REVIEW ARTICLE

Inactivation and risk control of pathogenic microorganisms in municipal sludge treatment: A review

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HIGHLIGHTS

- Diversity and detection methods of pathogenic microorganisms in sludge.
- Control performance of sludge treatment processes on pathogenic microorganisms.
- Risk of pathogen exposure in sludge treatment and land application.

ARTICLE INFO

GRAPHIC ABSTRACT
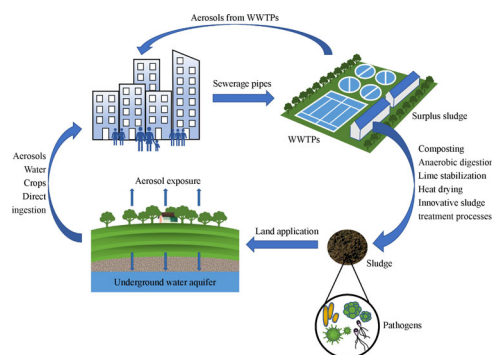
ABSTRACT

The rapid global spread of coronavirus disease 2019 (COVID-19) has promoted concern over human pathogens and their significant threats to public health security. The monitoring and control of human pathogens in public sanitation and health facilities are of great importance. Excessive sludge is an inevitable byproduct of sewage that contains human and animal feces in wastewater treatment plants (WWTPs). It is an important sink of different pollutants and pathogens, and the proper treatment and disposal of sludge are important to minimize potential risks to the environment and public health. However, there is a lack of comprehensive analysis of the diversity, exposure risks, assessment methods and inactivation techniques of pathogenic microorganisms in sludge. Based on this consideration, this review summarizes the control performance of pathogenic microorganisms such as enterovirus, *Salmonella* spp., and *Escherichia coli* by different sludge treatment technologies, including composting, anaerobic digestion, aerobic digestion, and microwave irradiation, and the mechanisms of pathogenic microorganism inactivation in sludge treatment processes are discussed. Additionally, this study reviews the diversity, detection methods, and exposure risks of pathogenic microorganisms in sludge. This review advances the quantitative assessment of pathogenic microorganism risks involved in sludge reuse and is practically valuable to optimize the treatment and disposal of sludge for pathogenic microorganism control.

1 Introduction

The treatment of urban sewage inevitably produces plentiful sludge as a byproduct (Hu et al., 2019). The sludge contains various pollutants, such as infectious pathogens, heavy metals, and micro-organic pollutants (Romdhana et al., 2009; Yang et al., 2015). Although sludge is the main sink of pathogenic microorganisms in WWTPs and a potential source of microorganisms in the environment (Fig. 1), it is also an important source of organic matter and nitrogen- and phosphorus-containing nutrients. Reuse of sludge is the general trend. To date,

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many wastewater treatment plants (WWTPs) install sludge treatment units, and treated sludge may be used as fertilizers and nutritional soils to improve fertility. In this case, pathogenic microorganisms should be carefully inactivated and controlled to minimize potential risks to water bodies, soils, and human health as much as possible (Pritchard et al., 2010).

The pathogens in sewage and WWTP effluents have been widely reported to threaten the health of humans and animals (Schöniger-Hekele et al., 2007; Sutherland et al., 2010; Boehm et al., 2018). However, relatively less attention has been given to the occurrence, concentrations, and risks of aerosol transmission and epidemiology of various pathogens in sludge (Viau et al., 2011). This makes considering the annual production of large amounts of sludge an important global issue. For example, in China, WWTPs produced 67.65 million tons of sludge with a moisture content of 80% in 2018, and the annual growth rate was expected to be in the range of 5% to 8% (Dai et al., 2020).

To achieve proper sludge treatment, different countries and regions have issued standards and documents (Table 1). However, most regulations on pathogenic microorganism control are dependent on several pathogens, such as coliforms and enteroviruses, and the risk of pathogen transmission via sludge can hardly be well characterized (Viau et al., 2011; Kelessidis and Stasinakis, 2012). In addition, the risk of pathogenic microorganisms carrying antibiotic resistance genes (ARGs) and thus having antibiotic resistance has not been considered by standards and regulations. Regulation standards and detection methods need to be updated and improved (Yang et al., 2015). It is important to promise a theoretical and technical basis to guarantee the update of relevant standards. In China, there is no virus indicator in the relevant standards for municipal sludge treatment and sludge agricultural use.

Recently, the risks of SARS-CoV-2 spreading through fecal-oral and fecal-respiratory transmission have received great attention, but transmission and spread via sludge is far from well-illustrated (Carraturo et al., 2020; Foladori et al., 2020; Li et al., 2020b). A recent study showed that because sludge contains more virus particles and longer residence times, it is easier to detect SARS-CoV-2 in sludge than in sewage (Balboa et al., 2021). Obviously, routine detection cannot adequately represent the risk of special pathogens in sludge during a particular period. In this case, the treatment and disposal of sludge concerning pathogenic microorganism control should be given more attention than before.

Conventional sludge treatment methods include anae-robic digestion, aerobic digestion, composting, lime stabilization, etc. (Yang et al., 2015; Tong et al., 2019; Major et al., 2020). Disposal methods include sanitary landfills, incineration, land application, production of building materials, etc. (Yang et al., 2015). Recently, resource reclamation and energy harvesting have been an essential focus in the process of upgrading existing WWTPs, and land application is an important way to reuse sludge. The inactivation efficiency of pathogenic microorganisms is largely dependent on the different physical, chemical, and biological processes (Kelessidis and Stasinakis, 2012; Dauknys et al., 2020; Jin et al., 2020). This strategy may potentially minimize potential pathogenic risks by reducing the sludge pathogenic load with optimized temperature, pH, residence time and other conditions (Goberna et al., 2018). To achieve promising sludge sanitation for land application, combined processes with two or more units are expected to be implemented; however, there is still a lack of sufficient data to propose feasible processes and optimized strategies to control pathogenic microorganisms.

Based on these considerations, this review aims to

Table 1   Pathogen requirements for sludge applied to land in China, the United States and the European Union

