Supplemental data for
Assessment of an anti-scale low-frequency electromagnetic field device on drinking water biofilms

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Table S1: Some physical-chemical and bacteriological characteristics of Nancy's tap water during the two series of analyses.

		Series 1						Series 2					
		Out « Assay »			Out « Control »			Out « Assay »			Out « Control »		
T (°C)	n = 3-15	**20.7**	±	1.1	**21.2**	±	0.8	**21.3**	±	1.3	**21.0**	±	1.2
pH	n = 3-15	**7.8**	±	0.2	**7.9**	±	0.2	**7.9**	±	0.1	**7.8**	±	0.1
Conductivity ($\mu S\ cm^{-1}$)	n = 3-14	**360**	±	19	**363**	±	18	**404**	±	57	**407**	±	64
CAT (°f)	n = 3-8	**8.4**	±	0.2	**8.3**	±	0.2	**8.3**	±	0.3	**8.3**	±	0.3
Mg ($mg\ L^{-1}$)	n = 8-9	**5.1**	±	0.7	**5.1**	±	0.6	**6.3**	±	2.2	**6.4**	±	2.2
Ca ($mg\ L^{-1}$)	n = 8-9	**45.6**	±	1.3	**45.7**	±	1.4	**47.5**	±	3.6	**47.6**	±	3.6
Cells (mL^{-1})	n = 20-22	**$3.7\ 10^5$**	±	$4.1\ 10^5$	**$4.9\ 10^5$**	±	$5.8\ 10^5$	**$1.0\ 10^6$**	±	$5.6\ 10^5$	**$1.1\ 10^6$**	±	$1.6\ 10^6$
CFU_{14d} (mL^{-1})	n = 20-22	**$4.1\ 10^4$**	±	$3.6\ 10^4$	**$5.1\ 10^4$**	±	$3.2\ 10^4$	**$5.2\ 10^4$**	±	$3.7\ 10^4$	**$3.7\ 10^4$**	±	$4.8\ 10^4$

		Series 1			Series 2		
		In					
T (°C)	n = 6-30	**19.5**	±	1.7	**20.1**	±	1.7
pH	n = 6-30	**8.0**	±	0.1	**7.9**	±	0.1
Conductivity ($\mu S\ cm^{-1}$)	n = 6-28	**358**	±	19	**399**	±	51
CAT (°f)	n = 4-16	**8.2**	±	0.2	**8.2**	±	0.5
Mg ($mg\ L^{-1}$)	n = 20-21	**5.4**	±	0.8	**5.7**	±	0.9
Ca ($mg\ L^{-1}$)	n = 20-21	**46.2**	±	2.3	**47.0**	±	2.4
Cells (mL^{-1})	n = 28-40	**$1.4\ 10^5$**	±	$1.8\ 10^5$	**$2.4\ 10^5$**	±	$7.2\ 10^5$
CFU_{14d} (mL^{-1})	n = 28-40	**$2\ 10^4$**	±	$3\ 10^4$	**$2\ 10^4$**	±	$4\ 10^4$

		Series 1			Series 2		
		Out « Carbonates »					
T (°C)	n = 3-15	**21.0**	±	1.0	**21.5**	±	0.7
pH	n = 13-63	**8.9**	±	0.4	**8.9**	±	0.2
Conductivity ($\mu S\ cm^{-1}$)	n = 3-14	**364**	±	19	**384**	±	55
CAT (°f)	n = 3-8	**9.4**	±	0.5	**9.4**	±	0.6
Mg ($mg\ L^{-1}$)	n = 7-11	**5.5**	±	1.4	**5.3**	±	0.9
Ca ($mg\ L^{-1}$)	n = 7-11	**50.4**	±	2.6	**49.7**	±	5.3
Cells (mL^{-1})	n = 11-18	**$3.1\ 10^5$**	±	$2.7\ 10^5$	**$5.9\ 10^5$**	±	$5.7\ 10^5$
CFU_{14d} (mL^{-1})	n = 11-18	**$5.2\ 10^4$**	±	$3.5\ 10^4$	**$4.0\ 10^4$**	±	$4.8\ 10^4$

Table S2: Value ranges of elemental biofilm analysis (minimal and maximal values on 4 - 5 coupons) in the reactors R1 and R3 alternatively used as "*Assay*" or "*Control*", and reactor R2 for "*Carbonates*" assay. Analyses were carried out by ICP-OES (SARM, UMR 7358 CNRS-UL, Nancy, FR) on dispersed biofilm acidified at pH near 0 with ultra-pure HNO_3 65 % (~ 2 % HNO_3, final concentration). Al, Mn and Ti were under the detection limit of quantitation (< LQ).

