Applied and Environmental Microbiology, Dec. 2010, p. 8102–8109 Vol. 76, No. 24
0099-2240/10/$12.00 doi:10.1128/AEM.02339-09

PCR Inhibitor Levels in Concentrates of Biosolid Samples Predicted by a New Method Based on Excitation-Emission Matrix Spectroscopy▽

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Received 28 September 2009/Accepted 14 October 2010

Biosolids contain a wide variety of organic contaminants that are known for their ability to inhibit PCR. During sample processing, these contaminants are coconcentrated with microorganisms. Elevated concentrations of these compounds in concentrates render samples unsuitable for molecular applications. Glycine-based elution and recovery methods have been shown to generate samples with fewer PCR inhibitory compounds than the current U.S. EPA-recommended method for pathogen recovery from biosolids. Even with glycine-based methods, PCR inhibitors still persist in concentrations that may interfere with nucleic acid amplification. This results in considerable loss of time and resources and increases the probability of false negatives. A method to estimate the degree of inhibition prior to application of molecular methods is desirable. Here we report fluorescence excitation-emission matrix (EEM) profiling as a tool for predicting levels of molecular inhibition in sample concentrates of biosolids.

Amplification of nucleic acid from pure microbial cultures is easily achieved; however, this is not the case when dealing with nucleic acid recovered from environmental samples such as biosolids. The relative difficulty in amplifying target nucleic acids in biosolid samples is due to the presence of a variety of inhibitors. An array of substances has been reported as PCR inhibitors. The most commonly reported biological inhibitors include humic acids, fulvic acids, fats, and proteins (8, 10, 22, 23, 25, 26). Environmental samples, especially urban sludge, may contain these substances in addition to numerous organic and inorganic compounds, such as polyphenols and heavy metals (24). These compounds are liable to form complexes with nucleic acids and inhibit amplification enzymes (18).

Reported strategies for the removal/mitigation of inhibitors from sample concentrates include the use of additives such as bovine serum albumin or the T4 gene 32 protein, which are directly added to the PCR (13), use of sample-washing steps to clean DNA, use of density gradient centrifugation using cesium chloride (15, 21), hexadecyltrimethylammonium bromide (CTAB) (7), and polyvinylpolypyrrolidone (PVPP) (9, 28), use of gel electrophoresis (28), and use of the Sephadex G-100 and G-200 columns (1, 17). The addition of $AlNH_4(SO_4)_2$ during the direct extraction of soil DNA by using the UltraClean soil DNA kit (MoBio, Carlsbad, CA) significantly reduces the copurification of PCR inhibitors, in addition to minimizing the loss of DNA yield (5). Other reports have also shown variable inhibitor removal efficiencies by different DNA extraction/purification methods (16, 27), while high rates of PCR inhibition in samples processed by beef extract (BE)-based methods have been reported (1, 2, 12). In addition to coconcentrating inhibitors, most of these methods are limited in their application by being time-consuming or expensive. Many of these methods also result in significant loss of DNA during recovery procedures (14, 19, 28) or even the complete elimination of some DNA templates of low-abundance microbes.

Fluorescence spectroscopy for organic matter characterization has been advanced by the use of excitation-emission matrix (EEM) spectroscopy, which measures emission spectra across a range of excitation wavelengths, resulting in a landscape surface defined by the fluorescence intensity at pairs of excitation and emission wavelengths (6). The EEM approach has been used to characterize dissolved organic matter (DOM) extracted from a variety of sources, such as leaf litter, crop residues, humic substances, and municipal wastewater treatment sludge (6).

EEM has been typically characterized by noting the locations of one or more peaks corresponding to maximum fluorescence intensities ("peak picking"). Two fluorophores frequently observed in DOM samples are located near the excitation-emission wavelength pairs corresponding to approximately 270 to 280 and 335 to 350 nm and also approximately 310 to 325 and 420 to 445 nm. These have been characterized as "protein-like" and "humic-like," respectively (6). Chen et al. (in 2003) operationally quantified EEM spectra by delineating the EEM signals into five regions and calculating the integrated volume under each region to characterize the DOM. The regions are characterized as corresponding to aromatic proteins (two regions), fulvic acid, microbial by-products, and humic acid. By analysis of these regions, it has been shown that humic acid is more relevant to the PCR inhibition in environmental samples, including soils and land-applied biosolids.

Application of molecular techniques on polluted samples

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▽ Published ahead of print on 22 October 2010.

