

Supporting Information

Table S1. Classification of the genera delineated in the fracturing water microbiome.

Bacteria	Gram-negative	% Total	Gram-positive	% Total
Non-Spore Forming	40	60	7	10
Spore Forming	9	13.5	5	7.5
Unknown	5	7.5	1	1.5
Total Bacteria	54 (of 67 Total)	81	13 (of 67 Total)	19

Table S2a

Table S2.a. Absorbance values for the bacterial liquid cultures at different dilutions and biocide concentrations.

BG Liquid Culture								
Boric acid	10 ⁷ /mL	STDEV	10 ⁶ /mL	STDEV	10 ⁵ /mL	STDEV	10 ⁴ /mL	STDEV
0 g/L	1	0.099177	1	0.067146	1	0.044583	1	0.070904
100 mg/L	0.87025	0.06761	0.77792	0.033431	0.787593	0.039387	0.834771	0.027385
250 mg/L	0.949698	0.090437	0.829318	0.034659	0.849561	0.004102	0.848714	0.039661
500 mg/L	0.945033	0.082846	0.909454	0.014901	0.904877	0.04329	0.912403	0.049175
1 g/L	0.916802	0.106878	0.98373	0.064558	0.912494	0.052995	0.92155	0.053525
5 g/L	0.371505	0.014303	0.575102	0.022662	0.652593	0.010479	0.695717	0.030874
EDTA	10 ⁷ /mL	STDEV	10 ⁶ /mL	STDEV	10 ⁵ /mL	STDEV	10 ⁴ /mL	STDEV
0 g/L	1	0.137554	1	0.051403	1	0.050196	1	0.030671
100 mg/L	0.71517	0.066214	0.6613	0.012626	0.78957	0.004257	0.79639	0.014949
250 mg/L	0.682366	0.022265	0.667875	0.02945	0.830555	0.072775	0.791715	0.0115
500 mg/L	0.533789	0.020564	0.594125	0.009905	0.825845	0.073811	0.834522	0.015647
1 g/L	0.330934	0.094834	0.1825	0.007366	0.212051	0.002571	0.197275	0.020095
5 g/L	0.153126	0.017682	0.17635	0.005923	0.214651	0.006153	0.196691	0.022054
Lactic acid	10 ⁷ /mL	STDEV	10 ⁶ /mL	STDEV	10 ⁵ /mL	STDEV	10 ⁴ /mL	STDEV
0 g/L	1	0.05149	1	0.092133	1	0.040746	1	0.016919
100 mg/L	0.914326	0.016051	0.741886	0.00982	0.74864	0.039914	0.817108	0.04968
250 mg/L	0.951409	0.056164	0.695432	0.015092	0.797509	0.02733	0.82361	0.037358
500 mg/L	0.950567	0.01331	0.748497	0.028588	0.804764	0.02087	0.81869	0.067186
1 g/L	0.968495	0.054487	0.781101	0.080356	0.828172	0.044808	0.817108	0.044834
5 g/L	0.791201	0.039467	0.745476	0.01238	0.832655	0.004735	0.791453	0.109027
Tannic acid	10 ⁷ /mL	STDEV	10 ⁶ /mL	STDEV	10 ⁵ /mL	STDEV	10 ⁴ /mL	STDEV
0 g/L	1	0.077673	1	0.049186	1	0.026534	1	0.057637
100 mg/L	0.735601	0.041032	0.919095	0.096527	0.732852	0.010175	0.804791	0.035261
250 mg/L	0.631727	0.04082	1.01271	0.041708	0.695572	0.031142	0.681352	0.046398
500 mg/L	0.465533	0.051464	0.634837	0.024307	0.644318	0.010168	0.622741	0.015578
1 g/L	0.382714	0.011563	0.715657	0.041352	0.505895	0.170448	0.308702	0.013058
5 g/L	0.169332	0.013021	0.281851	0.039944	0.314915	0.002466	0.428078	0.012495
E. coli Liquid Culture								
Boric acid	10 ⁷ /mL	STDEV	10 ⁶ /mL	STDEV	10 ⁵ /mL	STDEV	10 ⁴ /mL	STDEV
0 g/L	1	0.021128	1	0.061229	1	0.089756	1	0.032327
100 mg/L	0.924824	0.045092	0.873343	0.010594	0.917722	0.105835	0.934539	0.063375
250 mg/L	0.961739	0.063003	0.920984	0.037235	0.942791	0.042647	1.000327	0.027641
500 mg/L	0.910853	0.055921	0.911833	0.035461	0.96131	0.046338	0.972298	0.022882
1 g/L	0.899007	0.021113	0.910594	0.005925	0.928329	0.052673	0.977892	0.040217
5 g/L	0.886801	0.041408	0.904631	0.029664	0.917461	0.004477	0.977208	0.042556
EDTA	10 ⁷ /mL	STDEV	10 ⁶ /mL	STDEV	10 ⁵ /mL	STDEV	10 ⁴ /mL	STDEV
0 g/L	1	0.055292	1	0.036235	1	0.06221	1	0.033258
100 mg/L	0.838496	0.009223	0.826758	0.008391	0.768255	0.012698	0.857155	0.018509
250 mg/L	0.891915	0.058303	0.882676	0.009427	0.809559	0.016659	0.865737	0.019067
500 mg/L	0.899802	0.030464	0.897397	0.056851	0.777241	0.043724	0.94004	0.051612

