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Impacts of nitrogen-containing coagulants on the nitritation/denitrification of anaerobic digester centrate

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Nitrogen-containing coagulants are widely used in wastewater treatment to improve centrifugation of anaerobic digestate. When ammonia-rich centrate is nitrified, coagulant-derived particulates in the liquid phase select for heterotrophic ammonia-oxidizing bacteria and overdoses can adversely affect downstream nitrogen removal. Research is needed to determine coagulant impacts on different nitrogen removal processes and whether coagulant particulates retained in biosolids affect suitability for land application.

Impacts of nitrogen-containing coagulants on the nitritation/denitrification of anaerobic digester centrate

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Abstract

Nitritation of anaerobic digestion centrate reduces aeration energy demand by preventing oxidation to nitrate and can be affected by changes in upstream processing of anaerobic digestate. Here we report impacts of nitrogen-containing coagulants on autotrophic/heterotrophic nitritation and partial denitrification in a pilot-scale reactor treating anaerobic digester centrate. The pilot reactor selected for a stable microbial community with nitritation of 60-65% of influent TKN; ~30-35% nitrogen removal; low nitrate concentrations; and concurrent appearance of autotrophic and heterotrophic ammonia oxidizing bacteria (AOB). Dominant autotrophic AOB were *Nitrosomonadaceae*. Heterotrophic AOB included *Xanthomonadaceae* and *Chitinophagaceae.* Denitrifying bacteria included *Comamonadaceae* and *Actinomycetales*. The effects of coagulant dosage on nitritation were studied in bench-scale sequencing batch bioreactors (SBRs), where unclassified AOB were identified that had *amoA* sequences clustering between the autotrophic and heterotrophic clades. Heterotrophic nitritation was stimulated by glucose addition, especially in SBR biomass adapted to continuous coagulant addition, with elevated levels of *Xanthomonadaceae*, *Chitinophagaceae*, and *Rhodanobacteraceae*. Further research is needed to understand the effects of coagulants on downstream nitrogen removal unit operations and implications for land-application of treated biosolids.

Introduction

Domestic wastewater is a significant source of reactive nitrogen in the environment. Left untreated, this wasted nitrogen can adversely impact ecosystems due to ammonia toxicity, eutrophication, and nitrogenous oxygen demand, culminating in anoxic dead zones, incidental release of toxins, damaged fisheries, and harm to public health and the economy.[1] Conventional bioprocesses for nitrogen removal use a combination of nitrifying and denitrifying microorganisms for mainstream treatment. These processes have high energy requirements for delivery of the O_2 to oxidize ammonium to nitrate (nitrification) and high chemical costs for delivery of the reducing power needed to reduce nitrate to N_2 (denitrification), as, for example, by addition of methanol.[2] At many treatment plants, an additional in-plant source of nitrogen is anaerobic digestion, where anaerobic ammonification of proteinaceous organic matter results in ammonia-rich centrate.[3] While the flowrates of such digestate sidestreams are small compared to influent flowrates, nitrogen levels are an order of magnitude higher than influent values, with typical Kjeldahl nitrogen levels of 1-2 g N/L. These sidestreams are often recirculated back to the mainstream for treatment, increasing plant nitrogen loading by 15-30%.[4] An alternative is sidestream treatment. Use of "short-cut" nitrogen removal for sidestream treatment can enable savings of up to 50% of the energy required for nutrient removal[5]. In the Sharon process[6], for instance, limiting ammonia oxidation to nitrite decreases energy requirements for O_2 delivery, and less reducing power is needed for nitrite reduction to N_2. These beneficial outcomes are achieved by creating environments favorable for autotrophic ammonia-oxidizing bacteria (AOB) but unfavorable for nitrite-oxidizing bacteria (NOB)[7].

While autotrophic AOB are typically responsible for mainstream nitritation, ammonia-oxidizing

23 archaea (AOA) and heterotrophic AOB can also play a role[8]. AOA are present and active in
24 wastewater treatment plants operating at low concentrations of DO[8] and low ammonia[9]. Selection
25 conditions favorable for heterotrophic AOB are less clear but appear to involve oxidation of both
26 ammonia and organic nitrogen, with coupled anoxic oxidation of NAD(P)H[10]. Heterotrophic
27 nitrifiers reportedly do not obtain energy for cell growth from ammonia oxidation and are thought
28 to be limited to systems in which autotrophic nitrification is suppressed[11], as in acidic soils[12].

29 Despite the significant functional role of heterotrophic AOB in the natural nitrogen cycle[13], their
30 significance in engineered systems, such as systems that nitrify centrate from anaerobic digesters,
31 has received limited attention. Centrate contains organic and nitrogenous substances other than
32 ammonia that may affect the microbial community in a nitrification system. Nitrogen-containing
33 polymeric coagulants based on polyamine, polyacrylamide and polydiallyldimethylammonium
34 chloride (polyDADMAC), for instance, are added before centrifugation to improve dewatering.
35 Overdose of coagulants can result in charge reversal and re-stabilization of colloids, increasing the
36 concentrations of suspended proteins and polysaccharides and decreasing dewaterability.[14] The
37 change in centrate quality as a response to coagulant dosage can have negative impacts on
38 biological nitrogen removal. Increases in organic matter can select for heterotrophs over autotrophs
39 and may lead to a deterioration in reactor performance.[15] High concentrations of organic matter
40 are also inhibitory to anammox bacteria. [16]