		Assay (µg cm^{-2})		*Control* (µg cm^{-2})	
		R1 (series #1)	R3 (series #2)	R3 (series #1)	R1 (series #2)
Si	**PVC**	< LQ - 0.7	< LQ - 0.7	< LQ - 0.5	< LQ
	SS	< LQ - 0.7	< LQ	< LQ - 0.4	< LQ
Fe	**PVC**	< LQ - 1.1	3.7 - 8.6	< LQ - 0.8	2.7 - 2.7
	SS	< LQ - 1.2	4.7 - 6.4	< LQ - 1.0	1.3 - 3.6
Mg	**PVC**	<LQ	< LQ - 0.6	< LQ	< LQ
	SS	< LQ - 0.4	< LQ - 0.7	< LQ - 0.5	< LQ - 0.7
Ca	**PVC**	3.8 - 19.4	8.5 - 16.8	3.8 - 17.5	6.6 - 17.0
	SS	3.5 - 23.0	12.0 - 30.5	3.1 - 16.4	7.8 - 8.0
Na	**PVC**	1.3 - 3.7	1.9 - 3.0	1.3 - 2.7	1.5 - 2.5
	SS	1.1 - 1.9	1.5 - 1.8	1.0 - 2.3	2.4 - 2.7
K	**PVC**	< LQ - 6.5	1.8 - 3.6	< LQ - 4.2	< LQ - 2.2
	SS	< LQ - 2.3	< LQ - 1.2	< LQ - 2.2	< LQ - 3.6

		Carbonates	
		R2 series #1	R2 series #2
Si	**PVC**	0.7 - 1.5	< LQ - 31.8
	SS	0.8 - 3.3	1.5 - 33.4
Fe	**PVC**	0.5 - 1.6	< LQ - 5.7
	SS	0.5 - 1.4	< LQ - 9.4
Mg	**PVC**	< LQ - 0.8	0.7 - 42.5
	SS	< LQ - 0.7	2.0 - 64.5
Ca	**PVC**	8.1 - 14.7	35.9 - 529.0
	SS	9.0 - 40.7	58.3 - 588.2
Na	**PVC**	1.5 - 1.9	1.4 - 110.8
	SS	1.3 - 1.8	1.3 - 134.7
K	**PVC**	< LQ - 2.5	< LQ - 18.7
	SS	< LQ - 2.4	< LQ - 31.4

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Table S3. Statistical analysis (Wilcoxon-Mann-Whitney test) of the data sets from series #1 and #2 comparing the two reactors (R1 and R3) when they are connected or not to the electromagnetic field (EMF). Reactor exposed to EMF is indicated by an "*". The two sets of data are considered to be significantly different when p-value is < to 0.05. CFU = colony forming unit (cultivability); SYBR = fluorochrome used to assess the total cell number; PI = fluorochrome used to assess the cell integrity.

Treatment	Parameters	Material	Experimental series	Considered reactors	p-value	n
Preventive	CFU	pvc	Series #1	R1* vs R3	0.7	6
		SS	Series #1	R1* vs R3	0.1	6
		pvc	Series #2	R3* vs R1	0.1	6
		SS	Series #2	R3* vs R1	**0.02**	6
		pvc	Series #1 + series #2	R1* + R3* vs R1 + R3	0.5	12
		SS	Series #1 + series #2	R1* + R3* vs R1 + R3	0.1	12
		pvc	Series #1 + series #2	R1* + R1 vs R3* + R3	0.1	12
		SS	Series #1 + series #2	R1* + R1 vs R3* + R3	**0.004**	12
	Cells density (SYBR)	pvc	Series #1	R1* vs R3	0.2	6
		SS	Series #1	R1* vs R3	0.1	6
		pvc	Series #2	R3* vs R1	0.3	6
		SS	Series #2	R3* vs R1	**0.02**	6
		pvc	Series #1 + series #2	R1* + R3* vs R1 + R3	0.9	12
		SS	Series #1 + series #2	R1* + R3* vs R1 + R3	0.6	12
		pvc	Series #1 + series #2	R1* + R1 vs R3* + R3	0.1	12
		SS	Series #1 + series #2	R1* + R1 vs R3* + R3	**0.008**	12
	Damaged cells (PI)	pvc	Series #1	R1* vs R3	0.8	6
		SS	Series #1	R1* vs R3	0.1	6
		pvc	Series #2	R3* vs R1	0.2	6
		SS	Series #2	R3* vs R1	**0.015**	6
		pvc	Series #1 + series #2	R1* + R3* vs R1 + R3	0.4	12
		SS	Series #1 + series #2	R1* + R3* vs R1 + R3	0.7	12
		pvc	Series #1 + series #2	R1* + R1 vs R3* + R3	0.2	12
		SS	Series #1 + series #2	R1* + R1 vs R3* + R3	**0.07**	12

