Supplementary Materials

Influence of rhamnolipids and ionic cross-linking conditions on the mechanical properties of alginate hydrogels as a model bacterial biofilm

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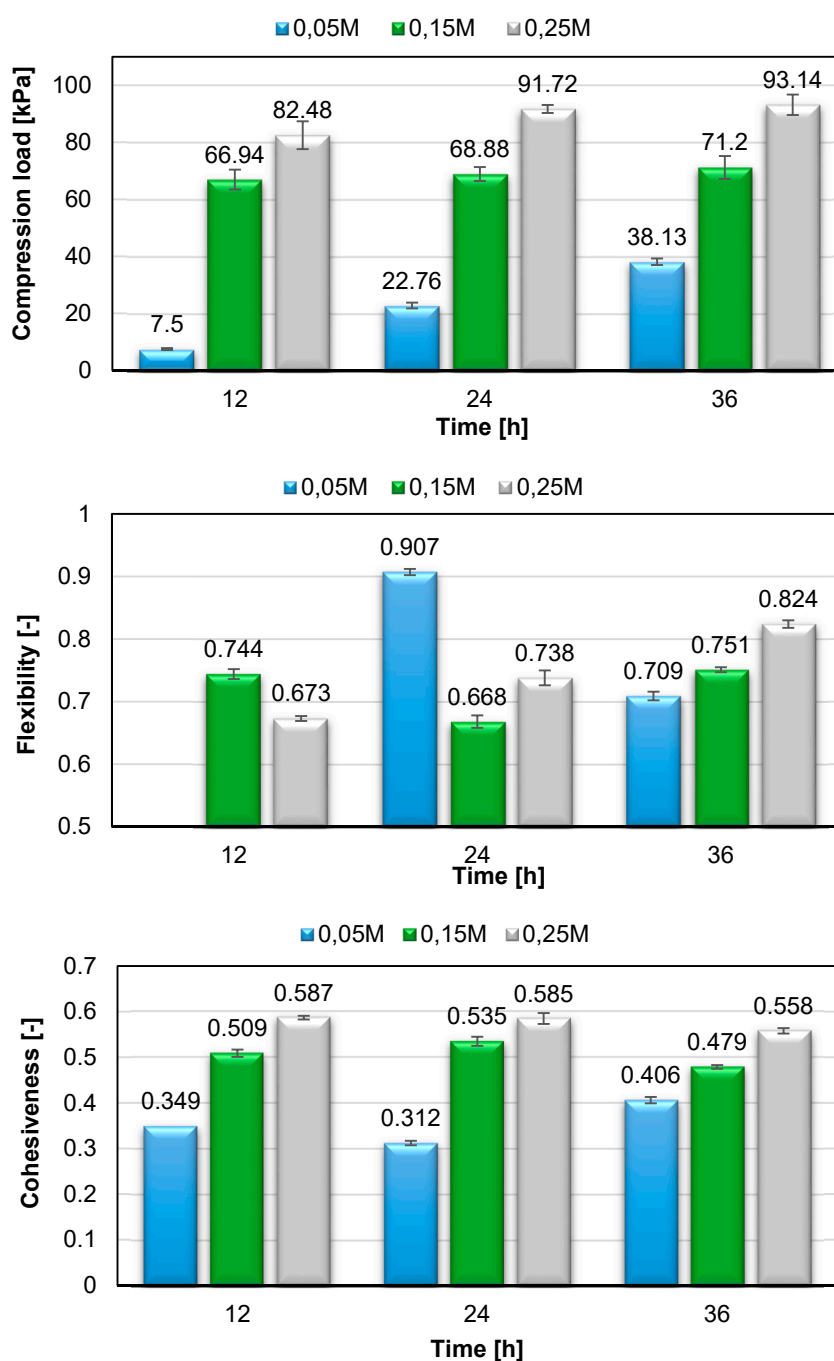* corresponding author: natalia.czaplicka@pg.edu.pl, tel. +48 58 347 21 14

Figure S1. Mechanical properties of the control hydrogels samples as a function of cross-linking time and $CaCl_2$ concentration.

