

Long-term, simultaneous impact of antimicrobials on the efficiency of anaerobic digestion of sewage sludge and changes in the microbial community

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Supplementary materials

Table S1. The mean values of pH, FOS/TAC ratio, TS, VS, TP and TN in each of the experimental series in the control (CB) and process (PB) bioreactors. TS-total solids, VS-volatile solids, TP-total phosphorus, TN- total nitrogen

	Digesters	pH	FOS/TAC [mg/g]	TS [mg/g]	VS [mg/g]	TP [mg/g]	TN [mg/g]
Experimental series 1	CB	7,55 ±	0,20 ±	30,55 ±	21,28 ±	2,02 ±	3,27 ±
		0,09	0,05	2,56	1,69	0,10	0,18
	PB	7,49 ±	0,23 ±	31,00 ±	21,48 ±	1,58 ±	3,43 ±
		0,07	0,06	1,71	1,23	0,24	0,44
Experimental series 2	CB	7,74 ±	0,19 ±	35,86 ±	25,47 ±	1,47 ±	3,72 ±
		0,08	0,03	1,14	0,70	0,21	0,21
	PB	7,73 ±	0,19 ±	35,77 ±	25,23 ±	1,55 ±	3,75 ±
		0,08	0,03	0,80	0,63	0,27	0,22
Experimental series 3	CB	7,87 ±	0,17 ±	38,31 ±	26,91 ±	1,68 ±	4,01 ±
		0,14	0,02	2,28	1,95	0,31	0,31
	PB	7,88 ±	0,17 ±	38,33 ±	26,75 ±	1,59 ±	3,99 ±
		0,10	0,02	3,17	2,82	0,18	0,34
Experimental series 4	CB	7,99 ±	0,15 ±	39,93 ±	27,94 ±	1,67 ±	4,08 ±
		0,12	0,01	0,57	0,92	0,20	0,21
	PB	7,94 ±	0,16 ±	40,24 ±	28,21 ±	1,77 ±	4,21 ±
		0,15	0,01	0,86	0,77	0,28	0,20
Experimental series 5	CB	7,85 ±	0,16 ±	47,05 ±	31,63 ±	1,85 ±	4,72 ±
		0,09	0,02	3,81	4,19	0,36	0,22
	PB	7,83 ±	0,16 ±	44,75 ±	30,21 ±	1,78 ±	4,33 ±
		0,09	0,01	2,85	1,16	0,30	0,22
Experimental series 6	CB	7,66 ±	0,17 ±	39,08 ±	28,25 ±	1,83 ±	4,26 ±
		0,08	0,02	3,22	1,09	0,32	0,33
	PB	7,67 ±	0,17 ±	38,09 ±	28,54 ±	1,44 ±	4,18 ±
		0,08	0,02	3,30	0,52	0,38	0,29

Table S2. Differences in methane production between samples from process and control bioreactors (two-way ANOVA; $p < 0.05$).

% of total variation	P-value	P-value summary	Significant	SS value	DF value	MS value	F-ratio (DFn, DFd)
0.5632	0.0265	*	Yes	532.2	1	532.1	F (1, 32) = 5.408

Table S3. Differences in VFA (volatile fatty acid) content between samples from process and control bioreactors (two-way ANOVA; $p < 0.05$).

VFA	% of total variation	P-value	P-value summary	Significant	SS value	DF value	MS value	F-ratio (DFn, DFd)
Acetic acid	1.292	0.2374	ns	No	0.2576	1	0.2576	F (1, 32) = 1.450
Propionic acid	3.123	0.0731	ns	No	0.05075	1	0.05075	F (1, 32) = 3.434
Isobutyric acid	2.423	0.1382	ns	No	0.03048	1	0.03048	F (1, 32) = 2.312
Butyric acid	0.8648	0.4376	ns	No	0.02573	1	0.02573	F (1, 32) = 0.6179
Isovaleric acid	0.2052	0.625	ns	No	0.02083	1	0.02083	F (1, 32) = 0.2436
Valeric acid	0.0793	0.8241	ns	No	0.001212	1	0.001212	F (1, 32) = 0.05024
Isocaproic acid	0.3301	0.6751	ns	No	0.02161	1	0.02161	F (1, 32) = 0.1789
Caproic acid	0.01006	0.9288	ns	No	0.001877	1	0.001877	F (1, 32) = 0.008120
Heptanoic acid	1.365	0.3604	ns	No	0.002855	1	0.002855	F (1, 32) = 0.8608

