Electronic Supplementary Material (ESI) for Environmental Science: Processes & Impacts.

Filamentous sludge bulking control by nano zero-valent iron in activated sludge treatment systems

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Supporting Information

Table S1. Primer used for filamentous bacteria detection

Target	Primer	Sequence (5'-3')	References
Most 021 N group strains	21Nf	CGTAGGCGGCTCTTTAAGTCRGAT	(Vervaeren et al., 2005)
Most 021 N group strains	21Nr	CCGACGGCTAGTTGACATCGTTTA	(Vervaeren et al., 2005)
Gordonia spp.	G268f	CGACCTGAGAGGGTGATCG	(Nielsen et al., 2004)
Gordonia spp	G1096r	ATAACCCGCTGGCAATACAG	(Nielsen et al., 2004)
Candidatus Microthrix parvicella	M1f	GGTGTGGGGAGAACTCAACTC	(Kumari et al., 2009)
Candidatus Microthrix parvicella	M2r	GACCCCGAAGGACACCG	(Kumari et al., 2009)
T.eikelboomii AP3	Cpn60 49/50f	ATAATAATGCGATTGCTCAAG	(Dumonceaux et al., 2006)
T.eikelboomii AP3	Cpn60 49/50r	ACGACTAAAGTGGCTAAGG	(Dumonceaux et al., 2006)
Bacterial 16S rRNA	1055f	ATGGCTGTCGTCAGCT	(Harms et al., 2003)
Bacterial 16S rRNA	1392r	ACGGGCGGTGTGTAC	(Harms et al., 2003)

Table S2. Primer used for nitrifiers in this study

Target	Primer	Sequence (5'-3')	References
Bacterial 16S rRNA	Eub338f	(6-FAM)-ACTCCTACGGGAGGCAGC	(Amann et al., 1990)
AOB 16S rRNA	Nso1225r	CGCCATTGTATTACGTGTGA	(Mobarry et al., 1996)
Nitrospira 16S rRNA	Ntspa685r	CGGGAATTCCGCGCTC	(Regan et al., 2002)

Table S3. Primer and DNA Concentrations used in q-PCR

Methods	Component in one well	Stock Conc.	Volume per rxn (µL/well)	Final Conc in a 25-µL PCR rxn
SYBR Green [1]	F/ R Primer	10 µM	1	0.4 µM
	DNA Template (with 100 time dilution)	0.9-2.3 ng/µL	5	0.2-0.5 ng/µL
	SYBR PCR Mix	X	12.5	0.5 X
	PCR grade water		3.75	
	EDTA	10 mM	1.25	0.5 mM
	$MgCl_2$	25 mM	0.5	0.5 mM
Taqman Probe[2]	F/ R Primer	10 µM	1	0.4 µM
	Probe	10 µM	0.5	0.2 µM
	DNA Template (with 100 time dilution)	0.9-2.3 ng/µL	5	0.2-0.5 ng/µL
	Tapman PCR Mix	X	12.5	0.5 X
	PCR grade water		3.25	
	EDTA	10 mM	1.25	0.5 mM
	$MgCl_2$	25 mM	0.5	0.5 mM

[1] SYBR green method was for Type 021N and total PCR volume was 25 µL.

[2] Taqman probe method was used for total bacteria determination and total PCR volume was 25 µL.