41 In this study, we monitored nitritation of anaerobic digester centrate in a low-oxygen pilot-scale
42 continuous stirred tank reactor (CSTR) and obtained evidence of simultaneous autotrophic and
43 heterotrophic nitritation. We also observed that an overdose of coagulants added to improve
44 dewatering of biosolids during centrifugation can result in dispersed black particulate matter that,

if not removed in the nitritation reactor, can adversely affect downstream denitification processes, such as CANDO[17] and Annamox[18]. We then carried out follow-up studies in bench-scale sequencing batch reactors (SBRs) to understand the effects of coagulants dosage on nitritation performance and microbial community. The results indicate that continuous dosing of coagulants selects for heterotrophic nitrification, likely mediated by *Xanthomonadaceae* and *Chitinophagaceae*, and this process can be stimulated by the presence of soluble, biodegradable organic matter, added as glucose in this study.

Experimental

Pilot-scale reactor operation

A pilot-scale nitritation CSTR with a working volume of 2.5 m^3 was operated for a six-month period as the first stage of a Coupled Aerobic–anoxic Nitrous Decomposition Operation (CANDO) nitrogen removal process[17] at the Delta Diablo Wastewater Treatment Plant (DDWTP, Antioch, CA, **Figure S1**). Feed for the CSTR was centrate generated daily by the centrifugation of anaerobic digestate dosed with about 156 mg/L of nitrogen-containing coagulants (Clarifloc^{TM} WE-223, Polydyne Inc., CA, 6% stock solution). The centrate was stored in a 3 m^3 tank with a mechanical stirrer (**Figure S2**). Composition of the centrate is provided in **Table S1**. Such dosage of coagulants led to the formation of black particulates in the centrate (**Figure 1**) during the pilot-scale nitritation reactor operation. The mechanical stirrer was stopped on Day 130 to add a settling step to reduce the particulates content in the feed.

The nitritation reactor was initially inoculated with 0.4 m^3 of returned activated sludge from DDWTP and 0.2 m^3 of nitrifying activated sludge from the City of Brentwood wastewater

treatment plant (Brentwood, CA). It was then batch-fed from the centrate storage tank for a month. For the following two months of operation, feed rates for centrate were increased from 0.1 to 1.0 m^3/d and aeration rates increased proportionally from 1.4 to 8.5 L/s. Programmable logic controllers (PLCs) set reactor temperature at 31.6 ± 2.3 °C with an immersion heater. PLCs also set reactor pH at 7.5 ± 0.5 by addition of NaOH solution (2% w/v) and reactor DO levels by intermittent aeration, alternating 1-minute aeration from zero to 0.1-2.4 mg DO/L (average of ~ 1mg DO/L), followed by 5 minutes without aeration (**Figure S3**). Steady state levels of nitrite were observed at a hydraulic retention time (HRT) of 2.5 d, and a steady state ammonia loading rate of 0.62-0.70 kg N/m^3-d (**Table S2**). On Day 178, the pilot-scale nitritation reactor was shut down due to failure of a recirculation pump on an external pH monitoring loop. The failed pump led to pH measurements within the loop that did not reflect conditions within the reactor. Alkali addition stopped, the reactor acidified, and operation was halted.

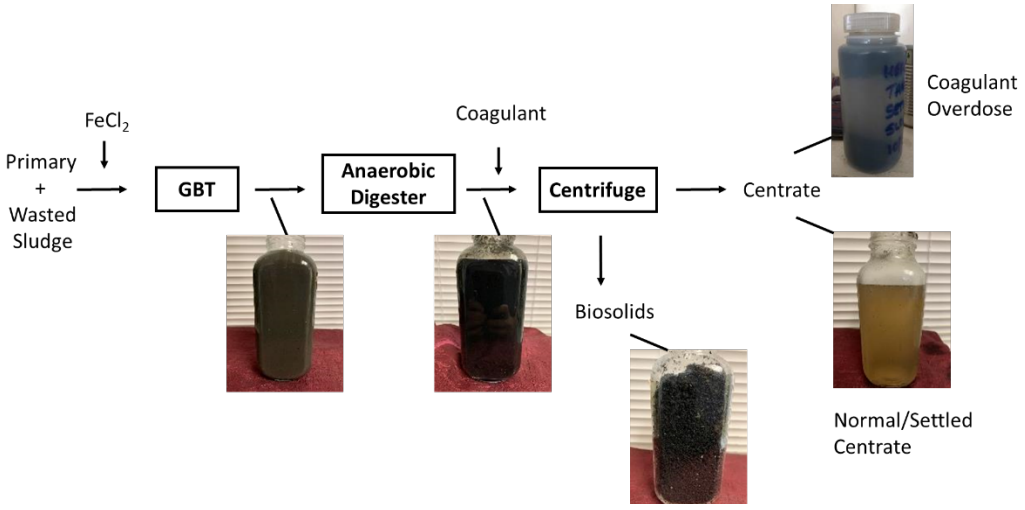


Figure 1. Biosolids processing steps at the DDWTP showing samples collected at each step. When coagulants were added in excess, black particulates were observed during operation of the pilot-scale nitritation reactor. These particulates interfered with downstream nitrogen removal[17]. On Day 130, a settling tank was added to the system for removal of the black precipitate.

