SUPPLEMENTARY MATERIAL

ZVI-Fenton based process affects the total load of human pathogenic bacteria in wastewater

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S1. Wastewater characterization

The wastewater samples were characterized for conductivity (HI2030 Multi-parameter probe, Hanna Instruments), pH (Checker HI98103, Hanna Instruments), total carbon (TC), inorganic carbon (IC), total organic carbon (computed as TOC = TC – IC), total nitrogen (TN, Shimadzu ON-LINE TOC-VCSH instrument, equipped with an ASI-V autosampler and fed with zero-grade air), and the anions Cl^-, NO_3^- and SO_4^{2-} (Dionex DX 500 ion chromatograph, Dionex Ion Pac AS9-HC column, elution with 9 mM K_2CO_3 at 1 mL min^{-1}). The results are reported in Supplementary Table S1 below.

Supplementary Table S1. Physico-chemical features of the studied wastewater samples. TC = total carbon, TOC = total organic carbon, IC = inorganic carbon (TOC = TC - IC), TN = total nitrogen.

Parameter	WW
Conductivity, $\mu S\ cm^{-1}$	267
pH	7.5
TC, $mg_C\ L^{-1}$	10.5
TOC, $mg_C\ L^{-1}$	7.8
IC, $mg_C\ L^{-1}$	2.7
TN, $mg_N\ L^{-1}$	5.8
Chloride (Cl^-), mg L^{-1}	35.6
Nitrate (NO_3^-), mg L^{-1}	34.7
Sulfate (SO_4^{2-}), mg L^{-1}	19.6

Supplementary Table S2. Operational conditions used in the ZVI-Fenton treatment of wastewater. In the case of the experiments with H_2O_2 alone, conditions were the same except that ZVI was not added.

Time, min	ZVI + H_2O_2		
	H_2SO_4, pH 5	H_3PO_4, pH 5	H_3PO_4, pH 6 checked and corrected during the reaction
0	H_2O_2 3×10^{-4} M ZVI 0.02 g L^{-1}	H_2O_2 3×10^{-4} M ZVI 0.02 g L^{-1}	H_2O_2 3×10^{-4} M ZVI 0.02 g L^{-1}
30	H_2O_2 5×10^{-5} M Correct pH to 5	H_2O_2 5×10^{-5} M Correct pH to 5	H_2O_2 5×10^{-5} M
60	H_2O_2 5×10^{-5} M Correct pH to 5	H_2O_2 5×10^{-5} M Correct pH to 5	H_2O_2 5×10^{-5} M
90	End	End	End

Supplementary Table S3. Primer pairs and annealing temperatures used to detect and quantify the 16S rRNA gene, ARGs, and *intI*1 gene by qPCR.

Target gene	Primer pairs	Primer sequence (5'- 3')	Annealing temperature (°C)	Amplicon size (bp)	Reference
16S rRNA	Bact1369F Prok1492R	CGGTGAATACGTTCYCGG GGHTACCTTGTTACGACTT	55	142	Di Cesare et al., 2015
*qnr*S	qnrSF qnrSR	GACGTGCTAACTTGCGTGAT TGGCATTGTTGGAAACTTG	62	118	Marti and Balcázar, 2013
*sul*2	sulIIF sulIIR	TCCGGTGGAGGCCGGTATCTGG CGGGAATGCCATCTGCCTTGAG	60	191	Pei et al., 2006
*tet*A	tet(A)F tet(A)R	GCTACATCCTGCTTGCCTTC CATAGATCGCCGTGAAGAGG	64	210	Ng et al., 2001
*intI*1	intI1LC5 intI1LC1	GATCGGTCGAATGCGTGT GCCTTGATGTTACCCGAGAG	60	196	Barraud et al., 2010

Supplementary Table S4. Statistical results for the analysis of variance (ANOVA) assessing the influence of the treatment on the cell abundance of samples from "experiment 1". Abbreviations: "PA"= phosphoric acid, "SA"= sulfuric acid, "HP"= hydrogen peroxide, and "FE"= ZVI-Fenton Process.

