Water Research 103 (2016) 245–255

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Review

Quantification of viable helminth eggs in samples of sewage sludge

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ARTICLE INFO

Article history:
Received 23 May 2016
Received in revised form
16 July 2016
Accepted 18 July 2016
Available online 19 July 2016

Keywords:
Domestic sludge
Quantification of viable helminth eggs
Pathogens in sludge
Biossolids
Agricultural use

ABSTRACT

For the application of sewage sludge as fertilizer, it is of fundamental importance the absence of pathogenic organisms, such as viable helminth eggs. Thus, the quantification of these organisms has to be carried out by means of the application of reliable and accurate methodologies. Nevertheless, until the present date, there is no consensus with regard to the adoption of a universal methodology for the detection and quantification of viable helminth eggs. It is therefore necessary to instigate a debate on the different protocols currently in use, as well as to assemble relevant information in order to assist in the development of a more comprehensive and accurate method to quantify viable helminth eggs in samples of sewage sludge and its derivatives.

Contents

1. Introduction

The production of sewage sludge is intrinsic to the treatment of domestic wastewaters, and its use as a fertilizer has been practiced as an alternative to incineration and landfilling. This form of waste

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http://dx.doi.org/10.1016/j.watres.2016.07.039
0043-1354/

allocation presents economic and environmental advantages and it has been adopted in several countries, for example: United States (USEPA, 1992; Harrison et al., 1999); China (Wang, 1997); Mexico (Jiménez et al., 1997; 2004); Spain (Antolín et al., 2005); Brazil (Andreoli et al., 2008; Ghiniand Bettiol and Ghini, 2011; Bittencourt et al., 2014), and Australia (Pritchard et al., 2010). However, after the application of these biosolids on soil, viable pathogenic organisms that may be present in the sludge can become potentially infectious.

The sludge from sewage treatment plants is usually stabilized by either aerobic or anaerobic digestion processes (Hartenstein, 1981). However, many pathogens remain viable and infectious after stabilization (Reimers et al., 1981; Black et al., 1982; O'Donnell et al., 1984; Reimers et al., 1986; Wéry et al., 2008), and their presence in the sludge could often be undetectable. In a study carried out by Wéry et al. (2008), who studied the inactivation of bacteria in municipal sludge after composting, it was observed that *Campylobacter jejuni*, an enteric pathogen present in the sludge, exhibits higher survival rate than *Escherichia coli*, which is commonly used as an indicator of sludge treatment efficiency. Furthermore, enteric parasite eggs of the genera *Ascaris, Trichuris* and *Toxocara*, which are also known as helminths, are highly resistant to sludge digestion and their removal, or even inactivation, requires subsequent disinfection steps. Nevertheless, some of these pathogens maintain their infective potential even after severe treatment conditions (Nelson and Darby, 2002). For instance, Maya et al. (2012), evaluated the inactivation rate of eggs of *Ascaris lumbricoides*, *Ascaris suum*, *Toxocara canis* and *Trichuris trichiura*, when submitted to 80 °C and pH of 12.1, and concluded that less than 25% of these parasites had been inactivated under these conditions.

Thus, when the aim is to apply the biosolids in soils and prevent the spread of these pathogens in the environment, regulatory agencies in several countries have established limits for the concentration of helminth eggs in sewage sludge (Swiss Government, 1992; USEPA, 1992 – USA; Journal Officiel, 1998 – France, European Comission, 2001; NOM-004-SEMARNAT, 2002 – Mexico; CONAMA, 2006 – Brazil; WHO, 2006). However, there is no general agreement among researchers, environmental agencies and other government agencies with regard to the most appropriate method for the determination of the number of viable eggs in sludge samples (Nelson and Darby, 2001; National Research Council, 2002; Szabo and Vargha, 2006; Barés, 2010). Hence, in order to produce more reliable results regarding the identification and quantification of viable helminth eggs, the methodologies currently in use have to be reassessed.

Aiming at contributing to the identification of key points that have mislead researchers to the production of biased results, one of the goals of this review is to present an evaluation of the methodologies currently in use for the determination of viable helminth eggs in sewage sludge. This paper also aims at providing a critical review of different protocols currently in use. In order to fulfill these aims it was taken into consideration each and every step necessary for the recovery of viable helminth eggs from sewage sludge.

