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Supplementary Material

Impact of ferrous iron dosing on iron and phosphorus solids speciation and transformation in a pilot scale membrane bioreactor

Hao Wu[1], Yuan Wang[1], Atsushi Ikeda-Ohno[2], Christopher J. Miller[1] and T. David Waite[1,*]

[1] Water Research Centre, School of Civil & Environmental Engineering, The University of New South Wales, Sydney 2052, Australia

[2] Collaborative Laboratories for Advanced Decommissioning Science (CLADS), Japan Atomic Energy Agency (JAEA), 2-4 Shirakata, Tokai-mura, Naka-gun, Ibaraki 319-1195, Japan

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*Corresponding author: Professor T. David Waite, Email: d.waite@unsw.edu.au

X-ray absorption spectroscopy (XAS)

Data collection

Iron speciation was studied by X-ray absorption spectroscopy including both X-ray absorption near-edge structure (XANES) and extended X-ray absorption fine structure (EXAFS) regions at the Fe K-edge (7112.0 eV). The X-ray absorption spectra were collected on beamline 17C1 at the National Synchrotron Radiation Research Center (NSRRC), Taiwan (Tsang et al. 1995) using a Si(111) double-crystal monochromator with a Rh-coated mirror. Samples were ground to fine powder, pressed into a pellet of 5 mm diameter and 1.0 to 2.5 mm in thickness, and sealed in plastic bags. For iron reference compounds and some sludge samples with high iron concentrations, a small amount of the sample was mixed with boron nitride to dilute the sample to achieve an appropriate edge jump at the Fe K-edge. The X-ray energy of the acquired spectra was calibrated by the simultaneous measurement of the Fe K-edge for Fe metal foil (defined as 7112.0 eV at the first inflection point). The measurement was performed in transmission mode using He-filled ionization chambers under ambient temperature and pressure. All the collected spectra were processed according to a standard procedure (Koningsberger and Prins 1988) using the programs Athena (Ravel and Newville 2005) and WinXAS (Ressler 1998). The threshold energy, $E_{k=0}$, was defined at 7128.5 eV. EXAFS spectra were extracted using the Autobk algorithm ($R_{\mathrm{bkg}} = 0.9$; k-weight = 3).

For determination of the speciation of P-containing solids, XAS spectra were collected at the P K-edge (2145.5 eV) on beamline 16A1 at NSRRC (Dann et al. 1998) using a Si(111) double-crystal monochromator with a Ni-coated mirror. The dried sample powder was ground and spread homogeneously on Kapton tape immediately before the measurement and transferred into a He-filled sample chamber at the beamline. Reference phosphorous compounds were diluted with boron nitride to obtain optimal fluorescence signals, as well as to minimize self-absorption effects associated with high P loadings. The X-ray energy was calibrated by measuring the L_{III}-edge of Nb foil (defined as 2370.5 eV at a first inflection point). The X-ray absorption data was collected in fluorescence mode using an ion chamber filled with He gas (for I_0) and a Lytle detector filled with N_2 gas (for I_F) at ambient temperature. The data treatment was undertaken in the same manner employed for the analysis of the Fe K-edge XAS data.

Principal component analysis (PCA), target transformation (TT) and linear combination fitting (LCF)

The Fe K-edge EXAFS spectra were analyzed in a two-step procedure. In the first step, the extracted EXAFS spectra of the sludge samples were analysed by principal component analysis (PCA) (Ressler et al. 2000) using the SIXPACK code (Webb 2005) to determine the number of independent components (i.e. principal components) in a set of the spectra, which corresponds to the maximum number of significant Fe species which enable the reproduction of the series of experimental data. Based on the principal components obtained from the PCA results, target transformation (TT) was further performed in order to find possible reference spectra which can be reasonably reproduced with the principal components of the system (Noël et al. 2014), that is, the reference spectra which, statistically, can be meaningfully used for the subsequent linear combination fitting (LCF) analysis. Based on the results from the PCA/TT, LCF analysis was performed in the second step using the software Athena (Ravel and Newville 2005) to calculate the fractions (%) of each Fe reference present in the sludge samples.

A range of reference compounds was selected to represent the Fe(II), Fe(III) and P compounds which were potentially present in the sludge. The following compounds were considered in this study: Fe oxyhydroxides (goethite (α-$Fe^{III}O(OH)$), lepidocrocite (γ-$Fe^{III}O(OH)$), ferrihydrite ($Fe^{III}_2O_3 \cdot 0.5H_2O$), akaganéite ($\beta$-$Fe^{III}O(OH)$), hematite ($\alpha$-$Fe^{III}_2O_3$), maghemite ($\gamma$-$Fe^{III}_2O_3$), magnetite ($Fe^{II,III}_3O_4$), schwertmannite ($Fe^{III}_8O_8(OH)_6SO_4$)), Fe phosphate minerals (strengite ($Fe^{III}PO_4 \cdot 2H_2O$), vivianite ($Fe^{II}_3(PO4)_2 \cdot 8H_2O$)), Fe sulfide minerals (pyrite (FeS_2), mackinawite (FeS)) and Fe(III) citrate ($C_6H_5FeO_7$). The above list of Fe compounds represents the potential Fe products that could be formed during the oxidation and transformation of the dosed iron. Fe(III) phosphate minerals of different composition could occur, however, their EXAFS spectra would be expected to be similar due to the exclusive presence of O shells (i.e. O_{PO4} and O_{H2O}, both coordinating in a monodentate fashion) in the primary coordination sphere of the Fe centre. In fact, the EXAFS spectrum for strengite (Figure S2 in SI) resembles the reported spectrum for santabarbaraite ($Fe^{III}_3(PO_4)_2(OH)_3 \cdot 5H_2O$) (Pratesi et al. 2003). Strengite has also been frequently used as a representative of Fe(III) phosphate minerals in other works (e.g. Voegelin et al. 2010). Therefore, strengite was used as a representative of the possible Fe(III) phosphate minerals present in the sludge in this study. Although the structure of organic matter (OM) is not well defined, it is believed that the major functional groups in OM are carboxyls, phenolic OH, quinones and alcohol OH. Spectroscopic data have confirmed that carboxyl groups are primarily responsible for metal-OM complexation (Boyd et al. 1981, Rose and Waite 2003). Therefore, carboxylic organic acids (OA) can be treated as simplified models for OM. Indeed, comparison of the EXAFS spectrum of Fe(III) citrate with the spectra of other Fe(III)-OM complexes (e.g. iron(III) complexed with fulvic acids (Sjöstedt et al. 2013)) revealed no significant spectroscopic difference. Therefore, Fe(III) citrate is used to represent the iron organic matter (Fe(III)-OM) complexes that may be present in the sludge.