Table S1. ANOVA results of the quadratic model for compression load

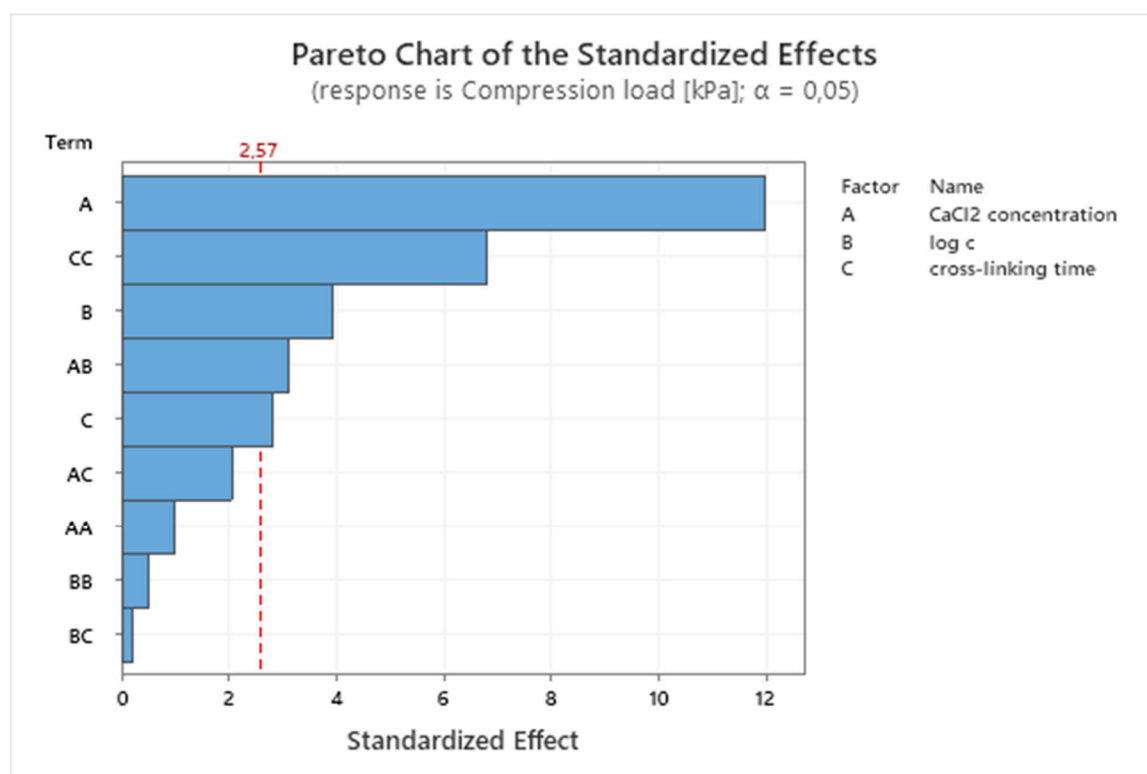| Source	Degrees of freedom (df)	Sum of squares (SS)	Mean square (MS)	F-value	p-value
Regression	9	11374.9	1263.87	**25.25**	**0.001**
Linear	3	8352.9	2784.29	**55.62**	**0.000**
A – $CaCl_2$ concentration	1	7194.0	7194.00	**143.72**	**0.000**
B – rhamnolipids concentration	1	767.1	767.14	**15.33**	**0.011**
C – ion cross-linking time	1	391.7	391.72	**7.83**	**0.038**
Square	3	2325.0	774.98	**15.48**	**0.006**
A^2	1	50.3	50.27	1.00	0.362
B^2	1	12.6	12.64	0.25	0.637
C^2	1	2313.2	2313.23	**46.21**	**0.001**
2-way interaction	3	697.1	232.35	4.64	0.066
AB	1	484.0	484.00	**9.67**	**0.027**
AC	1	210.8	210.83	4.21	0.095
BC	1	2.2	2.22	0.04	0.842
Residual error	5	250.3	50.06	-	-
Lack of fit	3	247.0	82.32	**49.47**	**0.020**
R^2			**0.9785**		

Figure S2. Pareto chart of the standardized effects for compression load.

