99 (±0.33)

[a] Indicates one standard deviation.

Molecular microbial ecology analysis

In both the coupled and decoupled feed strategies, bacteria were three orders of magnitude more abundant than Archaea as revealed by qPCR of 16S rDNA (Table 1). But genetic analyses revealed important differences. The *phaC*/16S rDNA ratio for the decoupled strategy was approximately 20 times greater than that for the coupled strategy. TEM imaging (Fig. 6) and GC measurements confirmed formation of PHB inclusion granules.

Bacterial genus-level composition was evaluated by 16S rDNA amplicon pyrosequencing (Fig. 7). Decoupled feeding of acetate/nitrite increased richness (from 53 genera to 66 genera) and diversity (Shannon index from 2.68 to 3.42) compared to coupled feeding. With coupled feeding, *Pseudomonas* and *Chryseobacterium* were more abundant, and patterns of denitrification were consistent with patterns reported for some *Pseudomonas*, with transcription and gene expression controlled by the level of denitrification intermediates.[49,57,65,66] With decoupled strategy richness and diversity decreased, unclassified *Xanthomonadaceae*, *Comamonas*, and *Paracoccus* increased in abundance and *pha*C abundance increased.

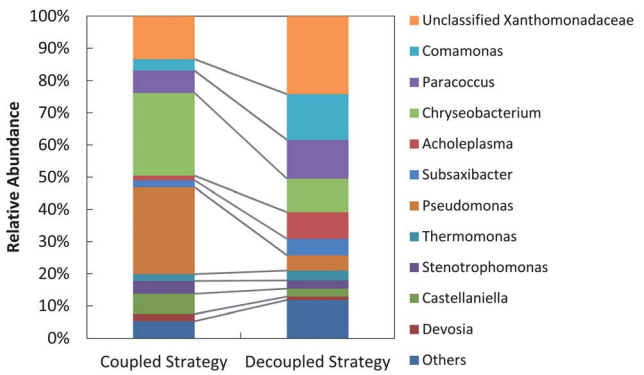

Fig. 7 Genus-level bacterial community structures for the coupled strategy (filtered sequences: 10881) and the decoupled strategy (filtered sequences: 9504). The category "Others" indicates populations with relative abundance <1%.

Complete denitrifiers (N_2-producing) such as *P. stutzeri*[66] were suppressed while incomplete denitrifiers (N_2O-producing) such as *Pseudoxanthomonas* sp.[67] and/or PHB-accumulating bacteria such as *Paracoccus* sp.[68] and *Diaphorobacter nitroreducens*[69] were stimulated.

Discussion

As noted previously, both the first step in CANDO – conversion of ammonium to nitrite – and the final step – the use of nitrous oxide as an oxidant in combustion – have been demonstrated at full-scale. This work establishes that the intermediate step – reduction of NO_2^- to N_2O – may be accomplished abiotically with Fe(II) or biotically with PHB storage granules as the source of electrons.

In the abiotic strategy, carbonate green rust efficiently and rapidly reduced NO_2^- to N_2O. The efficiency of nitrogen removal from the water was 98%, with >90% conversion of NO_2^- to N_2O. Deeper understanding of the N_2O production mechanism is needed. Oxidation of one Fe(II) atom yields just one electron, but reduction of NO_2^- to N_2O requires transfer of two. It is therefore likely that N_2O production requires near simultaneous transfer of two electrons from two Fe(II) atoms to NO_2^- adsorbed to the Fe(II)/Fe(III) solid. It is also likely that N_2 is produced from a second 2-electron reduction of the adsorbed N_2O. For this reaction to be sustained, the Fe(III) formed by reduction of NO_2^- will need to be reduced back to Fe(II). Such a regenerative cycle may be possible with heterotrophic Fe(III)-reducing bacteria, but additional research is needed to determine if such a cycle can be established and maintained. Previously researchers have demonstrated microbial oxidation of organic matter coupled to reduction of Fe(III) to produce carbonate green rust[70] and siderite[71] in systems containing high alkalinity (typical of anaerobic digester centrate).

In the biotic strategy, a decoupled feeding regime selected for organisms that store PHB then evidently use it as the source of reducing equivalents for nitrite reduction. The efficiency of nitrogen removal from the water was 98%, with 62% conversion of NO_2^- to N_2O.