The PCA/TT and LCF analysis was also applied to the P K-edge XANES spectra. The following compounds were considered as possible references: vivianite, strengite (Fe(III)-phosphate), sodium polyphosphate, sodium tripolyphosphate, phytic acid, adenosine triphosphate (ATP), phosphate adsorbed on AFO (P-adsorbed), $CaHPO_4$, $MgHPO_4$ and Na_3PO_4.

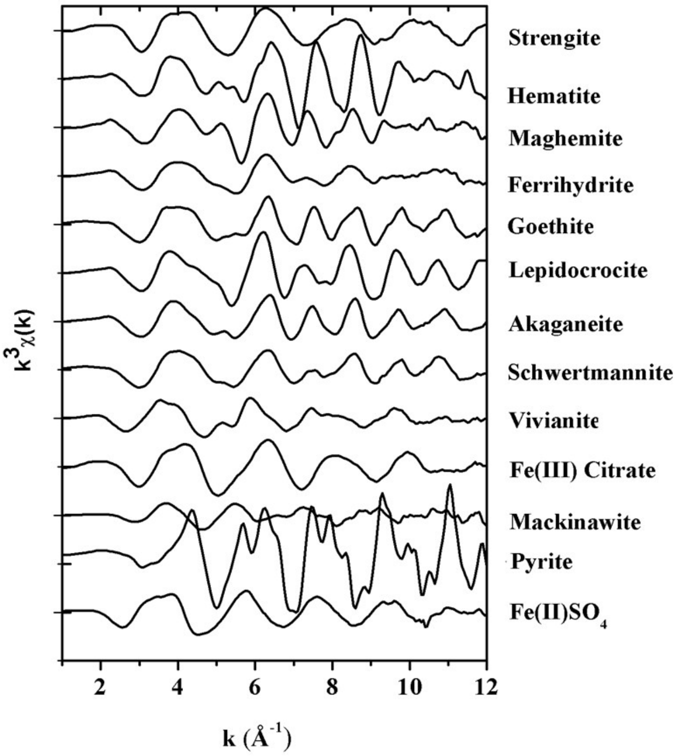

Figure S1. **k^3-weighted Fe K-edge EXAFS spectra of Fe references.**

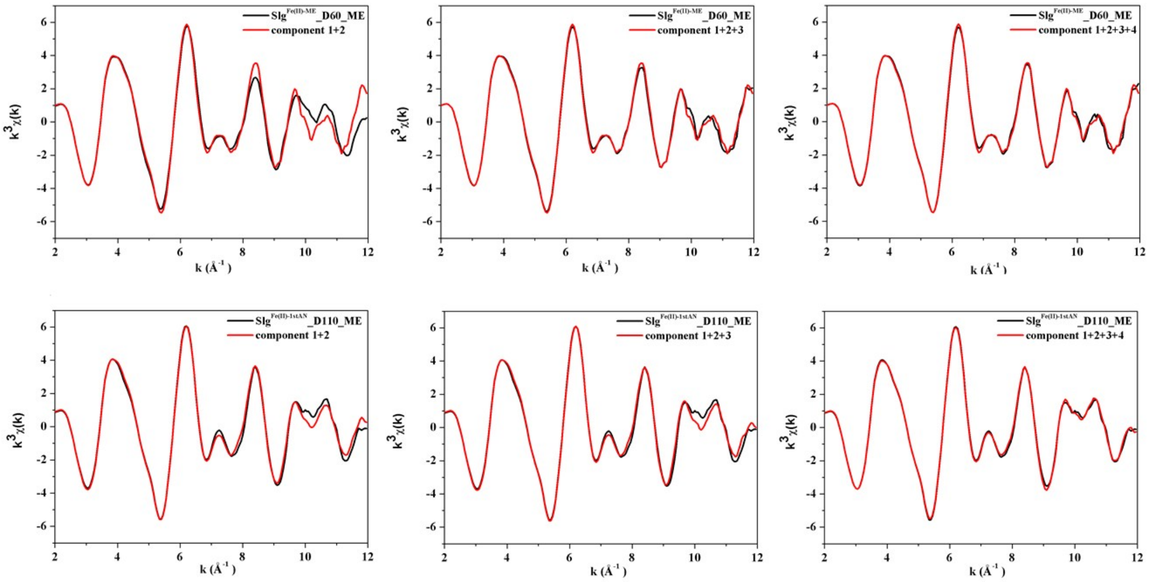

Figure S2. Reproduction of Fe K-edge EXAFS spectra (k^3-weighted) for Slg$^{Fe(II)-ME}$_D60_ME (top) and Slg$^{Fe(II)-1stAN}$_D110_ME (bottom) by principal components 1+2, components 1+2+3, and components 1+2+3+4.