ANOVA	Df	Sum Sq	Mean Sq	F-value	p-value
	13	0.7404	0.05695	14.25	4.77e-09
Pairwise comparison*	**Df**	**Lwr**	**Upr**	**p-value**	
PApH6T0-FEPApH5T90S	0.265467	0.076504	0.45443	0.00126320	
WWT0-FEPApH5T90S	0.239084	0.050121	0.428047	0.00472660	
PApH6T0-FEPApH6T90S	0.250923	0.06196	0.439885	0.00262410	
WWT0-FEPApH6T90S	0.22454	0.035577	0.413502	0.00962350	
HPPApH5T90S-FESApH5T90S	0.261353	0.07239	0.450316	0.00155450	
HPPApH6T90S-FESApH5T90S	0.286875	0.097912	0.475838	0.00042690	
PApH5T90S-FESApH5T90S	0.192186	0.003223	0.381149	0.04338000	
PApH6T0-FESApH5T90S	0.41964	0.230677	0.608602	0.00000060	
PApH6T90S-FESApH5T90S	0.28989	0.100927	0.478852	0.00036630	
WWT0-FESApH5T90S	0.393257	0.204294	0.582219	0.00000210	
WWT90-FESApH5T90S	0.32459	0.135627	0.513552	0.00006310	
PApH5T0-HPPApH5T90S	-0.23694	-0.4259	-0.04798	0.00525390	
SApH5T0-HPPApH5T90S	-0.27129	-0.46025	-0.08232	0.00094130	
PApH5T0-HPPApH6T90S	-0.26246	-0.45142	-0.0735	0.00147000	
SApH5T0-HPPApH6T90S	-0.29681	-0.48577	-0.10784	0.00025780	
PApH6T0-HPSApH5T90S	0.284198	0.095235	0.473161	0.00048910	
WWT0-HPSApH5T90S	0.257815	0.068852	0.446778	0.00185740	
WWT90-HPSApH5T90S	0.189148	0.000186	0.378111	0.04959510	
PApH6T0-PApH5T0	0.395226	0.206264	0.584189	0.00000190	
PApH6T90S-PApH5T0	0.265476	0.076514	0.454439	0.00126260	
WWT0-PApH5T0	0.368843	0.179881	0.557806	0.00000700	
WWT90-PApH5T0	0.300177	0.111214	0.489139	0.00021730	
PApH6T0-PApH5T90S	0.227454	0.038491	0.416417	0.00835650	
SApH5T0-PApH5T90S	-0.20212	-0.39108	-0.01316	0.02771370	
WWT0-PApH5T90S	0.201071	0.012108	0.390034	0.02907460	
SApH5T0-PApH6T0	-0.42957	-0.61853	-0.24061	0.00000040	
SApH5T90S-PApH6T0	-0.28377	-0.47273	-0.0948	0.00049990	
SApH5T0-PApH6T90S	-0.29982	-0.48878	-0.11086	0.00022120	
WWT0-SApH5T0	0.403189	0.214226	0.592152	0.00000130	
WWT90-SApH5T0	0.334522	0.145559	0.523485	0.00003830	
WWT0-SApH5T90S	0.257385	0.068422	0.446347	0.00189800	

*Only significant differences are reported

Supplementary Table S5. Statistical results for the analysis of variance (ANOVA) assessing the influence of the treatment on the cell vitality of samples from "experiment 1". Abbreviations: "PA"= phosphoric acid, "SA"= sulfuric acid, "HP"= hydrogen peroxide, and "FE"= ZVI-Fenton Process.