(to be continued)

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26 (Table S3, continuation and end)

Treatment	Parameters	Material	Experimental series	Considered reactors	p-value	n
Curative	CFU	pvc	Series #1	R1* vs R3	0.2	5
		SS	Series #1	R1* vs R3	0.5	5
		pvc	Series #2	R3* vs R1	0.7	4
		SS	Series #2	R3* vs R1	0.4	4
		pvc	Series #1 + series #2	R1* + R3* vs R1 + R3	0.5	9
		SS	Series #1 + series #2	R1* + R3* vs R1 + R3	0.6	9
		pvc	Series #1 + series #2	R1* + R1 vs R3* + R3	0.5	9
		SS	Series #1 + series #2	R1* + R1 vs R3* + R3	0.9	9
	Cells density (SYBR)	pvc	Series #1	R1* vs R3	1	5
		SS	Series #1	R1* vs R3	0.3	5
		pvc	Series #2	R3* vs R1	0.4	4
		SS	Series #2	R3* vs R1	0.1	4
		pvc	Series #1 + series #2	R1* + R3* vs R1 + R3	1	9
		SS	Series #1 + series #2	R1* + R3* vs R1 + R3	0.8	9
		pvc	Series #1 + series #2	R1* + R1 vs R3* + R3	0.9	9
		SS	Series #1 + series #2	R1* + R1 vs R3* + R3	0.7	9
	Damaged cells (PI)	pvc	Series #1	R1* vs R3	0.9	5
		SS	Series #1	R1* vs R3	0.2	5
		pvc	Series #2	R3* vs R1	0.4	4
		SS	Series #2	R3* vs R1	0.1	4
		pvc	Series #1 + series #2	R1* + R3* vs R1 + R3	0.8	8
		SS	Series #1 + series #2	R1* + R3* vs R1 + R3	**0.03**	8
		pvc	Series #1 + series #2	R1* + R1 vs R3* + R3	0.7	8
		SS	Series #1 + series #2	R1* + R1 vs R3* + R3	0.8	8

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Table S4. Statistical analysis (Wilcoxon-Mann-Whitney test) of the data sets from series #1 and #2 comparing the reactor R2 (exposed to limewater) with reactors R1 or R3 when they are connected or not to the electromagnetic field (EMF). Reactors exposed to EMF are indicated by an "*". The two sets of data are considered to be significantly different when p-value is < to 0.05. CFU = colony forming unit (cultivability); SYBR = fluorochrome used to assess the total cell number; PI = fluorochrome used to assess the cell integrity

Treatment	Parameters	Material	Experimental series	Considered reactors	p-value	*n*
Preventive	CFU	pvc	Lime water + series #1	R2* vs R3	0.7	6
		SS			0.4	6
		pvc	Lime water + series #2	R2* vs R1	0.05	7
		SS			**0.01**	7
		pvc	Lime water + series #1	R2* vs R1*	0.1	6
		SS			**0.004**	6
		pvc	Lime water + series #2	R2* vs R3*	0.7	7
		SS			0.4	7
	Cells density (SYBR)	pvc	Lime water + series #1	R2* vs R3	0.1	6
		SS			0.9	6
		pvc	Lime water + series #2	R2* vs R1	1	7
		SS			0.4	7
		pvc	Lime water + series #1	R2* vs R1*	0.8	6
		SS			0.2	6
		pvc	Lime water + series #2	R2* vs R3*	0.1	7
		SS			0.5	7
	Damaged cells (PI)	pvc	Lime water + series #1	R2* vs R3	0.05	6
		SS			0.3	6
		pvc	Lime water + series #2	R2* vs R1	0.9	7
		SS			0.3	7
		pvc	Lime water + series #1	R2* vs R1*	0.4	6
		SS			0.2	6
		pvc	Lime water + series #2	R2* vs R3*	0.2	7
		SS			0.3	7