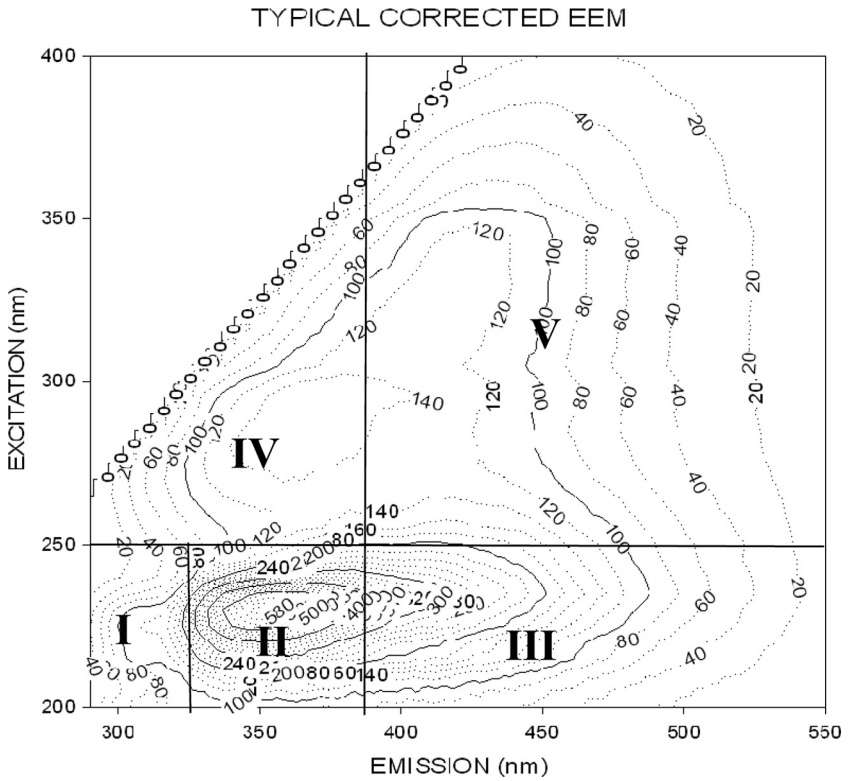

FIG. 1. Regions of the EEM corresponding to various groups of organic compounds. Zone I, aromatic protein type I; zone II, aromatic protein type II; zone III, fulvic acid-like compounds; zone IV, soluble microbial products; zone V, humic acid-like compounds.

such as biosolids may yield false-negative results. Therefore, a broadly applicable method to assess the level of nucleic acid inhibition in any sample concentrate can be helpful in minimizing the chances of false-negative results from molecular analyses. A method that enables the user to "predict" the success of a molecular reaction prior to onset will increase the likelihood of success later in the process. The objective of this study was to evaluate EEM profiling as a tool to predict the level of PCR inhibition in sample concentrates of biosolids.

MATERIALS AND METHODS

Sample collection. The biosolid samples were collected from various wastewater treatment plants utilizing different treatment processes. The utilities that participated in this study were as follows: the Green Valley Wastewater Treatment Plant, Green Valley, AZ, the Avra Valley Wastewater Treatment Facility, Avra Valley, AZ, the Stickney Water Reclamation Plant, Chicago, IL, and the Northwest Water Reclamation Plant (NWWRP), Mesa, AZ. At the Green Valley Wastewater Treatment Facility, the treatment train includes biological nutrient removal followed by filter press. The biosolids produced at this facility go through an aerobic digestion process. The Avra Valley Wastewater Treatment Facility uses an oxidation ditch, and biosolids are thickened by a polyacrylamide-based polymer. Thickened biosolids are filter pressed and air dried. At the Stickney Water Reclamation Plant, biosolids are produced by aerobic digestion and air drying. At the Northwest Water Reclamation Plant, biosolids are thickened by the addition of polymer, centrifuged, and anaerobically digested. The dehydrated biosolids from these utilities are used for land applications. The dry matter contents of all biosolid samples ranged from 25 to 36%. The biosolid samples collected from these utilities were stored at 4°C until used.

Sample processing. To analyze for viral pathogens, 100 grams of cake biosolid samples was added to clear polycarbonate bottles (Nalgene, Rochester, NY) and processed as follows using one of two elution protocols described as the beef extract (BE) method or the glycine method. Five hundred milliliters of beef extract elution buffer (3% beef extract, 3% citric acid, 3% Tween 20) or glycine elution buffer (0.1 M glycine, 3% citric acid, 0.3% NaCl) was added to the solids, and the pH was adjusted to 9.3 to 9.5. The samples were stirred for 30 min followed by centrifugation at 6,500 $\times$ *g* for 45 min. The supernatant was carefully poured into a clean, 1,000-ml clear polycarbonate bottle, and the pellet was discarded. The pH of the supernatant was adjusted to 3.5 $\pm$ 0.2 by using 1 N HCl. The sample was then mixed for 10 min at room temperature and centrifuged at 8,000 $\times$ *g* for 90 min. Following centrifugation, the supernatant was carefully discarded. The pellet was resuspended in 30 ml of 0.15 M Na_2HPO_4, and the pH was adjusted to 7.2 $\pm$ 0.2 with 1 N NaOH or 1 N HCl. The samples were stored at 4°C until further analysis.