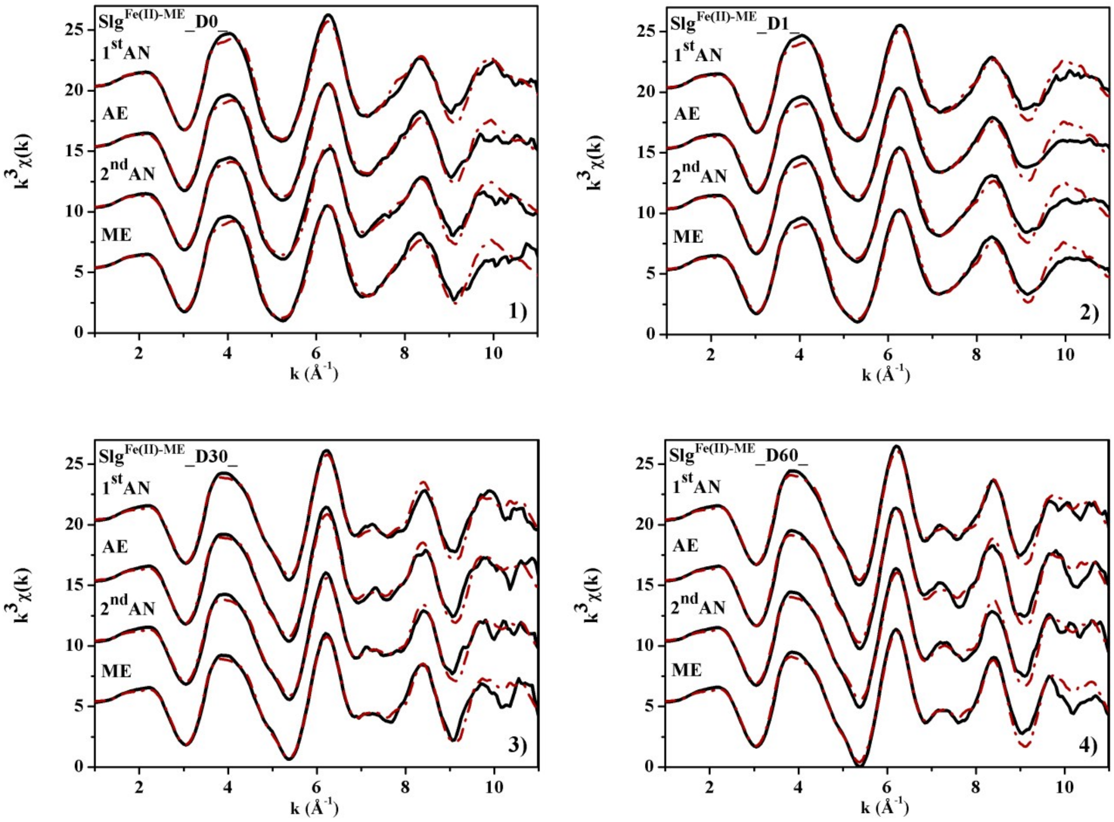

Figure S3. k^3-weighted Fe K-edge EXAFS spectra sludge samples from pilot scale MBRs with membrane chamber as the dosing position, 1) Slg$^{\text{Fe(II)-ME}}$_D0, 2) Slg$^{\text{Fe(II)-ME}}$_D1, 3) Slg$^{\text{Fe(II)-ME}}$_D30, 4) Slg$^{\text{Fe(II)-ME}}$_D60. The black solid lines represent the sample spectra, the red dash dot lines represent the reproduction by LCF.