| Countries and regions	Type of sludge	Indicator pathogens					Standards or documents
		Fecal coliforms	*Clostridium perfringens*	Helminth ova	*Salmonella*	Enteric viruses	
China	Agricultural sludge	Colititer is not less than 0.01	—	Mortality of Ascaris eggs is not less than 95%	—	—	Control standards of pollutants in sludge for agricultural use (2018)
United States	Class A	Less than 1000 MPN per gram of total solids (dry weight basis)	—	The alternative: Less than 1 viable helminth ova/4 grams of total solids (dry weight basis)	Less than 3 MPN per 4 grams of total solids (dry weight basis)	The alternative: Less than 1 PFU per 4 grams of total solids (dry weight basis)	USEPA, Environmental Regulations and Technology: Control of Pathogens and Vector Attraction in Sewage Sludge (2003). (Lloret et al., 2012)
European Union	Sludge applied to land	(*Escherichia coli*) 99.99% (4 log) reduction to less than 1×10^3 colony forming units per gram (dry weight)	No more than 3×10^3 spores per gram (dry weight)	—	No detectable *Salmonella* spp. in 50 g (wet weight)	—	European Commission, Proposal for a Directive of the European parliament and of the Council on the spreading of sludge on land (2003). (Lloret et al., 2012)
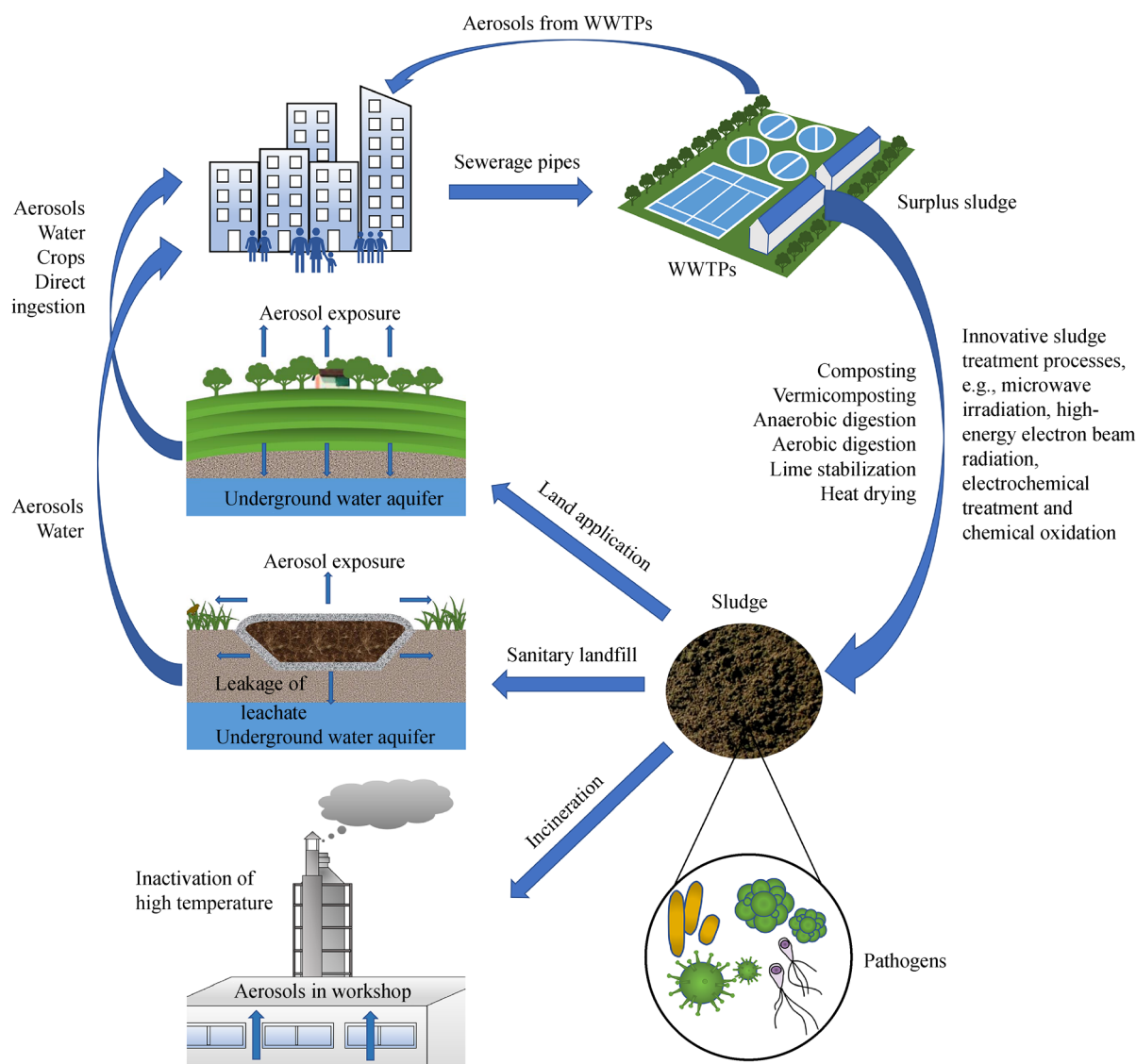
Fig. 1 Transfer and exposure pathways of pathogens involved in sludge treatment and disposal.

1) summarize the different species and methods of detecting pathogenic microorganisms in sludge; 2) investigate the efficiency and the dominant influencing factors involved in pathogen control by different sludge treatment processes; and 3) compare the advantages and disadvantageous of the currently used sludge treatment processes, as well as risks and potentials of sludge in land applications. This review provides insight into the treatment and disposal of sludge, and it is expected to be practically valuable with regard to pathogenic microorganism control in sludge.

2 Detection, diversity, and potential risks of pathogenic microorganisms in the sludge

Sewage sludge is reported to contain plentiful and diverse pathogenic microorganisms, such as pathogenic bacteria,

viruses, and pathogenic protozoa (Ye and Zhang, 2011; Bibby and Peccia, 2013; Amoah et al., 2018). Understanding of pathogen diversity and exposure risk benefits the establishment of treatment strategies and standards for pathogen control, although it is rather difficult to detect all pathogenic microorganisms in sludge (Levantesi et al., 2015).

2.1 Pathogenic bacteria

Bacterial pathogens can survive and reproduce rapidly in suitable environments (Cai and Zhang, 2013), and most studies have focused on human pathogenic bacteria in sludge. Total coliforms, fecal coliforms and *Salmonella* are usually used as indicators in standards (Viau et al., 2011; Kelessidis and Stasinakis, 2012). Generally, the detection method of inoculating and cultivating cultivable bacteria has obvious limitations because the infectious doses vary

greatly with different microorganism species (Shannon et al., 2007). Most bacteria in the environment are unculturable, which may cause deviations in test results (Lu et al., 2015). The quantitative real-time polymerase chain reaction (qPCR) method has the advantages of faster speed, higher sensitivity, and better specificity than other traditional methods. It is not limited by the cultivability of microorganisms. The good evident strengths enabled it to be widely used to detect pathogens and viruses in many studies (Jyoti et al., 2011; Jebri et al., 2014; Jahne et al., 2020). With the rapid development of molecular biology technology, 16S rRNA amplicon sequencing and metagenomic sequencing technologies have also been applied to study sewage sludge (Ye and Zhang, 2011; Lu et al., 2015; Ju et al., 2017; Huang et al., 2018). These technologies enable in-depth research to discover new pathogens in sewage sludge, explore the relationship between pathogenic and nonpathogenic microorganisms, and analyze the co-occurrence of pathogens and ARGs (Ju et al., 2016). However, these methods also have some limitations. For example, the results of 16S rRNA amplicon sequencing were strongly influenced by PCR primer choice and sample processing (Walker et al., 2015). Different DNA extraction methods may also cause significant differences in 16S rRNA gene sequencing (Kennedy et al., 2014). The selection of 16S rRNA gene variable regions potentially leads to different amplification biases (Claesson et al., 2010), which infers the importance of the experimental verification of primers. In addition, depth bias is characteristic of metagenomic approaches and one factor that may cause differences between test results and cell culture tests. Thus, it is critical to decide the appropriate sequencing depth in metagenomic sequencing (Quince et al., 2017). For cultivable microorganisms, cell culture more easily detects low-level bacteria than large-scale molecular detection because of sequencing detection thresholds (Lagier et al., 2012). In analyzing the sequencing results, in addition to the 16S rRNA gene sequence database, the virulence factor database is also often used to study pathogens. Virulence factors (VFs) are applied not only to study the pathogenesis of human pathogens but also as pathogenic indicators for the detection of human bacterial pathogens. For example, Cai *et al.* studied the 16S rRNA genes and the VF genes in AS, and *Mycobacterium tuberculosis* was reported to be the most dominant pathogen (Cai and Zhang, 2013). The use of these two databases may obtain more comprehensive results of pathogen diversity. The rapid development of metagenomic sequencing methods provides optimistic insight into the analysis of microbial diversity in sludge.

The US Environmental Protection Agency (USEPA) Office of Research and Development released information on the 24 types of pathogenic bacteria that may be found in Class B sludge: *Legionella, Aeromonas, Bacillus, Listeria, Brucella, Campylobacter, Proteus, Pseudomonas, Coxiella, Clostridium, Mycobacterium, Escherichia,*

Salmonella, Shigella, Citrobacter, Enterobacter, Serratia, Erysipelothrix, Staphylococcus, Klebsiella, Streptococcus, Francisella, Yersinia, and *Vibrio* (Lewis. and Gattie., 2002). In recent years, sequencing technology has continuously reported new pathogenic bacteria in sludge. Li et al. detected *Collinsella aerofaciens, Eubacterium rectale, Streptococcus salivarius,* and *Vibrio mimicus,* which were previously unreported in activated sludge (AS), and discovered *Bacteroides vulgatus, E. rectale, C. aerofaciens, Streptococcus suis,* and *S. salivarius,* which were previously unreported in anaerobic digestion sludge (ADS) (Li et al., 2015). Additionally, Ju et al. found that *C. aerofaciens, Arcobacter butzleri, S. salivarius, E. rectale, Acinetobacter johnsonii* and *S. suis* had high relative abundances in sludge digesters (Ju et al., 2016). It has been reported that *Oligella urethralis, Aeromonas hydrophila, Aeromonas veronii, Mycobacterium smegmatis, Vibrio cholerae,* and *Pseudomonas putida* have high relative abundances in AS (Zhang et al., 2021). Because more human bacterial pathogens were detected in the sludge, the risk of infection related to these pathogens should be taken seriously.