(to be continued)

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(Table S4, continuation and end)

Treatment	Parameters	Material	Experimental series	Considered reactors	p-value	*n*
Curative	CFU	pvc	Lime water + series #1	R2* vs R3	0.5	5
		SS			0.9	5
		pvc	Lime water + series #2	R2* vs R1	0.9	5
		SS			0.1	5
		pvc	Lime water + series #1	R2* vs R1*	0.2	4
		SS			0.2	5
		pvc	Lime water + series #2	R2* vs R3*	1	5
		SS			0.1	5
	Cells density (SYBR)	pvc	Lime water + series #1	R2* vs R3	0.1	5
		SS			0.6	5
		pvc	Lime water + series #2	R2* vs R1	0.7	5
		SS			**0.015**	5
		pvc	Lime water + series #1	R2* vs R1*	0.06	5
		SS			0.1	5
		pvc	Lime water + series #2	R2* vs R3*	0.7	5
		SS			0.2	5
	Damaged cells (PI)	pvc	Lime water + series #1	R2* vs R3	**0.03**	4
		SS			0.2	4
		pvc	Lime water + series #2	R2* vs R1	0.6	5
		SS			**0.015**	5
		pvc	Lime water + series #1	R2* vs R1*	**0.03**	4
		SS			0.2	4