Table S4. Sequencing analysis data presenting the abundance of dominant bacterial phyla in all sequenced samples, expressed in reads per million reads [ppm].

Sample ID	<i>Firmicutes</i>	<i>Nitrospirae</i>	<i>Chloroflexi</i>	<i>Tenericutes</i>	<i>Acidobacteria</i>	<i>Bacteroidetes</i>	<i>Actinobacteria</i>	<i>Proteobacteria</i>	<i>Candidatus Cloacimonetes</i>	<i>Archaea</i>
D1.1	21.62496	0.31316	1.33813	0.07013	45.51847	1.68763	16.10544	2.24161	0.42462	10.67584
D1.2	7.45391	0.39086	1.73622	0.02501	49.86144	2.78293	19.40291	4.47644	0.49421	13.37608
D1.4	4.74143	0.24791	1.84211	0.09987	46.17285	3.66507	19.06551	8.55573	0.5028	15.09418
D2.1	1.58303	6.91334	0.53073	0.16397	50.77718	3.71467	9.58201	7.41782	5.42605	13.89119
D2.3	2.75677	4.10148	0.29982	0.03093	47.5041	4.08178	7.43576	10.7416	7.82287	15.2249
D2.5	2.92099	2.48799	0.27388	0.03666	47.01472	2.81063	11.47948	12.96225	7.62673	12.38666
D3.1	2.7468	1.75426	0.18408	0.04593	45.26079	3.0166	5.16664	13.37711	10.79395	17.65384
D3.3	3.62197	0.63007	0.19703	0.09217	40.01094	5.15355	7.80937	9.17684	14.58717	18.72089
D3.4	3.6649	1.25128	0.02581	0.14557	35.19761	4.13275	7.92466	14.96819	19.41701	13.27222
D4.1	4.12036	1.76782	0.35746	0.20918	30.77452	5.1053	16.50538	15.0247	6.16551	19.96977
D4.4	5.54465	1.39268	0.15659	0.09338	39.06272	5.51519	9.81098	13.65583	6.84632	17.92165
D4.5	5.68011	2.74548	0.21031	0.1027	32.9642	3.81911	11.65063	14.93561	6.14817	21.74369
D5.1	3.1788	1.10734	0.17288	0.06603	42.8792	5.99212	8.09403	11.89138	7.74401	18.8608
D5.3	2.85418	1.49611	0.53846	0	40.94243	5.80337	12.25198	11.00974	6.78587	18.13682
D5.5	2.06849	1.1025	0	0.03751	53.93506	4.10642	2.90057	9.67525	8.54774	17.62646
D6.1	1.46702	1.69156	0.06762	0.33875	55.14094	2.81976	2.70574	7.28733	9.12501	19.35627
D6.3	2.47678	3.49117	0.43375	0.16861	37.93933	3.36762	9.62044	8.09879	7.59095	26.81257
D6.5	2.2153	3.65454	0.49121	0.19632	38.05324	5.92532	10.2654	4.98817	9.61102	24.59948
C1.1	4.06679	0.29829	1.47576	0	52.9495	3.67964	19.59078	3.17682	0.26118	14.50122
C4.1	5.13474	1.37716	0.42321	0	32.87294	3.89203	18.59797	12.37394	7.04387	18.28413
C6.5	0.96387	3.88559	0.01971	0.02864	68.48818	1.80641	3.51159	3.75938	9.94369	7.59294

D-dose ID, C-control samples

Table S5. Sequencing analysis data presenting the abundance of four orders of methanogens in all sequenced samples, expressed in reads per million reads [ppm].