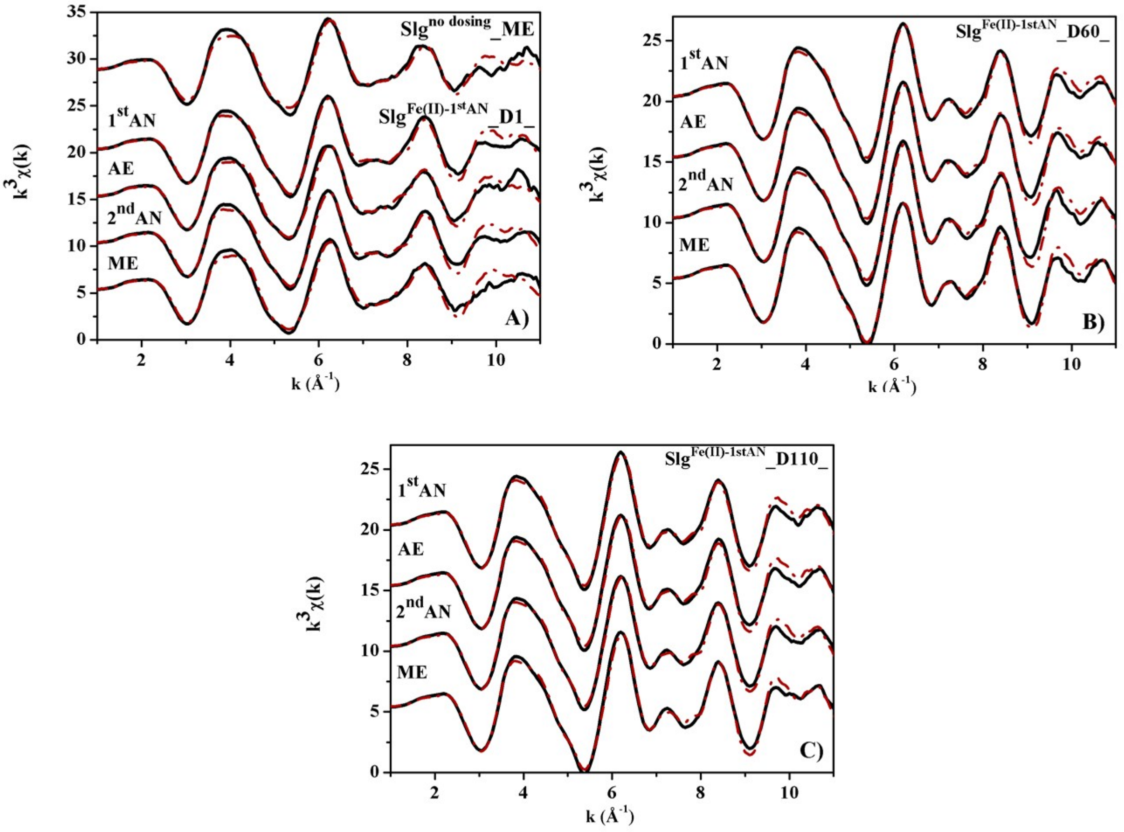

Figure S4. **k^3-weighted Fe K-edge EXAFS spectra sludge samples from pilot scale MBRs with 1st anoxic chamber as the dosing position, A) Slg$^{\text{no dosing}}$_ME and Slg$^{\text{Fe(II)-1stAN}}$_D1, B) Slg$^{\text{Fe(II)-1stAN}}$_D60, C) Slg$^{\text{Fe(II)-1stAN}}$_D110. The black solid lines represent the sample data while the red dash dot lines represent the reproduction by LCF.**

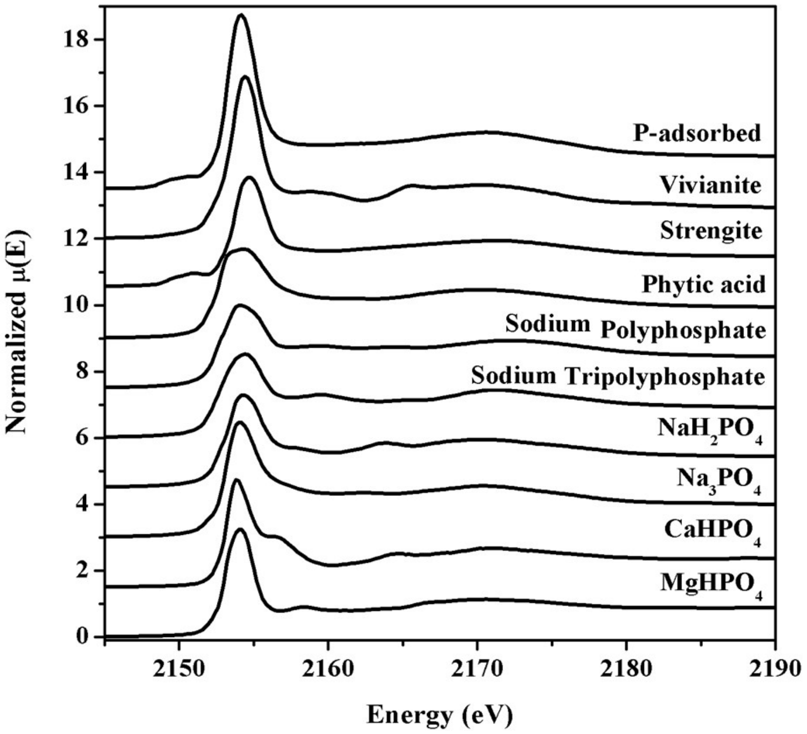

Figure S5. Normalized P K-edge XANES spectra of P references.