		pvc	Lime water + series #2	R2* vs R3*	0.4	5
		SS			0.2	5
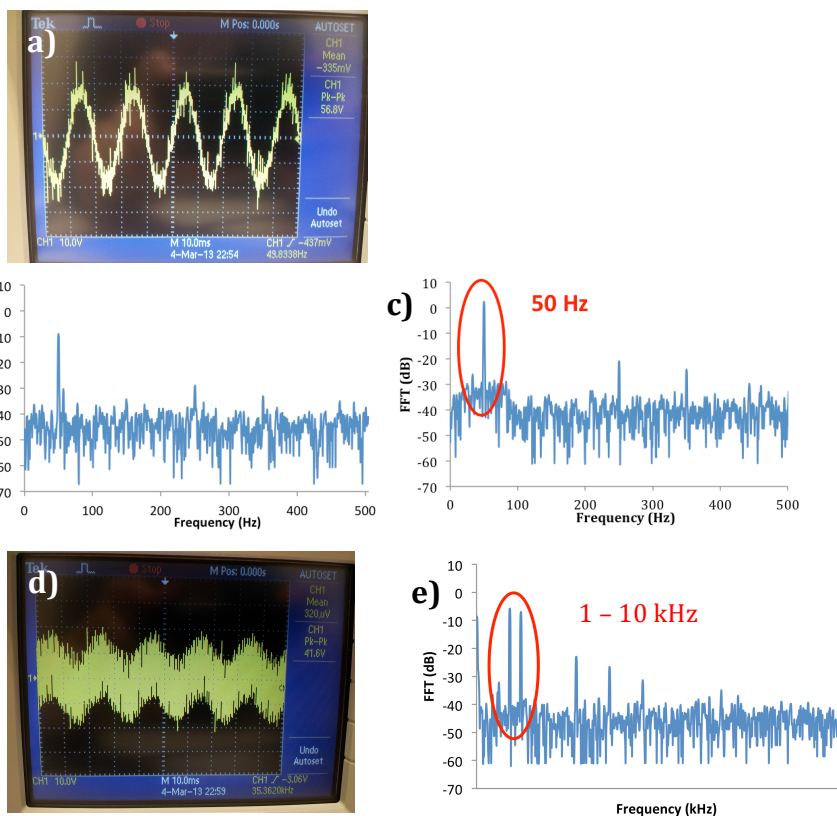
Figure S1. Measurements of the frequencies and intensities of the electromagnetic field generated by the Aqua-4D® tubes: a) screenshot of the oscilloscope indicating the electromagnetic signal background noise of the network when the device is switched is off (*Control*), b) and c) corresponding frequencies of the water in and out for the *Control*, respectively (the frequency of 50 Hz is due to the electrical network background), d) screenshot of the oscilloscope indicating the electromagnetic signal of the network when the device is switched on (*Assay*), and e) corresponding frequencies of the water out for the *Assay* (two frequencies between 1 - 10 kHz).
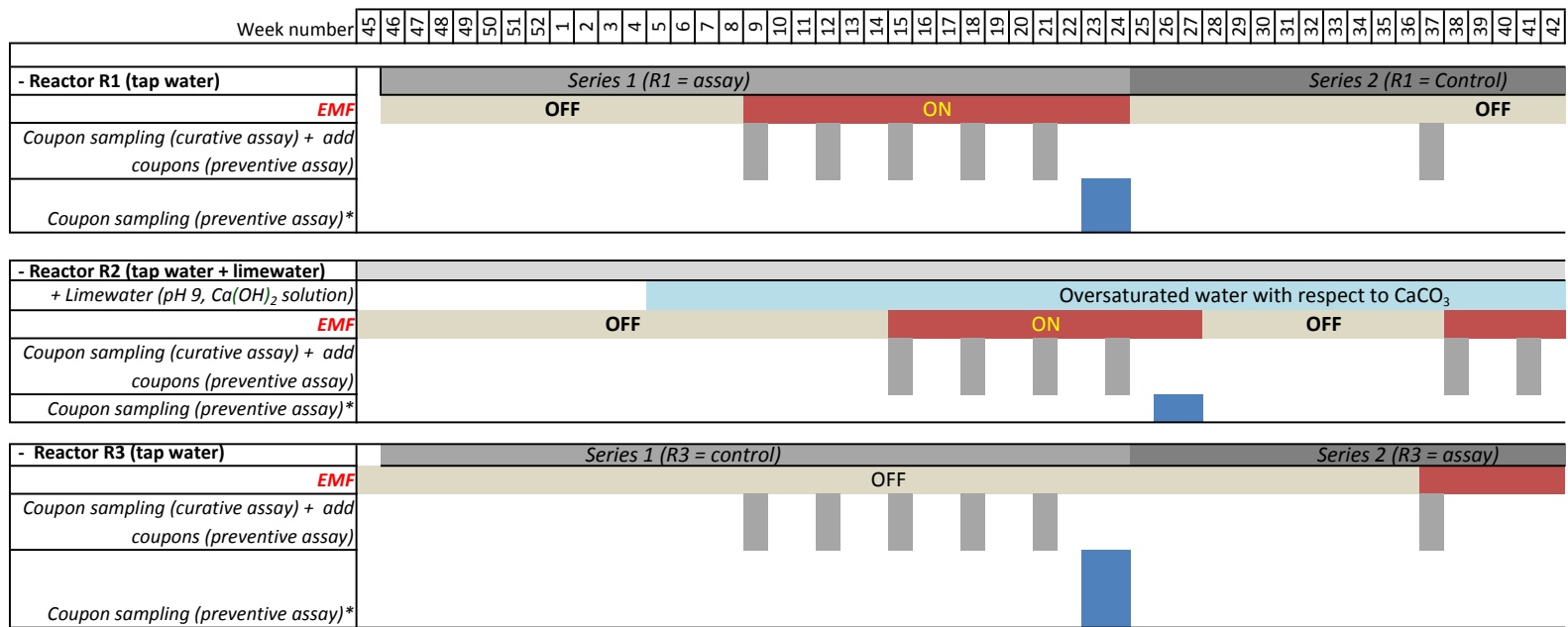
Coupons from preventive assay were sampled at the same time but exhibited different "ages" since they were installed at different time