Pre-PCR sample processing for inhibitor removal. Sample concentrates of biosolids processed by the methods described in the previous section were further cleaned using the following procedure. Three-milliliter volumes of the sample concentrates were extracted by using equal volumes of phenol solution (Sigma catalog no. P4557), followed by another extraction using equal volumes of phenol-chloroform (5:1; Sigma catalog no. P1944). The phenol-chloroform-extracted sample was further purified by use of the QIAamp DNA/RNA purification kit (Qiagen, Valencia, CA), following the manufacturer's instructions.

Characterization of natural organic matter (NOM) in sample concentrates. The Qiagen kit-purified sample concentrates (20 μl) were analyzed for the fluorescence EEM. Fluorescence excitation-emission matrix spectroscopy was used as a tool for rapid characterization of dissolved organic matter (DOM) in sample concentrates. The fluorescence spectra were recorded using a Perkin-Elmer LS50B luminescence spectrophotometer. The spectrophotometer was equipped with a xenon excitation source, and excitation-emission slits were set to a 10-nm band-pass. The fluorescence (EEM) was obtained by increasing the excitation wavelengths from 200 nm to 400 nm in 5-nm increments. With this technique, a three-dimensional picture of fluorescence intensity as a function of excitation and emission wavelengths is generated.

The emission spectra for all the samples were adjusted against blanks. The Qiagen buffer AVE, used as a carrier for all the samples, was used as a method blank. A typical EEM is shown in Fig. 1. Based on the literature, the

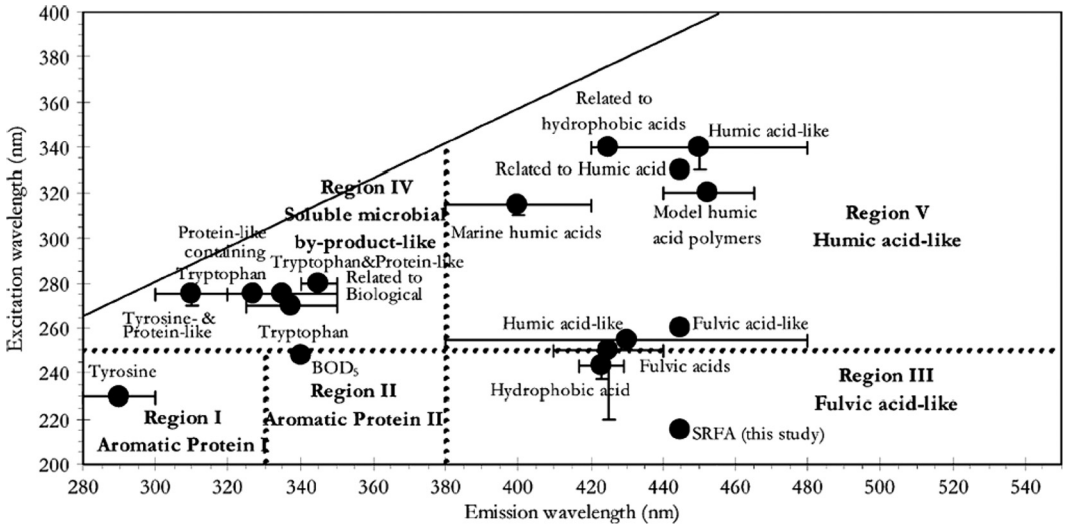

FIG. 2. Location of EEM peaks based on operationally defined excitation-emission wavelength boundaries for five EEM regions. (Reprinted from reference 6 with permission of the publisher. Copyright 2003 American Chemical Society.)

fluorescence of representative (model) compounds can be delineated into five excitation-emission regions using the EEM (6).

Each of the five EEM regions represents a specific class of organic compounds. Regions I and II represent aromatic proteins. Regions III, IV, and V represent fulvic acid-like, soluble microbial by-product-like, and humic acid-like substances, respectively. The excitation-emission wavelength boundaries defined in the literature were used for interpretation of the peaks noted for each sample (Fig. 2). The areas under the curves or peak intensities at specific wavelengths were used for purposes of comparison.

Validation of EEM results by quantitative RT-PCR. To evaluate EEM spectroscopy as a tool for predicting PCR inhibition, samples analyzed by EEM spectroscopy were spiked with the purified genome of poliovirus type 1 and analyzed by reverse transcription-quantitative PCR (RT-qPCR). The results of both analyses were compared to evaluate EEM spectroscopy as a tool for predicting PCR inhibition in biosolid sample concentrates.