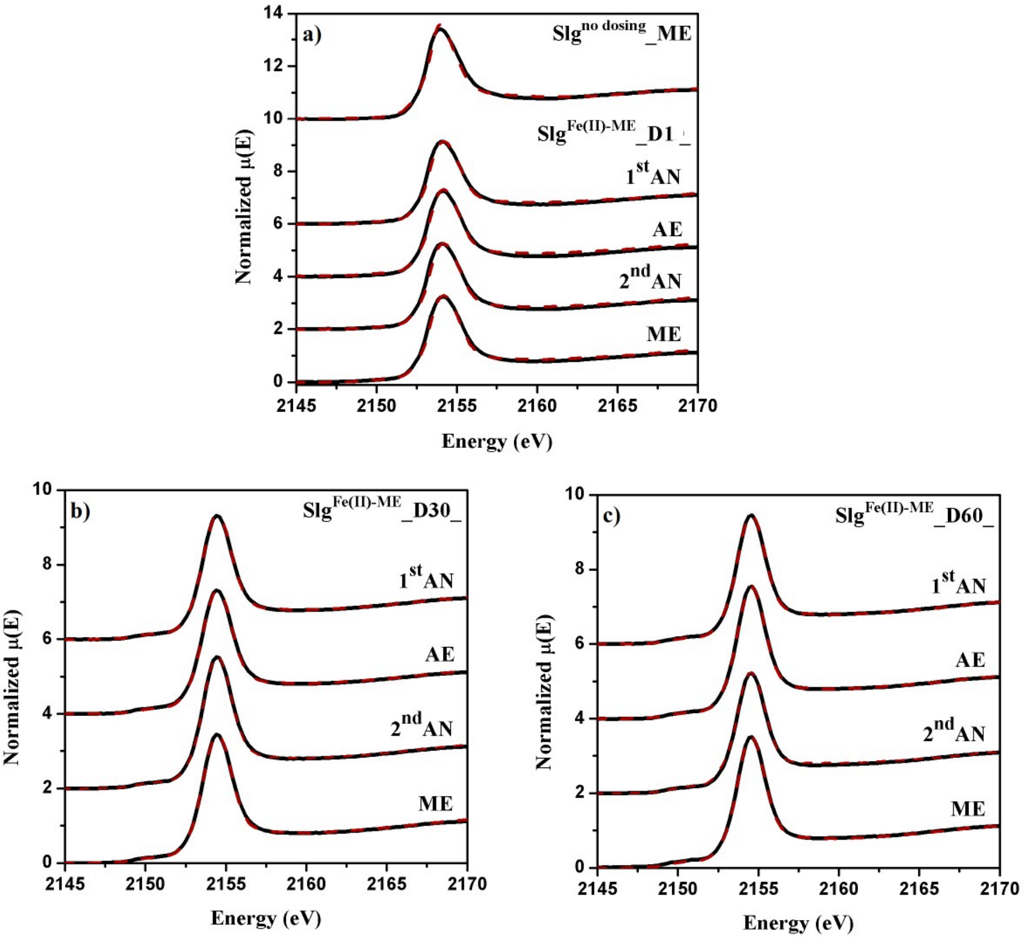

Figure S6. Normalized P K-edge XANES spectra of sludge samples from different chambers with membrane chamber as the dosing position: a) Slg$^{no\ dosing}$_ME and Slg$^{Fe(II)-ME}$_D1, b) Slg$^{Fe(II)-ME}$_D30, c) Slg$^{Fe(II)-ME}$_D60. The black line represents the experimental data while the grey dash line represents the reproduction by LCF.

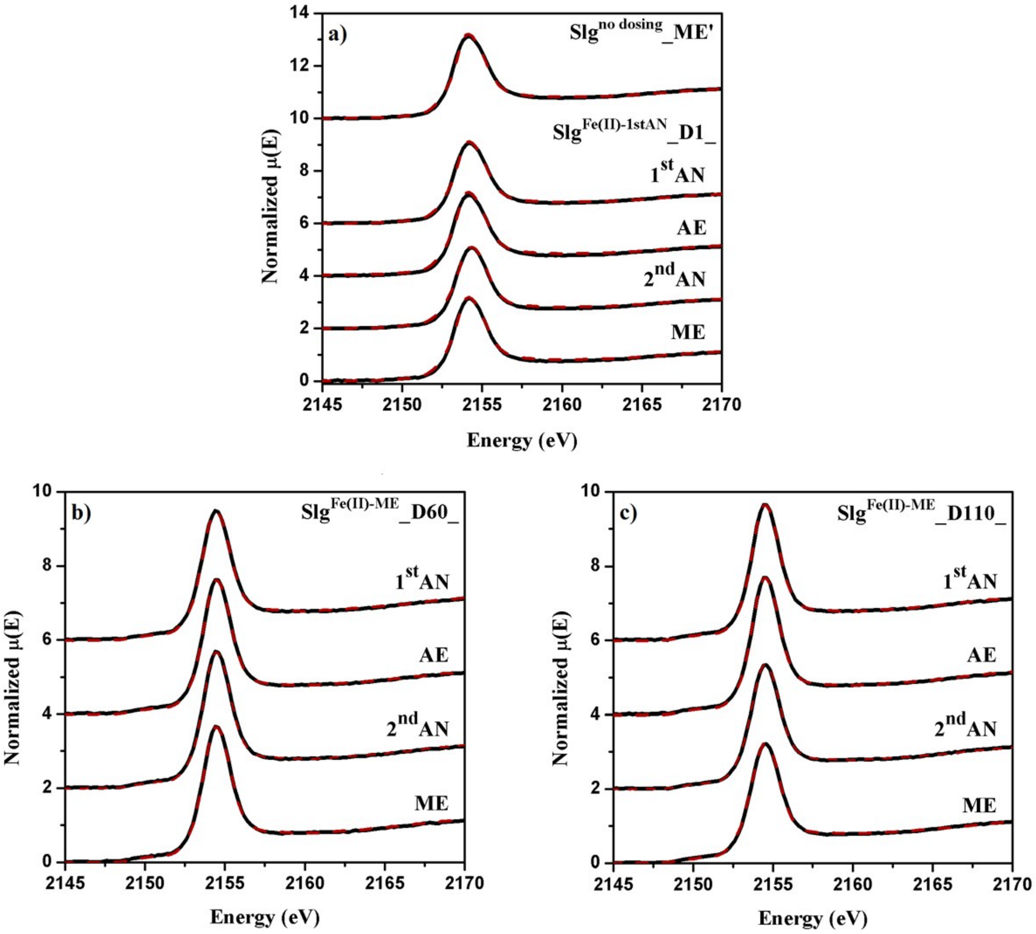

Figure S7. Normalized P K-edge XANES spectra of sludge samples from different chambers with 1st anoxic chamber as the dosing position: a) Slg$^{\text{no dosing}}$_ME’ and Slg$^{\text{Fe(II)-1stAN}}$_D1, b) Slg$^{\text{Fe(II)-1stAN}}$_D60, c) Slg$^{\text{Fe(II)-1stAN}}$_D110. The black line represents the experimental data while the grey dash line represents the reproduction by LCF.