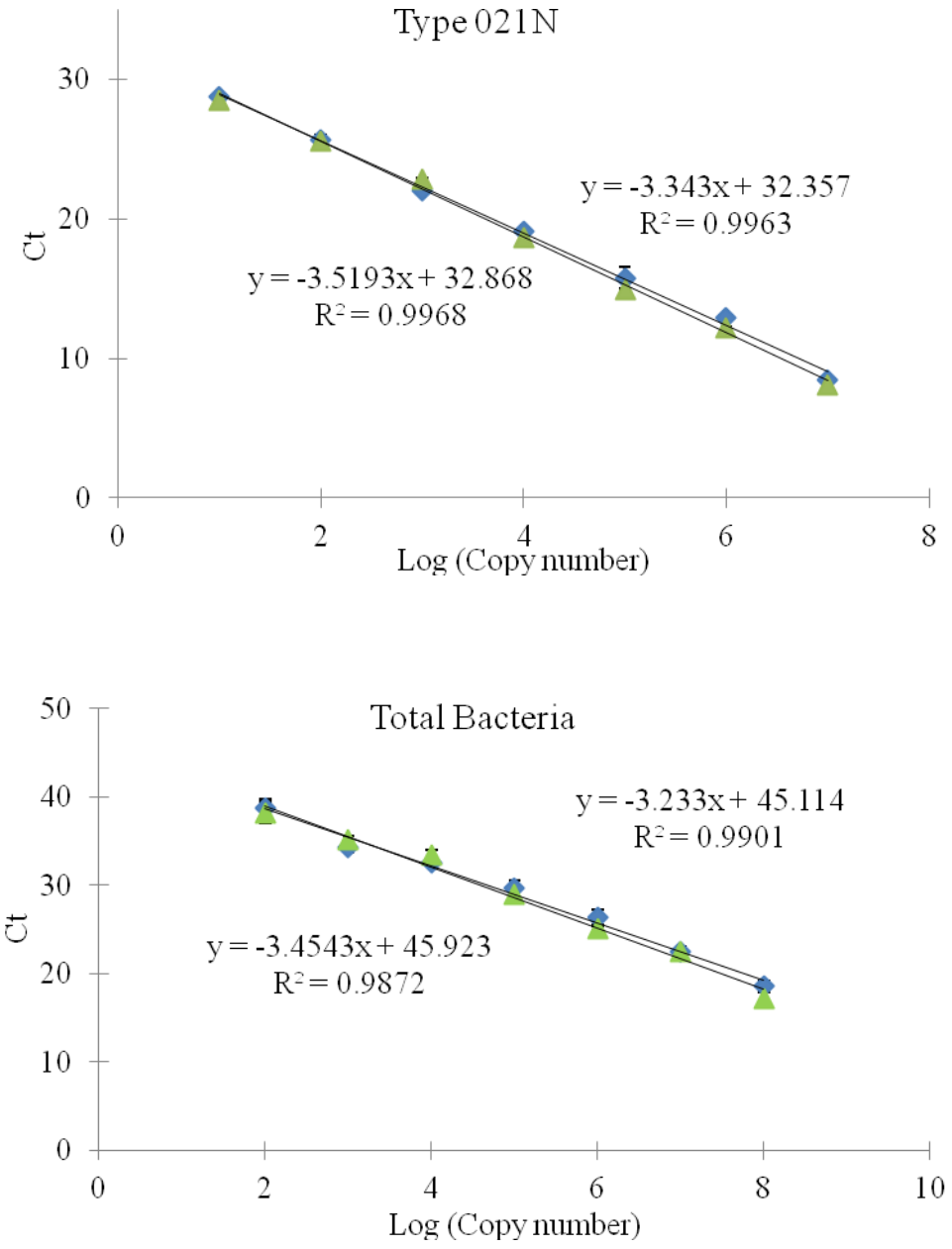


Figure S1. qPCR standard curves for Type 021N and total bacteria in the presence (◆) and absence (▲) of EDTA and $MgCl_2$ in PCR reactions. Error bars represent one standard deviation from the mean of triplicate samples.