ANOVA	Df	Sum Sq	Mean Sq	F-value	p-value
	13	0.19124	0.014711	16.1	1.15e-09
Pairwise comparison*		Df	Lwr	Upr	p-value
FEPApH6T90S-FEPApH5T90S		-0.14006	-0.23039	-0.04972	0.00031320
FESApH5T90S-FEPApH5T90S		-0.12327	-0.2136	-0.03293	0.00185400
HPPApH6T90S-FEPApH5T90S		-0.15496	-0.2453	-0.06463	0.00006450
HPSApH5T90S-FEPApH5T90S		-0.0977	-0.18803	-0.00737	0.02498560
PApH5T0-FEPApH5T90S		-0.14447	-0.23481	-0.05414	0.00019590
PApH5T90S-FEPApH5T90S		-0.09552	-0.18585	-0.00519	0.03079550
PApH6T0-FEPApH5T90S		-0.21188	-0.30222	-0.12155	0.00000020
PApH6T90S-FEPApH5T90S		-0.15111	-0.24145	-0.06078	0.00009690
SApH5T0-FEPApH5T90S		-0.13491	-0.22525	-0.04458	0.00054070
SApH5T90S-FEPApH5T90S		-0.09943	-0.18976	-0.0091	0.02112550
WWT0-FEPApH5T90S		-0.28935	-0.37968	-0.19902	0.00000000
WWT90-FEPApH5T90S		-0.20808	-0.29842	-0.11775	0.00000030
WWT0-FEPApH6T90S		-0.14929	-0.23963	-0.05896	0.00011750
WWT0-FESApH5T90S		-0.16608	-0.25642	-0.07575	0.00002010
WWT90-FESApH5T90S		-0.08482	-0.17515	0.005516	0.08184300
HPPApH6T90S-HPPApH5T90S		-0.09315	-0.18348	-0.00281	0.03852520
PApH6T0-HPPApH5T90S		-0.15007	-0.2404	-0.05973	0.00010820
WWT0-HPPApH5T90S		-0.22753	-0.31787	-0.1372	0.00000000
WWT90-HPPApH5T90S		-0.14627	-0.2366	-0.05593	0.00016200
WWT0-HPPApH6T90S		-0.13439	-0.22472	-0.04405	0.00057200
PApH6T0-HPSApH5T90S		-0.11419	-0.20452	-0.02385	0.00477950
WWT0-HPSApH5T90S		-0.19165	-0.28198	-0.10132	0.00000150
WWT90-HPSApH5T90S		-0.11038	-0.20072	-0.02005	0.00706330
WWT0-PApH5T0		-0.14487	-0.23521	-0.05454	0.00018770
PApH6T0-PApH5T90S		-0.11637	-0.2067	-0.02603	0.00381340
WWT0-PApH5T90S		-0.19383	-0.28416	-0.1035	0.00000120
WWT90-PApH5T90S		-0.11256	-0.2029	-0.02223	0.00564860
SApH5T90S-PApH6T0		0.112455	0.022123	0.202788	0.00571210
WWT0-PApH6T90S		-0.13824	-0.22857	-0.0479	0.00038000
WWT0-SApH5T0		-0.15443	-0.24477	-0.0641	0.00006820
WWT0-SApH5T90S		-0.18992	-0.28025	-0.09959	0.00000170
WWT90-SApH5T90S		-0.10865	-0.19899	-0.01832	0.00842440

*Only significant differences are reported

Supplementary Table S6. Statistical results for the analysis of variance (ANOVA) assessing the influence of the treatment on the cell abundance of samples from "experiment 2". Abbreviations: "PA"= phosphoric acid, "HP"= hydrogen peroxide, and "FE"= ZVI-Fenton Process.

ANOVA	Df	Sum Sq	Mean Sq	F-value	p-value
	4	0.8518	0.21296	36.23	6.36e-06
Pairwise comparison*		Df	Lwr	Upr	p-value
PAT0-FET90S		-0.50999	-0.71601	-0.30398	0.00007630
PAT0-HPT90S		-0.51742	-0.72343	-0.31141	0.00006730
PAT90S-PAT0		0.51242	0.306406	0.718433	0.00007320
WWT0-PAT0		0.713457	0.507443	0.91947	0.00000370

*Only significant differences are reported

Supplementary Table S7. Statistical results for the analysis of variance (ANOVA) assessing the influence of the treatment on the cell vitality of samples from "experiment 2". Abbreviations: "PA"= phosphoric acid, "HP"= hydrogen peroxide, and "FE"= ZVI-Fenton Process.

ANOVA	Df	Sum Sq	Mean Sq	F-value	p-value
	4	0.10125	0.025312	235.7	7.57e-10
Pairwise comparison*		Df	Lwr	Upr	p-value
PAT90S-FET90S		-0.07205	-0.0999	-0.0442	0.00005180
WWT0-FET90S		-0.20731	-0.23516	-0.17946	0.00000000
PAT90S-HPT90S		-0.06417	-0.09201	-0.03632	0.00014160
WWT0-HPT90S		-0.19943	-0.22727	-0.17158	0.00000000
PAT90S-PAT0		-0.08789	-0.11573	-0.06004	0.00000870
WWT0-PAT0		-0.22315	-0.25099	-0.1953	0.00000000
WWT0-PAT90S		-0.13526	-0.16311	-0.10741	0.00000020

*Only significant differences are reported

Supplementary Table S8. Statistical results for generalized linear model (GLM) assessing the influence of treatment on richness of whole bacterial community and potential pathogens.