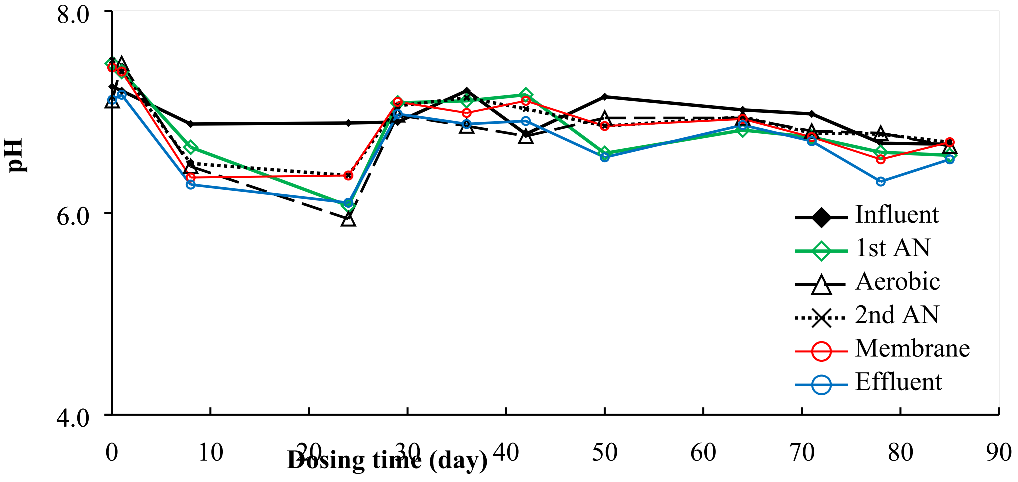

Figure S8. Changes of pH during Fe(II) dosing to the membrane zone.

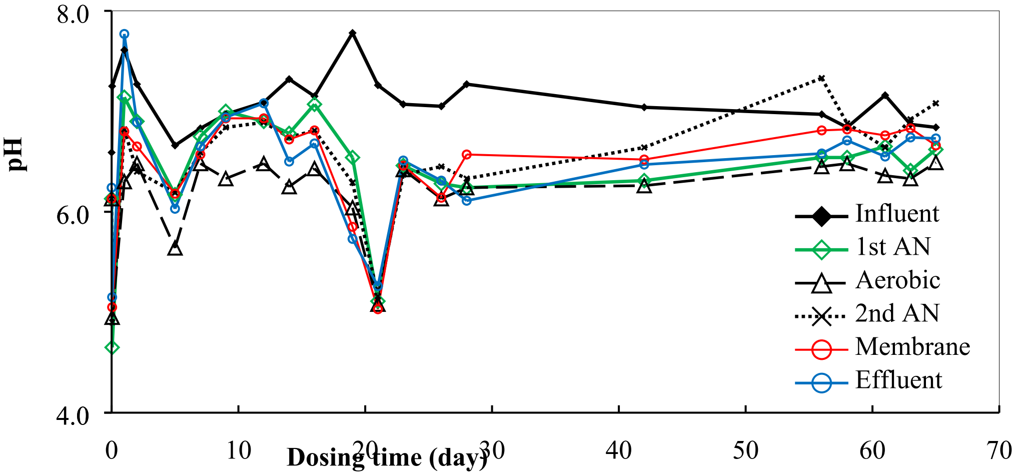

Figure S9. Changes of pH during Fe(II) dosing to the primary anoxic zone.

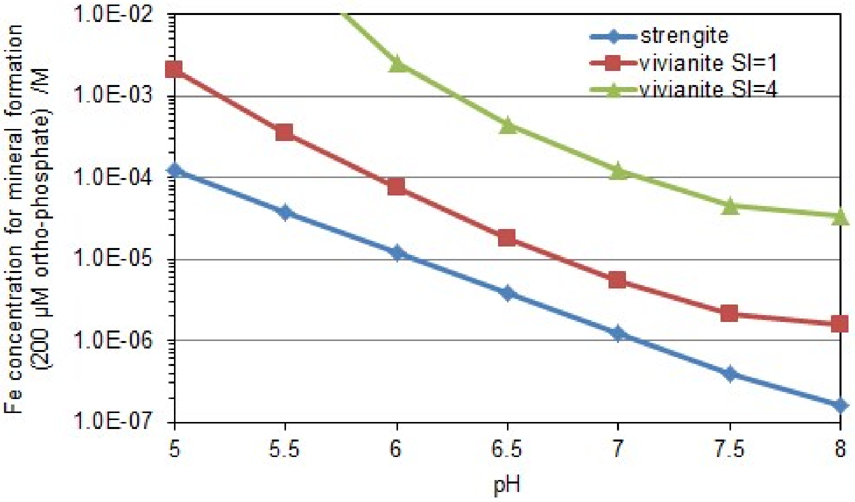

Figure S10. Fe concentrations required for mineral formation at reactor start up on dosing to the primary anoxic zone.

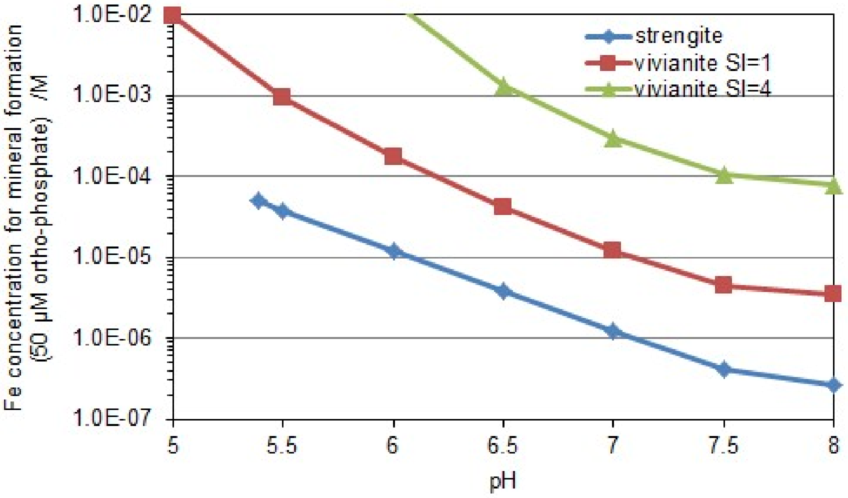

Figure S11. Fe concentrations required for mineral formation at reactor steady state on dosing to the primary anoxic zone. Note that data for strengite only shown from pH 5.39 upwards as the presence of dissolved Fe(III)HPO_4 makes strengite precipitation impossible at lower pHs at this low total phosphate concentration).