In addition, the risk of bacterial pathogens carrying ARGs leading to drug resistance cannot be ignored. The large-scale use of human and animal antibiotics may further complicate pathogenic risks to environments and humans, and ARGs in sludge have become a research hotspot (Zhang et al., 2009; Su et al., 2015; Liao et al., 2018). It has been reported that most ARGs in AS were carried by chromosomes (Zhang et al., 2021), which proved the possibility that bacterial pathogens in the sludge had antibiotic resistance. Bacterial pathogens may serve as ARG hosts, and the ARGs of multidrug and macrolide-lincosamide-streptogramin are most likely to co-occur with human pathogens in sludge digesters (Ju et al., 2016). The hosting relationship is considered the most direct origin of ARG species co-occurring events (Ju et al., 2016), which undoubtedly increases the risks of resistant pathogens to environments and humans. In aerobic granular sludge (AGS) cultivation, abundant AGS may lead to the proliferation of inactivated ARGs (Li et al., 2020a), and much effort has been made to determine the potential hosts of ARGs in sludge and the corresponding control strategy.

2.2 Viruses, pathogenic protozoa, and worms

Due to the limitation on virus cultivability and the high detection costs, only the indicators of a few viruses were regulated by most standards for virus control. However, the exposure and environmental risks of viruses should be given more attention, especially during the coronavirus disease 2019 (COVID-19) pandemic. *Somatic coliphages* (SOMCPH) can be used as a good indicator of viruses to illustrate their fate in the sludge treatment process (Mandilara et al., 2006); however, a single indicator can hardly provide full insight into the fate and risks of viruses.

The diversity of human viruses in sludge is expected to be much higher than in wastewater and other environmental samples. Unfortunately, the methods used to explore the diversity of human viruses in sludge are far from well developed. The application of traditional cell culture methods is primarily restricted by their time consumption (Monpoeho et al., 2004), low adaptation to various virus species, and difficulty in culturing viruses such as noroviruses and astroviruses. (Sano et al., 2003). Immunological detection methods have the disadvantages of a high detection limit and low sensitivity (Graff et al., 1993). Metagenomic sequencing, gene chip, and qPCR methods can also be applied to virus detection (Soueidan et al., 2015; Li et al., 2016b; Zou et al., 2017). However, unlike bacterial pathogens, viruses have two types of genetic material, DNA and RNA. Viral metagenomics, which is more suitable for detecting viruses, has been widely used for viruses in environmental samples such as sewage and soil (Yu et al., 2019b; Guajardo-Leiva et al., 2020). However, its application in sludge is rare because people have paid less attention to viruses in sludge in the past. Compared to qPCR, metagenomic sequencing provided less sufficient data to promisingly represent human viruses (Bibby and Peccia, 2013). In addition, the viral genome database contains only a small portion of all viruses, and the genomes of most viruses remain to be studied.

Sludge may contain multiple viruses, such as astroviruses, norwalk viruses, hepatitis viruses, rotaviruses and enteroviruses (Wong et al., 2010; Prado et al., 2014; Lizasoain et al., 2018). With the continuous development of detection technologies, an increasing number of new viruses have been discovered in sludge, and virus-related databases have been updated accordingly. The risk of bacteriophages in sludge as a resistance gene carrier was also assessed, and the densities of the two resistance genes of *blaTEM* and *sul1* in sludge phages were as high as 5.5 and 4.4 $\log_{10}$ gene copies (GC)/g (Calero-Cáceres et al., 2014). For the viruses in sludge, most studies have investigated nonenveloped enteroviruses, which are more difficult than enveloped viruses to inactivate in sewage sludge (Sano et al., 2003; Monpoeho et al., 2004; Jebri et al., 2014). Enteroviruses are also adopted as the virus indicator in the standard. While being used for land, the risk estimates, including those of adenoviruses and noroviruses, proved that the risk values of enteroviruses significantly underestimate the total infection risk of pathogenic microorganisms in sludge (Viau et al., 2011). By using a metagenomic sequencing method, 43 different types of human viruses, i.e., 26 DNA viruses and 17 RNA viruses, were detected in sewage sludge, and newly emerging viruses, such as coronavirus HKU1, the cosavirus, and the klassevirus, have high relative abundance, and the detection rate of coronavirus HKU1 exceeds 80% (Bibby and Peccia, 2013). The SARS-CoV-2 virus that causes pneumonia is an enveloped virus (Foladori et al., 2020), and there is still limited knowledge about the inactivation efficiency of enveloped viruses in the treatment processes of sludge. A recent study showed that different temperatures of anaerobic digestion play an important role in the inactivation of the SARS-CoV-2 virus (Bardi and Oliaee, 2021). However, the high pathogenicity and rare data on its fate and transportation advocate concern for its ecological and health risks. In addition, the current virus enrichment methods are mainly for nonenveloped viruses, such as enteroviruses, and further studies must be done on the enrichment methods of enveloped viruses in sludge (Yang et al., 2020). The occurrence and risks of enveloped viruses in sludge treatment and reclamation should be carefully studied in the future.

Pathogenic protozoa that may be present in sludge include *Giardia*, *Cryptosporidium*, *Entamoeba*, *Toxoplasma*, and so on, and their infection may cause different symptoms, such as intermittent diarrhea, abdominal pain, cramps, bloody stools, weight loss, and dehydration (Amorós et al., 2016; Benito et al., 2020). *Giardia* and *Cryptosporidium* are often used as indicator protozoans in sewage. For parasitic worms, the inactivation rate of worm eggs is usually used as a biological indicator to evaluate sludge treatment performance, and worm egg concentrations are also used to assess the health risks of composted sludge to be applied to agricultural soil (Navarro et al., 2009; Amoah et al., 2018).

3 Inactivation of pathogenic microorganisms by different sludge treatment processes

3.1 Composting and vermicomposting

Sludge composting is a typical exothermic aerobic process and is one of the most widely used methods for sludge treatment (Liao et al., 2018). The efficiency of reducing the pathogenic load of sludge by composting is compared in Table 2 (Wéry et al., 2008). In the thermophilic phase, the composting temperature is in the range of 55°C to 70°C, the majority of pathogenic microorganisms are inactivated, and sludge sanitation is achieved (Lung et al., 2001; Mehta et al., 2014). High temperature may cause enzyme denaturation, RNA inactivation, and protoplast membrane damage, and these effects lead to the death of microbial cells. Comparatively, hyperthermophilic composting may achieve a rapid rise in temperature to as high as 90°C within 24 h, and a significantly higher capability to inactivate pathogenic microorganisms is expected compared to conventional composting (Liao et al., 2018). It was reported that 91% of resistance genes and 88% of mobile genetic elements were removed after 21 days of hyperthermophilic composting, and the ratios of conventional composting were observed to be 39% and 51%, respectively (Liao et al., 2018). In addition, the interspecific competition of microorganisms was reported to play an important role in the inactivation of most
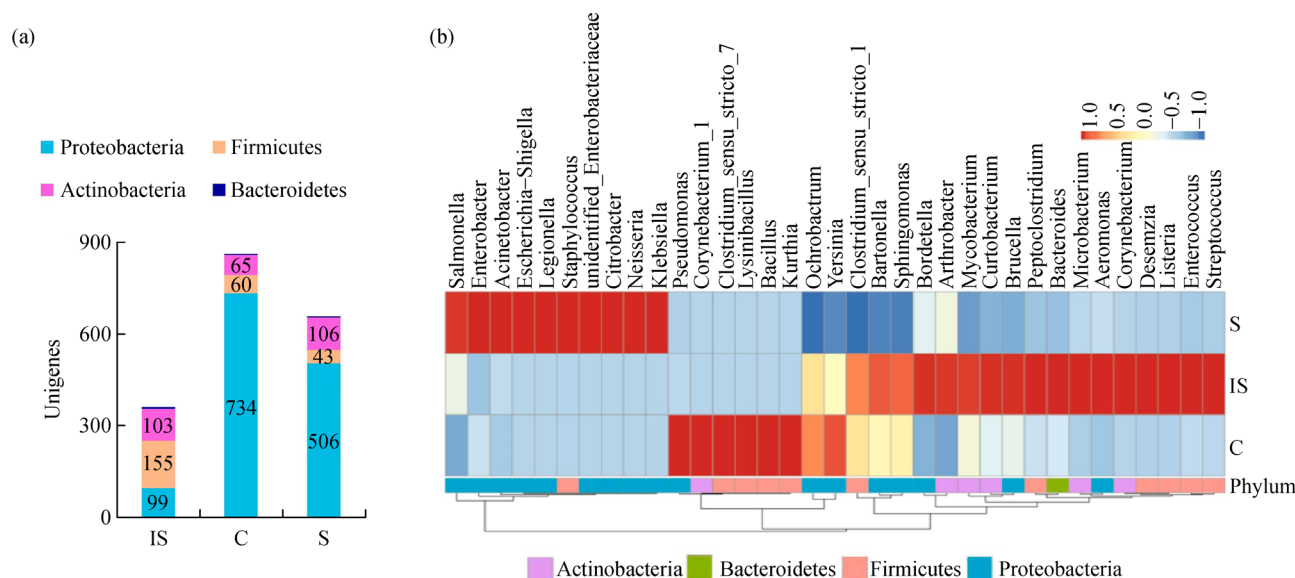
Fig. 2 Unigenes of human pathogenic bacteria at the phylum level (a) and their relative abundances (log10-transformed) at the genus level (b) in different samples obtained from initial sludge (IS), control (C), and sludge vermicompost (S). Reprinted with permission from Elsevier (Huang et al., 2020).