	Df	Chisq	p-value
BACTERIAL COMMUNITY	2	85.694	< 2.2e-16
Pairwise comparison			
PA - FE			< 0.0001
PA - HP			0.0512
FE - HP			< 0.0001
POTENTIAL PATHOGENS	2	1.7685	0.413

a)
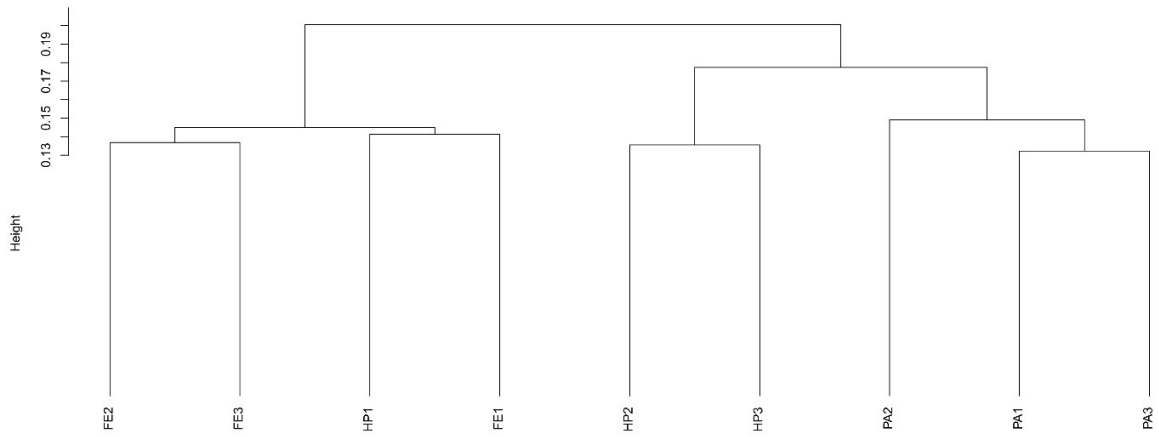
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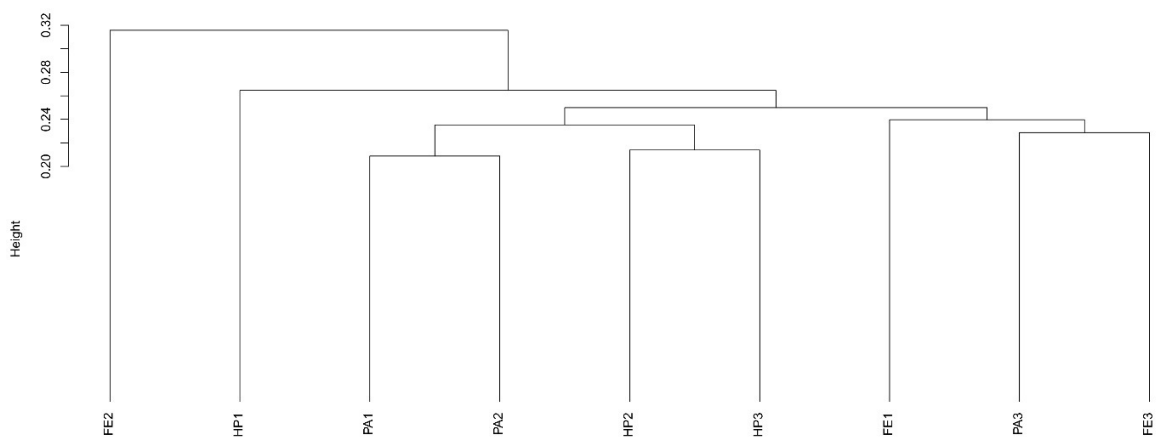
Supplementary Figure S1. Dendrogram of average linkage clustering based on Bray–Curtis distances between the different samples. **a)** total bacterial community, **b)** potentially pathogenic genera. Abbreviations: "PA"= phosphoric, "HP"= hydrogen peroxide, and "FE"= ZVI-Fenton Process.

Supplementary Table S9. Results of the analysis of variance (PERMANOVA) of the beta diversity of bacterial community and potential pathogens as function of treatment.

	Df	Sum Sq	Mean Sq	F-value	R^2	p-value
Bacterial community						
	2	0.064994	0.032497	2.8189	0.48444	0.007
Potential Pathogens						
	2	0.086173	0.043086	1.3342	0.30783	0.023

Supplementary Table S10. Statistical results for generalized linear model (GLM) assessing the influence of treatment on the number of total reads associated to potential pathogens.

	Df	Chisq	p-value
GLM	2	9.3775	0.009198
Pairwise comparison			
PA - FE			0.0252
PA - HP			0.9937
FE - HP			0.0186

Supplementary Table S11. Statistical results for generalized linear model (GLM) assessing the influence of treatment on the number of reads associated to potentially pathogenic genus.