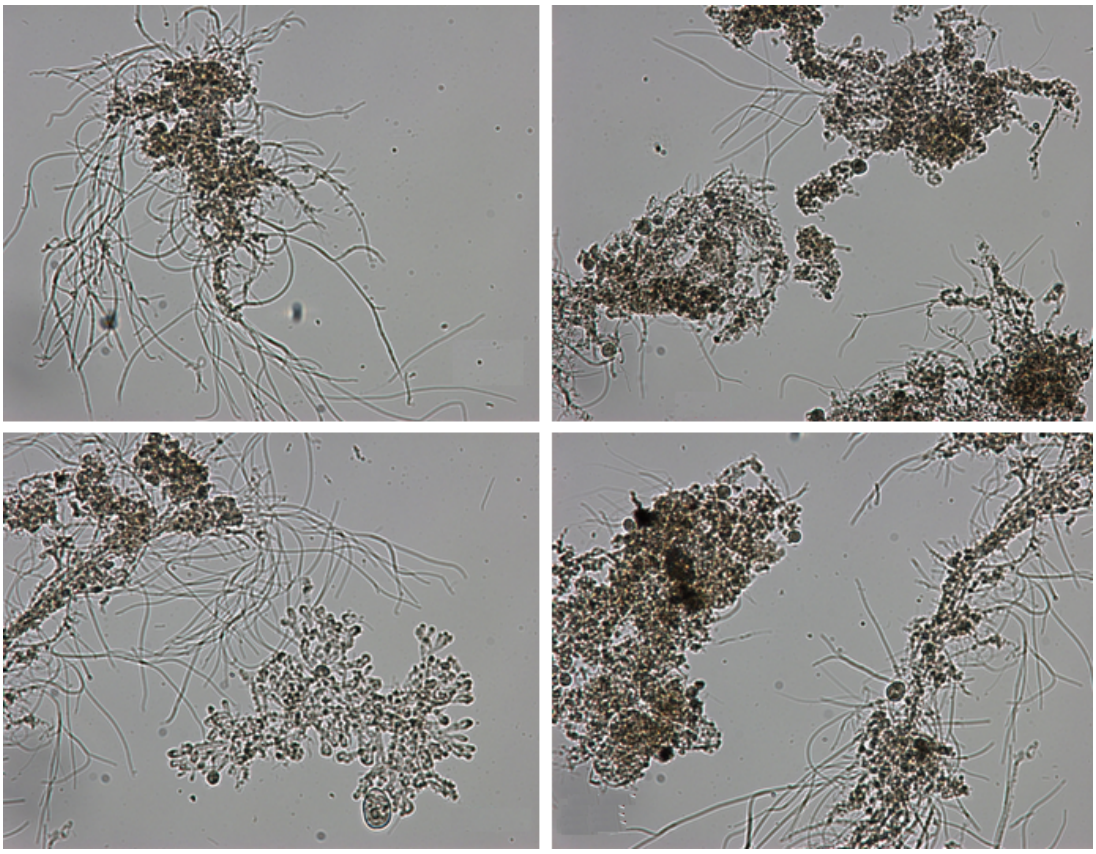


Figure S2. Micrographs of light microscopy of activated sludge samples from Tank #1 (left panel) and Tank #2 (right panel) taken on day 80 and 100, respectively.

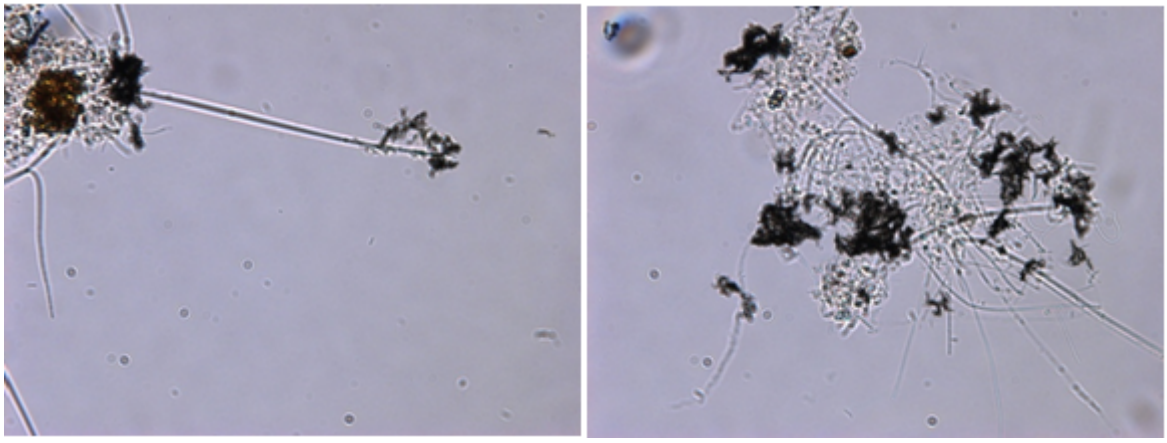


Figure S3. Micrographs of light microscopy of activated sludge samples from Tank #1 (left panel) and Tank #2 (right panel) taken 1 h after NZVI dosing in the MLE systems with agglomerated NZVI structure shown in black.