1 g/L	0.813752	0.008979	0.800671	0.040584	0.787251	0.018218	0.850128	0.022696
5 g/L	0.700816	0.010808	0.745169	0.021845	0.734307	0.02982	0.80883	0.035143
Lactic acid	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.088683	1	0.036544	1	0.023007	1	0.065177
100 mg/L	1.038784	0.129678	0.918636	0.01921	0.917382	0.078907	0.99004	0.026787
250 mg/L	0.999301	0.027587	0.962385	0.010429	0.832192	0.025796	0.919431	0.089412
500 mg/L	1.005667	0.011842	0.882809	0.02656	0.838237	0.070646	0.80773	0.017501
1 g/L	1.038453	0.033466	0.967258	0.033187	0.865086	0.064945	0.869072	0.031736
5 g/L	0.998105	0.012088	0.908627	0.040608	0.875088	0.037024	0.889701	0.033744
Tannic acid	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.021462	1	0.034799	1	0.027238	1	0.061004
100 mg/L	0.932311	0.165901	0.841789	0.040633	0.820451	0.016743	0.830613	0.032117
250 mg/L	0.727987	0.036571	0.791178	0.013846	0.756426	0.005052	0.782127	0.009708
500 mg/L	0.74385	0.086473	0.762683	0.019897	0.713249	0.01219	0.765386	0.036078
1 g/L	0.751154	0.050724	0.747585	0.037273	0.752428	0.013081	0.756371	0.061288
5 g/L	0.597838	0.032633	0.601707	0.031119	0.630419	0.060248	0.638855	0.104062
Fracturing Water Liquid Culture								
Boric acid	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.139851	1	0.570557	1	0.575045	1	0.023625
100 mg/L	0.699312	0.204925	0.991086	0.769262	0.24718	0.000436	0.781481	0.001039
250 mg/L	0.901616	0.328686	0.289909	0.002419	0.830805	0.443507	0.985185	0.026001
500 mg/L	1.009339	0.1665	1.138196	0.847406	0.325749	0.003219	0.920782	0.017307
1 g/L	0.947321	0.082283	0.289618	0.002722	0.33463	0.004876	0.970165	0.022073
5 g/L	0.722448	0.142806	0.914822	0.612112	0.327823	0.003745	0.94465	0.020085
EDTA	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.176823	1	0.007988	1	0.386695	1	0.022806
100 mg/L	0.815967	0.087199	1.436531	0.2388	0.717947	0.270902	0.808647	0.002401
250 mg/L	0.89772	0.137093	3.40696	0.459755	0.424816	0.002406	0.901755	0.013204
500 mg/L	0.821578	0.34176	2.615759	0.539996	0.423411	0.002859	0.921661	0.015611
1 g/L	0.828913	0.169143	0.932023	0.009364	0.437546	0.002603	0.975599	0.022407
5 g/L	0.122725	0.026594	0.936351	0.002951	0.437216	0.008402	0.997003	0.016266
Lactic acid	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.109642	1	0.418667	1	0.006848	1	0.017229
100 mg/L	0.869032	0.250561	0.991872	0.505122	0.839626	0.002425	0.861349	0.008879
250 mg/L	0.847949	0.141126	1.02382	0.449068	0.958034	0.002524	0.948684	0.008199
500 mg/L	0.908471	0.173625	0.449392	0.003765	0.929017	0.004279	0.916776	0.01369
1 g/L	0.892683	0.71733	0.440557	0.004532	0.963027	0.004966	0.968586	0.009057
5 g/L	0.363198	0.28887	0.650905	0.178473	0.931981	0.007165	0.939967	0.007114
Tannic acid	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.228952	1	0.460064	1	0.000889	1	0.012011
100 mg/L	0.985515	0.275855	0.441268	0.007703	1.009264	0.006461	1.042321	0.019032
250 mg/L	0.652217	0.188908	0.660563	0.067223	2.344345	0.345315	1.32125	0.006358
500 mg/L	0.45089	0.207618	0.619744	0.002875	1.495193	0.004341	1.46375	0.011092
1 g/L	0.381248	0.025232	0.753782	0.01117	1.787275	0.00542	1.736429	0.006806
5 g/L	0.303793	0.016877	0.81986	0.056291	1.604964	0.029905	1.819643	0.0269

Table S2. b

Table S2b. Absorbance values for bacterial biofilms at different dilutions and biocide concentrations.

BG Biofilm								
Boric acid	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.047604	1	0.276341	1	0.431664	1	0.137193
100 mg/L	1.002609	0.01908	1.018605	0.102942	1.107388	0.126402	1.110406	0.003814
250 mg/L	1.011306	0.042407	0.811489	0.257727	0.935849	0.168992	0.949587	0.292658
500 mg/L	1.003354	0.061481	1.095156	0.021968	1.175932	0.075875	0.97344	0.142745