pathogenic microorganisms, and the rapid propagation of dominant bacteria inhibited the survival of pathogenic microorganisms accordingly (Millner et al., 1987; Pietronave et al., 2004). Dehydration can also lead to the rupture of the protein shell of viruses (Ward and Ashley, 1978). Further studies are required to illustrate the contribution of various factors, the optimized parameters, and the inactivation efficiency of different pathogenic microorganisms.

Conventional composting technology has disadvantages with regard to the removal of pathogenic microorganisms. For example, the presence of heat-resistant mutants greatly affects the inactivation of pathogens (Wichuk and Mccartney, 2007; Elving et al., 2010; Inglis et al., 2010), and this tends to increase the proportion of heat-resistant mutants in environments. Additionally, some specific bacteriophages or plant viruses, e.g., cucumber green mottle mosaic viruses, were reported to be highly resistant to conventional composting (Robledo-Mahón et al., 2019). At the end of thermophilic composting, the abundance of *Pseudomonas* spp. and *Streptomyces* spp. in sludge was detected to be near 1% of the entire community, which did induce the disease in crops such as potatoes (Robledo-Mahón et al., 2020).

In contrast to conventional composting, vermicomposting is a mesophilic process with a temperature below 35°C, and this range enables the growth of worms and pathogenic microorganisms (Khwairakpam and Bhargava, 2009; Swati and Hait, 2018). Vermicomposting achieves sludge stabilization by the synergistic effect between worms and microbial populations (Table 2), and it has been reported that the enzyme activity and endosymbiotic microorganisms of earthworms play an important role (Monroy et al.,

2009; Sen and Chandra, 2009; Swati and Hait, 2018). Furthermore, the humate in sludge and gut transport of worms are also involved in pathogen inactivation (Soobhany et al., 2017). Comparatively, mesophilic vermicomposting was better than vermicomposting at controlling heat-resistant mutants, and the contrary result has also been reported before (Huang et al., 2020). The worm presentation significantly changed the community structure of pathogenic microorganisms, and their abundance was rarely reduced (Fig. 2). Most studies suggest that vermicomposting is reliable for inactivating pathogenic microorganisms in sludge, and there are still conflicts to be well illustrated.

3.2 Anaerobic digestion and aerobic digestion

Anaerobic digestion is one of the most popular techniques used to achieve sludge stabilization, and the inactivation efficiency of pathogenic microorganisms by different anaerobic digestion methods has been reported in various studies (Table 2). In general, the reduction in pathogenic load by thermophilic anaerobic digestion (TAD) was over 3 log units, whereas that by mesophilic anaerobic digestion (MAD) was below 2 log units (Traub et al., 1986; Astals et al., 2012; Grübel and Suschka, 2015; Levantesi et al., 2015). The occurrence rates of human adenoviruses, enteroviruses, and human polyomaviruses in biosolids upon MAD were reported to be as high as 83%, 42%, and 58%, respectively (Wong et al., 2010). Conventional MAD can only meet the USEPA Class B biosolid standard with regard to pathogen control (USEPA, 1994; Lewis. and Gattie., 2002; Forster-Carneiro et al., 2010; Wong et al., 2010; Simmons and Xagoraraki, 2011) and can hardly

achieve the Class A standard for agricultural purposes because it cannot ensure that the treated sludge is virtually pathogen-free. TAD improves the inactivation of pathogenic microorganisms to a greater extent; however, after TAD, the detection of some pathogenic microorganisms has also been reported in many studies (Kearney et al., 1993; Astals et al., 2012; Levantesi et al., 2015). To achieve promising control of pathogenic microorganisms, the combination of conventional anaerobic digestion and other sanitation technologies, such as thermophilic pre-treatment and sludge disinfection, may be practical to meet the Class A biosolid standard (Cheunbarn and Pagilla, 2000).

The mechanisms of pathogenic microorganism inactivation involved in anaerobic digestion are far from well illustrated, and the efficiency is highly dependent on different factors, such as temperature and solid retention time (Zhao and Liu, 2019). TAD at temperatures higher than 55°C is recognized to be efficient in obtaining Class A biosolids in the United States, and high temperature plays a determining role in the inactivation of pathogenic microorganisms (Kabrick and Jewell, 1982; Liu et al., 2011; Benito et al., 2020; López et al., 2020). A higher temperature improved inactivation, and TAD showed better performance than MAD to remove pathogenic microorganisms and ARGs (Diehl and LaPara, 2010; Pandey and Soupir, 2011; Rizzo et al., 2013; Forbis-Stokes et al., 2016). However, different pathogenic microorganism species have different tolerances to temperature (Watcharasukarn et al., 2009). Some pathogenic microorganisms, e.g., *Bacillus cereus, L. monocytogenes*, and some spores and eggs, exhibit strong resistance to

temperature and can survive upon TAD treatment (Elmerdahl Olsen and Errebo Larsen, 1987; Kearney et al., 1993; Orzi et al., 2015; Zhao and Liu, 2019). Moreover, interspecific competition between pathogenic microorganisms and anaerobic bacteria also contributed to the lowered pathogenic load of sludge (Orzi et al., 2015). Additionally, the produced volatile fatty acids (VFAs) and free ammonia in anaerobic digestion also positively improved the inactivation of pathogenic microorganisms (Sahlström, 2003; Lloret et al., 2013; Fidjeland et al., 2015; Magri et al., 2015; Orzi et al., 2015). Free ammonia may penetrate the cell membrane and result in proton imbalance, potassium ion (K^+) deficiency, and intracellular pH variation and adversely affect the normal physiologic function of cells (Rajagopal et al., 2013; Yenigün and Demirel, 2013; Chen et al., 2014).

Autothermal thermophilic aerobic digestion is reported to completely remove total coliforms of *E. coli* and *Salmonella* spp., and unfortunately, the removal efficiency of *C. perfringens* spores is as low as 1.97 log units (Lloret et al., 2012). The elevated pH during aerobic digestion also showed inactivating effects toward pathogenic microorganisms (Kabrick and Jewell, 1982; Lloret et al., 2012). Additionally, two-phase anaerobic–aerobic digestion improved sludge digestion and methane production and positively favored sludge sanitation (Min Jang et al., 2019). Upon anaerobic digestion, thermophilic aerobic digestion further decreased the species of human bacterial pathogens from 44 to 16, and the relative abundance of human bacterial pathogens was reduced from 2.42% to 0.77% (Fig. 3) (Min Jang et al., 2019).
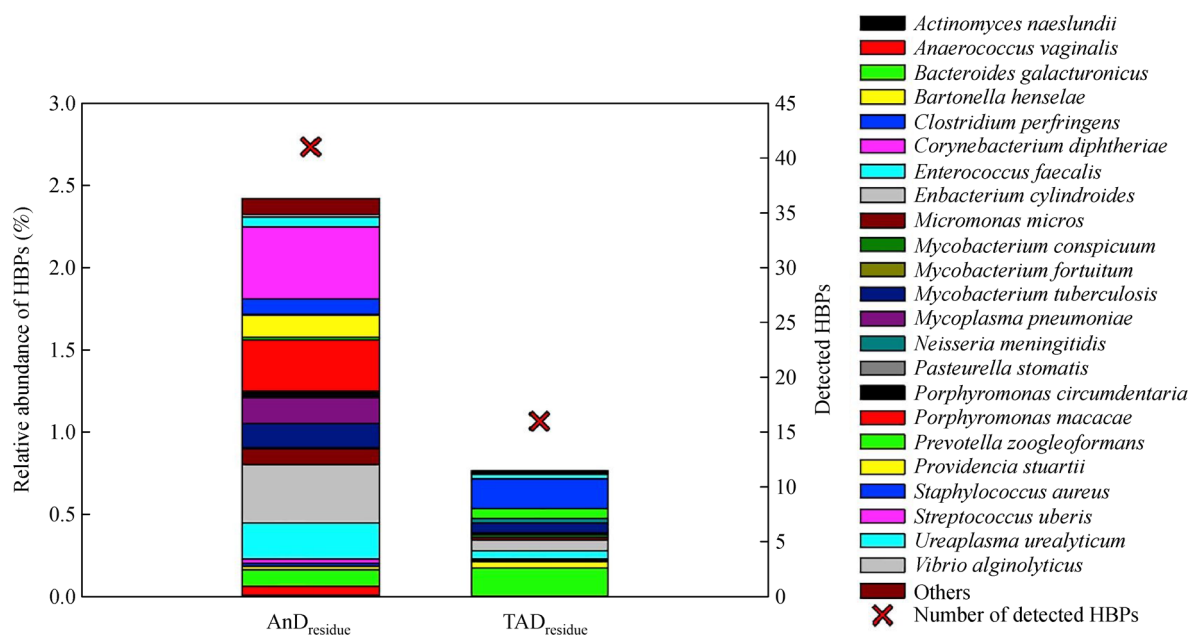