Pilot reactor mixed liquor analyses

Samples of raw centrate and mixed liquor from the pilot reactor were stored frozen until thawed for analysis. Alkalinity, chemical oxygen demand (COD), total suspended solids (TSS), volatile suspended solids (VSS), and total Kjeldahl nitrogen (TKN) assays were carried out as per Standard Methods[19]. Thawed samples were filtered with 0.45 μm Nylon filters for analysis of soluble substrates. Concentrations of ammonia, nitrite and nitrate in filtered samples were determined using a DR2800 spectrophotometer (Hach Company, Loveland, CO). Nitrogen mass balances (sum of TKN, nitrite-N, nitrate-N) were conducted on influent and effluent samples. To eliminate interference due to high nitrite concentrations in nitrate assays, sulfamic acid (10 g per g-N) was added to remove nitrite prior to analysis[19].

Pilot reactor community analyses

After extracting genomic DNA from the pilot reactor mixed liquor using a FastDNA Spin Kit for Soil (MP Biomedicals, Solon, OH), the V3-V4 region of bacterial 16S rRNA genes were amplified using primer set 341F and 785R.[20] PCR and cloning was carried out as previously described.[21] Topomize Amplicon Library Prep Kits (MCLAB, South San Francisco, CA) were used to add adapters and barcodes to the amplicons. The PCR products were measured by a Bioanalyzer 2100 (Agilent Technologies, Santa Clara, CA) using the MCNext™ SYBR® Fast qPCR Library Quantification Kit (MCLAB, South San Francisco, CA) before sequencing with MiSeq Reagent Nano Kit v2 (500-cycles) (Illumina, San Diego, CA) on a MiSeq instrument. Sequences were filtered through a MOTHUR[22] pipeline with OTUs defined at 97% identity level. OTUs with abundance less than 1% of the total sequence numbers were excluded from the relative abundance plot. Raw sequences were submitted to NCBI SRA database (BioProject PRJNA559928).

Bench-scale bioreactor operations

Follow-up bench-scale studies were performed to better understand the effects of coagulants dosage. Two laboratory-scale SBRs were fed DDWTP anaerobic digester centrate that was collected bimonthly after the installation of the setting tank. One SBR received centrate alone (Control), and the second received centrate supplemented with additional 30 mg/L of nitrogen-containing coagulants (Test). Both reactors were seeded with 2 L of coagulant-adapted inoculum, and both reactors commenced operation at the same time. The inoculum was prepared by mixing together two pre-adapted nitrifying cultures (details in **Supplementary Information**) to create a diverse mixed inoculum capable of tolerating a coagulants overdose. Each reactor had a 2-L working volume and operated on a 48-h cycle (0.5-h settle, 0.3-h decant, 0.2-h fill, 47.0-h react). Alkali stock solution (200 mL of 80 g$NaHCO_3$/L) was added at hour 24. The HRT was 2.6 days, and solids retention time (SRT) was 20 days. Each SBR was operated with intermittent aeration (30 min on; 30 min off) at 22 °C, and DO levels alternated between 0 and 3 mg/L.

Preparation of 16S rRNA and amoA clone libraries and phylogenetic analysis

Genomic DNA from biomass samples were extracted from the inoculum of bench-scale SBR and both SBR on Day 100. The primer set 8F and 1492R were used to amplify full-length 16S rRNA from extracted genomic DNA, whereas bacterial *amoA* genes were amplified using primer set amoA-1F and amoA-2R primers with the genomic DNA extracted.[23] PCR and cloning were conducted as previously described.[8,23] One hundred forty nine 16S rRNA clones were retrieved and sequenced by MCLAB (NCBI GenBank accession number SUB8325720). A phylogenetic tree was then constructed using sequences from the NCBI GenBank database. The maximum-likelihood method with bootstrap values based on 1000 replications was used in the MEGA 7

program (Saitou, Tamura, Kumar) using near full-length (~430 bp) *amoA* gene clones and sequences. The retrieved 60 *amoA* clones were deposited to GenBank under accession numbers MT242413 - MT242446, MT242455 - MT242477, MT242479 - MT242481.

Inhibition of autotrophic AOB and stimulation of heterotrophic AOB

To assess heterotrophic nitritation in the bench-scale SBR fed with centrate and the SBR receiving additional nitrogen-containing coagulants, ammonia oxidation rates were measured in batch tests on Day 100 after addition of allylthiourea (ATU)[24] or acetylene[25], inhibitors of autotrophic AOB. Settled centrate without the presence of black particulates was used in the assays. For the ATU inhibition assays, 80 mL of mixed liquor from the SBR was added to 160-mL serum bottles along with 16 mL of digester centrate and 4 mL of ATU stock solution (125 mg ATU/L), leaving 60 mL of air headspace. For acetylene inhibition assays, 16 ml of digester centrate was added to 84 mL of mixed liquor to give 100 ml of liquid in 160-mL serum bottles. The remaining gas volume consisted of 6 mL of stock acetylene (1 mg/L) and 54 mL air. Final inhibitor concentrations were 5 mg/L for ATU and 0.1 mg/L for acetylene at equilibrium (Henry's law constant of 0.039 mol/L-atm[26]). To assess the effect of added organic substrate, 4 mL of a glucose stock solution (1.25 gCOD/L) was added. Initial added ammonium concentrations were ~200 mgN/L. Triplicate assays were incubated at 22 °C for 24 hours, with the headspace replenished with air after 12 hours.