2. Sanitization of sewage sludge

The stabilization of organic matter and the assessment of the biological safety of the biosolids are required for the recycling and use of sewage sludge in soils (USEPA, 2003; CONAMA, 2006; Arthurson, 2008; Sidhu and Toze, 2009). Therefore, sludge stabilization processes aim to accelerate the degradation of organic matter, reduce odors and vector attraction, and ensure that, if applied to soils, the sludge does not compete for natural resources in the environment during the degradation of its organic compounds (Hartenstein, 1981; Singh and Agrawal, 2008).

In order to pose no risk of biological contamination to the environment, the sludge must be properly sanitized (Reilly, 2001; Arthurson, 2008). Procedures for the sanitization of sewage sludge include composting (Pourcher et al., 2005; Cofie et al., 2009), liming (Czechowski and Marcinkowski, 2006; Tamanini et al., 2008) and heat treatment (Piterina et al., 2010; Rubio-Loza and Noyola, 2010). Concerning the correct treatment and final disposal of sewage sludge, the United States Environmental Protection Agency, in its guidance *Control of Pathogens and Vector Attraction in Sewage Sludge*, defines processes to significantly reduce pathogens (PSRPs), and processes to further reduce pathogens (PFRPs) in samples of sewage sludge (USEPA, 2003). In the case of the application of these processes, the reduction of pathogens in the sludge to below detectable levels is expected (Caballero, 1984; Farrel et al., 1996; USEPA, 2003). Composting, using either in-vessel, static aerated pile or windrow method, and lime stabilization can be cited as examples of PSRPs. In the case of composting, the temperature of the process will reach 70 °C and the total period of the process will vary between 90 and 120 days (Pereira Neto, 1996). In the second case, sufficient lime will be added to the sewage sludge to raise the pH to 12, after a 2-h contact time. A sewage sludge treated by these means is qualified as Class A biosolid with respect to helminth ova, enteric viruses, and pathogenic bacteria, among other parameters. Thus, the biosolids produced by both methods may be used in soils with no site restrictions in the United States, because the resulting material complies with the pollutant concentration limits established in part 503 of the USEPA guidance (USEPA, 2003). Regarding the PFRPs some techniques can be mentioned, such as: i) heat drying, in which sludge is dried by direct or indirect contact with hot gases to reduce the moisture content to 10% or less; ii) heat treatment, in which liquid sewage sludge is heated to 180 °C or higher, for 30 min; and iii) pasteurization, in which the temperature of the sewage sludge is maintained at 70 °C, or higher, for 30 min or longer (USEPA, 2003; Lang and Smith, 2008; Romdhana et al., 2009).

However, to determine whether a disinfection treatment is effective to remove pathogens, it is necessary to quantify the number of viable organisms present in the sludge samples before and after any sanitization treatment. The pathogens that must be monitored in sewage sludge samples are enteric viruses, primarily adenoviruses and enteroviruses, thermotolerant coliforms, mainly *Escherichia coli*, *Salmonella*, and helminths in the form of viable *Ascaris* eggs, which can become infective under appropriate medium conditions (USEPA, 1992; Straub et al., 1993; Sidhu and Toze, 2009).

Although many pathogens can be found in sludge samples, their presence does not necessarily indicate that there is a risk of contamination (Lewis and Gattie, 2002). The risk of contamination depends on the infectious dose that is required for that organism to become pathogenic in an individual (Gerba and Smith, 2005). For instance, the minimum infective dose for helminths is very low and the contact with or the ingestion of a single viable egg can lead to the development of a parasite-associated disease (Navarro et al., 2009). Therefore, the low infectious dose associated with the resistant structure of helminth eggs makes helminths the primary target of sewage sludge cleaning techniques. Thus, it is essential to accurately and rapidly quantify the number of viable helminth eggs in sludge samples intended for agricultural use.

3. Helminth eggs in sewage sludge

In the field of Sanitary Engineering, helminths are related to the group of intestinal parasites in which man is the definitive host. The diseases caused by these organisms are helminthiases and,