Fig. 3   Species and relative abundance of human bacterial pathogens (HBPs) found in sludge treated by anaerobic digestion (AnD$_{residue}$) and thermophilic aerobic digestion (TAD$_{residue}$). Reprinted with permission from Elsevier (Min Jang et al., 2019).

3.3　Lime stabilization

Convenient and cost-effective lime stabilization is widely used for sewage sludge sanitation and can significantly reduce the pathogenic load in sludge in USEPA guidelines (USEPA, 1994). Many studies have reported that after lime stabilization, pathogenic bacteria, indicator bacteria such as *E. coli* and *Salmonella*, and human viruses such as adenovirus and rotavirus can be reduced to below the detection limit (Table 2) (Gantzer et al., 2001; Plachá et al., 2008; Wong and Selvam, 2009; Yin et al., 2017; Martín-Díaz et al., 2020). However, the risks of bacterial spores and helminth eggs with strong resistance to lime stabilization should also be considered (Capizzi-Banas et al., 2004). Bean et al. reported an insignificant difference in the numbers of *Ascaris* lumbricoides ova before and after lime stabilization (Bean et al., 2007). Lime stabilization can hardly inactivate nematode eggs in a short time, and the storage of sludge for over 6 months at pH>11.5 may achieve this goal (Gantzer et al., 2001). For lime stabilization, other sanitation strategies should be introduced to strengthen pathogen inactivation.

In lime stabilization, the contributive effects on pathogen inactivation include high pH, high temperature, dehydration, and ammonia toxicity, and extremely high pH is regarded to be the dominant factor (Mignotte-Cadiergues et al., 2002; Capizzi-Banas et al., 2004). The addition of plentiful calcium oxide (CaO) and calcium hydroxide [$Ca(OH)_2$] remarkably increased the sludge pH to as high as 12 and the sludge temperature to above 65°C (Pecson et al., 2007; Valderrama et al., 2013). Under strong alkaline conditions, the hydrolysis of nitrogen-containing organic matter led to increased concentrations of free ammonia, with toxic effects on microbial cells (Pecson et al., 2007; Magri et al., 2015). Additionally, CaO strongly absorbed water in sludge and gradually stabilized the physical and chemical properties by rapid water loss and inhibited microorganism survival. However, to achieve sludge stabilization and sanitation, extremely high lime doses and pH are both required, which adversely increase the inorganic contents and is detrimental to sludge disposal for land use and incineration.

3.4　Heat drying

Heat drying can greatly reduce the volume of sludge and improve the performance of sludge (Font et al., 2011; Deng et al., 2013). As the USEPA mentioned, sludge heating to over 80°C by hot gas may reduce the water content to below 10%, which significantly reduces the pathogenic load and accordingly minimizes the negative effects (USEPA, 1994). However, the extent of pathogenic load reduction varies with the operating conditions (Gantzer et al., 2001; Mocé-Llivina et al., 2003; Monpoeho et al., 2004). Romdhana *et al.* compared four heat drying

processes on hepatitis A virus control in sewage sludge; the agitated conductive process (120°C), fry-drying process (vacuum, oil temperature: 95°C) and drum drying process (112°C–137°C) exhibited complete inactivation of hepatitis A virus within 20 min, 10 min, and 10 s, respectively, and solar drying showed a much lower efficiency (Romdhana et al., 2009). High temperature plays a determining role in the inactivation of pathogenic microorganisms (Romdhana et al., 2009; Naidoo et al., 2019; Espinosa et al., 2020; Gomes et al., 2020), and the efficiency is highly dependent on the heat transfer rate. Thin-layer drying with rapid heat transfer required much less time to achieve sludge drying and sanitation (Romdhana et al., 2009). The dehydration effect also greatly contributed to the inactivation of pathogenic microorganisms, and this was supported by the remarkable decrease in pathogenicity in air drying at natural temperature (Mondal et al., 2015; Kong et al., 2018).

3.5　Innovative sludge treatment processes

Recently, an increasing number of studies have proposed innovative processes such as microwave treatment, radiation, and chemical oxidation for sludge treatment and the control of pathogenic microorganisms.

3.5.1　Microwave irradiation

Microwave irradiation is often used as a pretreatment for anaerobic digestion because of the effective inactivation of pathogenic microorganisms (Hong et al., 2006; Coelho et al., 2011; Afolabi and Sohail, 2017; Mawioo et al., 2017; Gil et al., 2018). Microwaves showed the complete removal of *E. coli*, *coliforms*, *Staphylococcus aureus*, and *Enterococcus faecalis,* and the sludge volume was reduced by more than 60% due to the thermal effect of microwave radiation (Mawioo et al., 2017). Additionally, microwave pretreatment increased biogas production in anaerobic digestion by 35% (Uma Rani et al., 2013).

The inactivation mechanism toward pathogenic microorganisms includes thermal effects as the dominant factor and electromagnetic radiation. In microwave radiation, elevated temperatures over 60°C effectively inactivate pathogenic microorganisms, and the efficiency is positively correlated with higher microwave energy (Mawioo et al., 2017). Microwave radiation also destroys the cell membrane, causes the exclusion of intracellular species (Cosgun and Semerci, 2019), and causes DNA damage to pathogenic microorganisms (Hong et al., 2004).

3.5.2　High-energy electron beam radiation

The radiation of γ-rays and high-energy electron beams (*β*-rays) is produced by an electron accelerator, and it was reported to reduce the sludge volume and improve sludge

dissolution and organic pollutant degradation (Wang and Wang, 2007; Kim et al., 2011; Wu et al., 2017). Radiation is also effective in inactivating pathogens in sludge (Borrely et al., 1998; Chmielewski and Han, 2016). The pathogenic load may be promisingly reduced to a safe level at ray radiation doses above 2 kGy (AL-Ghonaiem et al., 2010). Additionally, different microbial species have different sensitivities toward electron beam radiation. γ-ray radiation at a 1 kGy dose contributed to the complete inactivation of *E. coli* in raw waste sludge, whereas the decrease ratio of the mold abundance was as low as 0.6 log units (Ranković et al., 2020).

The inactivation of microorganisms by radiation is attributed to direct irradiation and indirect effects. In the irradiation of sewage sludge, radiation tends to destroy the structure of biological macromolecules such as nucleic acids and proteins and to cause ionization and destruction of intercellular substances (Wang and Wang, 2007; Chmielewski and Han, 2016). On the other hand, high-energy rays induce chemical processes such as sensitizer reactions and the generation of free radicals such as $\cdot$OH, e_{aq}^-, and H$\cdot$ (Wang and Wang, 2007; Chmielewski and Han, 2016). The formed radiation products may further interact with nucleic acids, proteins, and enzymes and hinder the normal physiologic process of cells.