Figure S2. Summary of the operation sequence for sampling, addition of new coupons, and sketch of EMF on/off.

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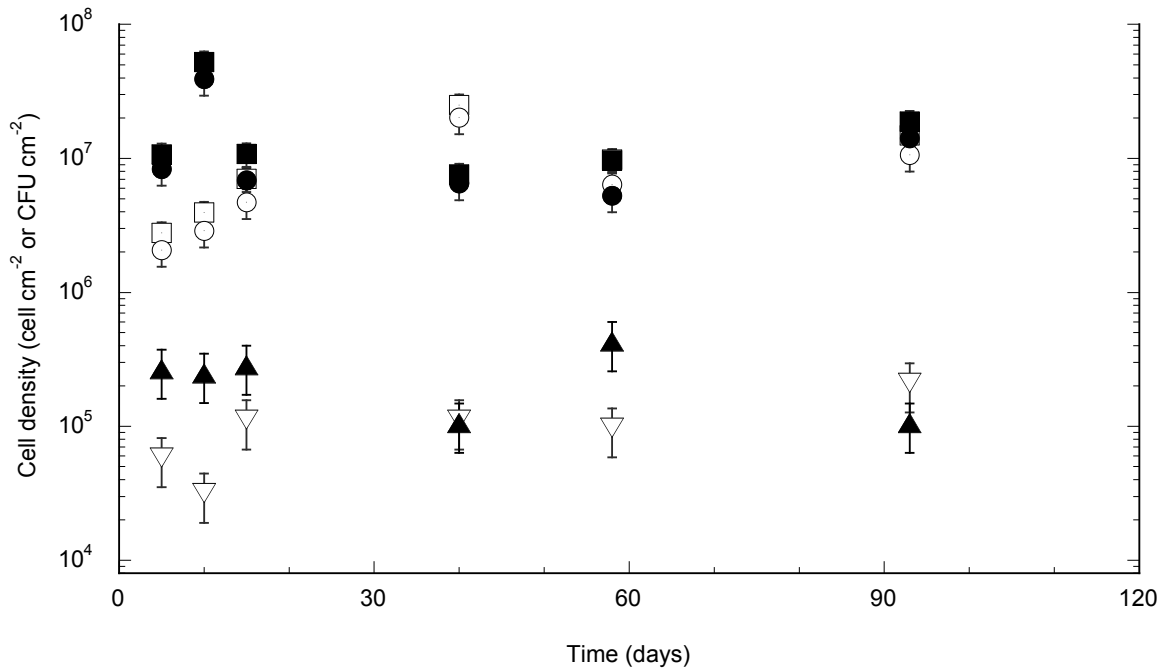79
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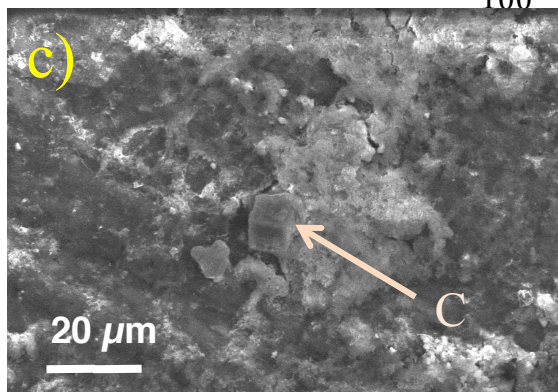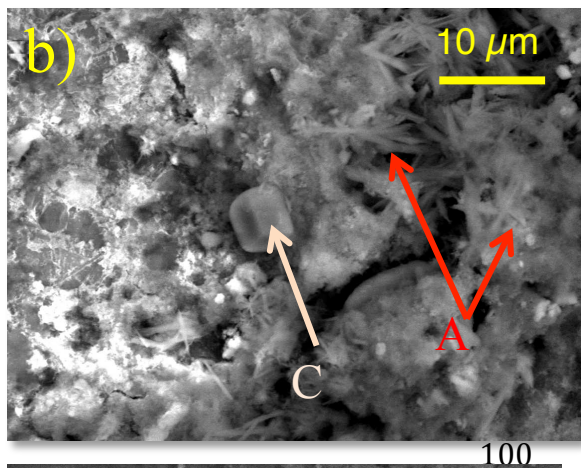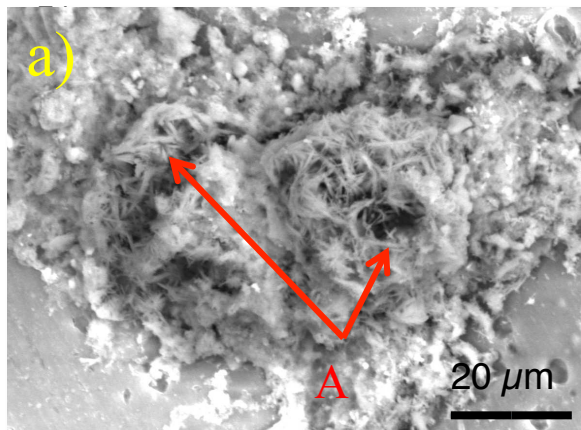
112 **Figure S3**. Images by scanning electron microscopy of
113 the PVC coupons from Reactor R2 (supplemented with
114 limewater): a) preventive treatment + EMF; b) curative
115 treatment + EMF, c) control before exposition to EMF
116 (2 month-old biofilm). A = aragonite, C = calcite.

a) (PVC coupons, 2nd test with Reactor P3 as Assay)

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**b)** (SS coupons, 2nd test with Reactor P3 as Assay)