Results and Discussion

Pilot-scale reactor performance

The centrate-fed pilot-scale reactor was operated for six months and achieved stable and continuous nitritation over a four-month period. During the first month of batch operation,

ammonia persisted. Upon initiating continuous feeding, nitrite concentrations increased rapidly, as shown in the mass balance of **Figure 2**. After a second month of operation, nitritation stabilized at high levels (790-960 mg nitrite-N /L), with nitrate at relatively low levels (<30 mg-N/L). Under steady state operational conditions (days 71 to 178), ammonia-N was present at 135±58 mg/L, nitrite-N at 870±89 mg/L, and nitrate-N at 18±15 mg/L. Average DO from Day 15 to Day 150 was 1.1 ± 0.5 mg/L, based on a daily grab sample. A heater malfunction occurred on Day 161, and temperature dropped to 25 °C twice on Day 161 and 176. On Day 178, the experiment was terminated due to a recirculation pump failure that resulted in loss of pH control and reactor acidification.

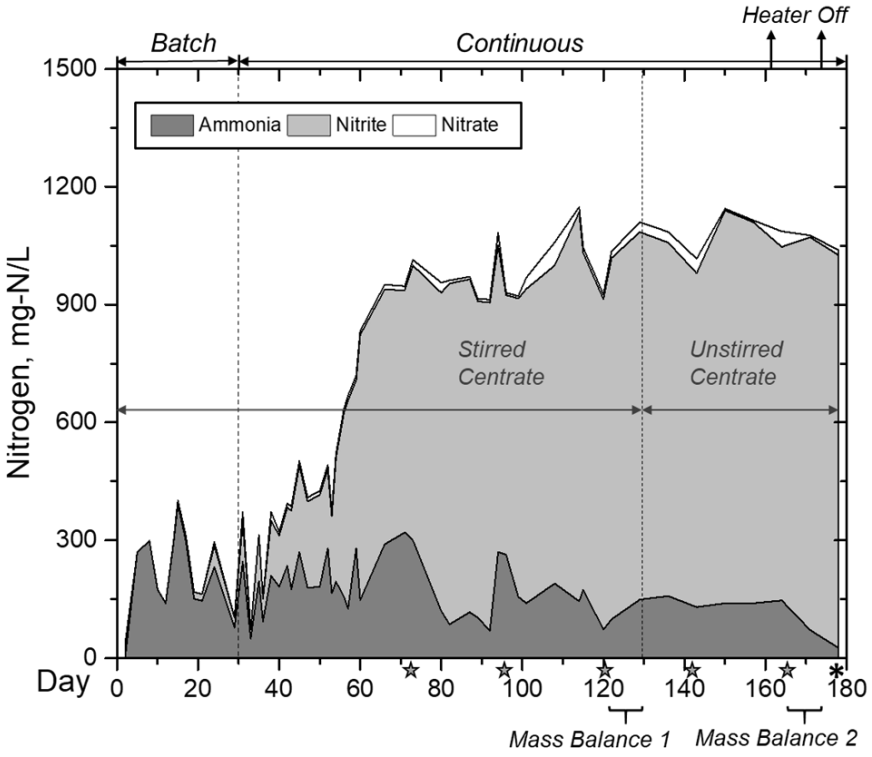
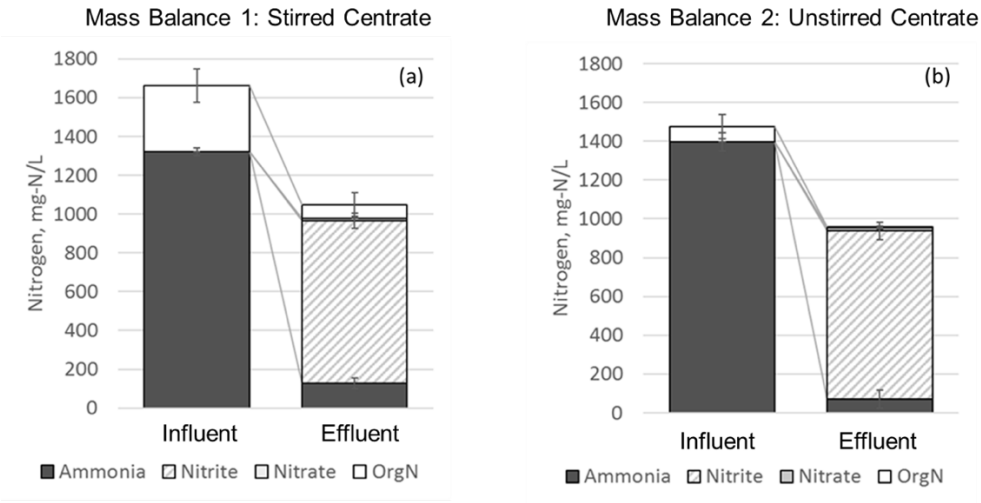


Figure 2 Concentrations of soluble nitrogen species in nitritation reactor effluent. Dashed lines marked the operational changes and daily samples for mass balance analyses. Stars indicate biomass sample dates. The asterisk indicates the date where there was loss of pH control. TKN concentrations in the influent were 1660±99 and 1480±71 mgN/L on Day 120-127 and Day 165-172, respectively.

