

# Evaluating Removal and Inactivation Efficiency of Adenovirus during Soil Aquifer Treatment for Quantitative Microbial Risk Assessment

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Key Words: *Water reclamation, Soil aquifer treatment, Microbial risk reduction, Adenovirus, Removal efficiency*

## 1. Background

Water reclamation system has been desired especially in urban area because of highly concern about instability of water resources in quantity and quality with climate change. One of the candidate alternative water sources is secondary-treated wastewater. In many countries, membrane-based water reclamation system has been in operation, but they have disadvantages in cost and energy consumption. In this research, soil aquifer treatment (SAT) process with a framework of an advanced risk management was focused, and its removal ability of pathogens was evaluated.

## 2. Objectives

This research aims to establish procedures for collecting adenovirus (AdV) data toward Quantitative Microbial Risk Assessment. Specific purposes are 1) to establish concentration procedure for water samples containing pathogens at very low level; 2) to estimate removal efficiency of pathogens using pilot-scale SAT column; 3) to evaluate inactivation efficiency of pathogens during SAT process.

## 3. Methods

One litre SAT influent samples (Secondary-treated sewage) and 50 L SAT effluent samples were collected regularly. After concentration of the SAT effluent to 1 L by ultrafiltration with membrane of MWCO 50,000, both SAT influent and the primarily-concentrated SAT effluent samples were further concentrated by polyethylene glycol (PEG) precipitation. The recovery rate during each concentration procedure was also determined by seeding AdV40. AdV concentrations were determined using a quantitative PCR (qPCR) method. Then, the removal efficiencies during SAT process were calculated. At the same time, the removal efficiencies of fecal coliform were also determined. At last, the virus infection assay using CaCO-2 cell line and the following PCR detection of intracellular virus genome was also examined to estimate most probable number (MPN) of infectious AdV in each concentrated sample.

## 4. Result and Discussion

The recovery rates of virus were determined as 2.4 and 28% for PEG precipitation and ultrafiltration, respectively. The removal efficiencies of AdV during SAT column ranged 0.56 – 1.52 log, while fecal coliform removal of approximately 6 log in average were quite high and more stable. The AdV data of  $10^4$  -  $10^5$  and  $10^3$  -  $10^4$  genome copies/ $\mu$ L for the SAT influent and effluent, respectively, may include virus particle that lost infectivity to human or cell-free genome in samples, because that kind of molecular approach cannot discriminate infectious virus particles from the others. Therefore, the virus infection assay was performed using 4 concentrated water samples. AdV infection was confirmed in only one SAT effluent sample, and the MPN can be calculated. Compared to the AdV data determined by qPCR method, the MPN of infectious AdV indicates 1/50 smaller value. This result suggests that a large contribution of inactivation mechanisms to microbial risk reduction can be expected in SAT process.

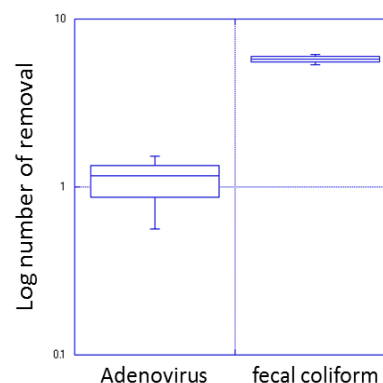


Figure 1 Comparison of removal of fecal coliform and adenovirus during SAT process