Spiking description. For the spiking experiments, viral stocks were lysed and RNA extracted using the Qiagen kit (as previously described). Concentrations of nucleic acid (nanograms/μl) were calculated using the NanoDrop system (Wilmington, DE). Purified and extracted biosolid sample concentrates were serially diluted in DNase/RNase-free water. For each sample type, the 0-, 10-, and 100-fold dilutions were spiked with 16, 160, and 1,600 pg of purified genome of poliovirus type 1. All spiked dilutions were analyzed in triplicate. A negative control was included in each set of RT-PCR analyses. Since humic acid is the most relevant PCR-inhibiting agent in biosolids, a control study of PCR inhibition by defined quantities of humic acids in amplification reaction mixtures containing a 10-fold dilution series of the target genome was performed. In addition to the experiment with humic acids, an independent experiment using bile salt, a representative of a biological metabolite, was also included in the inhibition study (data not shown).

Quantitative real-time RT-PCR. The primers (Table 1) used in this study were designed based on a nontranslated conserved region of the poliovirus genome by using Primer Express software (Applied Biosystems, Foster City, CA). The fluorogenic probe was labeled with a 6-carboxyfluorescein (FAM) reporter at the 5′ end and a 6-carboxytetramethylrhodamine (TAMRA) quencher at the 3′ end.

A one-step RT-PCR using the TaqMan One Step RT qPCR master mix kit (Eurogentec, San Diego, CA) was carried out in the ABI Prism 7900 HT sequence detection system (Applied Biosystems). The one-step RT-PCR was performed in a 20-μl volume containing 10 μl TaqMan One Step RT-qPCR master mix (2× strength), a 300 nM concentration of each primer, 300 nM TaqMan probe, 0.1 μl of EuroScript plus RT and RNase inhibitor, and 4 μl of target. The temperature profile was 48°C for 30 min and 95°C for 10 min followed by 40 cycles of 95°C for 15 s and 60°C for 1 min. Real-time measurements were taken, and a threshold cycle (C_T) value for each sample was calculated by determining the point at which the fluorescence exceeded a threshold limit of 0.2.

Ranking system. PCR success rates for different elution buffers and sample types were calculated by ascribing an interpolated $\log_{10}$ value to the dilution step and average success rate in each dilution series. In this study, undiluted sample and 1:10 and 1:100 dilutions of sample were analyzed by PCR, and 3, 2, and 1 were assigned as the dilution factors, respectively. The areas under the peaks in each region of EEM for each sample were used for ranking. The highest rank was ascribed to the peak with the smallest area. For each sample type, ranking for PCR and EEM was corroborated and homogeneity of ranking order was used as a tool to predict PCR inhibition.

RESULTS

Impact of sample-processing method on the amplification of poliovirus genome. Biosolid samples concentrated by BE and glycine elutions were spiked with poliovirus genome, and spiked samples were subjected to quantitative real-time RT-PCR. Samples were analyzed in triplicate, and PCR success rates are presented in Table 2. Results show variation in

TABLE 1. Poliovirus type 1-specific oligonucleotide primers and probe for TaqMan RT-qPCR assays

Primer/probe	Position[a]	Sequence (5′→3′)[b]	T_m (°C)[c]
Forward	2401–2422	GGTTTTGTGTCAGCGTGTAATGA	65.8
Reverse	2475–2451	GCTAGCGCTTTTTGCTCTATATGTG	65.6
Probe	2428–2446	FAM-CGTGCGCTTGTTGCGAGAT-TAMRA	69.8

[a] Nucleotide position based on GenBank accession number NC002058 (polyprotein gene of poliovirus type 1).
[b] FAM, 6-carboxyfluorescein (fluorescence receptor dye); TAMRA, 6-carboxytetramethylrhodamine (fluorescence quencher).
[c] T_m, annealing temperature.

TABLE 2. Comparison of different elution buffers for the removal of PCR inhibitors in aerobically digested biosolids

Type of elution buffer	Template amt (pg)	No. of positive PCRs/total PCRs (by sample dilution)		
		1:1	1:10	1:100
Beef extract	1,600	0/3	0/3	3/3
	160	0/3	0/3	3/3
	16	0/3	0/3	3/3
Glycine	1,600	0/3	1/3	3/3
	160	0/3	1/3	3/3
	16	0/3	1/3	3/3

PCR amplification efficiencies with the two elution buffers. Although amplification was seen at the smallest amount of spiked poliovirus, 16 pg, no nucleic acid amplification was achieved in nondiluted sample concentrates from either method. Each additional dilution increased the proportion of positive amplification results until the last dilution, for which all tested samples had at least one positive amplification reaction.

The glycine-based elution method resulted in amplification in the 10-fold-diluted sample, whereas no amplification was achieved in the equivalent sample processed by the BE-based method. From these results, it is evident that the glycine elution method resulted in higher amplification success rates measured by positive reactions than those with the BE elution method.