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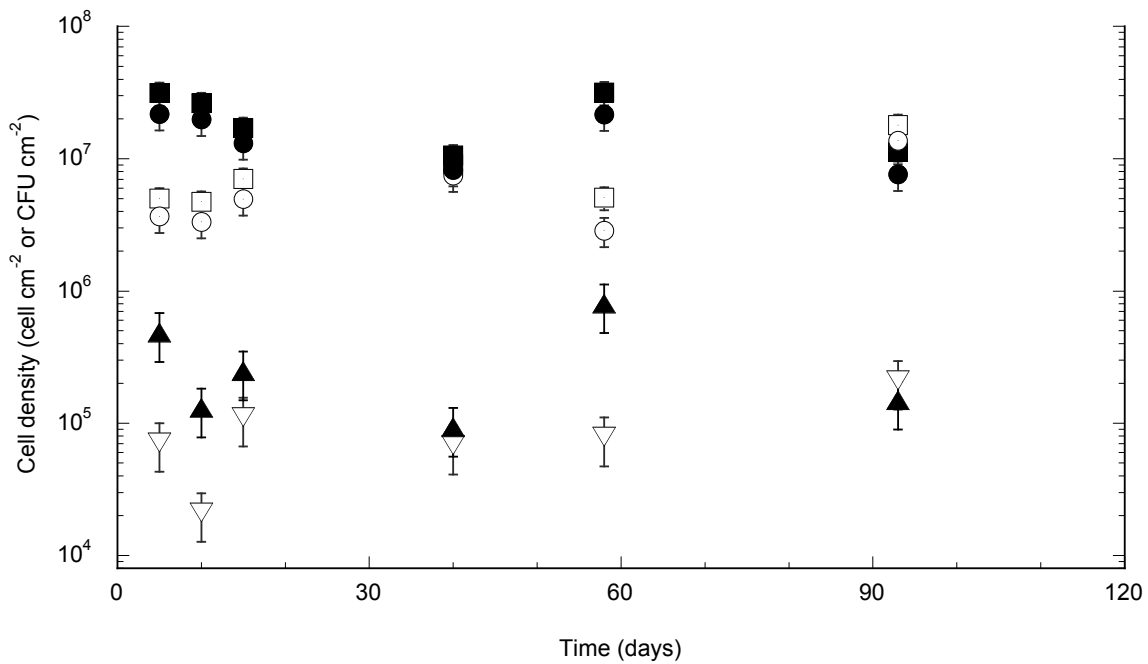
119 **Figure S4:** Number of total cells (square), membrane-damaged cells (circle), and cultivable
120 cells (CFU) (triangle) along the time in biofilms on PVC (a) and SS (b) coupons of Reactor
121 R1 (open symbols) and Reactor R3* (closed symbols). Reactor R1 was fed with tap water not
122 treated to EMF (*Control*), Reactor R3* was fed with the same tap water constantly exposed to
123 EMF (*Assay*). The experiment started with blank coupons (« preventive treatment »).
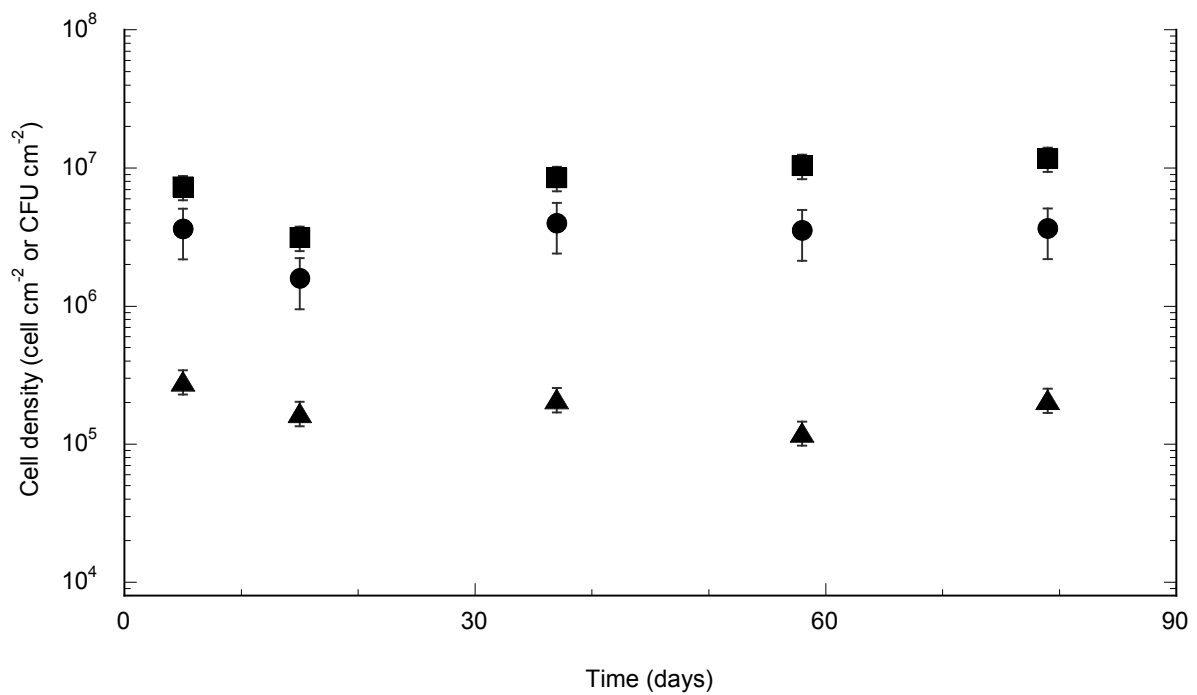124