1 g/L	0.979639	0.02861	1.075783	0.095901	1.196623	0.023042	0.907407	0.306366
5 g/L	0.766972	0.309529	1.05035	0.088302	1.201256	0.005302	0.928185	0.440915
EDTA	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.072819	1	0.027573	1	0.014174	1	0.019135
100 mg/L	10.00865	0.028162	18.26242	0.031449	19.99856	0.065462	17.46811	0.020665
250 mg/L	3.962254	0.025047	7.424955	0.011857	8.212738	0.026117	7.553994	0.105342
500 mg/L	3.92233	0.095862	7.551963	0.016483	8.427281	0.020394	7.337801	0.230478
1 g/L	3.879725	0.057676	7.721519	0.058239	8.553817	0.012758	6.68846	0.09192
5 g/L	3.990005	0.271989	1.261568	0.035867	0.962557	0.030456	0.957502	0.04888
Lactic acid	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.125688	1	0.033889	1	0.019415	1	0.167423
100 mg/L	0.93492	0.009753	0.994839	0.010294	0.992609	0.060367	1.113839	0.04062
250 mg/L	0.943117	0.024051	0.853123	0.297169	0.90241	0.256808	1.010932	0.308026
500 mg/L	0.932914	0.076449	0.99206	0.014614	0.999002	0.017197	0.949065	0.28609
1 g/L	0.941911	0.017379	0.998046	0.004583	0.99706	0.022285	1.067804	0.03822
5 g/L	0.927179	0.025546	0.99517	0.009494	0.999764	0.016702	1.052914	0.153809
Tannic acid	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.022774	1	0.008147	1	0.005254	1	0.025571
100 mg/L	0.908771	0.016456	0.986102	0.0051	0.995477	0.029673	0.998803	0.005823
250 mg/L	0.925909	0.038784	1.006155	0.01022	1.000112	0.017328	1.017162	0.034698
500 mg/L	0.911621	0.036945	1.007181	0.019881	0.999874	0.009582	1.001682	0.003831
1 g/L	0.90162	0.02155	1.002825	0.018023	0.772381	0.924173	0.494206	0.787956
5 g/L	0.599723	0.149141	0.84036	0.448825	1.022672	0.031568	0.968228	0.179039
E. coli Biofilm								
Boric acid	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.003338	1	0.007544	1	0.003758	1	0.013372
100 mg/L	0.991785	0.00611	0.984753	0.003362	0.986135	0.006396	0.998085	0.035127
250 mg/L	1.000157	0.023044	1.00586	0.021508	0.997016	0.002797	0.995828	0.012344
500 mg/L	1.002378	0.00418	0.997582	0.007158	1.00217	0.008697	0.996699	0.006915
1 g/L	1.003374	0.003525	0.998933	0.00151	0.999443	0.006096	1.000157	0.011279
5 g/L	1.001438	0.020471	1.006372	0.016438	1.004727	0.005581	0.9983	0.005255
EDTA	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.012279	1	0.009802	1	0.015215	1	0.008472
100 mg/L	0.985003	0.014627	0.985717	0.006596	0.974187	0.009378	0.9871	0.026885
250 mg/L	0.996798	0.01937	0.996958	0.009417	0.990376	0.006255	0.997576	0.011694
500 mg/L	1.009338	0.025441	1.009629	0.015096	0.993924	0.018429	0.996073	0.041635
1 g/L	0.995085	0.011317	0.994828	0.007674	0.991046	0.014809	1.006478	0.070187
5 g/L	0.995872	0.014362	0.99871	0.010458	0.995614	0.009297	1.012049	0.060651
Lactic acid	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.031778	1	0.018113	1	0.008599	1	0.037164
100 mg/L	0.980114	0.030934	0.978789	0.011643	0.971945	0.016249	0.979555	0.013203
250 mg/L	0.998221	0.012911	1.000014	0.010324	0.999986	0.003308	0.997782	0.035133
500 mg/L	0.997531	0.037511	1.003129	0.007315	1.004587	0.002138	0.997006	0.015924
1 g/L	0.996359	0.034424	1.009882	0.009789	1.003255	0.005853	1.004851	0.049557
5 g/L	0.989754	0.022043	1.002881	0.011801	1.002458	0.012795	0.999071	0.032846
Tannic acid	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.029151	1	0.01885	1	0.006897	1	0.036074
100 mg/L	0.983585	0.008723	0.974584	0.006486	0.970392	0.004004	0.974057	0.008514
250 mg/L	0.957549	0.108072	1.012129	0.010825	1.005963	0.014174	0.882555	0.343183
500 mg/L	0.929248	0.290621	1.013844	0.012056	1.021245	0.041586	0.821978	0.388277
1 g/L	0.918642	0.158531	0.996194	0.005519	0.995812	0.015168	0.913116	0.219422
5 g/L	0.996413	0.017936	1.067242	0.096908	1.078158	0.032871	1.049466	0.097682
Fracturing Water Biofilm								
Boric acid	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.210817	1	0.070437	1	0.06507	1	0.015053
100 mg/L	0.855964	0.180951	0.852082	0.01513	0.902678	0.019352	0.984809	0.029752

250 mg/L	0.421375	0.073176	0.801819	0.020162	0.807598	0.004879	0.933964	0.016357
500 mg/L	0.576144	0.164833	1.00742	0.096661	0.883105	0.020247	1.027748	0.027321
1 g/L	0.717814	0.196413	1.366084	0.28023	0.871959	0.012606	0.923733	0.010728
5 g/L	0.537077	0.176712	1.281355	0.261109	0.838657	0.005452	0.937219	0.015938
EDTA	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.231158	1	0.004706	1	0.369842	1	0.016501
100 mg/L	0.916078	0.345587	0.862117	0.006088	0.464534	0.012816	0.912881	0.025753
250 mg/L	1.366677	0.420158	2.996096	0.486942	0.541787	0.023403	0.982413	0.030389
500 mg/L	0.722062	0.058493	1.189725	0.051055	0.510105	0.008952	1.115291	0.025321
1 g/L	0.612128	0.116591	1.108682	0.012638	0.558096	0.019695	1.106497	0.011915
5 g/L	0.355384	0.010516	1.113367	0.016951	0.544518	0.019463	1.105683	0.011573
Lactic acid	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.050763	1	0.131484	1	0.006951	1	0.010821
100 mg/L	1.209065	0.070867	0.643571	0.084064	1.394213	0.086286	1.00914	0.00446
250 mg/L	1.518193	0.150279	0.749064	0.115874	0.948292	0.002684	1.040761	0.006661
500 mg/L	1.195021	0.04249	0.470662	0.014232	0.974383	0.004202	1.042984	0.006408
1 g/L	0.594319	0.544005	0.503745	0.00756	1.016603	0.011254	1.076087	0.006065
5 g/L	0.861315	0.103915	0.479775	0.015698	1.092979	0.0104	1.194417	0.009701
Tannic acid	10⁷/mL	STDEV	10⁶/mL	STDEV	10⁵/mL	STDEV	10⁴/mL	STDEV
0 g/L	1	0.170342	1	0.542422	1	0.024014	1	0.008195
100 mg/L	1.083677	0.4278	0.30624	0.016267	1.082533	0.01347	1.153732	0.002869
250 mg/L	0.891866	0.172586	0.712267	0.343067	3.397373	0.675452	1.214039	0.011125
500 mg/L	0.59934	0.100656	0.395963	0.018583	1.644972	0.037152	1.532872	0.008208
1 g/L	0.58615	0.01277	0.448785	0.001646	1.596942	0.031122	1.74518	0.022084
5 g/L	0.839929	0.04016	0.921734	0.053893	2.585964	0.0133	3.107266	0.043432

