Correlation Between Planktonic and Attached Growth Bacteria Densities in Bioaugmented Flow-through Column Test

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BACKGROUND

Recent commercial availability of molecular biological tests to identify and quantify environmentally relevant subsurface bacteria (and their genes for production of various enzymes) has changed the way stakeholders and regulators view assessment and interpretation of natural and enhanced bioremediation initiatives.
Users are faced with the decision of whether to test groundwater to garner measurement of water borne planktonic bacteria or to test soil (or passive sampler) to garner measurement of attached growth (sessile) bacteria. Interest in correlation between attached growth bacteria densities and their planktonic counterparts is increasing[1]. Knowledge about correlation between planktonic and sessile bacteria density in the subsurface could help stakeholders with their decision making.

[1] Natalie L. Capiro, Yonggang Wang, Janet K. Hatt, Carmen A. Lebron, Kurt D. Pennell, and Frank E. Löffler. Distribution of Organohalide-Respiring Bacteria between Solid and Aqueous Phases. Environ. Sci. Technol. 2014, 48, 10878−10887.

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APPROACH

Flow-through sand and gravel soil/groundwater test columns were amended with commercial ZVI+electron donor EHC-F, inoculated with commercial dechlorinating bacteria cultures SDC-9 and TCA-20, and then fed PCE- and 1,1,1-TCA-spiked groundwater for 350 days at a rate 5.6 mL/hour to achieve linear velocities of 0.5 ft./day for a 2-day column residence time.

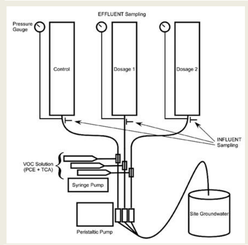
Column 1: Control (no amendment); PCE in inlet water = 2,172 ± 315 µg/L; TCA in inlet water = 347 ± 93 µg/L.

Column 2: 0.25% EHC-F + SDC-9 (3E+03 DHC cells/gram of column soils) + TCA-20 (3E+03 DHBt cells/gram of column soils); PCE in inlet water = 2,172 ± 315 µg/L; TCA in inlet water = 347 ± 93 µg/L.

Column 3: 1.0% EHC-F + SDC-9 (3E+03 DHC cells/gram of column soils) + TCA-20 (3E+03 DHBt cells/gram of column soils); PCE inlet = 2,172 ± 315 µg/L; TCA inlet = 347 ± 93 µg/L.

End-of-run water samples (day 344) were collected at inlet, middle, and outlet of each of the 3 columns (#1 column is control). Companion (co-located) end-of-run soil samples (day 350) were collected upon tear down of the columns.

Co-located water and soil samples were analyzed for total eubacteria (EBAC), Dehalobacter spp. (DHBt), and Dehalococcoides spp. (DHC) by DNA CENSUS qPCR method. Detection limits in water was ~7E-01 cells/mL. Detection limits in soil was 2E+02 cells/gram.