Characterization of natural organic matter (NOM). In order to evaluate the relative molecular applicability of biosolid sample concentrates by EEM spectroscopy, samples processed by both elution methods (BE and glycine) were evaluated. The EEM profiles of Qiagen kit-purified sample concentrates of biosolids are presented in Fig. 3. For each sample, the area under the curve or peak intensity at a specific wavelength was used for purposes of comparison. Initially, the effect of the sample elution method was investigated by processing anaerobically digested biosolids by using BE- and glycine-based methods.

The EEM profiles from both methods are presented in Fig. 3a and b. The comparison of these EEM scans illustrates the impact of the sample-processing technique on the levels and types of inhibitors present in sample concentrates. The sample concentrates generated from the two methods appear to have similar levels of tyrosine-like proteins and fulvic acid-like compounds. However, the BE-based elution process appears to concentrate greater amounts of humic materials (peaks IV and V in Fig. 3a). The samples processed by the BE method contained higher levels of natural organic matter (NOM) species than the samples processed by the glycine method. Based on these results, samples processed by the BE-based method are expected to show higher levels of PCR inhibition than the samples processed by the glycine-based method. Comparison of the EEM profiles of samples processed by both (BE- and glycine-based) methods to the amplification results for the poliovirus genome (Table 2) confirmed that considerably higher levels of inhibitory compounds coconcentrated in samples processed by the BE-based method, which resulted in decreased molecular sensitivity.

This not only indicates the potential advantage of glycine-based methods for molecular applications but also the effectiveness of EEM profiling to characterize inhibitor variations within sample concentrates. Independent experiments using humic acid and bile salts as positive controls for PCR inhibition were conducted. These agents were selected because other biosolid-related PCR-inhibitory compounds, such as tyrosine and fulvic acid, were determined to have lesser contributions in PCR

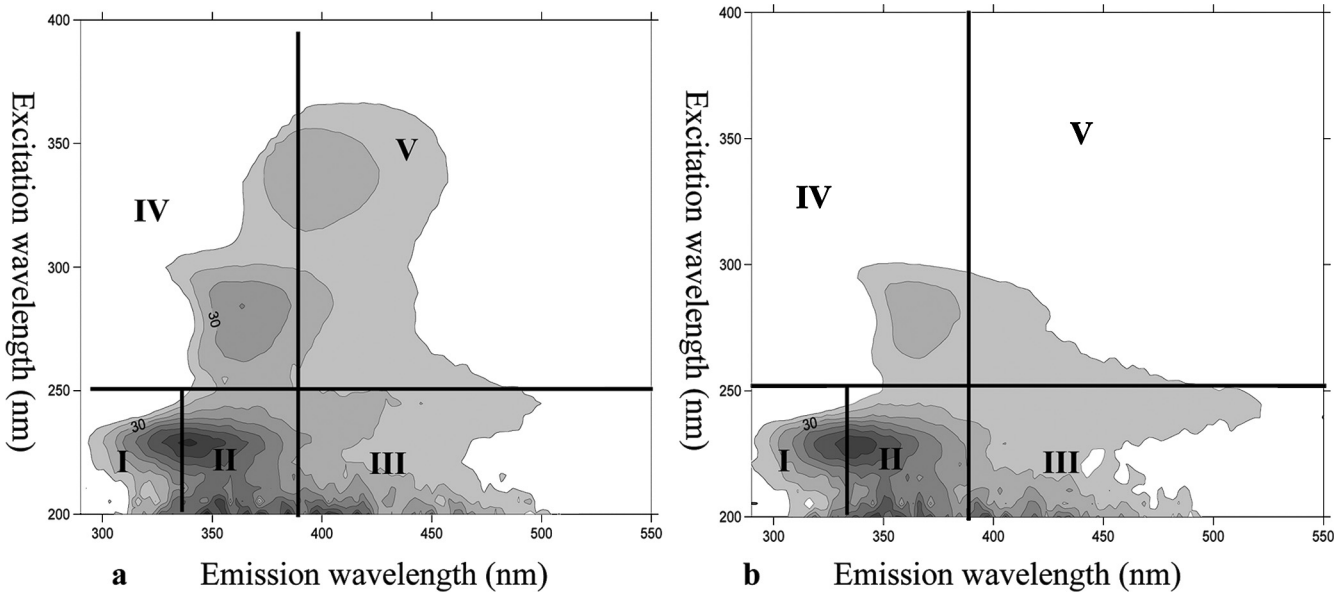

FIG. 3. The EEM of Qiagen kit-purified sample concentrates of biosolids from the NWWRP (Mesa, AZ). (a) Elution by a beef extract (BE)-based method. (b) Elution by glycine-based method.