Figures S1-S14

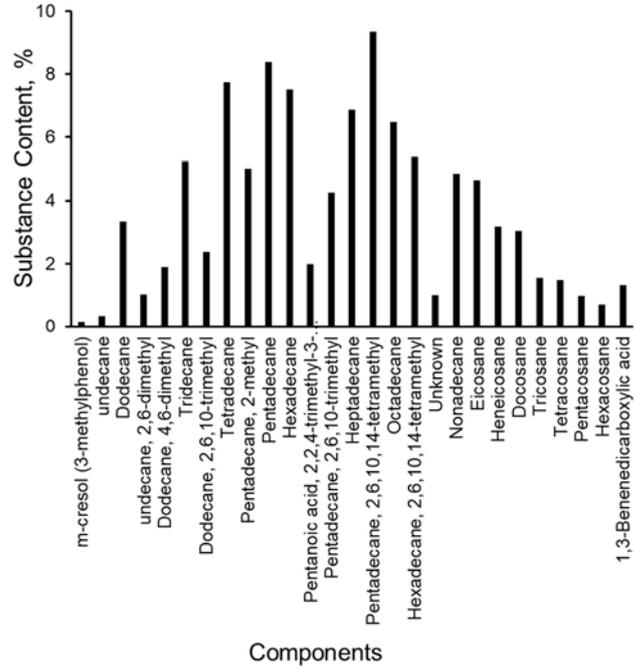


Figure S1. GC-MS analysis of fracturing water sample.

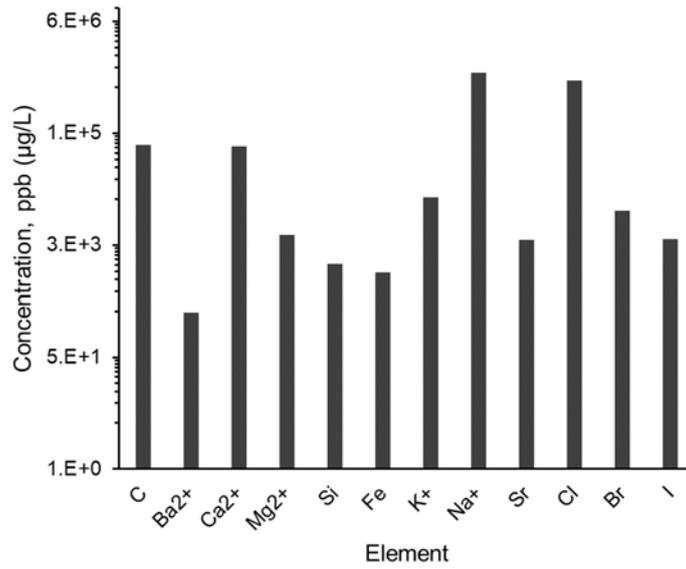
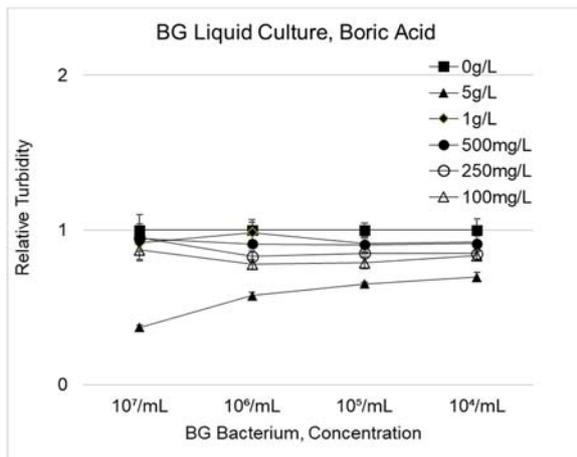
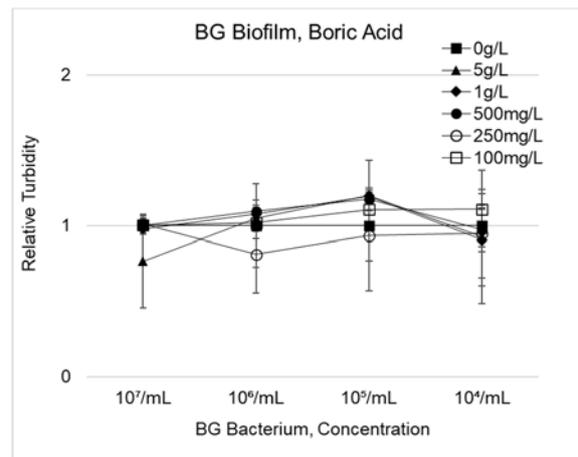


Figure S2. Aqueous elemental composition of the back-produced fracturing water collected at Ford Shale, TX. All concentrations are reported in µg/L (ppb).