Throughout the 6-month test period, anaerobic digester centrate was held in a storage tank before transfer to the nitritation reactor. The composition of the centrate was affected by upstream centrifugation protocols, notably addition of coagulants and $FeCl_2$, which required mixing in the storage tank. For Days 1-129, the storage tank was stirred with a mixer, and a suspension of black particulates (~4 g/L, 65% volatile) was present in the centrate. The nitrogen content of the filtered and dried solids was as high as 14% by weight. After steady state was achieved, a week of daily monitoring of reactor influent and effluent was conducted to assess the nitrogen mass balance (**Figure 3a**). Eighty five percent of the organic nitrogen in the influent was removed. Influent nitrogen levels exceeded effluent levels, suggesting removal of ~35% of the nitrogen by denitrification as N_2. Dissolved N_2O levels in the reactor were less than the detection limit of an industrial Clark-type sensor (5 µg N/L, Unisense, Denmark), implying that the major product was N_2. Separate batch assays confirmed negligible N_2O production. Centrate containing black particulate matter entered and passed through the nitritation reactor into a pilot-scale CANDO reactor, adversely impacting its operation, as discussed elsewhere[17]. The overdose of coagulants likely resulted in re-stabilization of colloids and associated small particles with an increase in total suspended solids. To remove the black particulates, the storage tank mixer was turned off on Day 130. Without mixing, the black particulate matter settled and was drained from the bottom of the tank, resulting in a 95% decrease in suspended solids loading and a 77% decrease in COD loading on the nitritation reactor. After re-establishment of steady state, a second week of daily monitoring was performed to obtain a mass balance on nitrogen in the absence of the black precipitate (**Figure 3b**). Total nitrogen loading on the reactor decreased from 0.70 to 0.62 kg/m^3-d because the settling tank removed 75% of the influent organic nitrogen. As expected, ammonia removal increased from 90% to 95%. Biodegradability assays indicated that the residual effluent soluble COD (~200 mg/L)

185 was recalcitrant.



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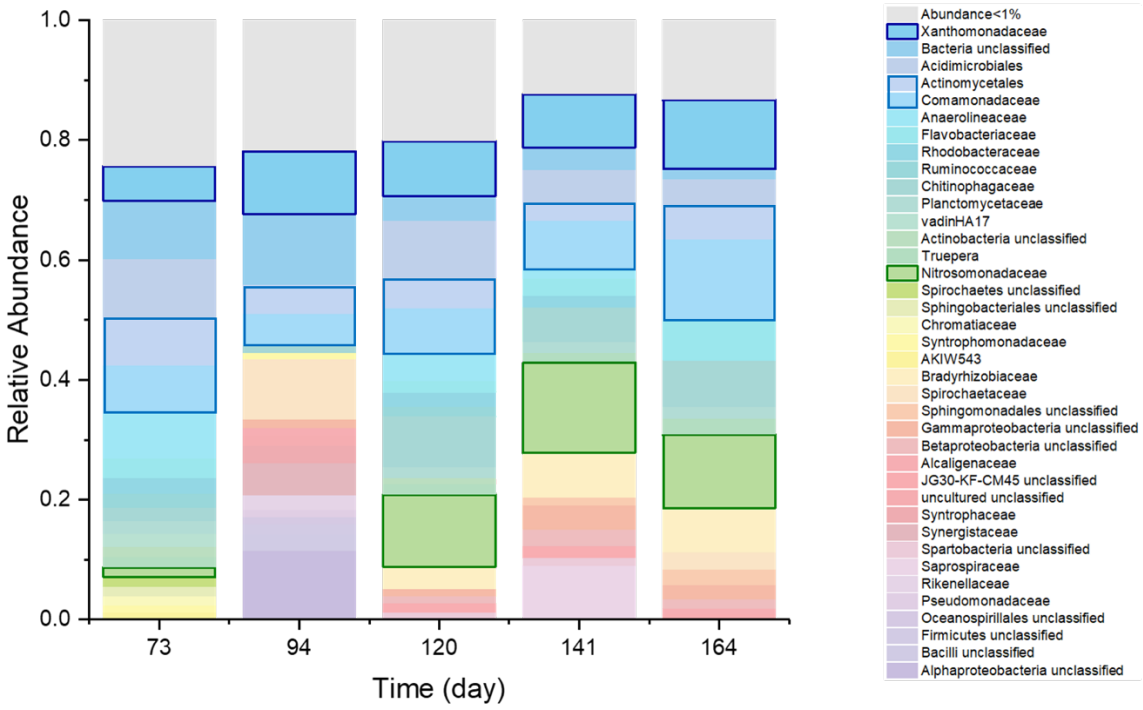
187 **Figure 3** Nitrogen mass balance (including soluble and particulate) for the influent and effluent of the pilot-scale
188 nitritation reactor (a) before the mixer in the centrate storage tank was turned off (Days 120-127, Mass Balance 1),
189 and (b) after (Days 165-172, Mass Balance 2) after the mixer in centrate storage tank was turned off the mixer in
190 centrate storage tank was turned off. The centrate mixer was turned off on Day 130.