TABLE 3. Results of PCR amplification (success rates) in different types of biosolid samples processed by glycine elution buffer

Sample source	Treatment processes for biosolid production	Template amt (pg)	No. of positive PCRs/total PCRs (by sample dilution)		
			1:1	1:10	1:100
Green Valley Wastewater Treatment Plant, AZ	Biological nutrient removal and filter press and aerobic digestion of biosolids	1,600	0/3	1/3	3/3
		160	0/3	1/3	3/3
		16	0/3	1/3	3/3
Avra Valley Wastewater Treatment Facility, AZ	Oxidation ditch, polymer thickening, and filter press and air drying of biosolids	1,600	0/3	0/3	1/3
		160	0/3	0/3	1/3
		16	0/3	0/3	0/3
Stickney Water Reclamation Plant, Chicago, IL	Aerobic digestion and air drying of biosolids	1,600	1/3	3/3	3/3
		160	0/3	3/3	3/3
		16	0/3	1/3	3/3
NWWRP, Mesa, AZ	Polymer thickening and centrifugation and anaerobic digestion of biosolids	1,600	0/3	3/3	3/3
		160	0/3	3/3	3/3
		16	0/3	3/3	2/3

inhibition (peaks IV and V in Fig. 3a correspond to most inhibitory compounds), and similar results have been previously reported (16). In PCR amplification reaction mixtures containing picogram to femtogram amounts of target nucleic acids, bile salts exhibited one order of magnitude less inhibition than humic acids when added at concentrations between 10^{-7} to 10^{-10} grams per reaction (data not shown).

Evaluation of the EEM method to predict PCR inhibition in different types of biosolids. After the selection of glycine as an appropriate elution buffer, the level of PCR inhibition in different types of biosolid samples was compared with the respective EEM profile. A two-dimensional inhibition gradient approach was used to characterize molecular inhibition in different types of biosolid sample concentrates. A wide variation in nucleic acid amplification was observed in biosolids from sources produced by different treatment processes (Table 3). In general, little or no nucleic acid amplification was achieved in nondiluted sample concentrates from Avra Valley, Green Valley, and Chicago. However, more consistent nucleic acid amplification was noticed in the 10-fold- and 100-fold-diluted samples from all sources. Amplification success ranged from 0 to 100%, and the success rate increase was directly proportional to spike concentration in all samples. In general, the highest level of inhibition was noted in samples from Avra Valley, and samples from Chicago showed the lowest level of inhibition.

The applicability of the EEM method for assessing PCR inhibition in different types of samples was then investigated. EEM profiles of Qiagen kit-purified sample concentrates from Green Valley, AZ (aerobically digested), Chicago, IL (aerobic polymer added), and Avra Valley, AZ (filter-pressed and air-dried) biosolids are presented in Fig. 4a, b, and c, respectively.

Biosolid samples from different sources showed different concentrations of NOM species. In general, the aerobically digested biosolids had the least humic material, followed by the anaerobically digested biosolid samples. The humic material found in biosolid sample concentrates from Green Valley, AZ (aerobically digested), and Avra Valley, AZ (filter pressed, air dried), corresponds to aquatic/marine humic species (see peaks in Fig. 4a and c). The Qiagen kit used in this study appears to be less efficient at removing aquatic/marine humic species than at removing other types of humic material. The highest level of NOM species was detected in sample concentrates from Avra Valley (filter pressed and air dried), followed by samples from Green Valley (aerobically digested and filter pressed) and Chicago (aerobically digested and polymer added). These samples, as expected, showed similar trends in PCR inhibition.

In addition to visualization of the EEM images, the areas calculated under the peaks of the five EEM regions for different samples are provided in Table 4. After evaluation of the region areas, it was determined that regions III, IV, and V most accurately depicted the PCR success rate and applicability of the samples analyzed. These regions, III, IV, and V, correspond to fulvic acid-like, soluble microbial by-product-like, and humic acid-like compounds, respectively. When evaluated, the samples that contained the greatest areas in regions I and II corresponded to the highest level of inhibitory impact on the molecular success of the sample (Table 5). These areas were then ranked for PCR success rate, with a score of 3 for the worst PCR amplification success rate. Each preceding number corresponds to increased amplification success. Comparison of the quantification cycle (Cq) values for quantitative PCR (qPCR) performed on samples spiked with different levels of target and inhibitor concentrations yielded lower Cq values (greater detection limit) for samples processed using glycine buffer compared to the samples processed using beef extract (Fig. 5).