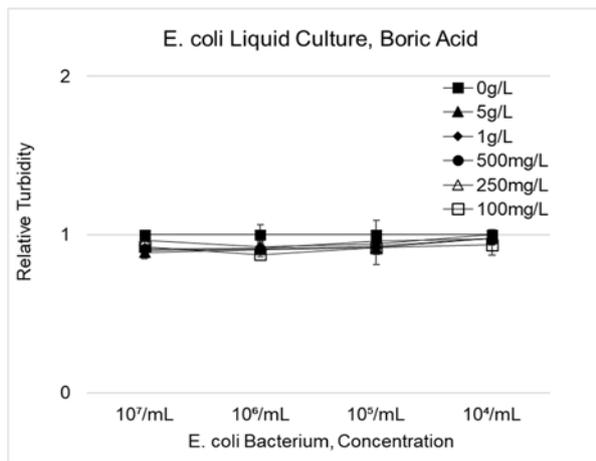


(a)

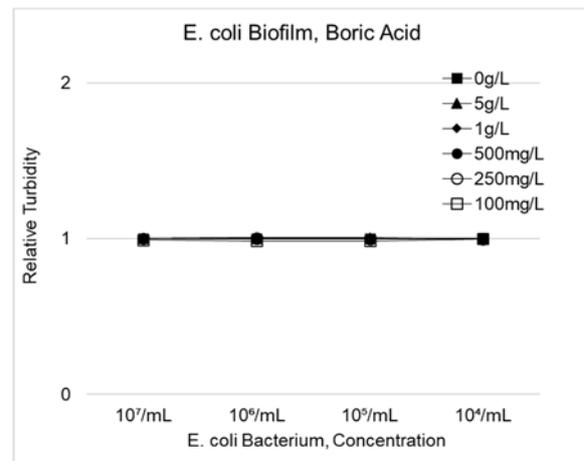


(b)

Figure S3. (a) The effect of increasing boric acid concentrations on the growth of BG liquid cultures and (b) on the formation of BG biofilms.

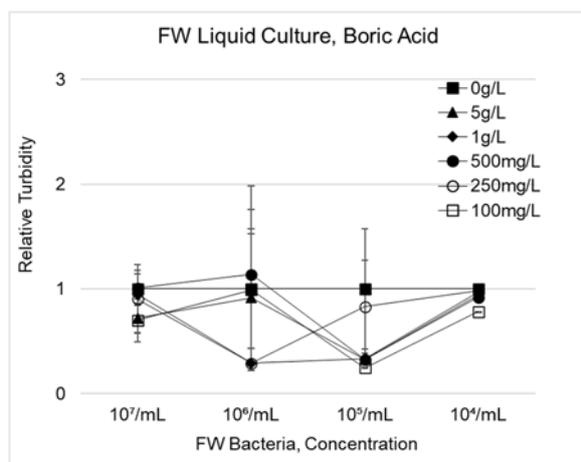


(a)

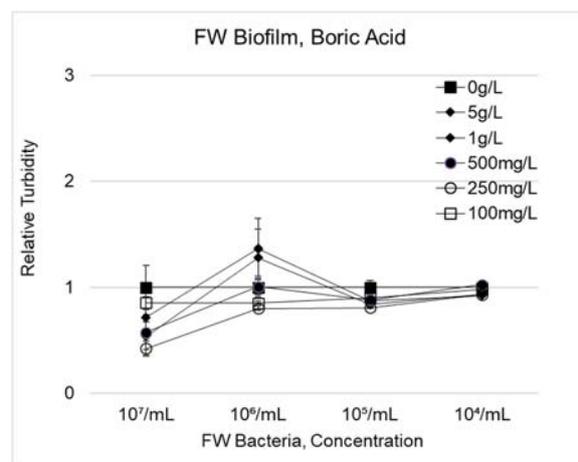


(b)

Figure S4. (a) The effect of increasing boric acid concentrations on the growth of *E. coli* liquid cultures and (b) on the formation of *E. coli* biofilms.



(a)



(b)

Figure S5. (a) The effect of increasing boric acid concentrations on the growth of FW liquid cultures and (b) on the formation of FW biofilms.

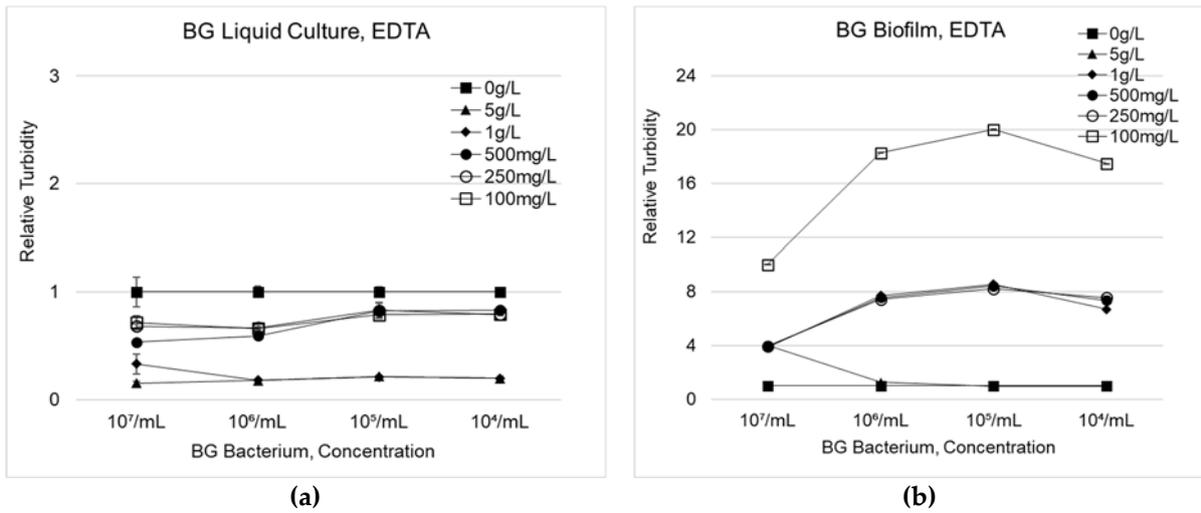


Figure S6. (a) The effect of increasing EDTA concentrations on the growth of BG liquid cultures and (b) on the formation of BG biofilms.