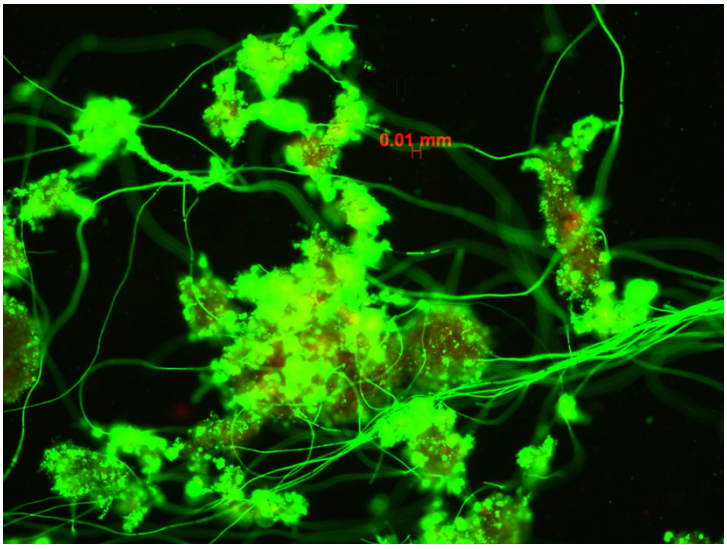


Figure S4. The viability of bulking activated sludge before NZVI treatment. Under florescence microscopy, living cells were stained green and dead cells were stained red.

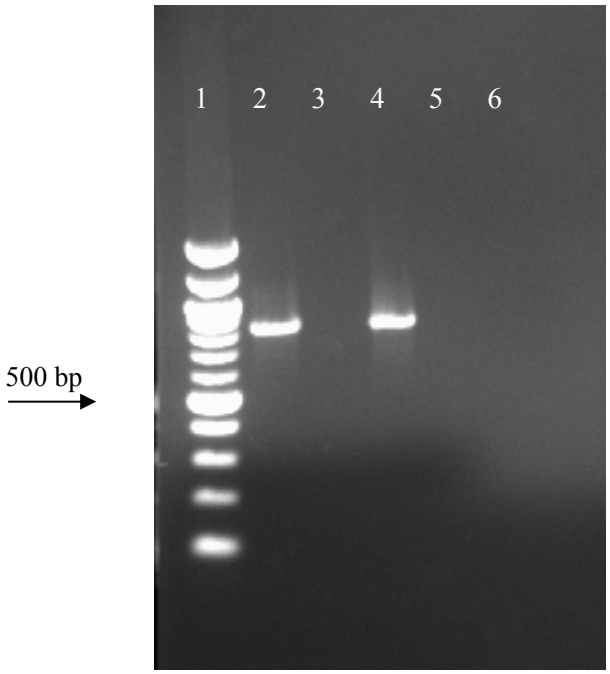
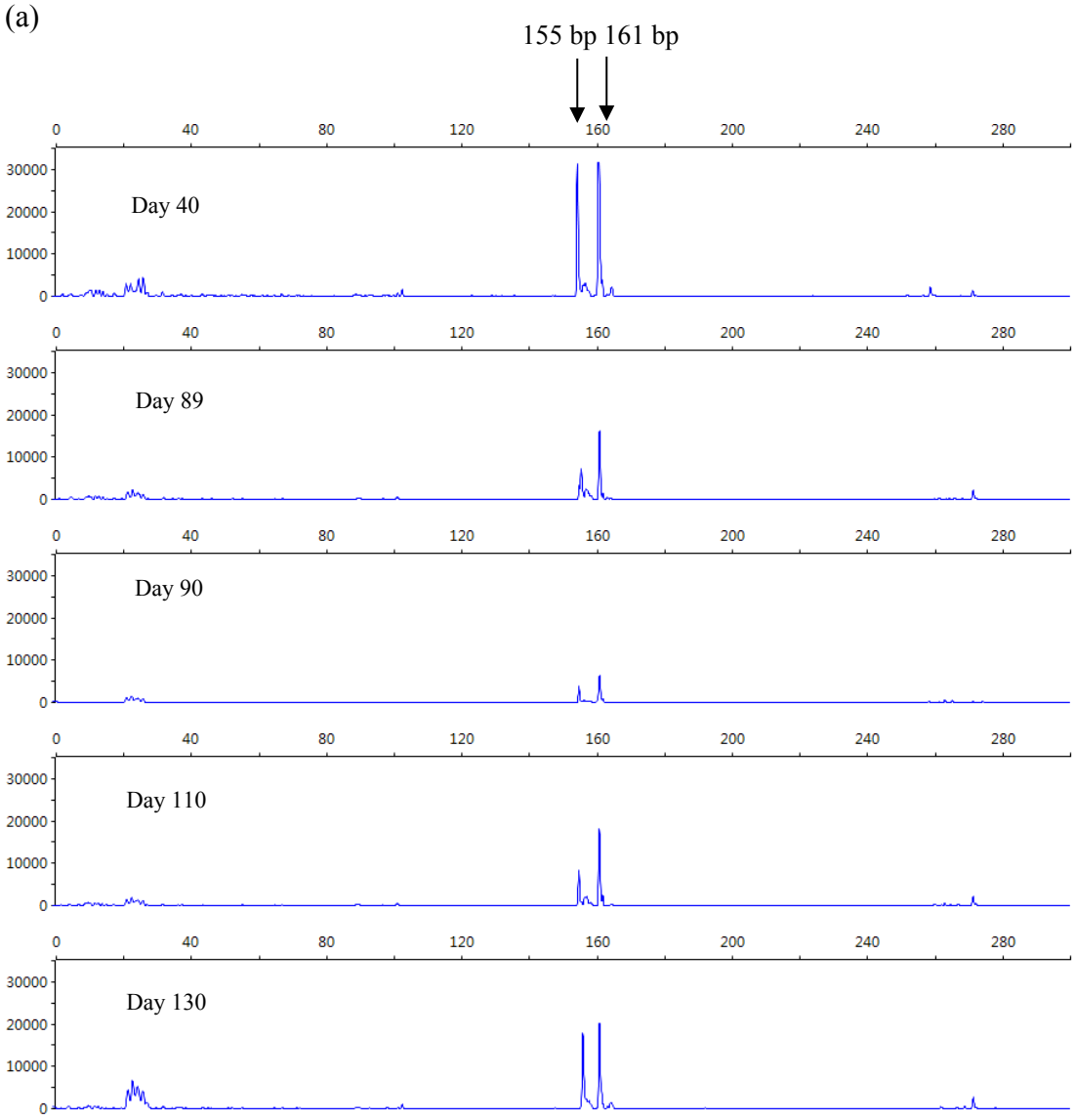