3.5.3 Electrochemical treatment

Many studies have investigated the coagulation and disinfection effect of electrochemical treatment toward sewage and sludge (Huang et al., 2008; Cui et al., 2013; Lei et al., 2020), and the sanitation efficiency to produce Class A biosolids has been investigated recently (Navab Daneshmand et al., 2012; Rumky et al., 2020). In electrocoagulation, the in situ-produced coagulants reduce sewage turbidity by destabilization and flocculation effects, and most pathogens are captured and enter the sludge with flocs thereafter (Buzzini et al., 2007). Reactive oxygen and chlorine species were also produced during electrochemical treatment (Bakheet et al., 2020), which may destroy the microbial cell structure and inactivate the microorganisms in sludge (Fig. 4) (Zeng et al., 2019). At a voltage of 15 V, the extent of the *E. coli* decrease was more than 3 log units, and the extents of *Salmonella* spp. and *Streptococcus faecalis* decrease were nearly 5 log units. At an anode current density of 4.71 A/dm^2, the removal rate of *E. coli* and fecal coliform in municipal sewage sludge was reported to be as high as 5 log units (Drogui et al., 2013). In addition, the combined use of a low-energy input electrochemical system and alkaline digestion may promisingly remove fecal coliform and *E. coli* in sewage
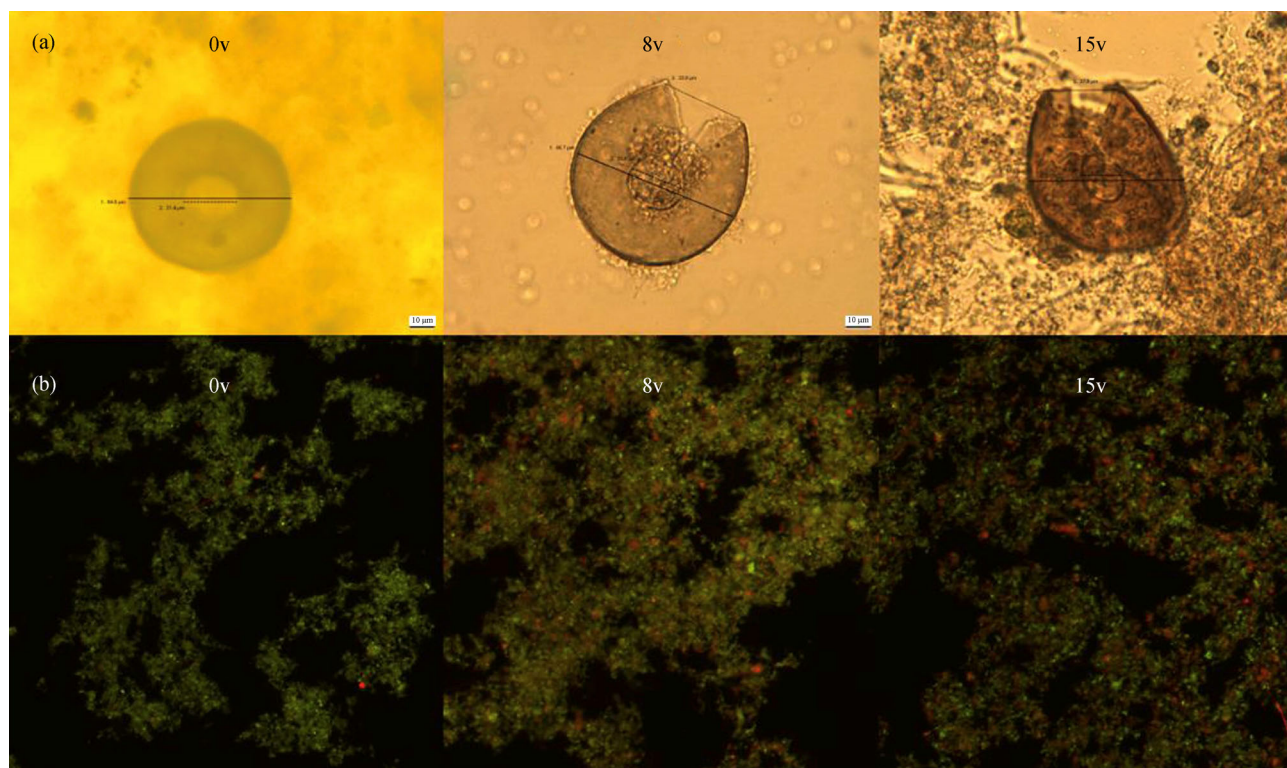

Fig. 4   (a) Images of microorganisms in sludge before and after electrochemical treatment at 8 V and 15 V and (b) distribution of the live and dead bacterial consortia observed with a confocal laser scanning microscope (CLSM). Reprinted with permission from Elsevier (Zeng et al., 2019).

sludge and achieve the Biosolid Class A standard (Jafari and Botte, 2021).

To date, the inactivation mechanism of pathogenic microorganisms involved in the electrochemical process is far from well understood, and three positive effects were proposed (Navab Daneshmand et al., 2012; Yin et al., 2018; Zeng et al., 2019): 1) the generation of ohmic heat in electrochemical reactions; 2) the formation of different oxidants, such as free chlorine and reactive oxygen species; and 3) the extremely high or low pH at the interfaces of electrode plates. The quantitative contribution of these factors to sludge sanitization is not clear, and most studies suggest that ohmic heat is the primary contribution (Navab Daneshmand et al., 2012; Yin et al., 2018; Zeng et al., 2019). Future studies may focus on the other factors, and the molecular biological mechanisms involved in pathogen inactivation and the effects of electrochemical treatment on the post biological units for pathogen control.

3.5.4 Chemical oxidation

Strong oxidants with high redox potential tend to destroy the microorganism structure and degrade biological macromolecules such as enzymes and genetic materials, thus inactivating pathogenic microorganisms accordingly. Common oxidants include peracetic acid and chlorine-containing disinfectants, e.g., HOCl and $Ca(ClO)_2$ (Yu et al., 2010; Yu et al., 2019d; Hu et al., 2021; Luukkonen et al., 2020). At peracetic acid doses over 480 mg/L, the complete inactivation of *E. coli* and *Salmonella spp.* in sludge was observed (Luukkonen et al., 2020). NaClO at a dose of 2.2 g/L decreased the most probable number of fecal coliform in sludge from 6.9 log units to 0.8 log units, and positive effects on improving dewatering and heavy metal leaching were also observed (Zhang et al., 2020). Higher doses of chemical oxidants are required to achieve the complete inactivation of pathogens in sludge than in sewage (Hu et al., 2021), owing to the protection of microbial cells and extracellular polymeric substances.

Advanced oxidation processes (AOPs) generate various free radicals, and recently, AOPs have been proposed to treat sewage and sludge. Fenton and Fenton-like processes have been indicated to effectively remove pathogenic microorganisms in sewage (Tong et al., 2020; Venieri et al., 2020; Wang et al., 2020). The persulfate at 0.5 mM activated by solar energy may contribute to a 6.0 log unit reduction in bacteria in sewage after 2 h of reaction (Ferreira et al., 2020). The combined use of ozone and zerovalent iron (ZVI) at doses of 30 and 63 mg/g TS may contribute to the reduction in the total coliforms and *E. coli* in sludge by 98.6% and 97.7%, respectively, and •OH was reported to play an important role (Yu et al., 2019c). Wang *et al.* compared five pretreatment approaches for pathogen inactivation in sewage sludge, and electric-Fenton reactions exhibited the best performance and contributed to the removal of coliform and *E. coli* by 4.84 log units and 3.86

log units after 60 min (Wang et al., 2021). Furthermore, H_2O_2 at a low dose can hardly improve the removal of pathogenic microorganisms in sludge due to its decomposition into water and oxygen instead of free radicals with its exposure to the organic matter in sludge (Yu et al., 2010). In addition to pathogen inactivation, AOPs were reported to improve sludge conditioning and dewatering performance (Maqbool et al., 2019; Yu et al., 2019d; Ge et al., 2020). The effects of AOPs on the degradation of micropollutants, the inactivation of ARGs, and sludge ecotoxicity in post land application should be further confirmed.