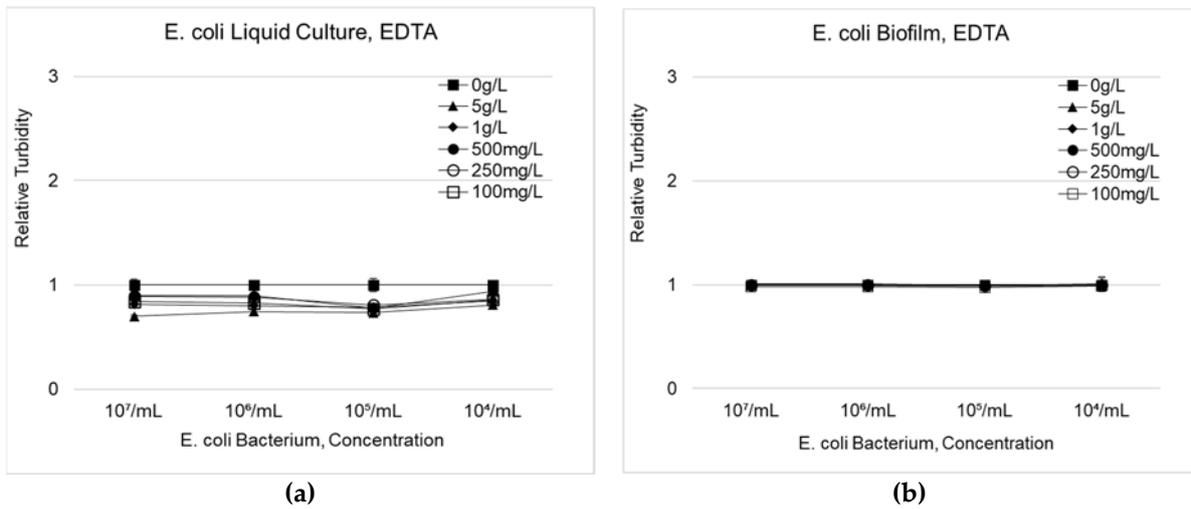


Figure S7. (a) The effect of increasing EDTA concentrations on the growth of *E. coli* liquid cultures and (b) on the formation of *E. coli* biofilms.

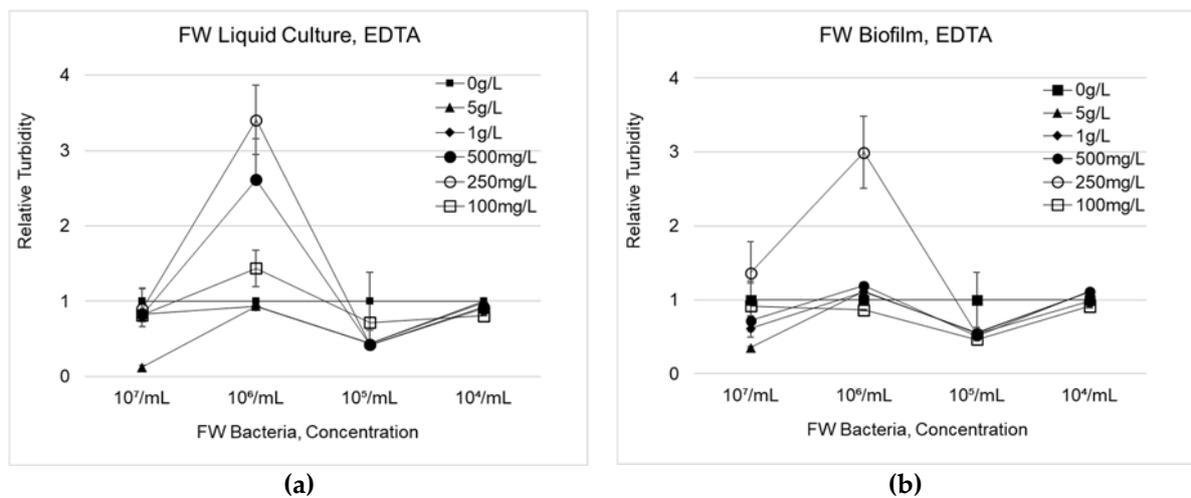
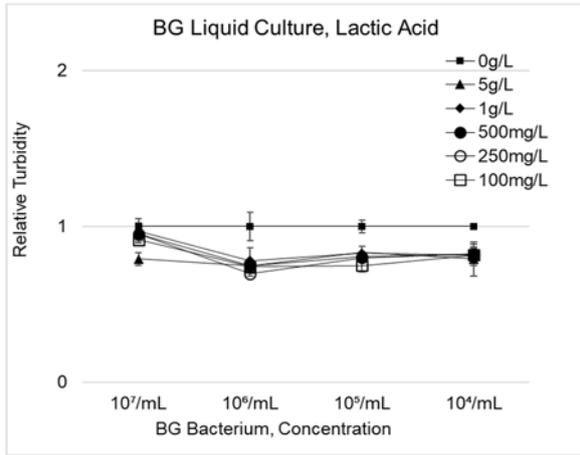
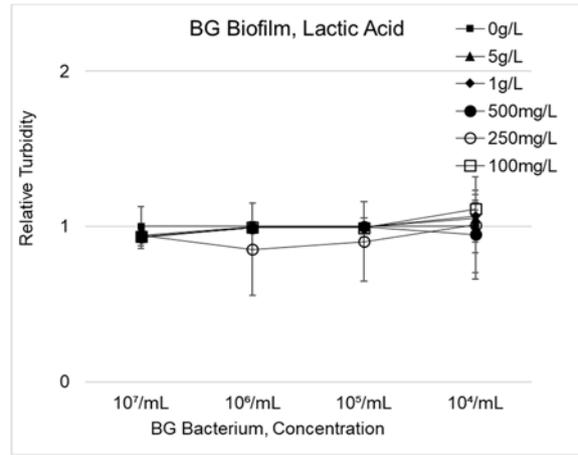