Figure S5. Conventional PCR analysis of the sludge samples targeting genomic DNA (829 bp) of *Gordonia* spp. Lane 1, molecular mass marker (100 bp plus DNA ladder); Lane 2, DNA template from Tank #1 before NZVI dosing; Lane 3, DNA template from Tank #1 one day after NZVI dosing; Lane 4, DNA template from Tank #2 before NZVI dosing; Lane 5, DNA template from Tank #2 one day after NZVI dosing; Lane 6, negative control (no DNA template).

(a)


155 bp 161 bp
0
40
80
120
160
200
240
280
30000
20000
10000
Day 40
Day 89
Day 90
Day 110
Day 130

(b)

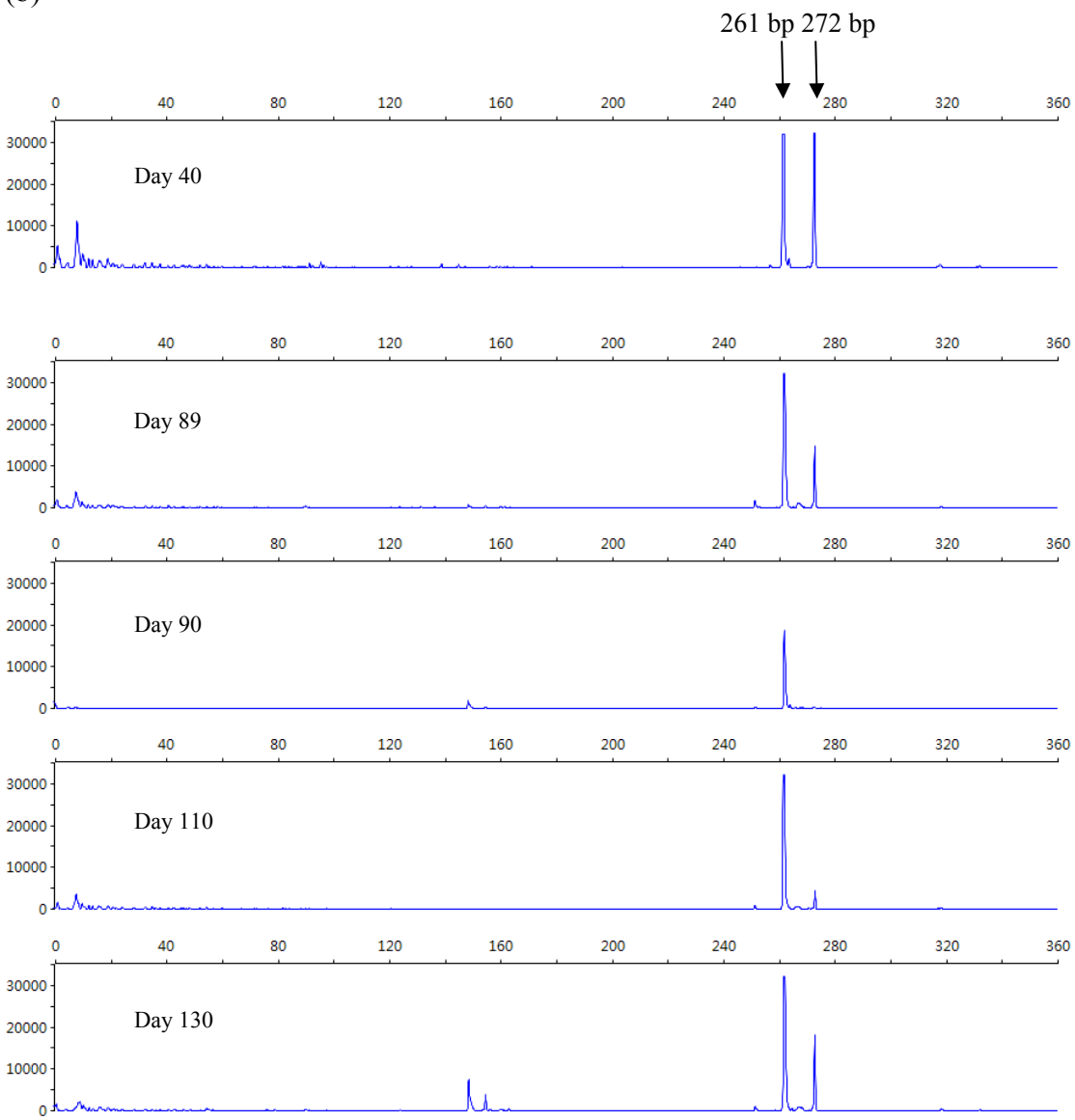