Table S2. ANOVA results of the quadratic model for flexibility

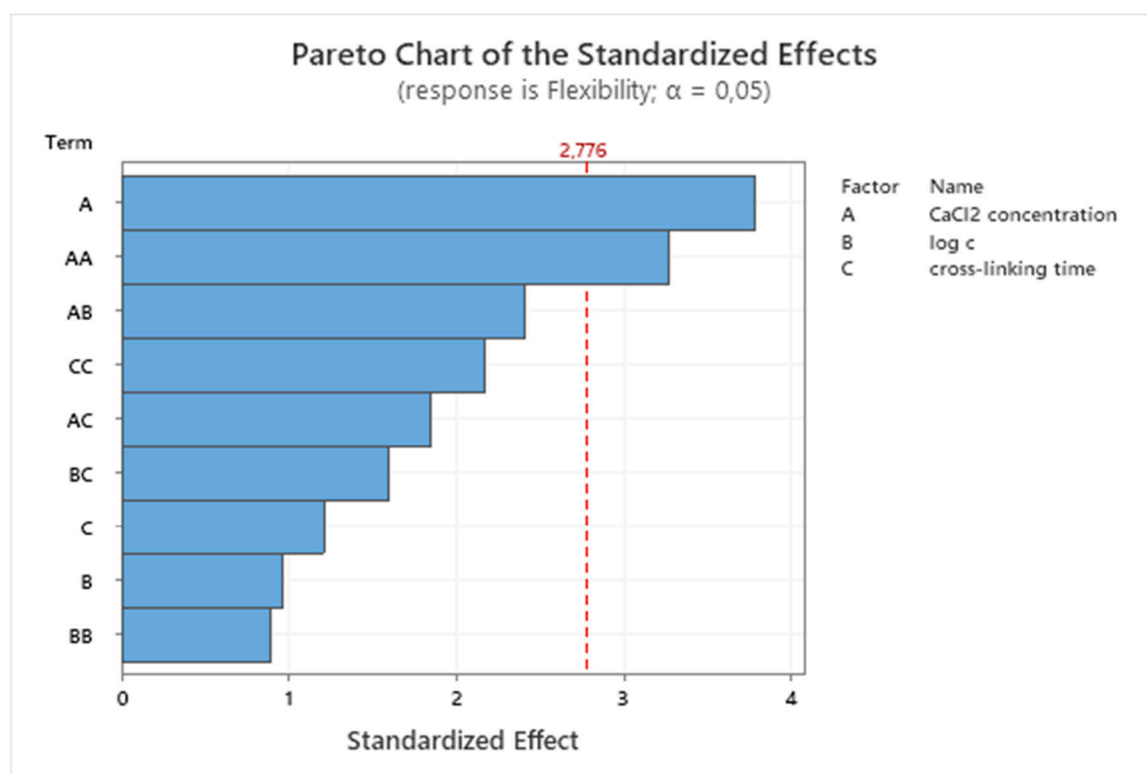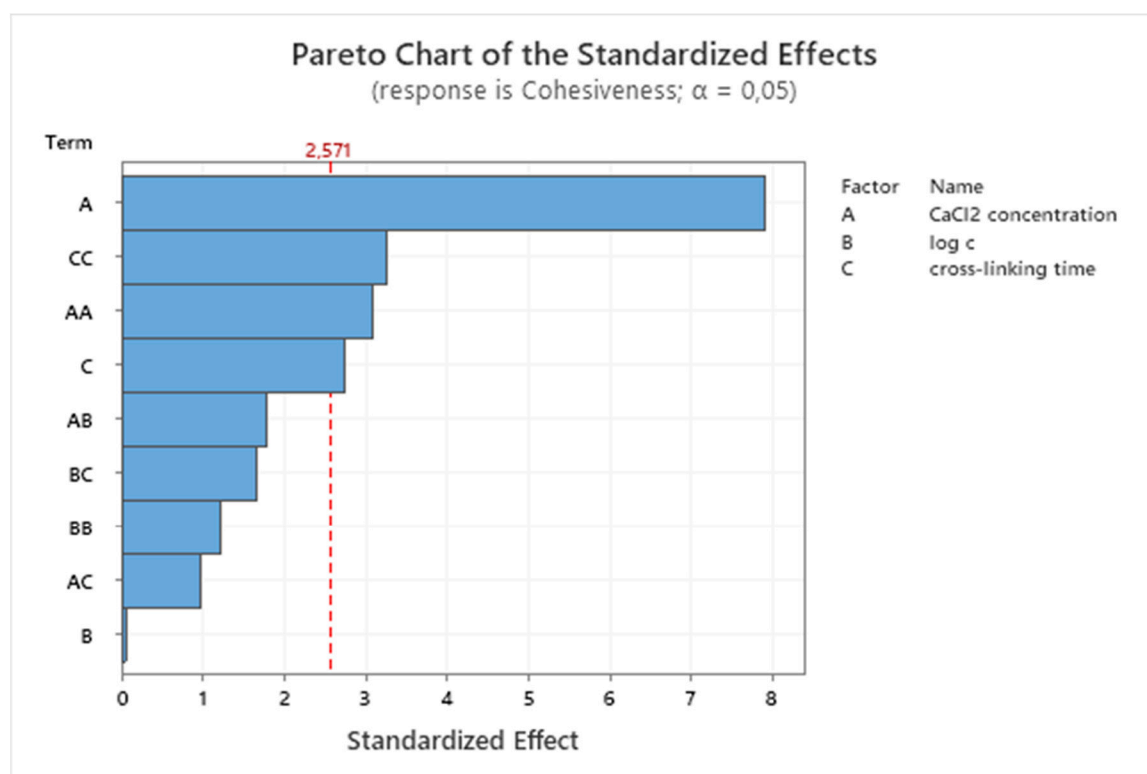| Source	Degrees of freedom (df)	Sum of squares (SS)	Mean square (MS)	F-value	p-value
Regression	9	0.042461	0.004718	3.85	0.104
Linear	3	0.018727	0.006242	5.09	0.075
A – CaCl$_2$ concentration	1	0.017595	0.017595	**14.35**	**0.019**
B – rhamnolipids concentration	1	0.001128	0.001128	0.92	0.392
C – ion cross-linking time	1	0.001789	0.001789	1.46	0.294
Square	3	0.018144	0.006048	4.93	0.079
A^2	1	0.013142	0.013142	**10.72**	**0.031**
B^2	1	0.000979	0.000979	0.80	0.422
C^2	1	0.005792	0.005792	4.72	0.095
2-way interaction	3	0.014440	0.004813	3.93	0.110
AB	1	0.007140	0.007140	5.82	0.073
AC	1	0.004163	0.004163	3.40	0.139
BC	1	0.003136	0.003136	2.56	0.185
Residual error	4	0.004904	0.001226	-	-
Lack of fit	2	0.004704	0.002352	**23.52**	**0.041**
R^2		**0.8965**			