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a) (PVC coupons with Reactor R2)
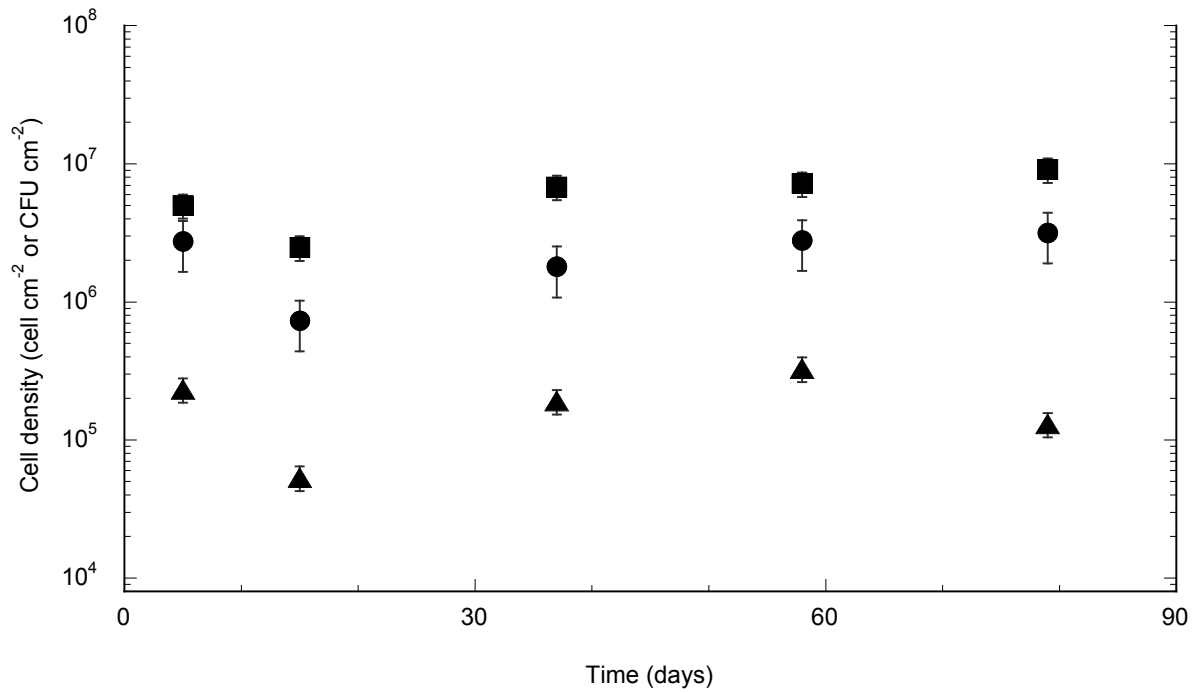
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b) (SS coupons with Reactor R2)

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130 **Figure S5:** Change of total cell number (square), cultivable cells (CFU) (triangle), and
131 membrane-damaged cells (circle) along the time in biofilms on PVC and SS coupons (n=1)
132 for Reactor R2 when it was fed with a tap water supplemented in $CaCO_3$ and constantly
133 exposed to EMF. The experiment started with blank coupons (preventive treatment).