Figure S6. Nitrifying bacterial community composition reflected by T-RFLP profiles targeting 16S rRNA genes of *Nitrosomonas* (a) and *Nitrospria* (b) in Tank #1 before and after NZVI dosing on day 89. Arrows indicate significant T-RFLPs of AOB: 161bp for AOB Group-1 and 155 bp could belong to the uncharacterized AOB. Arrows also correspond to significant T-RFLPs of Nitrospira, 261 bp, and 272 bp.

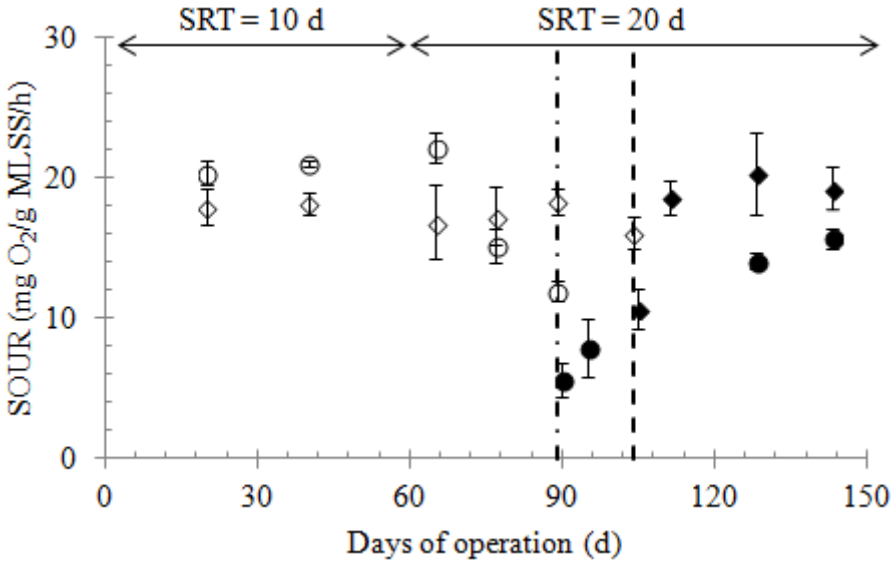


Figure S7. Autotrophic SOUR in Tank #1 (○) and #2 (◇) before NZVI dosing and in Tank #1 (●) and #2 (◆) after NZVI dosing on day 89 and day 104, respectively. Two vertical hashed lines show the days of NZVI addition in Tanks #1 and #2, respectively.