Sample ID	c_ <i>Methanobacteria</i> o_ <i>Methanobacteriales</i>	c_ <i>Methanomicrobia</i> o_ <i>Methanomicrobiales</i>	c_ <i>Methanomicrobia</i> o_ <i>Methanosarcinales</i>	c_ <i>Thermoplasmata</i> o_ <i>Methanomassiliicoccales</i>
D1.1	0.35787	0	10.19512	0.12285
D1.2	0.48116	0	12.58544	0.30948
D1.4	0.57622	0	14.51796	0
D2.1	0.20555	0.00907	7.67928	5.99729
D2.3	0.23513	0	6.88437	8.1054
D2.5	0.43578	0	9.56336	2.38753
D3.1	0.18818	0.0883	7.05033	10.32703
D3.3	0.71645	0	13.24716	4.75727
D3.4	0.92917	0	10.2155	2.12755
D4.1	0.53003	0	18.42586	1.01388
D4.4	0.40739	0	13.7039	3.81036
D4.5	0.45345	0	16.73308	4.55715
D5.1	0.61743	0	17.2107	1.03267
D5.3	0.32637	0	16.40069	1.40977
D5.5	0.41561	0	6.63666	10.57418
D6.1	0.38356	0	6.29201	12.6807
D6.3	0.5426	0	9.72868	16.54128
D6.5	0.32881	0	9.24552	15.02515
C1.1	0.35953	0	14.06668	0.07501
C4.1	0.50199	0	16.48515	1.29699
C6.5	0.17611	0	1.8877	5.52912

D-dose ID, C-control samples

Table S6. Correlations between the occurrence of *Archaea* in the process bioreactor (Spearman's rank correlation coefficient; significant results are marked in red, $p < 0.05$).

	c__Methanobacteria o__Methanobacteriales	c__Methanomicrobia o__Methanomicrobiales	c__Methanomicrobia o__Methanosarcinales	c__Thermoplasmata o__Methanomassiliicoccales
c__Methanobacteria o__Methanobacteriales	1	-0.54612	0.539732	-0.34985
c__Methanomicrobia o__Methanomicrobiales	-0.54612	1	-0.34203	0.24188
c__Methanomicrobia o__Methanosarcinales	0.539732	-0.34203	1	-0.68215
c__Thermoplasmata o__Methanomassiliicoccales	-0.34985	0.24188	-0.68215	1

Table S7. Correlations between the occurrence of bacterial phyla in the process bioreactor (Spearman's rank correlation coefficient; significant results are marked in red, $p < 0.05$).

	<i>Firmicutes</i>	<i>Nitrospirae</i>	<i>Chloroflexi</i>	<i>Tenericutes</i>	<i>Acidobacteria</i>	<i>Bacteroidetes</i>	<i>Actinobacteria</i>	<i>Proteobacteria</i>	<i>Candidatus Cloacimonetes</i>	<i>Archaea</i>
<i>Firmicutes</i>	1	-0.53973	0.287926	-0.21362	-0.38287	-0.10836	0.682147	0.195046	-0.511	-0.28999
<i>Nitrospirae</i>	-0.53973	1	-0.0258	0.30031	-0.14964	0.102167	-0.22188	0.162023	0.160	0.374613
<i>Chloroflexi</i>	0.287926	-0.0258	1	-0.11868	0.038184	-0.28173	0.770898	-0.50464	-0.717	-0.16615
<i>Tenericutes</i>	-0.21362	0.30031	-0.11868	1	-0.3581	0.083591	-0.07946	-0.01754	0.110	0.496388
<i>Acidobacteria</i>	-0.38287	-0.14964	0.038184	-0.3581	1	-0.47368	-0.29412	-0.54386	-0.102	-0.4902
<i>Bacteroidetes</i>	-0.10836	0.102167	-0.28173	0.083591	-0.47368	1	-0.13519	0.395253	0.368	0.477812
<i>Actinobacteria</i>	0.682147	-0.22188	0.770898	-0.07946	-0.29412	-0.13519	1	-0.08566	-0.783	-0.13725
<i>Proteobacteria</i>	0.195046	0.162023	-0.50464	-0.01754	-0.54386	0.395253	-0.08566	1	0.261	0.124871
<i>Candidatus Cloacimonetes</i>	-0.511	0.160	-0.717	0.110	-0.102	0.368	-0.783	0.261	1	0.238
<i>Archaea</i>	-0.28999	0.374613	-0.16615	0.496388	-0.4902	0.477812	-0.13725	0.124871	0.238	1