191 *Pilot-scale reactor community structure*

192 Sharon-type processes typically enrich *Nitrosomonas*-related autotrophic AOB.[27] However,
193 amplicon sequencing of 16S rRNA genes revealed significant *Xanthomonadaceae* during all
194 periods of nitritation, as noted in other nitrifying systems (**Figure 4**)[28]. Also present were
195 *Chitinophagaceae*, heterotrophic AOB recently reported as dominant nitrifiers in a bench-scale
196 SBR treating anaerobic digestate for total nitrogen removal[29]. Autotrophic AOB
197 *Nitrosomonadaceae* were also present, except on Day 94, when an increase in centrate feed rate
198 coupled to decreased aeration and low DO (**Figure S3**) may have led to a surge in denitrifying
199 populations (*Pseudomonaceae*, Bacilli, Firmicutes) and washout of autotrophic AOB. The
200 increase in free ammonia concentration from 1.6 to 5.4 mgN/L due to loading increase might also
201 affected the growth of *Nitrosomonadaceae*. A similar shift in denitrifying bacteria occurred on

202 Day 141, but without loss of autotrophic AOB.



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204 **Figure 4.** Shifts in bacterial community structure (family level) during the period of stable nitritation (Days 72 -168)
205 in the pilot-scale reactor as determined by Illumina sequencing of 16S rRNA . The relative abundance of known
206 heterotrophic AOB (*Xanthomonadaceae*, *Chitinophagaceaea*), autotrophic AOB (*Nitrosomonadaceae*), and
207 heterotrophic denitrifying bacteria (*Comamonadaceae, Actinomycetales*) are highlighted.

208 For the pilot-scale reactor, nitrogen mass balances indicated removal of ~30-35% of influent
209 nitrogen, likely by denitrification (**Figure 3**). At low DO (0.1-0.2 mg/L), simultaneous
210 nitrification/denitrification confers a competitive advantage on heterotrophic nitrifiers, which have
211 low rates of nitritation, but higher specific growth rates[30] as reducing power can be diverted to
212 denitrifying enzymes[10]. *Comamonadaceae*, a family known to harbor many denitrifying species,
213 was present at a relative 16S gene abundance of 8% (**Figure 4**) and likely contributed to
214 denitrification[17]. An additional factor contributing to heterotrophic AOB activity was the overdose
215 of nitrogen-containing coagulants and resulting particulates in the feed. This factor was evaluated

in follow-up bench scale studies.

Follow-up bench-scale SBR studies: performance and community structure

Two lab-scale SBRs treating DDWTP anaerobic digester centrate were used to assess the effects of coagulants dosage on nitritation. The inoculum for both SBRs was pre-adapted to varied levels of coagulant and contained significant *Nitrosomonas eutropha*, *Xanthomonadaceae* (KC252880), *Rhodanobacter sp.* (FJ821729), and *Trueperaceae*. Both SBRs carried out efficient and stable oxidation of centrate ammonia to nitrite (**Figure S5**), and both SBRs included autotrophic AOB (*Nitrosomonadaceae*) and heterotrophic AOB (*Xanthomonadaceae*, *Chitinophagaceae*) (**Figure 5**). Control SBR received centrate only, and *Rhodanobacteraceae* decreased in relative abundance from 10% after initiation of the SBR to negligible levels by the end of the test period. By contrast, *Rhodanobacteraceaea* persisted at a relative abundance of 10-20% in the Test SBR fed centrate supplemented with 30 mg/L coagulants. This observation and the dramatic increase of *Rhodanobacteraceae* observed in the inoculum when spiked with coagulants at a high level (300 mg/L) suggest that the growth of this strain was stimulated by coagulant addition, but efforts to isolate the strain were not successful. One other notable difference was increased dominance of *Comamonadaceaea* in the SBR fed centrate alone, but loss of *Comamonadaceaea* in the SBR fed centrate plus coagulants.