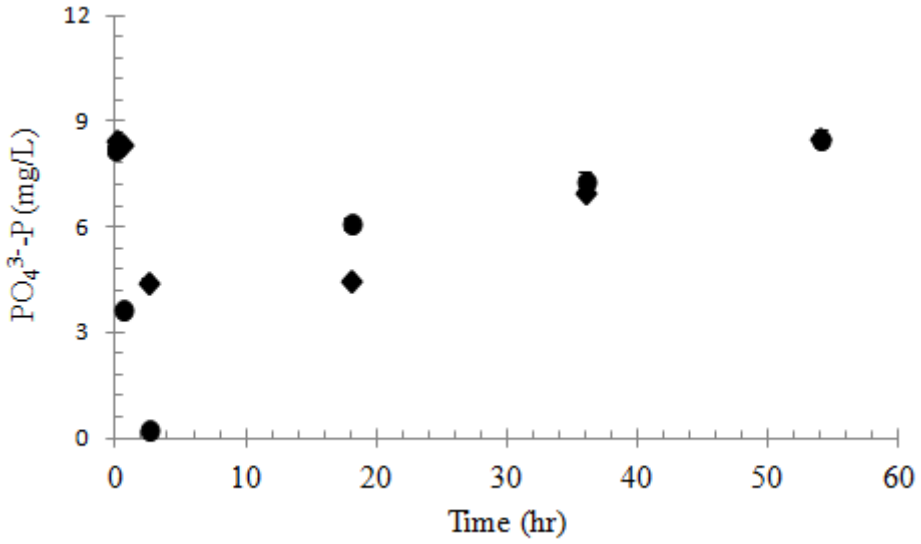


Figure S8. Effluent PO_4^{3-}-P concentrations in Tank #1 (●) and Tank #2 (♦) right after NZVI dosing. Error bars represent one standard deviation from the mean of duplicate samples.

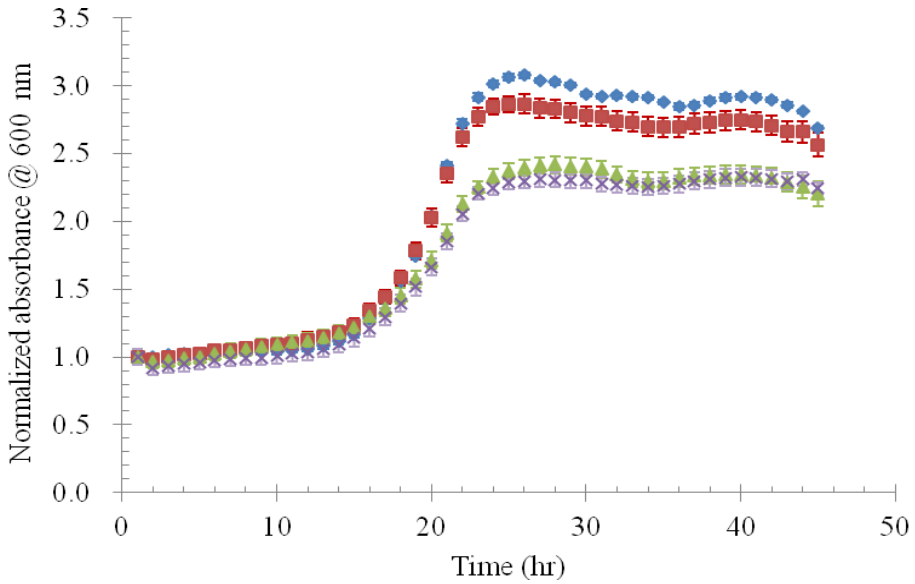


Figure S9. Aerobic bacterial growth, as indicated by optical density measurments at 600 nm, in the groups treated with different NZVI concentrations: Control (◆), 20 mg/L (■), 100 mg/L (▲) and 200 mg/L (×). Activated sludge from the Columbia WWTP was used as a seed culture. The error bars represent one standard error of the mean (n = 8).