The RT-qPCR data for biosolid samples processed by glycine- and BE-based methods are consistent with the total areas in the EEM profiles of these samples (Table 4). Based on the data, it can be concluded that application of the EEM method to biosolid sample concentrates can accurately characterize and quantify organic contaminants. Additionally, these data suggest that molecular inhibition in biosolid samples processed by different methods can be accurately predicted by EEM profiling. It is important to point out that these samples contained low copy numbers (16 pg) of purified viral genome, which indicates the potential application

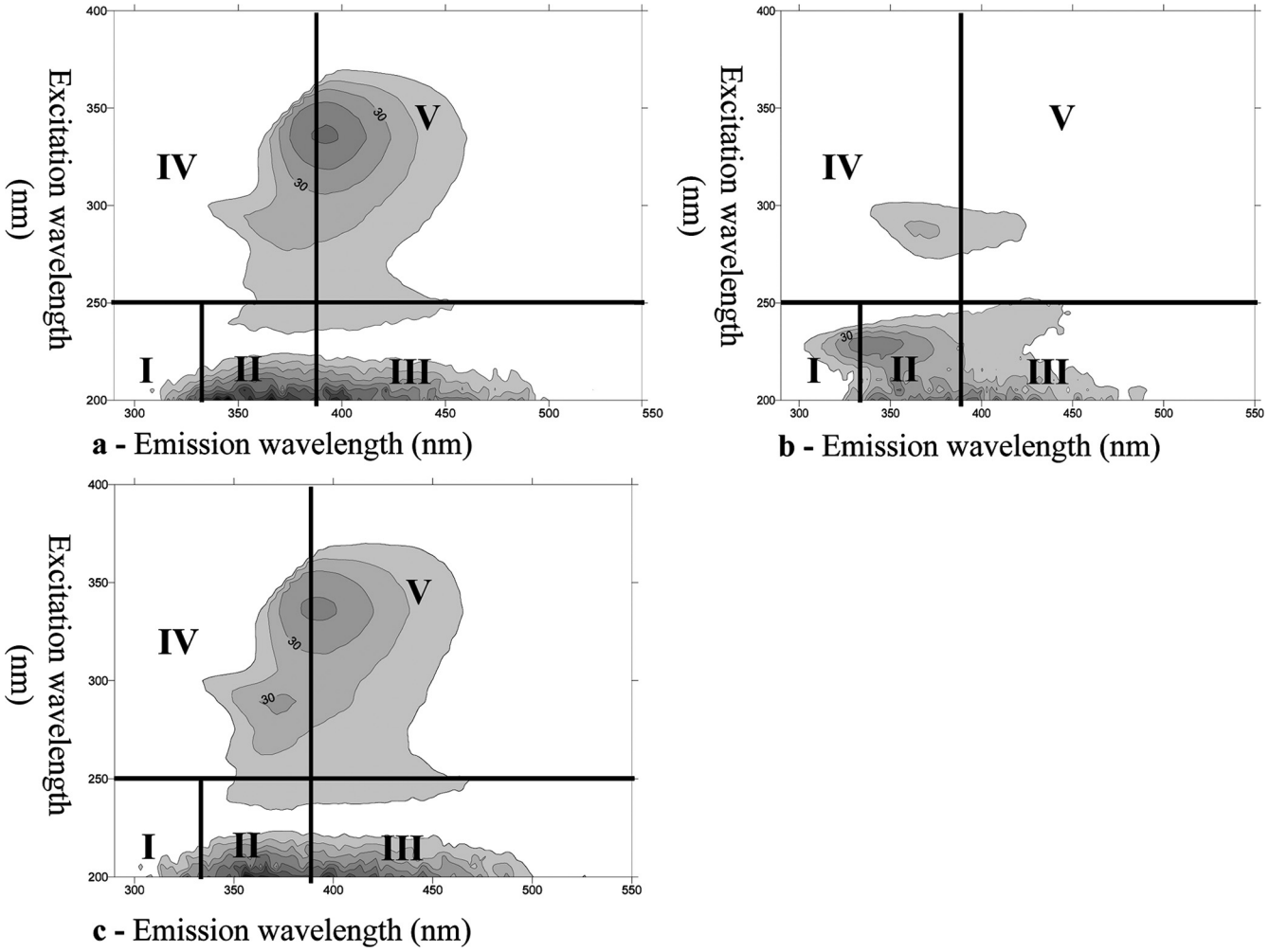

FIG. 4. The EEM of Qiagen kit-purified sample concentrates eluted by the glycine-based method. (a) Biosolids from Green Valley, AZ. (b) Biosolids from Chicago, IL. (c) Biosolids from Avra Valley, AZ.

of the method in pathogen occurrence/monitoring studies using molecular techniques.