3.6 Comparison and summary of sludge treatment processes

The efficiency of reducing the pathogenic load of sludge by different sludge treatment processes is compared in Table 2. Lime stabilization is superior to conventional composting and mesophilic anaerobic digestion in inactivating pathogens, and several innovative sludge treatment processes can also reduce the pathogenic load in sludge to the sanitary level (Drogui et al., 2013; Mawioo et al., 2017; Luukkonen et al., 2020; Ranković et al., 2020). The reduction in the pathogenic load of sludge by conventional composting and anaerobic digestion obviously depends on the operating temperature (Liao et al., 2018; López et al., 2020), and the potential of thermophilic anaerobic digestion in the suppression and elimination of pathogens is significantly greater than that of mesophilic anaerobic digestion (Astals et al., 2012; Grübel and Suschka, 2015; Levantesi et al., 2015). However, the vast majority of sludge treatment processes are ineffective in controlling heat-resistant mutants, and the spores or ova of pathogens can also survive due to resistance to high temperatures and extreme pH. Comparatively, vermicomposting is a more promising method to control their spread through sludge, but interaction mechanisms between worms, their endo-symbiotic microorganisms and pathogens should be further studied from the perspective of microbial ecology. Furthermore, to more thoroughly inactivate pathogens in sludge and control the spread of heat-resistant mutants, a combination of two or more sludge treatment processes is recommended.

The mechanisms of pathogen inactivation by different sludge treatment processes are summarized in Table 3. High temperature is the dominant factor for the inactivation of pathogens in most sludge treatment processes, and dehydration also plays an important role in the inactivation of pathogenic microorganisms. In innovative sludge treatment processes, some special pathogenic inactivation mechanisms, such as direct irradiation by a high-energy electron beam, have been reported (Wang and Wang, 2007; Chmielewski and Han, 2016). Different mechanisms may cause changes in the properties of sludge and affect the land application of sludge. Sludge after lime stabilization

Table 2 Pathogenic load control by different sludge treatment processes

Treatment	Duration of the process; maximum temperature	Pathogenic microorganism or microbial indicator	Change in the pathogenic load	Reference
Composting	NA [a]; NA	Enteric viruses	ND [b]	Watanabe et al., 2002
	4 weeks of the active phase, matured for 2 months and stored for 2−3 months; 60°C−70 °C	E. coli	ND	Wéry et al., 2008
		C. perfringens	ND	Wéry et al., 2008
		Salmonella spp.	ND	Watanabe et al., 2002; Wéry et al., 2008
		Enterococcus spp.	↓(2.9 $\log_{10}$ gene copies/g) [c]	Wéry et al., 2008
Vermicomposting	4 weeks; NA	Fecal coliforms	↓(2.98 $\log_{10}$ MPN/g)	Hait and Tare, 2011
		Enterococcus	↓(2.21 $\log_{10}$ MPN/g)	Hait and Tare, 2011
		Salmonella	↓(1.82 $\log_{10}$ MPN/g)	Hait and Tare, 2011
		Helminths ova	ND	Hait and Tare, 2011
	40 days; NA	Ochrobactrum anthropi	ND	Lv et al., 2018
		Brevundimonas diminuta	ND	Lv et al., 2018
		Eubacterium tenue	ND	Lv et al., 2018
		Bacillus thuringiensis	ND	Lv et al., 2018
Mesophilic anaerobic digestion	NA; 34.5 °C	Bacteriophage f2	↓(0.04 $\log_{10}$ PFU/Lper h)	Traub et al., 1986
	14 days; 36 °C	Enterovirus	↓(1 $\log_{10}$ gene copies/g)	Monpoeho et al., 2004
	20 days; 37 °C	Somatic coliphages	↓(1 $\log_{10}$)	Astals et al., 2012
		F-specific RNA-bacteriophages	↓(2.7 $\log_{10}$)	Astals et al., 2012
		E. coli	↓(2.2 $\log_{10}$)	Astals et al., 2012
Thermophilic anaerobic digestion	15 days; 55 °C	Somatic coliphages	↓(4.2 $\log_{10}$)	Astals et al., 2012
		E. coli	↓(2.3 $\log_{10}$)	Astals et al., 2012
		F-specific RNA-bacteriophages	↓(3.4 $\log_{10}$)	Astals et al., 2012
Aerobic digestion	30 days; Winter: 25 °C; Other season: 48 °C	E. coli	↓(3.5±0.9 $\log_{10}$ MPN/g)	Gantzer et al., 2001
		Enterococci	↓(2.1±0.5 $\log_{10}$ MPN/g)	Gantzer et al., 2001
		Spores of sulfite-reducing anaerobic bacteria	↓(1.3±0.5 $\log_{10}$ MPN/g)	Gantzer et al., 2001
	14.6 days; 62 °C	Total coliforms	ND	Lloret et al., 2012
		Salmonella spp.	ND	Liu et al., 2011; Lloret et al., 2012
		C. perfringens spores	↓(1.97 $\log_{10}$ spores/mL)	Lloret et al., 2012
Lime stabilization	24 hours; NA	Bacteriophage MS2	ND	Hansen et al., 2007
		Adenovirus type 5	ND	Bean et al., 2007; Hansen et al., 2007
		Rotavirus	ND	Bean et al., 2007; Hansen et al., 2007
	NA; NA	Enteroviruses	ND	Monpoeho et al., 2004
	24 hours; NA	E. coli	↓(>6 $\log_{10}$ MPN/mL)	Bean et al., 2007; Santos et al., 2020
		Salmonella	ND	Bean et al., 2007
		Ascaris lumbricoides ova	No significant difference	Bean et al., 2007
Heat drying	Indirect drying; 10 hours; 108 °C	E. coli	↓(3.7±0.3 $\log_{10}$ MPN/g)	Gantzer et al., 2001
		Enterococci	↓(3.9±0.6 $\log_{10}$ MPN/g)	Gantzer et al., 2001
		Spores of sulfite-reducing anaerobic bacteria	↓(3.2±0.1 $\log_{10}$ MPN/g)	Gantzer et al., 2001

Treatment	Duration of the process; maximum temperature	Pathogenic microorganism or microbial indicator	Change in the pathogenic load	Reference
Microwave technology	MW energy: 3.4 kWh	E. coli	ND	Mawioo et al., 2017
		Coliforms	ND	Mawioo et al., 2017
		S. aureus	ND	Mawioo et al., 2017
		E. faecalis	ND	Mawioo et al., 2017
Electrochemical pretreatment	Applied voltage: 15 V; Time: 1 hour	E. coli	↓ (3.12 ± 0.2 log$_{10}$ CFU/g TS)	Zeng et al., 2019
		Salmonella spp.	↓(>4 log$_{10}$ CFU/g TS)	Zeng et al., 2019
		S. faecalis	↓(>4 log$_{10}$ CFU/g TS)	Zeng et al., 2019
Gamma radiation	Dose of gamma irradiation: 2 kGy	Fecal coliforms	ND	AL-Ghonaiem et al., 2010
		Salmonella spp.	ND	AL-Ghonaiem et al., 2010
Peracetic acid (PAA) oxidation	480 mg of 100% PAA per L of sludge	E. coli	ND	Luukkonen et al., 2020
		Salmonella spp.	ND	Luukkonen et al., 2020

Note: a) Not available; b) Not detected; c) Signifies the decrease in pathogenic load with the log value of the decrease in brackets.

Table 3 Mechanisms of pathogen inactivation by different sludge treatment processes

Treatment	Mechanisms	Reference
Composting	High temperature in the thermophilic phase	Mehta et al., 2014; Liao et al., 2018
	Interspecific competition of microorganisms	Pietronave et al., 2004
	Dehydration	Ward and Ashley, 1978
Vermicomposting	Enzyme activity and endosymbiotic microorganisms of earthworms	Monroy et al., 2009; Swati and Hait, 2018
	Humate in sludge and gut transport of worms	Soobhany et al., 2017
Anaerobic digestion and aerobic digestion	High temperature	López et al., 2020
	Elevated pH	Kabrick and Jewell, 1982; Lloret et al., 2012
	Interspecific competition between pathogens and anaerobic bacteria	Orzi et al., 2015
	Produced VFAs and free ammonia	Sahlström, 2003; Lloret et al., 2013; Fidjeland et al., 2015; Magri et al., 2015
Lime stabilization	Extremely high pH	Pecson et al., 2007; Valderrama et al., 2013
	High temperature	Pecson et al., 2007; Valderrama et al., 2013
	Dehydration	Capizzi-Banas et al., 2004
	Ammonia toxicity	Capizzi-Banas et al., 2004
Heat drying	High temperature	Naidoo et al., 2019; Gomes et al., 2020
	Dehydration	Mondal et al., 2015; Kong et al., 2018
Microwave technology	Thermal effect	Mawioo et al., 2017
	Cell membrane destruction and the exclusion of intracellular species	Cosgun and Semerci, 2019
	DNA damage	Hong et al., 2004
High-energy electron beam radiation	Direct irradiation (inactivation of biomacromolecules and the ionization and destruction of the intercellular substance)	Wang and Wang, 2007; Chmielewski and Han, 2016
	Indirect effects (sensitizer reaction and the generation of free radical)	Wang and Wang, 2007; Chmielewski and Han, 2016