Figure S8. (a) The effect of EDTA solutions on the growth of back-produced fracturing water (FW) liquid cultures and (b) on the formation of FW biofilm.

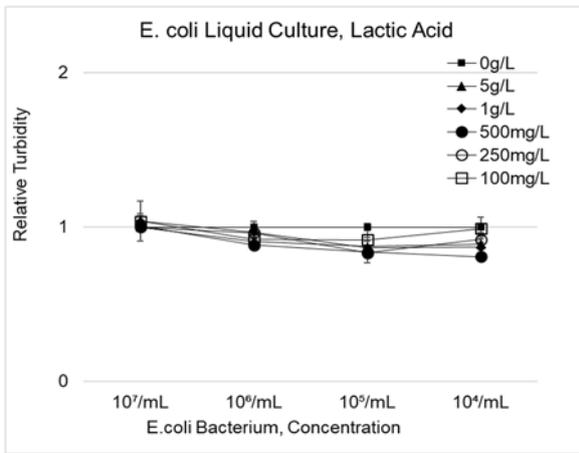


(a)

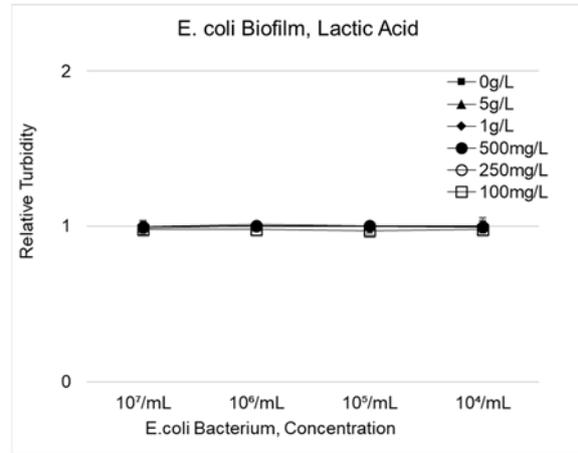


(b)

Figure S9. (a) The effect of increasing lactic acid concentrations on the growth of BG liquid cultures and (b) on the formation of BG biofilms.

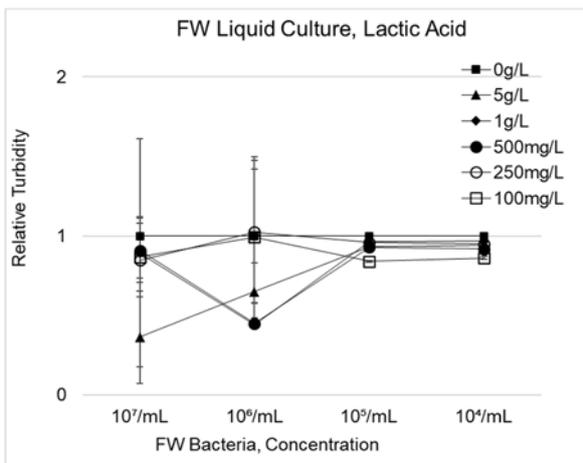


(a)

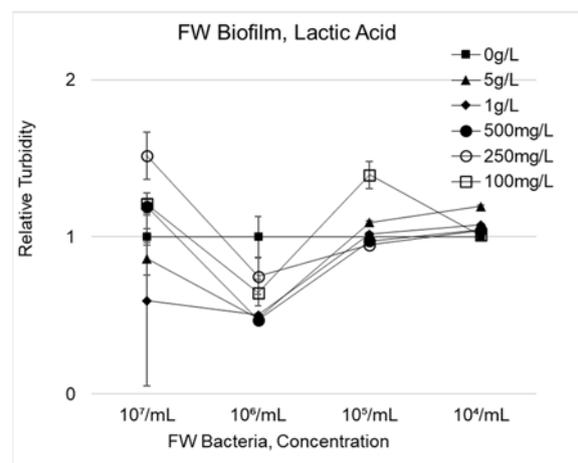


(b)

Figure S10. (a) The effect of increasing lactic acid concentrations on the growth of *E. coli* liquid cultures and (b) on the formation of *E. coli* biofilms.