DISCUSSION

Municipal wastewater and biosolids are known to contain large amounts of different fractions, which include (i) a fraction degradable only under aerobic conditions, (ii) a fraction degradable only under anaerobic conditions, (iii) a fraction degradable under both anaerobic and aerobic conditions, and (iv) a nondegradable fraction. Because of this, biosolids produced by different treatment process are qualitatively different, maintaining various fractional components. For example, the activated sludge stabilization processes by anaerobic-aerobic digestion and aerobic-anaerobic digestion are known to have different effects on the levels of pathogens, volatile solids, and odors (20). These variations can impact the results of downstream analysis, and these impacts can vary from location to location. Therefore, proper evaluation of each fractional component of dissolved organic carbon (DOC) is crucial for estimation of its inhib-

TABLE 4. Calculated areas under EEM peaks in different regions

Sample analyzed in Fig.:	Area under EEM peak					
	Zone I	Zone II	Zone III	Zone IV	Zone V	Total
3a	25,644.06	120,539.60	130,637.20	−13,153.60	189,574.70	453,241.89
3b	33,294.43	107,036.40	112,031.00	−51,100.50	103,996.20	305,257.44
4b	−14,218.00	62,132.64	77,356.26	−105,183.00	93,193.09	113,280.76
4a	−24,392.90	54,560.11	90,837.74	−54,461.50	210,031.10	276,574.60
4c	−26,011.60	49,322.89	92,190.44	−45,716.20	212,604.40	282,389.92

TABLE 5. Comparison of rankings for PCR success rate and the calculated area under EEM peaks in different regions

Sample source	Elution buffer	Ranking[a]						
		PCR	EEM					
			Zone I	Zone II	Zone III	Zone IV	Zone V	Total
NWWRP, Mesa, AZ	Glycine	1	2	1	1	2	1	1
	Beef extract	2	1	2	2	1	2	2
Chicago, IL	Glycine	1	1	3	1	3	1	1
Green Valley, AZ	Glycine	2	3	2	2	2	2	2
Avra Valley, AZ	Glycine	3	2	1	3	1	3	3

[a] See the text for a description of the ranking system. Zone I, aromatic protein I-like compounds; zone II, aromatic protein II-like compounds; zone III, fulvic acid-like compounds; zone IV, soluble microbial by-product-like compounds; zone V, humic acid-like compounds.

itory role. Additionally, municipal wastewaters are known to contain high concentrations of nitrogen, which can be substantially removed by anaerobic-aerobic sequential treatment (3). Results of the present study are consistent with previous reports that have identified differences in the quality and quantity of organic pollutants in biosolids produced by various treatment methods.

Capability to predict PCR inhibition in any sample can be valuable, especially in the case of sample dearth (insufficiency). In the peer-reviewed literature, little information on the methods currently available to predict PCR inhibition is available. A previous study attempted to correlate sample inhibition of PCR assays with high or low levels of aquatic humic materials in environmental water samples by using the specific UV absorbance (SUVA). In this study, SUVA was delineated by calculating the absorbance at 254 nm (expressed per meter of absorbance) divided by the DOC concentration (in milligrams per liter). Although an association seems intuitive, no association between the relation of SUVAs and PCR inhibition was reported (2). Various researchers have used real-time PCR amplification efficiencies to detect PCR inhibitors in clinical samples (4, 11). However, there is no reported method that can be used to estimate inhibition before performing molecular assays on any sample. This is the first report of accurate estimation of PCR inhibition patterns in environmental samples. The proposed method can potentially minimize the chances of false-negative results while saving valuable time and resources.

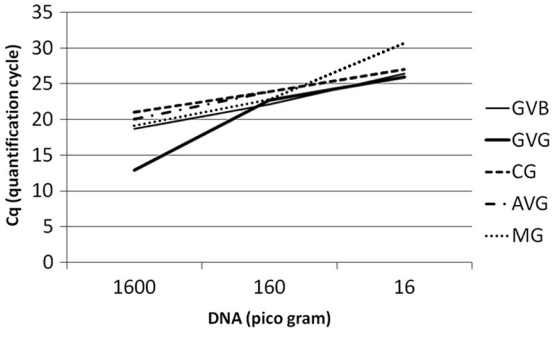

FIG. 5. Quantification cycle (Cq) correlations for biosolid sample concentrates processed by glycine and beef extract at the 1:100 dilution. GVB, biosolids from Green Valley (AZ) processed with BE; GVG, biosolids from Green Valley processed with glycine; CG, biosolids from Chicago (IL) processed with glycine; AVG, biosolids from Avra Valley (AZ) processed with glycine; MG, biosolids from Mesa (AZ) processed with glycine.

ACKNOWLEDGMENTS

Financial support for this work was provided by a grant from the Water Environmental Federation (project 02-HHE-2) and the NSF Water and Environmental Technology Center at Arizona State University.

We also acknowledge Erin Daughtery, Aaron Dotson, and Paul Westerhoff for helping with the EEM analyses.

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