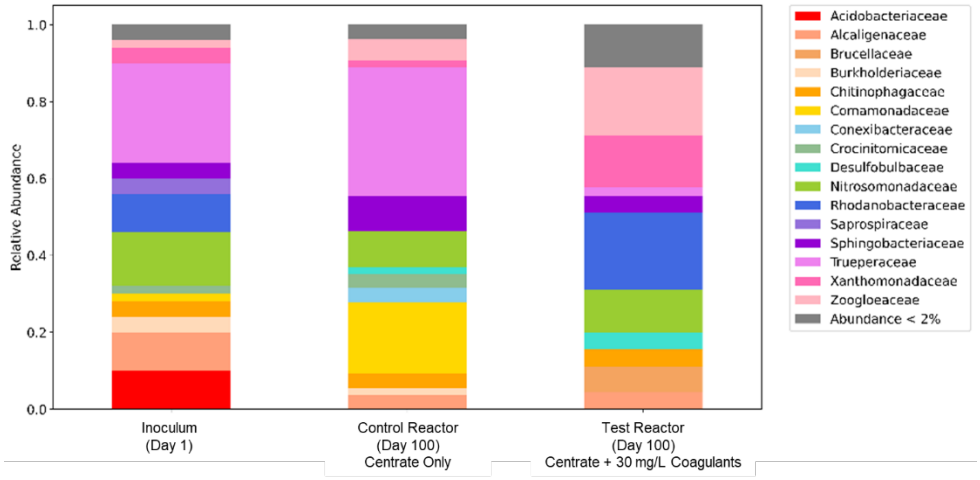
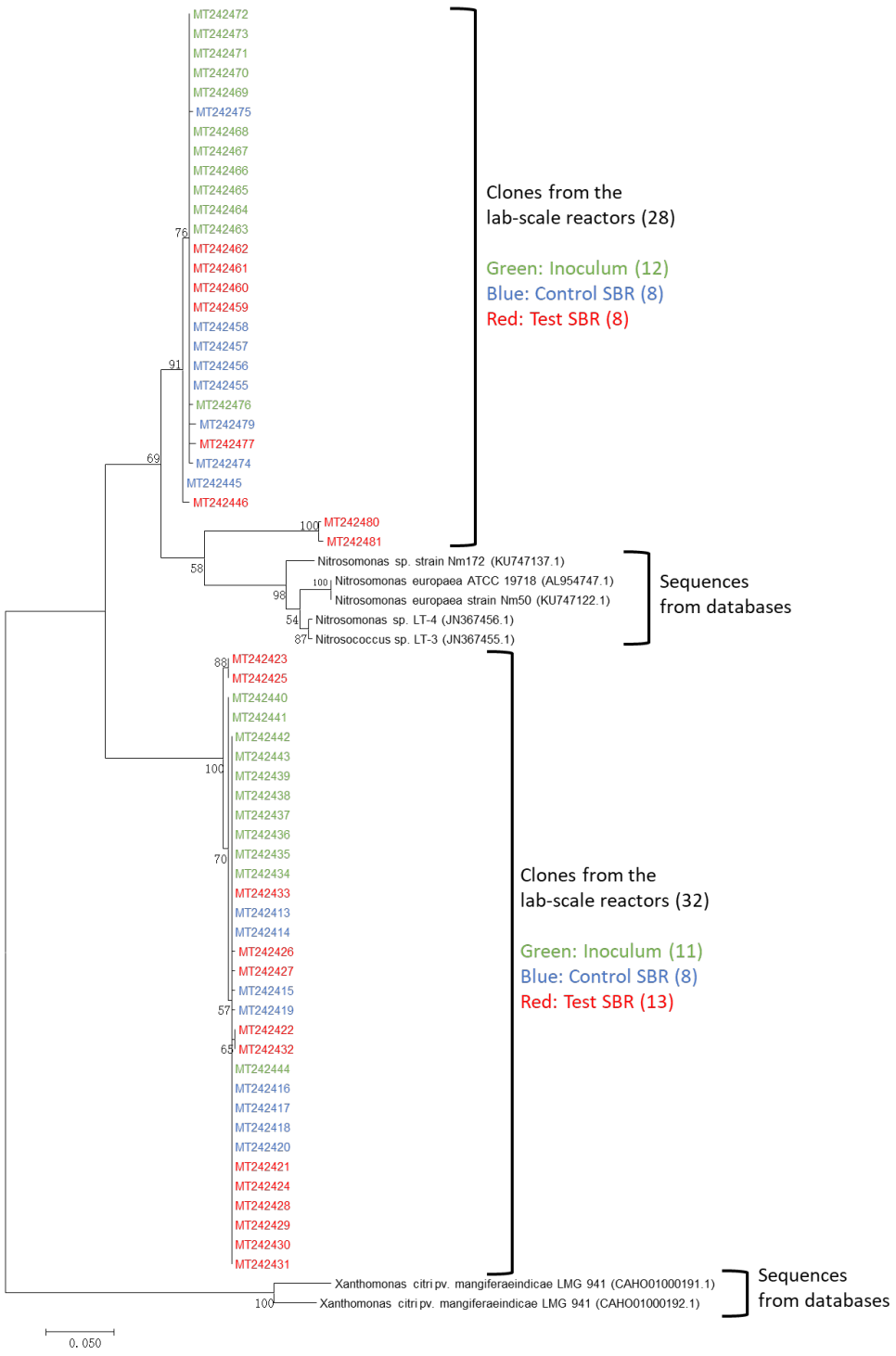


Figure 5. 16S rRNA clone library analyses of bench-scale nitritation reactors on Day 1 (initial) and Day 100 (final). The Control and Test Reactors were inoculated with the same inoculum.

The primer set used to assess *amoA* diversity in lab-scale SBRs captured 60 clones with novel *amoA* sequences. A phylogenetic tree was constructed using these sequences and *amoA* sequences for autotrophic and heterotrophic AOB (**Figure 6**). The lower part of the tree contained 13 of 21 clones (62%) from the Test SBR fed centrate plus coagulants, 8 of 16 clones (50%) from the Control SBR fed centrate alone, and 11 of 23 clones (48%) from the inoculum. The upper part of the tree contained sequences from 32 clones including 13 from the Test SBR. This section was more closely related to known sequences for *Nitrosomonas*, *Nitrosococcus* and *Xanthomona.* Interestingly, sequences for two clones (MT242480 and MT242481) from the Test SBR clustered in a unique location, and their *amoA* genes appear far less related to available *amoA* genes from *Nitrosomonas* strains in Genbank (< 89% similarity).



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247 **Figure 6** Evolutionary relationship of 67 *amoA* sequences using the maximum-likelihood method. The percentage of
248 replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to
249 the branches. Sixty clones were sequenced.

Batch assays to assess heterotrophic nitrification

Acetylene and allylthiourea are inhibitors of autotrophic ammonia oxidation and are commonly used to distinguish autotrophic from heterotrophic ammonia oxidation[24, 25]. To determine the stimulation effect of nitrogen-containing coagulants to heterotrophic nitritation, acetylene and allylthiourea were added to mixed liquor from each SBR in batch assays. From particulate COD measurements, the black particulates in centrate contained significant amount of organics. Therefore, glucose was added to assess the enhancement of heterotrophic nitritation by organics. The results are summarized in **Figure 7**.

