Q-419

A Two Dimensional Gradient Approach for the Detection of Pathogens in Class B Biosolids using Quantitative Real Time RT-PCR

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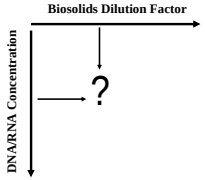
Abstract

Although the qRT-PCR technique has been extensively employed for virus detection from various environmental samples, the application of qRT-PCR to detect pathogens in biosolids is an arduous task due to inhibitory substances. The results of molecular techniques therefore depend on the efficacy with which the viral extraction technique used removes such compounds. The objective of this study was to evaluate sample detection methods that facilitate the application of molecular techniques to biosolids. In this study we evaluated the amplification of both viral DNA and RNA in class B biosolids. Four biosolid types were collected and processed using our newly developed laboratory procedure. Each sample concentrate was purified with the QIAamp DNA/RNA mini kit with an equivalent sample volume of 10 g of biosolids. Biosolid concentrates were spiked with known concentrations of viral nucleic acids. A one step RT-PCR reaction using the TaqMan One Step RT qPCR Master Mix kit was carried out with an ABI Prism 7900 HT Sequence Detection System. Each sample as well as its 10 and 100 fold dilutions was run in triplicate to test both the limit of detection and reproducibility (n=108). Through our analysis it was determined that the threshold limit of determination was different for each biosolid type tested, with anaerobically digested biosolids having the least inhibitory effect on amplification with 74% percent of samples tested resulting in positive reactions and aerobically digested having the most inhibitory effect with only 11% positives for Poliovirus RNA. With respect to dilutions it was seen that at a 100 fold dilution there was an 82% success rate, followed by 42% and 9% at the 10 and 0 fold dilutions respectively. It should be stressed that any research group dealing with biosolid samples must analyze method efficiencies in order to fully understand the dynamics of the sample that they are dealing with before determining the numbers of microbes present in the sample. The newly developed technique increases the applicability of quantitative Real Time PCR to quantify pathogens in biosolids.

Background

- Biosolids are rich in Humic and Fulvic acids
- Humic and Fulvic acids are mixtures of polyphenolics.
- Phenols bind to proteins by forming hydrogen bonds with peptide bond oxygens.
- Phenolic compounds can bind to and inactivate proteins of interest.

- SEM Image of Poliovirus Type 1



Materials & Methods

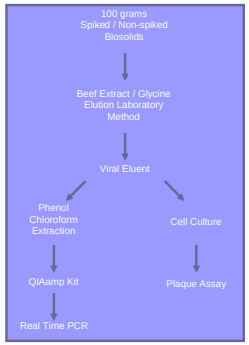
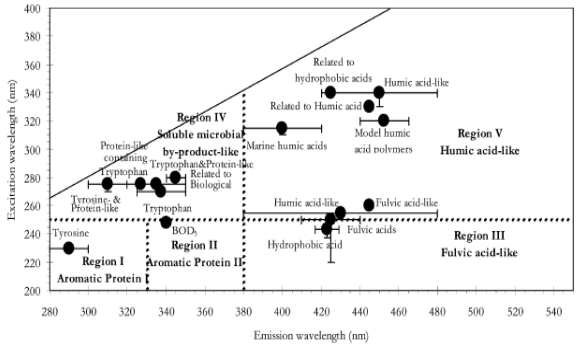


Figure 1. Virus Infected Cell Culture Monolayer

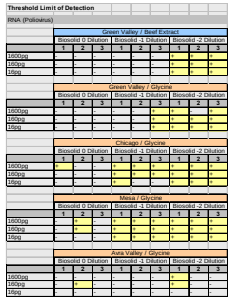
Primer/Probe	Nucleotide Sequence (5'→3')
Polio F	GGTTTTGTGTCAGCGTGTAATGA
Polio R	GCTAGCGCTTTTTGCTCTATATGTG
Polio Probe	CGTGCGCTTGTTGCGAGAT

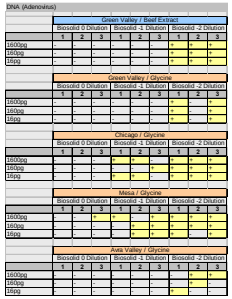
Characterization of NOM in Sample Concentrates



- Fluorescence excitation–emission matrix spectroscopy provides more detailed information about the fluorescence properties of natural/dissolved organic matter.
- Perkin-Elmer LS50B luminescence spectrophotometer, creates a three-dimensional picture based on fluorescence intensity as a function of excitation and emission wavelength.

Results





Why do different Biosolid types behave so differently?

Biosolid Processing Details

Mesa, AZ
-Anaerobic Digested / Polymer Added

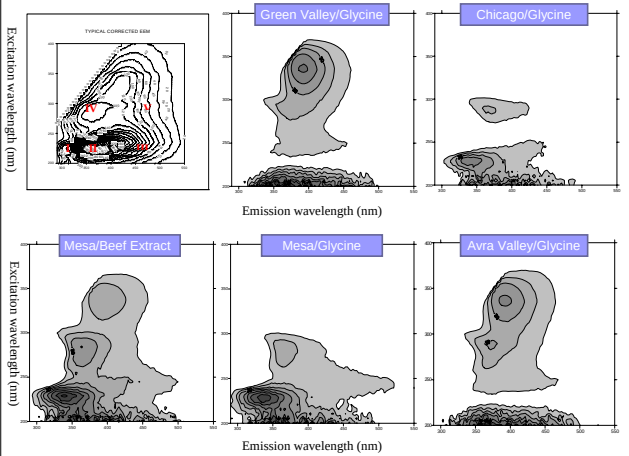
Chicago, IL
-Aerobic Digested/ Polymer Added

Green Valley, AZ
-Anaerobic Digested / Filter Pressed

Avra Valley, AZ
-Anaerobic Digested / Composted

Biosolid Type	Amplification Success Rate	
	DNA	RNA
Mesa	55%	74%
Chicago	52%	63%
Green Valley	22%	41%
Avra Valley	15%	11%

Biosolids Sample Characterization



Conclusions

- The performance of glycine based method was consistent for different type of biosolids, also the sample concentrates by this method have remarkably less amounts of humic acid like materials then those samples processed using Beef Extract.
- These results may indicate a possible reason for the success of the method in amplification of target DNA in Real Time PCR reactions.
- The sample concentrates from Chicago (Aerobic/Polymer) and Mesa (Anaerobic/Polymer) biosolids had the least amount of humic material followed by the biosolids from Green Valley (Anaerobic/Filter Pressed) and Avra Valley (Anaerobic/Compost).
- The humic material found in biosolids sample concentrates from Green Valley and Avra Valley correspond to aquatic/marine humic-like species
- EEM analysis of sample concentrates corresponded to specific biosolids amplification success rates and thus may be an alternative method for sample analysis
- Applicability of our molecular method can offer a better classification tool for biosolids in respect to microbial pathogens (Class A and Class B)

Significance

- Application of cutting edge molecular techniques to biosolids depends on the efficacy of sample processing protocols to recover microbes and remove inhibitory compounds from sample concentrates.
- The study provides new strategy of sample processing and detection methods that facilitate the application of molecular techniques for characterization of biosolids with respect to microbial pathogens.

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