	Df	Chisq	p-value
Acinetobacter	2	13.899	0.0009593
FE-HP			0.0039
FE-PA			1
HP-PA			0.0039
Bacteroides	2	37.653	6.664e-09
FE-HP			0.0001
FE-PA			<0.0001
HP-PA			0.1187
Clostridium_sensu_stricto_1	2	101.77	< 2.2e-16
FE-HP			<0.0001
FE-PA			<0.0001
HP-PA			0.0318
Klebsiella	2	14.338	0.00077
FE-HP			0.1972
FE-PA			0.0007
HP-PA			0.1012
Legionella	2	9.7538	0.007621
FE-HP			0.0177
FE-PA			0.992
HP-PA			0.0249
Mycobacterium	2	10.349	0.00566
FE-HP			0.8087
FE-PA			0.0408
HP-PA			0.0075
Prevotella_1	2	21.901	1.755e-05
FE-HP			0.9511
FE-PA			0.0005
HP-PA			0.0001
Prevotella_9	2	14.951	0.0005667
FE-HP			0.2445
FE-PA			0.0004
HP-PA			0.0647
Sphingomonas	2	77.285	< 2.2e-16
FE-HP			<0.0001
FE-PA			0.8046
HP-PA			<0.0001
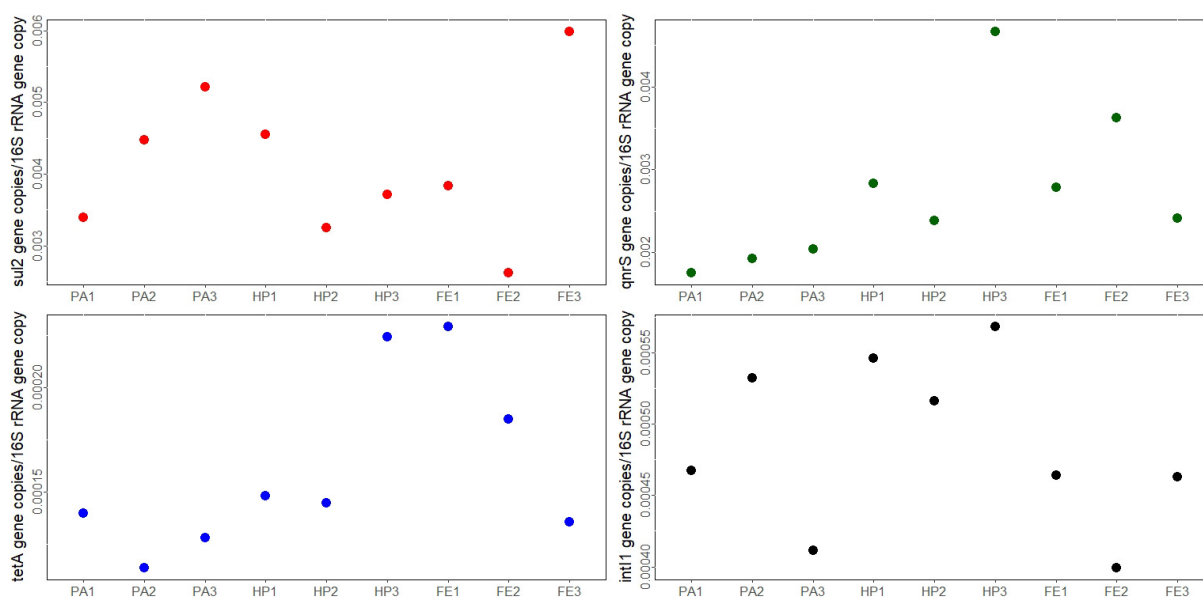
Supplementary Figure S2. Normalized abundances of the antibiotic resistance genes (*sul*2, *qnr*S, *tet*A) and *intI*1 gene. Abbreviations: "PA"= phosphoric acid, "HP"= hydrogen peroxide, and "FE"= ZVI Fenton based Process.

Supplementary Table S12. Statistical results for the analysis of variance (ANOVA) assessing the influence of the treatment on antibiotic resistance and *intI*1 genes abundance.

	Df	Sum Sq	Mean Sq	F-value	p-value
*sul*2	2	2.4380e-09	1.2189e-09	0.1453	0.8677
*tet*A	2	8.1970e-10	4.0985e-10	1.998	0.2163
*qnr*S	2	2.8954e-08	1.4477e-08	3.1034	0.1188
*intI*1	2	8.4080e-10	4.2040e-10	4.1251	0.07464

Supplementary Table S13. Assessment of treatment costs for chemical reagents. That mentioned in the table is the initial pH value: the final one was in the range of 5.7-6. Note that conventional wastewater treatment would cost around 0.4 $ m^{-3}, to which these are added costs.

	Cost, $ m^{-3}
	pH 5, H_3PO_4
ZVI	0.016
H_2O_2	0.012
H_3PO_4	0.121
Total cost	***0.149***

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