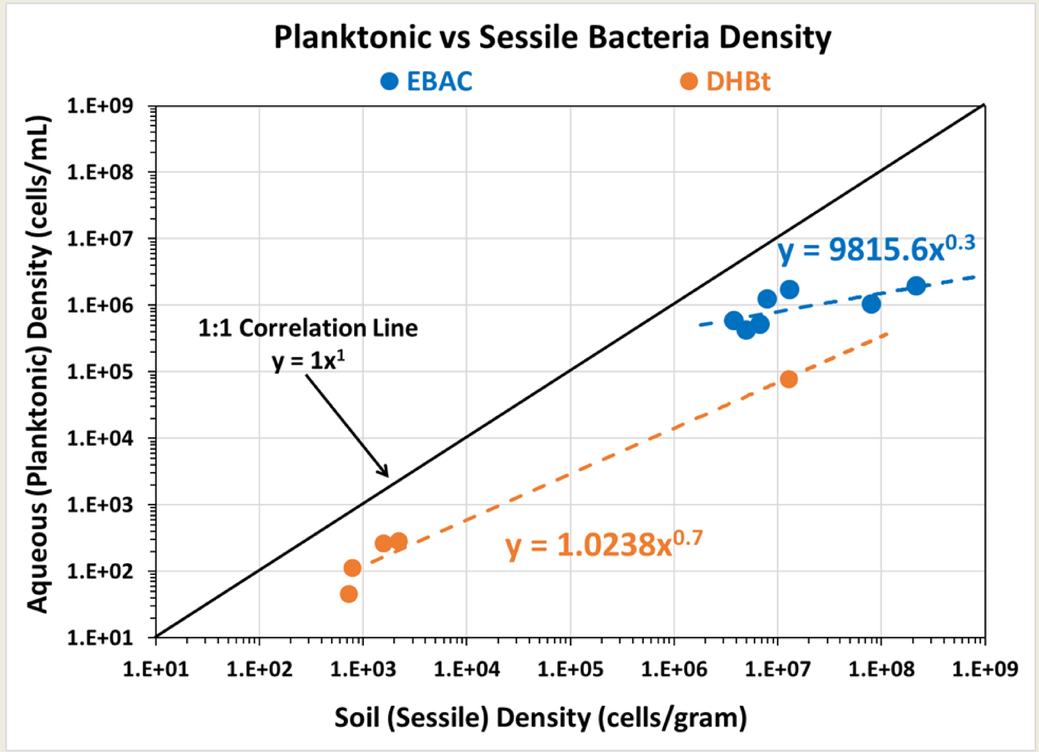


EHC-F Dosage (weight %)	Mass (mg)		Concentration (mg/kg)	
	ZVI	Dextrose	ZVI	Dextrose
0.25	1,000	1,450	400	580
1.0	4,000	5,800	1,600	2,230

RESULTS

Sessile vs Planktonic Microbial Densities at Termination of Column Tests

Column ID	Position	EBAC Total Eubacteria		DHBt *Dehalobacter* spp.		DHC *Dehalococcoides* spp.	
		Sessile (cells/g)	Planktonic (cells/mL)	Sessile (cells/g)	Planktonic (cells/mL)	Sessile (cells/g)	Planktonic (cells/mL)
Control	IN	5.E+06	4.E+05	8.E+02	1.E+02	<1E+03	8.E+04
Control	MID		7.E+05		5.E+01		1.E+02
Control	OUT		3.E+06		1.E+02		<4.E+00
0.25% EHC-F	IN	4.E+06	6.E+05	<1.E+04	1.E+01	<1E+03	2.E+03
0.25% EHC-F	MID	1.E+07	2.E+06	2.E+03	3.E+02	<1E+03	2.E+00
0.25% EHC-F	OUT	8.E+06	1.E+06	2.E+03	3.E+02	<1E+03	4.E+01
1.0% EHC-F	IN	7.E+06	5.E+05	7.E+02	5.E+01	<1E+03	8.E+03
1.0% EHC-F	MID	8.E+07	1.E+06	5.E+05	4.E+01	<1E+03	9.E+01
1.0% EHC-F	OUT	2.E+08	2.E+06	1.E+07	8.E+04	5.E+06	8.E+04



DISCUSSION and CONCLUSIONS

Behavior of, and relationship between, sessile attached-growth bacteria and their aqueous planktonic counterparts has important implications for practical bioremediation and its performance monitoring. The topic is much debated within the bioremediation community. Yet, relatively little is known about it because, in part, there are few documented field studies with paired quantification of sessile and planktonic members of the microbial community.

Thus, the paired results in this study may be of interest to others.

Readers are cautioned that this dataset is too small for meaningful statistical confidence in its apparent correlation between sessile EBAC and DHBt bacteria and their planktonic counterparts. Reasonable consensus opinion seems to be that behavior of sessile vs. planktonic bacteria is organism-specific and site-specific, depending in part on the character of the soil matrix involved and local biogeochemical conditions such as bioavailability of various nutrients, substrates, and hospitality factors such as pH, ORP, temperature, and toxic inhibitions.

An apparent linear correlation (n = 7) was observed between sessile and planktonic densities of EBAC for the measurement units used. A linear correlation (n = 5) was also observed for DHBt, albeit less convincingly than that for EBAC. Correlation between sessile and planktonic DHC was not available because DHC was detected in only one soil sample.

Water and co-located soil samples both illuminated occurrence of bacteria of interest.

Detection in water was more frequent than detection in soil, especially for DHC.

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