Figure S3. Pareto chart of the standardized effects for flexibility.

Table S3. ANOVA results of the quadratic model for cohesiveness

Source	Degrees of freedom (df)	Sum of squares (SS)	Mean square (MS)	F-value	p-value
Regression	9	0.167702	0.018634	**11.14**	**0.008**
Linear	3	0.117219	0.039073	**23.36**	**0.002**
A – CaCl$_2$ concentration	1	0.104653	0.104653	**62.57**	**0.001**
B – rhamnolipids concentration	1	0.000004	0.000004	0.00	0.961
C – ion cross-linking time	1	0.012561	0.012561	7.51	0.041
Square	3	0.038970	0.012990	**7.77**	**0.025**
A^2	1	0.015901	0.015901	**9.51**	**0.027**
B^2	1	0.002520	0.002520	1.51	0.274
C^2	1	0.017771	0.017771	**10.62**	**0.022**
2-way interaction	3	0.011513	0.003838	2.29	0.195
AB	1	0.005329	0.005329	3.19	0.134
AC	1	0.001560	0.001560	0.93	0.378
BC	1	0.004624	0.004624	2.76	0.157
Residual error	5	0.008363	0.001673	-	-
Lack of fit	3	0.008075	0.002692	18.69	0.051
R^2			**0.9525**		

Figure S4. Pareto chart of the standardized effects for cohesiveness.