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Comparing extraction buffers to identify optimal method to extract somatic coliphages from sewage sludges

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HIGHLIGHTS

- Somatic coliphages can be used as process indicators of sewage treatment efficacy.
- Efficient methods to extract coliphages from sewage sludges for enumeration are lacking.
- Twelve different extraction procedures were compared.
- Highest recovery was 16% using 10% beef extract at pH 9.0.
- Additional research is needed to develop better coliphage extraction methods.

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ABSTRACT

Somatic coliphages are present in high numbers in sewage sludge. Since they are conservative indicators of viruses during wastewater treatment processes, they are being used to evaluate the effectiveness of sludge treatment processes. However, efficient methods to extract them from sludge are lacking. The objective was to compare different virus extraction procedures and develop a method to extract coliphages from sewage sludge. Twelve different extraction buffers and procedures varying in composition, pH, and sonication were compared in their ability to recover indigenous phages from sludges. The 3% buffered beef extract (BBE) (pH 9.0), the 10% BBE (pH 9.0), and the 10% BBE (pH 7.0) with sonication were short-listed and their recovery efficiency was determined using coliphage-spiked samples. The highest recovery was 16% for the extraction that involved 10% BBE at pH 9.0. There is a need to develop methods to extract somatic phages from sludges for monitoring sludge treatment processes.

1. Introduction

Globally, there is an increasing need to find environmentally sustainable methods to dispose large volumes of municipal sewage wastes. In the United States, more than 8 million tons of sewage sludge is land applied. The US EPA has strict regulations governing the disposal of sewage sludges based on the levels of specific pathogens and indicator organisms. Sewage sludges are termed as Class A or Class B based on specific titers of pathogens such *Salmonella* spp., enteric viruses, helminth ova, and indicator organisms such as fecal coliforms (EPA, 1993). The National Academy of Sciences recommended that the EPA should review the risk-assessment methods used to determine the safety of sewage sludges leading to concerns that there are gaps in data on whether stabilization technologies sufficiently achieve desired pathogen destruction (NRC, 2002). Thus, there is a need to employ a wide variety of microbial target organisms that could be used to evaluate the efficiency of the treatment process.

There are a number of studies which indicate that somatic coliphages are present in relatively large quantities in sludge material (Guzman et al., 2007; Rouch et al., 2011; Zhang and Farahbakhsh, 2007). Thus, coliphages have been frequently used as surrogates or process indicators of the efficiency of treatment processes to remove pathogenic viruses (Arraj et al., 2005; Nappier et al., 2006; Tanji et al., 2002). Several methods have been described for extracting enteric viruses from sewage sludges (Mignotte et al., 1999; Safferman et al., 1988; Scheuerman et al., 1986; Schwartzbrod and Mathieu, 1986; Shimohara et al., 1986; Stetler et al., 1992; Straub et al., 1994a,b). Most often, methods used for extracting enteric viruses from sludges and sediments have also been used for assaying coliphages (Ahmed and Sorensen, 1995; Albert and Schwartzbrod, 1991; Guzman et al., 2007; Jofre et al., 1989; Lasobras et al., 1999; Mignotte et al., 1999; Soares et al., 1994).

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