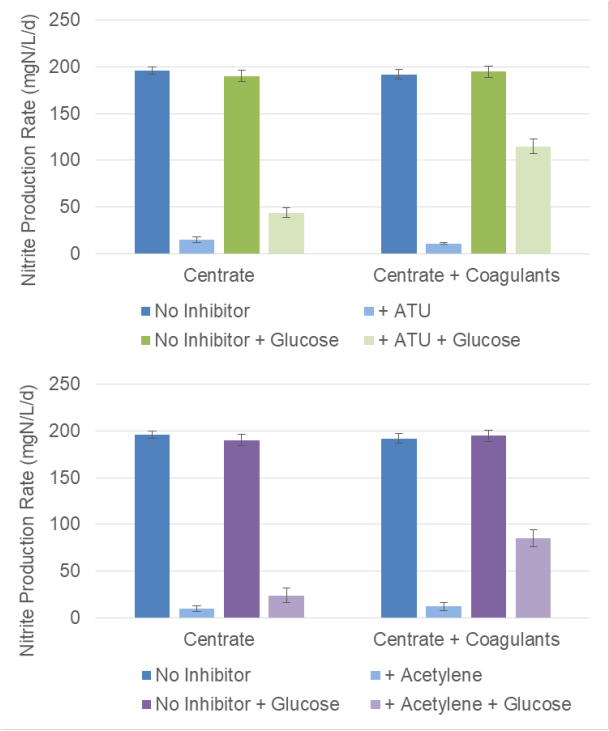

Figure 7 Effects of inhibitors (acetylene, allylthiourea) on autotrophic ammonia oxidation (uninhibited/inhibited), effects of glucose addition on heterotrophic nitritation (stimulated/not stimulated) with comparison of Control and Test SBR biomass. Initial ammonia concentration was 200 mgN/L. No nitrate was detected.

Highest rates of nitritation were observed in the absence of autotrophic AOB inhibitors (acetylene and ATU); lowest rates were observed in the presence of autotrophic AOB inhibitors with no added

glucose. Glucose addition stimulated heterotrophic nitritation when autotrophic nitritation was inhibited. The stimulatory effect of glucose addition on heterotrophic nitritation was most dramatic when glucose was added to Test SBR biomass adapted to 30 mg/L coagulants. This increase correlated with increased prevalence of *Rhodanobacteriaceae* in the Test SBR (**Figure 5**).

Effects of coagulants overdose on downstream treatment and biosolids

Further investigations were carried out to characterize the black particulates produced by coagulants overdose. To assess metal content, the particulates were filtered out then dissolved in nitric acid and analyzed by inductively coupled plasma atomic emission spectroscopy (ICP-OES)[31]. Major metal elements detected in the dried solids were iron (3.8%), aluminum (2.9%), calcium (2.9%) and magnesium (1.8%). The dried particulate contained organic nitrogen at 0.14 gN/g dry solids and organic matter at 0.75 g COD per g dry solids, which may have promoted heterotrophic growth. The performance of biological nitrogen removal processes, such as Anammox and CANDO, can be adversely affected by fluctuations in particulate organic loadings[16, 17].

Another important factor affecting nitritation of centrate is the nitrogen-containing coagulants. The coagulants used in the field study is a proprietary commercial mixture of polyamine, polyamide and polyDADMAC-based compounds. The coagulants themselves can be a significant source of organic nitrogen, with nitrogen content ranging from 9% in polyDADMAC to 20% in polyacrylamide. Heterotrophic bacteria are known to hydrolyze amide and release ammonia from polyacrylamide.[32] The carbon backbone of the polymer, on the other hand, likely resists biological depolymerization.[33, 34] A recalcitrant polyacrylate residue may thus remain in the centrate effluent and contribute to COD.

Many *Xanthomonas*-related bacteria are plant pathogens by virtue of metabolic pathways that enable them to synthesize and degrade polyamines, which are secreted by plant hosts as a defense response to infections[35]. In fact, polyamine synthesis profiles have been used to classify *Xanthomonas*[36]. *Xanthomonas maltophilia* can hydrolyze acrylamide, the monomer of polyacrylamide, releasing ammonia and acrylic acid[37]. Amidase is a key enzyme within heterotrophic nitrifiers and can potentially enable utilization of polyacrylamide as a nitrogen source. Research is needed to determine whether selection for *Xanthomonas* and other plant pathogens, such as some species of *Burkholderia*, occurs in soils containing coagulant-treated biosolids[38]. Soil-mediated nitrification of coagulant-treated biosolids could also select for *Rhodanobacter*, some of which confer benefits for phytopathogen control.[39, 40]

Conclusions

The pilot-scale nitritation reactor enabled stable production of nitrite with 35% total nitrogen removal, likely through denitrification to N_2. During steady-state operation, the microbial community contained *Nitrosomonadaceae*, a family of autotrophic AOB, and *Xanthomonadaceae*, and *Chitinophagaceae*, families known to include heterotrophic nitrifying bacteria, and denitrifying bacteria *Comamonadaceae* and *Actinomycetales*. Follow-up bench-scale studies established that heterotrophic nitritation is promoted by the presence of soluble biodegradable organic matter and selection pressures resulting from the presence of nitrogen-containing polymeric coagulants. Heterotrophic AOB rely upon *amoA* genes that differ from those of autotrophic AOB. Further study is needed to clarify the association between coagulant dosage and heterotrophic nitritation, and its potential impacts on the quality of biosolids for land application.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

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