If this process can be scaled up and its efficiency improved, or another N_2O-producing strategy developed, CANDO would be an attractive option for nitrogen removal. Fig. 8 compares conventional nitrification–denitrification to CANDO (partial nitrification/partial denitrification) for removal of 1 mole of NH_4^+ assuming that 111 grams of biodegradable COD (BOD_L) is potentially available for reduction of nitrogen oxides to N_2 and for reduction of CO_2 to methane (based on a BOD_L/N ratio of 7.9, typical of U.S. medium strength wastewater[72]). Oxygen requirements and energy recovery were calculated assuming the use of COD for stoichiometric production of N_2O (100% conversion efficiency) and anaerobic conversion of any remaining COD to CH_4. These calculations indicated that CANDO could theoretically decrease oxygen requirements by

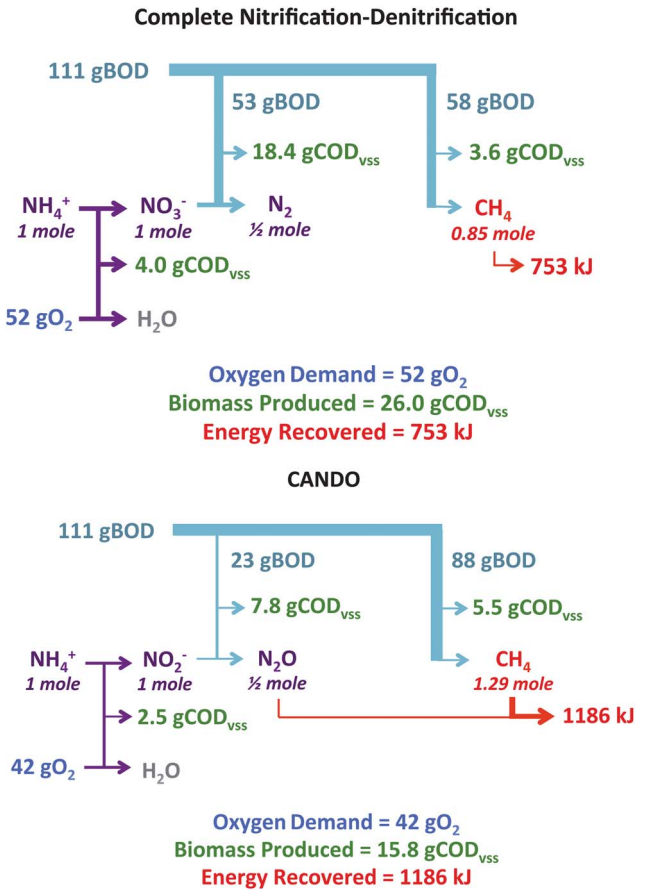

Fig. 8 Comparison of complete nitrification–denitrification to CANDO with respect to oxygen demand, biomass production, and energy recovery.

Table 2 Theoretical upper bound for four N removal processes treating U.S. *per capita* nitrogen and BOD_L loads of 13.3 g-N/p/d and 142 g-BOD_L/p/d, respectively.[72a]

Process	SRT denit.	f_s^o denit.	Oxygen (g/p/d)	Biomass (g COD/p/d)	Energy (MJ/p/d)
Complete nit.–denit.	5	0.58	48	37	1.06
SHARON	5	0.58	37	27	1.36
CANON	60[7]	0.14	21	13	1.78
CANDO	5	0.58	38	22	1.56[b]

[a] *Assumptions*: $T = 25$ °C, NH_3 is the N-source for cell synthesis. For partial nitrification, SRT = 6 days and $f_s^o = 0.14$. For complete nitrification, SRT = 10 and $f_s^o = 0.11$. f_s^o of denitrification for CANON corresponds to Anammox. f_s^o is defined as the maximum biomass yield expressed in dimensionless units (*e.g.* oxygen demand of biomass produced divided by the oxygen demand of the electron donor consumed) and calculated by the free energy protocol of Rittmann and McCarty.[43] f_s is the observed yield expressed in dimensionless units and adjusted for decay: $f_s = f_s^o\{(1 + 0.2bSRT)/(1 + bSRT)\}$. Analysis assumes energy recovery from soluble and particulate BOD with complete conversion of organic nitrogen and free ammonia. [b] Recovered energy is derived from COD (as CH_4) and NOD (as N_2O).

20%, decrease biomass production by 40%, and increase energy production by 60%.

Table 2 compares oxygen requirements, biomass production, and energy recovery for three short-circuit nitrogen removal processes and complete nitrification–denitrification. In full-scale installations, nitritation and anaerobic digestion are mostly limited to treatment of concentrated side streams; however, recent advances are enabling application of these processes to dilute main streams.[73–78] The analysis of Table 2 provides an upper bound for wastewater treatment efficiency by assuming nitritation and anaerobic digestion of both concentrated side and dilute main streams. Theoretical maximum process efficiency was determined by the demand in oxygen and organic reducing power required to completely treat the average U.S. *per capita* nitrogen load of 13.3 g-N/p/d with an average U.S. *per capita* BOD_L load of 142 g-O_2/p/d.[72] The BOD that remains after nitrogen removal is recovered as biogas CH_4 and converted to energy with 100% conversion efficiency. From this analysis, the CANON process with Anammox bacteria has the highest theoretical energy recovery, the lowest O_2 requirement, and the lowest biomass production. This is followed by CANDO. It should be noted, however, that CANDO mitigates the release of N_2O to the atmosphere and provides an additional option for nitrogen removal that may be attractive in terms of footprint, robustness, and ease of retrofit. Pilot-scale testing is needed to determine whether the expected short SRT values for CANDO are achieved under field conditions. Future work will also seek to optimize the NO_2^- to N_2O conversion fraction.

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