Abstract

Using Whole Cell Lysate from Dechloromonas agitata str CKB to Detect Perchlorate in the ppb Range

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Recognition of perchlorate as a widespread contaminant across the United States poses an adverse health threat and has motivated its placement on the contamination list. Current methods of detection require use of expensive and time-consuming ion chromatography equipment that require highly trained personnel. Our studies have focused on the development of an alternative mechanism for perchlorate determination. The goal of these studies is to develop a simple, highly sensitive, specific bioassay for perchlorate that can be performed on-site with the basic equipment. Our bioassay takes advantage of the unique biochemical of the perchlorate reductase (PCR) purified from the perchlorate reductase-producing Dechloromonas agitata strain CKB. Our results indicated that the biochemical electron donor, NADH, combined with the dnp-N-methylphenazinium methosulfate (DMP) provides the optimum system for supplying electrons to the PCR to reduce perchlorate. Monitoring NADH concentrations by absorbance at 340 nm revealed a strong reproducible correlation between the perchlorate concentration and the NADH reduced at PCR under anaerobic conditions. A similar correlation was determined when the assay was conducted aerobically on a benchtop under mineral oil. To bring the detection limit to below California regulatory limits, a perchlorate concentration step was added to the protocol. In our procedure, modified from a protocol published by the US Army Corps of Engineers, the perchlorate sample is loaded onto a three-disk column before conditioning with DTPA and eluting using MOPS buffer. Interfering ions, such as nitrate or chloride, are differentially eluted to avoid false positives. These combined techniques quantitatively detected perchlorate in samples that contain concentrations less than 5 ppb. More importantly, the developed bioassay, detected perchlorate in 3 of 4 contaminated groundwater samples and the results were verified with excellent confidence by the ion chromatography method.

These studies have resulted in the successful development of a highly sensitive and robust colorimetric bioassay for the specific determination of perchlorate concentrations. This assay has proven to be cost effective and has already been reliably applied to the determination of perchlorate contamination in diverse environmental groundwater samples.

Analyzing Perchlorate in the ppb Range

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As shown above, the first step in our perchlorate assay is a purification step using a solid phase extraction column (SPE). As previously published (Thoma (EDC/CRRL TR 04-04)), a dicylino-diphenyl ether (SDV) cartridge conditioned with dicyclohexylaminium bromide (DABT) can separate perchlorate from chloride and nitrate (ions that interfere with our bioassay). This purification can also result in as much as a 100 fold concentration. The perchlorate is eluted in acetonitrile, a solvent that can denature many enzymes. It has been demonstrated that oxygen can interfere with the activity of PCR. So a step in this assay needs to remove oxygen contamination. The easiest way to address this problem is to design an assay in the anaerobic chamber. However, this design would have a major limitation if the user would require an expensive and cumbersome piece of equipment. This poster deals with the creation of a field ready assay that detects perchlorate under aerobic conditions with a lower detection limit of 5 ppb.

Conclusions

NADH and PMS can be used as an electron donor/shuttle system to reduce perchlorate reduction. There is a linear correlation between the amount of NADH oxidized and the amount of perchlorate reduced under anaerobic and aerobic conditions. Soluble cell lysates can be substituted for perchlorate reductase to reduce the cost of the assay to $50-60 per sample.

In groundwater, perchlorate can be concentrated on SPE columns between 100 and 500 fold. This perchlorate can be diluted with MOPS, a biological buffer that has no effect on the bioassay.

The percentage of perchlorate retained on our SPE columns is affected by salts, however, there is no effect on the linearity of the response. Using the standard additions method, we have analyzed groundwater from contaminated sites. - The percentage of perchlorate retained on our SPE columns is affected by salts, however, there is no effect on the linearity of the response. Using the standard additions method, we have analyzed groundwater from contaminated sites.

Table 1: Calculated SPE recoveries of perchlorate from groundwater samples. Samples were analyzed using both ion chromatography and the perchlorate bioassay.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Determined by ion chromatography</th>
<th>Determined by bioassay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>8.49 ± 0.97 ppb</td>
<td>7.38 ± 0.94 ppb</td>
</tr>
<tr>
<td>Sample 2</td>
<td>7.13 ± 1.73 ppb</td>
<td>21.1 ± 4.9 ppb</td>
</tr>
<tr>
<td>Sample 3</td>
<td>7.04 ± 3.2 ppb</td>
<td>82.1 ± 7.12 ppb</td>
</tr>
<tr>
<td>Sample 4</td>
<td>21.78 ± 5.4 ppb</td>
<td>19.74 ± 2.98 ppb</td>
</tr>
</tbody>
</table>

Table 2: Comparison of perchlorate concentrations detected using the bioassay and ion chromatography. Concentrations detected by ion chromatography in both soil and groundwater samples are shown in blue. Perchlorate concentrations detected in soil samples are shown in red. Perchlorate concentrations detected in soil samples are shown in red. For the perchlorate bioassay, there is a linear relationship between the amount of perchlorate reduced and the amount of NADH oxidized.

Figure 4 - High concentrations of salt were added to the samples prior to analysis. NADH oxidizes, drives the amount of signal produced in solution and shows the decrease in signal produced as perchlorate is reduced. A similar trend is observed when perchlorate is reduced by PCR in the presence of the DMP. The results show that perchlorate concentrations can be reduced by PCR in the presence of the DMP.

Figure 5 - The perchlorate concentration is determined by the amount of NADH oxidized. A similar trend is observed when perchlorate is reduced by PCR in the presence of the DMP.

Figure 6 - The perchlorate concentration is determined by the amount of NADH oxidized. A similar trend is observed when perchlorate is reduced by PCR in the presence of the DMP.

Figure 7 - The perchlorate concentration is determined by the amount of NADH oxidized. A similar trend is observed when perchlorate is reduced by PCR in the presence of the DMP.

Figure 8 - The perchlorate concentration is determined by the amount of NADH oxidized. A similar trend is observed when perchlorate is reduced by PCR in the presence of the DMP.