Methods

1. Detailed procedures of PCR

PCR reactions were carried out in MicroAmp optical reaction plates (Applied Biosystems, Branchburg, NJ) containing 1 μL of each forward primer and reverse primer (stock concentration of 10 μM), 12.5 μL of SYBR Green PCR master Mix (Applied Biosystems, CA), 1.25 μL EDTA (10 mM), 0.5 μL $MgCl_2$ (25 mM), 3.75 μL of PCR water, and 5 μL sample DNA (a total of 4.3–11.6 ng DNA) in a 25 μL total volume. The qPCR reactions were performed starting at 50 °C for 2 min, followed by an initial denaturation at 95 °C for 10 min, and then 40 cycles of 95 °C for 15 s and 62 °C for 1 min. The dissociation step at 95 °C for 15 s and 60 °C for 1 min was added at the end to check the specificity of the PCR results (SI Table S3). For comparison purposes, qPCR was also applied to quantify total bacterial 16S rRNA gene copy number using primers 1055f and 1392r (SI Table S1). To reduce potential false-positive signals in total bacterial count, TaqMan-based detection was applied and the TaqMan probe 16S Taq1115 (6-FAM)-CAACGAGCGCAACCC-(TAMRA) was modified from the 1114f primer. The PCR Mix had a total volume of 25 μL consisting of 12.5 μL of TaqMan Universal PCR Master Mix (Applied Biosystems, CA), 1 μL of each forward primer and reverse primer (stock concentration of 20 μM), 0.5 μL of TaqMan probe, 1.25 μL EDTA (10 mM), 0.5 μL $MgCl_2$ (25 mM), 3.25 μL of PCR water, and 5 μL sample DNA.

The program for 16S rRNA gene amplification was set as follows: 10 min at 95 ˚C, 45 cycles of 30 s at 95 ˚C, 60 s at 50 ˚C, and 45 s at 72 ˚C.

2. NZVI toxicity evaluation

A turbidimetric microtiter assay was applied to evaluate the toxicity of NZVI on the growth of active sludge bacteria (at the concentration of about 2000 mg/L of biomass COD) at different concentrations (20, 100 and 200 mg Fe/L). Activated sludge samples from the Columbia WWTPs were washed with 1 × phosphate buffered saline (PBS) three times to remove the residue organic matter and nutrients before use. Aliquots (20 μL) of sludge samples in 8 replicates were added to the microplate wells followed by the addition of 180 μL sterile basal mineral medium in each well. The absorbance of cell culture in each microwell was measured at 600 nm every 1 hour for about 48 h by a microreader (VICTOR3, PerkinElmer, Shelton, USA). Activated sludge sample with no NZVI treatment served as control.

3. SOUR measurement

Aliquots (120 mL) of biomass sample were collected from the aeration chamber of each bioreactor and poured into two 50 mL respirometric bottles. After 3 min of aeration with pure oxygen gas, the respirometric bottles were tightly capped with no air space. MOPs (3-(N-morpholino) propanesulfonic acid) were added at a final concentration of 20 mM to maintain a constant pH at 7.5. At a predetermined time, an aliquot of substrate (10 mg N/L NH_4^+-N) was injected using a 10-μL syringe. A decrease of dissolved oxygen (DO) level in the respirometric bottles due to substrate oxidation was measured by the DO probe and continuously monitored at 4 Hz by an interfaced computer. Oxygen uptake rates were calculated based on a linear regression analysis because zero-order reactions were observed

for a long period of time. SOUR was calculated by dividing OUR by biomass concentration of each sample. All SOUR experiments were carried out in at least duplicate.

Results

Figure S9 shows bacterial growth curves in the presence of NZVI using a microtiter assay. Compared with the control having the specific bacterial growth rate of 2.36 ± 0.37 d^{-1}, the presence of NZVI at 20, 100 and 200 mg/L reduced the specific growth rates to 2.16 ± 0.52 d^{-1}, 1.61 ± 0.28 d^{-1} and 1.59 ± 0.43 d^{-1}, respectively. By increasing the NZVI concentration from 20 to 100 mg/L, the reduction in bacterial growth was increased from 8% to 32%. However, further reduction was not observed when NZVI concentration was increased to 200 mg/L. Therefore, an instantaneous, one-time dose of NZVI at the final concentration of 100 mg Fe/L in the mixed liquor was applied in the MLE bioreactors for sludge bulking control.