Table S1. Design parameters of pilot plant MBR.

	Pilot scale MBR
Reactor volume	1.25 m^3
Membrane nominal pore size	0.3 µm
Total membrane area	10.4 m^2
Filtration/Relaxation ratio	7 min : 1 min
Filtration flux	15.6 L/m^2 h (LMH)
Hydraulic retention time	10 h
Sludge retention time	30 days
Mixed liquor return flowrate (RAS)	RAS 1 = 300%; RAS 2 = 400%
DO (in aeration zone)	1.5-2.5 mg/L
Temperature	20-25°C
COD:N:P	100:15:2
Aeration for bioreactor	0.18 Nm^3/min
Specific aeration demand for membrane scouring	0.08 Nm^3/m^2 min

Table S2. Characteristics of MBR influent.

Parameter	**Unit**	**Mean value**[a]	**Maximum**[a]	**Minimum**[a]
TSS	mg/L	112.3	156	72
TDS	mg/L	938.2	1502	602
Carbonaceous BOD	mg/L	211	266	108
COD	mg/L	391	449	307
TOC	mg/L	83.2	97.1	71.8
DOC	mg/L	60.9		
Total nitrogen (as N)	mg/L	53.6	67.0	27.0
Ammonia-N	mg/L	33.2	40.9	28.5
Total phosphorus (as P)	mg/L	8.12	10.46	4.87
Alkalinity	mg $CaCO_3$/L	202	231	169
Ca	mg/L	24.5	28.2	20.1
K	mg/L	20.8	26.7	16.6
Mg	mg/L	25.6	44.5	15.5
Na	mg/L	218.4	369.0	132.0
Al	mg/L	0.40	0.61	0.27
Fe	mg/L	1.1	3.5	0.5
pH	-	7.08	7.78	6.16
Temperature	°C	23		

[a] between April 2012–July 2013

Table S3. Experimental details of ferrous dosing to the pilot scale MBR.

	No dosing	**To membrane zone**	**To primary anoxic zone**
Period of study	04/2012–10/2012	11/2012–02/2013	05/2013–08/2013
Average flowrate (m^3/day)	3.46	3.43	3.74
MLSS (g/L)	5.3–8.4	5.9–13.5	5.8–12.9
MLVSS/MLSS	0.85–0.95	0.92–0.67	0.86–0.66
pH	5.92–7.33	5.05–6.82	4.95–6.90
Fe(II):PO_4 molar ratio	–		
Min		2.01	2.01
Max		4.10	3.51
Average		2.99	2.64

Table S4. Results of the Principal Component Analysis (PCA) on Fe K-edge EXAFS spectra of the sludges.

Group 1

Component	Eingenvalue	Variance	Cumulative Variance	IND[a]
Comp 1	127.936	0.625	0.625	0.03032
Comp 2	19.517	0.095	0.720	0.02428
Comp 3	9.700	0.047	0.768	0.02451
Comp 4	7.895	0.038	0.806	0.02542
Comp 5	6.418	0.031	0.838	0.02725
Comp 6	5.266	0.025	0.863	0.03032
Comp 7	4.768	0.023	0.887	0.03423
Comp 8	4.075	0.019	0.907	0.04006
Comp 9	3.519	0.017	0.924	0.04890
Comp 10	3.445	0.016	0.941	0.06036
Comp 11	3.230	0.015	0.956	0.07567
Comp 12	2.985	0.014	0.971	0.09366
Comp 13	1.943	0.009	0.981	0.14638
Comp 14	1.574	0.007	0.988	0.29202
Comp 15	1.340	0.006	0.995	0.96550
Comp 16	0.965	0.004	1.0	NA

Group 2

Component	Eingenvalue	Variance	Cumulative Variance	IND[a]
Comp 1	119.573	0.749	0.749	0.03531
Comp 2	14.940	0.093	0.743	0.02325
Comp 3	5.899	0.096	0.880	0.02286
Comp 4	4.692	0.029	0.909	0.02264
Comp 5	3.551	0.022	0.931	0.02322
Comp 6	2.576	0.016	0.948	0.02562
Comp 7	1.965	0.012	0.930	0.03036
Comp 8	1.534	0.009	0.970	0.03924
Comp 9	1.301	0.008	0.978	0.05520
Comp 10	1.061	0.006	0.984	0.09057
Comp 11	0.987	0.006	0.991	0.17845
Comp 12	0.786	0.004	0.993	0.63240
Comp 13	0.632	0.003	1.0	NA

[a] Indicator function

Table S5. Target testing of reference spectra using the first four components obtained by PCA in Table S3.

Group 1

References	R value	SPOIL[a]
Lepidocrocite	0.02664	1.2595
Fe(III)-phosphate	0.03681	2.3516
Fe(III) Citrate	0.04905	2.3755
Ferrihydrite	0.11178	4.1450
Schwertmannite	0.10028	4.4156
Maghemite	0.33260	5.8078
Pyrite	0.78223	6.4522
Hematite	0.66772	7.0713
Mackinawite	0.88135	7.1356
Goethite	0.35349	7.2940
Vivianite	0.76533	9.4486
Akaganéite	0.34520	13.0248
Magnetite	0.56750	14.3142

Group 2

References	R value	SPOIL[a]
Lepidocrocite	0.04069	3.2768
Fe(III)-phosphate	0.07868	3.5119
Ferrihydrite	0.10889	4.2936
Fe(III) Citrate	0.16725	4.9382
Goethite	0.30425	7.0568
Pyrite	0.78421	7.6277
Maghemite	0.40133	9.0762
Magnetite	0.52867	9.6556
Schwertmannite	0.18467	11.0923
Akaganéite	0.32801	11.2514
Mackinawite	0.88322	12.5276
Hematite	0.72444	15.4574
Vivianite	0.7713	15.9071

[a] SPOIL classification (Malinowski 2002):
0–1.5, excellent; 1.5– 3, good; 3–4.5, fair; 4.5–6, acceptable; and >6, unacceptable reference.