Table S8. Sequencing analysis data presenting the abundance of ARG classes in all sequenced samples, expressed in reads per million reads [ppm].

Sample ID	TOTAL ARGs	aminoglycoside	bacitracin	beta-lactam	macrolide-lincosamide-streptogramin	multidrug	polymyxin	rifamycin	sulfonamide	tetracycline	vancomycin
D1.1	93.61712	3.432852	6.846525	2.05204	32.61209	26.12036	0.565749	1.534235	4.305448	11.59307	0.757529
D1.2	94.36849	2.471946	8.323507	2.809908	35.74666	23.46418	0.550394	1.48703	3.418238	11.32654	0.608331
D1.4	100.0212	2.657332	10.96149	4.777335	34.93609	24.28723	0.576406	1.182122	3.18489	11.68444	0.996499
D2.1	95.64991	3.371546	8.0936	3.125999	30.41947	29.31451	0.406097	1.019964	3.635981	12.25845	0.443873
D2.3	92.62204	3.152807	7.324799	2.876579	29.88975	25.37486	0.638181	0.99061	3.49571	14.59245	0.495305
D2.5	96.71919	2.451343	7.420281	2.574383	35.35044	24.63647	0.577343	1.73203	3.956221	13.94142	0.511091
D3.1	104.5119	3.358969	8.618154	3.301387	30.47065	32.53401	0.758167	1.190035	8.099913	10.78709	0.451062
D3.3	102.0107	2.270058	6.791258	1.825505	42.91356	20.53457	0.482387	1.664709	3.840182	17.91454	0.567515
D3.4	97.45341	2.670347	8.676252	2.052651	33.46962	25.45857	0.456145	1.976627	5.378706	13.17118	0.627199
D4.1	87.5149	2.93044	9.047379	1.744987	27.52148	24.92297	0.512116	1.735503	4.371951	10.60269	0.606952
D4.4	79.67867	2.311647	7.442138	2.52623	20.90236	24.43323	0.516951	1.892234	4.97443	10.88522	0.575473
D4.5	81.46714	2.427416	8.85575	2.456199	16.85759	28.77399	0.345403	1.73661	5.891041	9.728852	0.556483
D5.1	72.34629	1.671016	6.519859	1.516471	23.24934	20.69935	0.347726	1.516471	3.428963	9.784618	0.444316
D5.3	86.41608	2.804981	7.895502	2.474428	20.05042	30.7509	0.406108	1.520545	6.223846	9.312159	0.53833
D5.5	85.97485	2.332048	6.743507	2.26403	26.52705	25.0015	0.281789	1.136874	6.043892	11.45619	0.417825
D6.1	87.9707	2.558798	6.781776	2.366407	29.74362	25.9439	0.394401	1.317877	5.44466	9.475248	0.240489
D6.3	95.63269	2.527951	8.433011	2.527951	28.96895	31.32122	0.370896	1.3567	5.212069	10.03372	0.536824
D6.5	88.06147	2.473637	8.191543	2.76857	24.32727	29.03669	0.28542	0.884801	3.643857	11.67366	0.38056
C1.1	95.70167	3.636512	7.839754	2.578618	30.7734	28.22311	0.528947	1.473496	4.241024	10.91898	1.020113
C4.1	90.9142	2.211089	8.479044	2.038047	30.62838	25.08144	0.67294	1.701577	5.633469	10.33444	0.826755
C6.5	90.86236	1.929096	5.952917	2.542899	32.53157	27.38732	0.652775	0.828147	6.693378	9.031676	0.448174