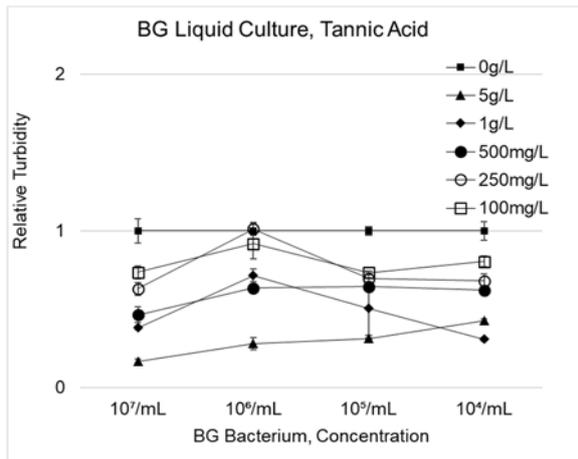


(a)

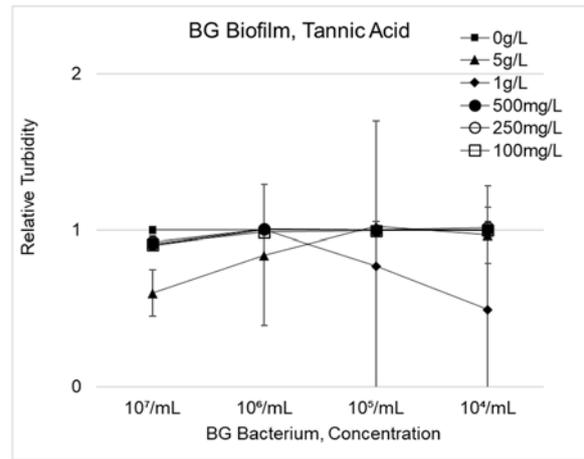


(b)

Figure S11. (a) The effect of increasing lactic acid concentrations on the growth of FW liquid cultures and (b) on the formation of FW biofilms.

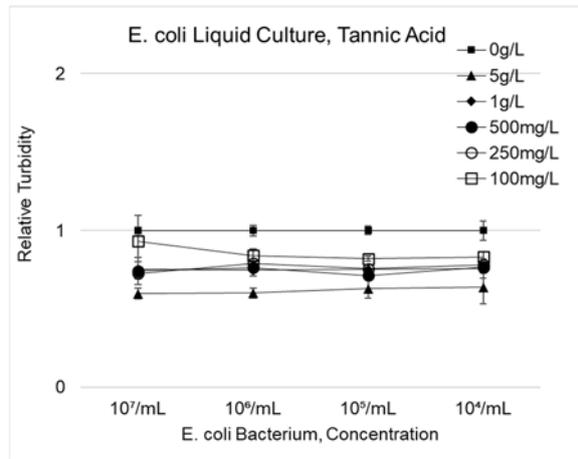


(a)

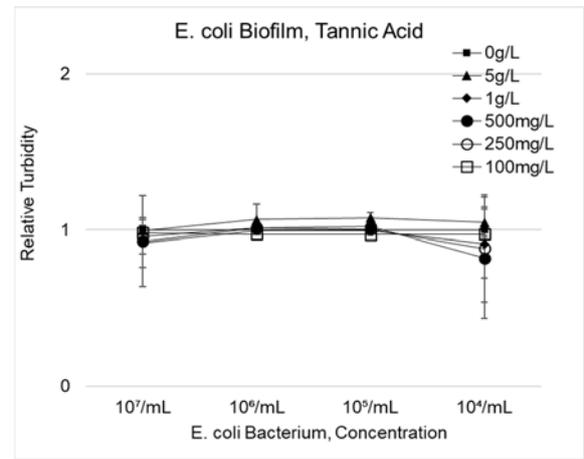


(b)

Figure S12. (a) The effect of increasing tannic acid concentrations on the growth of BG liquid cultures and (b) on the formation of BG biofilms.

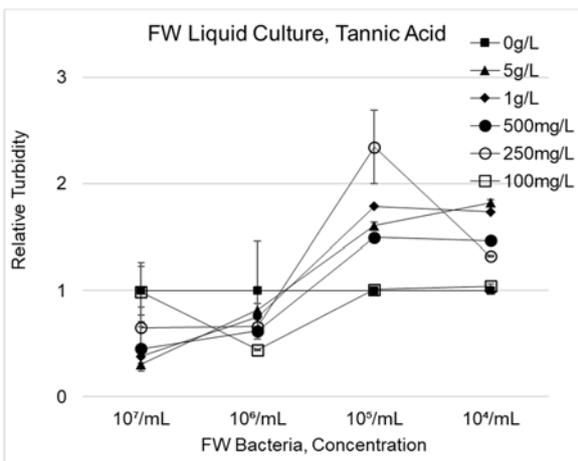


(a)

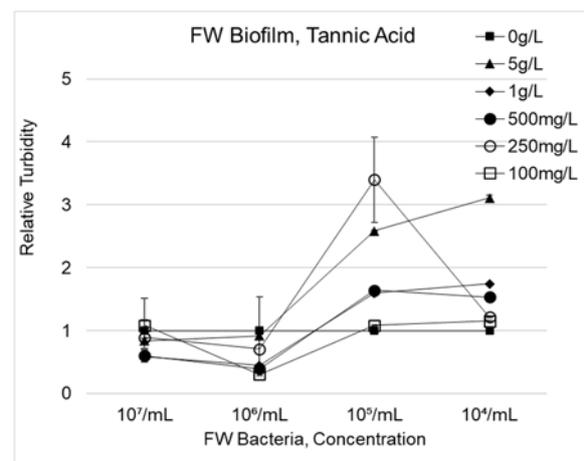


(b)

Figure S13. (a) The effect of increasing tannic acid concentrations on the growth of *E. coli* liquid cultures and (b) on the formation of *E. coli* biofilms.



(a)



(b)

Figure S14. (a) The effect of increasing tannic acid concentrations on the growth of FW liquid cultures and (b) on the formation of FW biofilms.