Table S6. Results of the Principal Component Analysis (PCA) on P K-edge XANES spectra of the sludges.

Group 1

Component	Eingenvalue	Variance	Cumulative Variance	IND[a]
Comp 1	140.689	0.890	0.890	0.00814
Comp 2	12.629	0.079	0.969	0.00157
Comp 3	1.874	0.011	0.981	0.00090
Comp 4	0.816	0.005	0.986	0.00069
Comp 5	0.464	0.002	0.989	0.00061
Comp 6	0.402	0.002	0.992	0.00048
Comp 7	0.193	0.001	0.993	0.00051
Comp 8	0.146	0.000	0.004	0.00055
Comp 9	0.123	0.000	0.995	0.00062
Comp 10	0.115	0.000	0.995	0.00069
Comp 11	0.108	0.000	0.996	0.00078
Comp 12	0.091	0.000	0.997	0.00093
Comp 13	0.089	0.000	0.997	0.00115
Comp 14	0.069	0.000	0.998	0.00149
Comp 15	0.068	0.000	0.998	0.00202
Comp 16	0.063	0.000	0.998	0.00293
Comp 17	0.060	0.000	0.999	0.00461
Comp 18	0.052	0.000	0.999	0.00877
Comp 19	0.049	0.000	1.0	6.68×10^{-18}
Comp 20	6.68×10^{-18}	0.000	1.0	NA

Group 2

Component	Eingenvalue	Variance	Cumulative Variance	IND[a]
Comp 1	59.108	0.910	0.910	0.00846
Comp 2	4.136	0.063	0.974	0.00208
Comp 3	0.787	0.012	0.986	0.00090
Comp 4	0.150	0.002	0.989	0.00100
Comp 5	0.144	0.002	0.990	0.00119
Comp 6	0.101	0.001	0.992	0.00147
Comp 7	0.090	0.001	0.993	0.00191
Comp 8	0.087	0.001	0.995	0.00257
Comp 9	0.080	0.001	0.996	0.00374
Comp 10	0.079	0.001	0.997	0.00577
Comp 11	0.064	0.000	0.998	0.01105
Comp 12	0.050	0.000	0.999	0.03748
Comp 13	0.037	0.000	1.0	NA

[a] Indicator function

Table S7. Target testing of reference spectra using the first four components obtained by PCA in Table S5).

Group 1

Reference	R value	SPOIL[a]
Na_3PO_4	0.00464	1.3666
ATP	0.00124	1.5705
Sodium polyphosphate	0.00802	1.9685
Sodium tripolyphosphate	0.01412	2.5898
Fe(III)-phosphate	0.00057	2.7181
Phytic acid	0.01709	3.2135
P-adsorbed	0.00508	3.3067
Vivianite	0.00395	6.5483
$MgHPO_4$	0.00375	7.1686
$CaHPO_4$	0.01488	8.1627

Group 2

Reference	R value	SPOIL[a]
Fe(III)-phosphate	0.00218	1.7445
Sodium polyphosphate	0.01055	4.4662
ATP	0.00173	4.5061
Phytic acid	0.01334	4.5719
Na_3PO_4	0.00905	4.6014
P-adsorbed	0.00905	4.6014
Sodium tripolyphosphate	0.01757	5.2831
Vivianite	0.0034	8.0803
$CaHPO_4$	0.01769	24.2125
$MgHPO_4$	0.00485	41.7923

[a] SPOIL classification (Malinowski 2002):
0–1.5, excellent; 1.5– 3, good; 3–4.5, fair; 4.5–6, acceptable; and >6, unacceptable reference.

Table S8. Concentration of ortho-P in the supernatant in different chambers when Fe(II) dosed to the 1st anoxic chamber.

Dosing period	**Concentration of ortho-P (μM)**				
	influent	**1st anoxic**	**Aerobic**	**2nd anoxic**	**Membrane**
Before dosing	192 ± 36	173.0	176	207	185.0
1 day		62.7	13	22	14.0
Within 1 SRT		10.1 ± 0.6	15 ± 6	21 ± 13	12.6 ± 0.2
Steady state (i.e. after 3 SRT)		3.2 ± 0.9	3 ± 1	3 ± 2	2 ± 1

Table S9. Ferrous and ferric iron concentrations in the supernatant from different chambers of the pilot plant MBR when Fe(II) dosed to the 1st anoxic chamber.

Dosing period	Concentration of Fe(II) and Fe(III) (μM)							
	1st Anoxic		Aerobic		2nd Anoxic		Membrane	
	Fe(II)	Fe(III)	Fe(II)	Fe(III)	Fe(II)	Fe(III)	Fe(II)	Fe(III)
1hr	11.45	1.40	7.59	2.01	7.43	2.07	4.58	0.82
1 day	1.68	0.97	0.76	1.77	1.24	1.39	1.79	0.64
Within 1 SRT	1.72±0.36	0.78±0.55	1.64±0.33	0.54±0.16	2.08±0.39	0.82±0.64	1.88±0.57	0.73±0.45
Steady-state (after 3 SRT)	1.73±0.21	0.59±0.80	0.86±0.50	1.54±0.68	1.67±0.14	0.89±1.21	1.94±0.22	1.04±0.66

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