D-dose ID, C-control samples

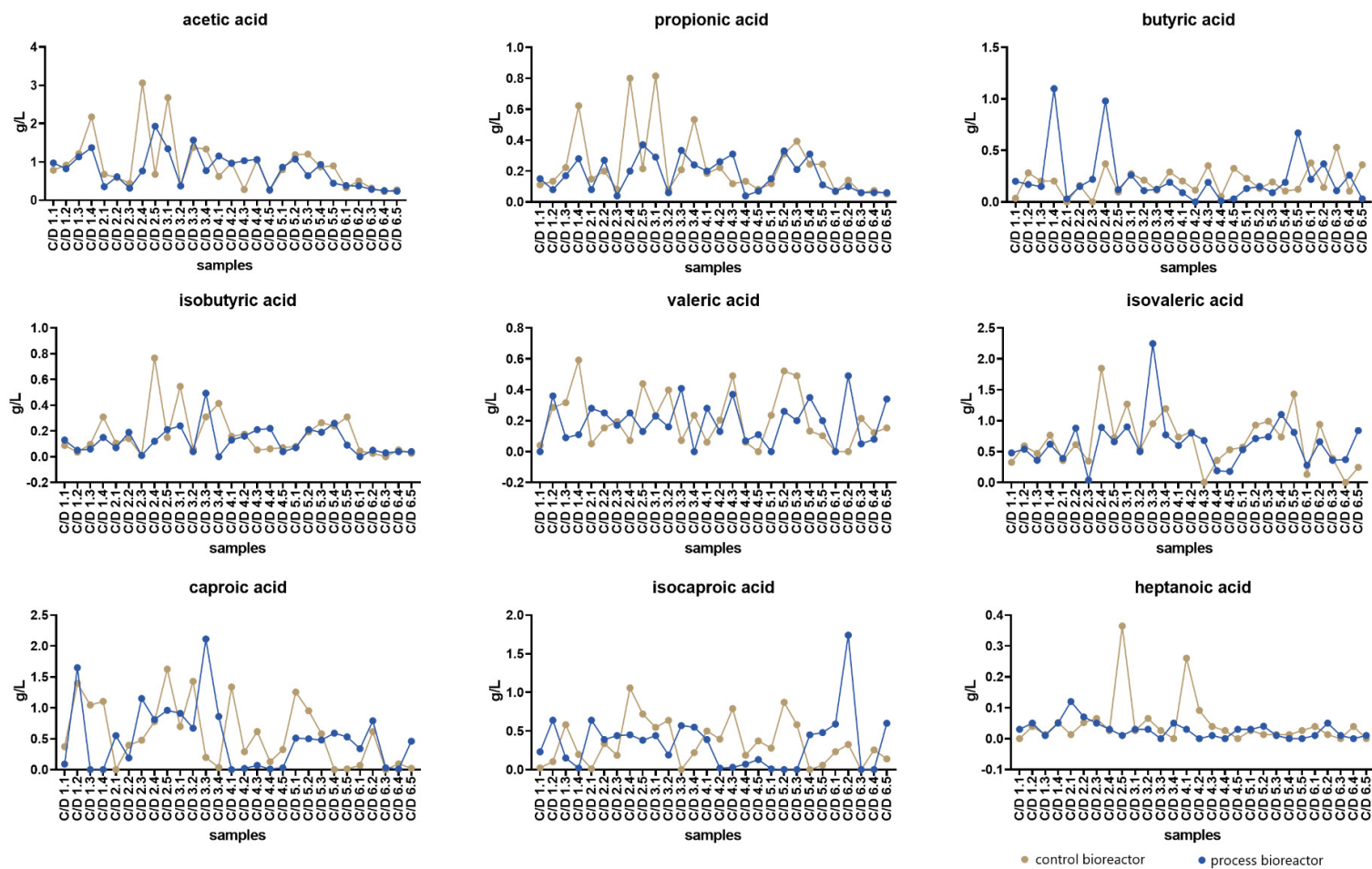


Figure S1. Content of VFAs (g/L) in bioreactors containing antimicrobials and in the control bioreactor. C-control samples, D-dose ID, VFAs-volatile fatty acids. For example, “C/D 1.1” indicates samples C1.1 and D1.1 (green and blue points on the line graph, respectively).

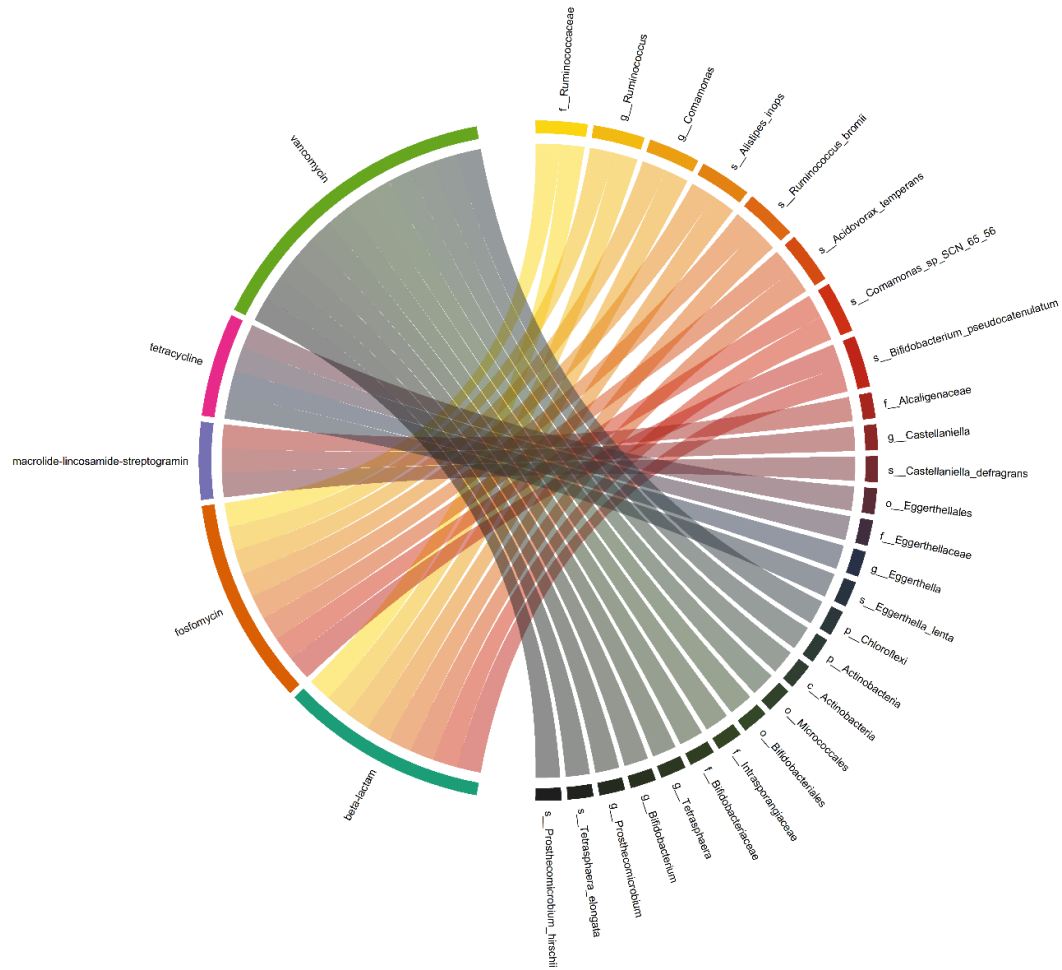


Figure S2. The taxonomic distribution of microbial diversity and ARG abundance in all sequenced samples of sewage sludge. The links describe the Pearson correlation (>0.7 , $p < 0.05$) between the abundance of ARGs and the relative abundance of the bacterial community on different taxonomic levels.