Treatment	Mechanisms	Reference
Electrochemical pretreatment	Generation of ohmic heat in electrochemical reactions	Navab Daneshmand et al., 2012; Yin et al., 2018; Zeng et al., 2019
	Formation of different oxidants such as free chlorine and reactive oxygen species	Navab Daneshmand et al., 2012; Yin et al., 2018; Zeng et al., 2019
	Extremely high or low pH at the interfaces of electrode plates	Navab Daneshmand et al., 2012; Yin et al., 2018; Zeng et al., 2019
Chemical oxidation	Destruction of the microorganism structure and degradation of biomacromolecules	Hu et al., 2020; Luukkonen et al., 2020

has an extremely high pH (Mosquera-Losada et al., 2012). Oxidants can change the oxidation-reduction potential of sludge. Microwave irradiation or high-energy electron beam radiation may induce mutated genes (Sado et al., 2001; Sutherland et al., 2010). These are not conducive to the subsequent land application of sludge. Moreover, due to the input of energy or the addition of a large amount of oxidant, innovative sludge treatment processes may be expensive with regard to the cost of implementation and operation, and the cost estimation of innovative sludge treatment processes, especially microwave irradiation and high-energy electron beam radiation (Zimek, 2020), should be further studied. Comparatively, composting, anaerobic digestion and lime stabilization are more cost-effective, biofuels such as CH_4 can be produced by anaerobic digestion, and mature and sanitary compost can be an effective soil conditioner.

4   Pathogen risk in sludge treatment and land application

4.1   Pathogen risk during sludge treatment

Sludge treatment involves concentration, conditioning, dehydration, transportation and other links, and includes aerobic and anaerobic digestion, composting, heat treatment, and other processes (Kelessidis and Stasinakis, 2012; Yang et al., 2015; Zhang et al., 2016; Yu et al., 2017; Wang et al., 2019; Yu et al., 2019a). The exposure of pathogenic microorganisms via contact and air inhalation in these processes should be evaluated and minimized as much as possible for the operators (Viau and Peccia, 2009; Han et al., 2021). In the process of sludge treatment, the exposure and health risk via bioaerosols should be well considered. The colony-counting method was proposed to detect bioaerosols, and the aerosol levels in the sludge thickening house were observed to be the highest at a fungal concentration of 8775±406 CFU/m³, which was remarkably higher than the acceptable guideline of 500 CFU/m³ (Xu et al., 2020). In the areas near the sludge thickening basin, the contents of culturable bacterial

aerosols and fungal aerosols were also the highest at 1697 CFU/m³ and 930 CFU/m³, respectively (Li et al., 2016a). Aerosols containing *S. aureus* may be used as an indicator of health risk. In residual sludge storage yards, the particle size of *S. aureus* bioaerosols was observed to be in the range of 3.3 to 4.7 mm, and the contents of respirable bioaerosols < 4.7 mm in size were also higher than those in aeration tanks (Yan et al., 2021). *Arcobacter* was observed to be the dominant taxon in aerosols, and the pathogens in indoor bioaerosols mainly came from sewage and sludge (Yang et al., 2019). Although a study showed that ARGs in liquid sludge from aeration tanks could diffuse through aerosols (Gaviria-Figueroa et al., 2019), there is still a lack of evidence suggesting that ARGs can diffuse through bioaerosols during sludge treatment.

In addition, there is also the risk of pathogens spreading in the process of sludge stabilization. The higher risk of bioaerosol exposure in sludge treatment units may be due to external forces such as dehydration applied to sludge (Xu et al., 2020). An aerosolization experiment using sludge of anaerobic digesters showed that some opportunistic pathogens were more likely to be aerosolized (Moletta-Denat et al., 2010), so attention should be given to the risk of pathogenic microorganisms that may exist in the biogas produced by the anaerobic digestion of sludge. It has been proven that long-term occupational exposure to bioaerosols in composting sites can adversely affect health (Schlosser et al., 2009). A recent study on bioaerosols found that *Fusarium graminerum* and *Stenotrophomonas rhizophila* had high bioaerosolization indexes during sludge biostabilization, which might cause risks to human health (Lu et al., 2021). In addition, inhalable dust could also be used as an indicator of culturable bacterial concentrations in the air during sludge composting (Schlosser et al., 2018). Although many studies have focused on the exposure risk of bioaerosols in sludge treatment units, there is still a lack of comprehensive data to assess the transmission and exposure pathways and health risks of pathogenic microorganisms. Additionally, the characterization and assessment of health risk by nonindicative pathogenic microorganisms should also be considered.

4.2 Pathogen risk associated with the land application of sludge

The ecological and health risks involved in the post application of sludge should be carefully evaluated. Currently, sludge landfills are prohibited in an increasing number of cities, and many WWTPs are planning to upgrade sludge treatment facilities to harvest the resources and energy in sludge. Land application has been widely implemented for global sludge reclamation and disposal. Sludge has been widely used as fertilizer on land after composting (Major et al., 2020). The process of concentration, anaerobic digestion, dehydration, and land application is considered to be one of the priority technical routes in China (Yang et al., 2015). The risks of different pathogen infections toward plants, animals and humans involved in land application have received great concern. While introducing undisinfected sludge on land, the desorption and leaching of adsorbed viruses may occur and potentially pollute water sources (Chetochine et al., 2006). Additionally, the risks of pathogenic aerosol inhalation occur while putting sludge into containers, spraying sludge, and mixing the soil (Brooks et al., 2005; Paez-Rubio et al., 2007). Brooks *et al.* used coliphage MS-2 as an indicator virus and reported the low risks of one-time and annual infection by inhaling sludge aerosols at a downwind distance above 30.5 m (Brooks et al., 2005). Unfortunately, there is still much uncertainty due to insufficient information on pathogen species and concentrations, exposure pathways, and corresponding dose–response effects. In terms of the risk of different exposure routes, direct ingestion is generally considered greater than aerosol transmission, indirectly contaminating groundwater and plants (Brooks et al., 2012). However, the possibility of people directly ingesting contaminated soil is low, so bioaerosols are considered the most concerning exposure risk related to the land application of sludge (Tanner et al., 2008; Viau et al., 2011).

In addition, the ingestion of agricultural crops using sludge as fertilizer sources and soil conditioners may still have pathogen infection risks. The risk of infection by worm eggs is relatively high while eating lettuce grown on sludge-improved soil. It may take as long as 30 to 40 days to reduce this risk to the WHO tolerable risk value of 10^{-4} (Amoah et al., 2018). The application of sewage sludge on land increased the exposure risk of root crops to *Cryptosporidium* oocysts and *Salmonellas* by counts of 0.033 and 0.070 kg^{-1}, respectively (Gale, 2003). However, the infection risk encountered by eating vegetables on soil treated with sludge application was reported to be low based on the intake of 283.7 g of vegetables per person per day (Gale, 2005). Biosolids upon anaerobic digestion and aerobic digestion were used for farmland, and ARGs were observed by qPCR and high-throughput sequencing analysis to rarely transfer to vegetables during harvest (Lau et al., 2017). Comparatively, the use of raw and anaerobically digested sludge may greatly increase the ARG abundance in harvested vegetables compared to land without sludge application, and this phenomenon disappears after one year of application (Rahube et al., 2014). Therefore, the spreading risk of pathogens and ARGs from sludge to crops and vegetables may be affected by many factors, such as the sludge treatment processes, the amount of applied sludge, and the time intervals between sludge application and harvesting.

As mentioned in the USEPA 503 regulation, Class A biosolids must be virtually pathogen-free, whereas Class B biosolids may contain pathogens (Iranpour and Cox, 2007). When Class B biosolids are applied to land, the risk to public health and the environment may be minimized if the guidelines to reduce exposure are followed, e.g., restricting site entry and reducing vector attractants (USEPA, 1994). However, the standards for Class A biosolids only include the detection of fecal coliform and *Salmonella*, so Class A biosolids may have other risks in considering the various pathogens and the large number of unknown microorganism species in sludge. It was reported that by measuring the concentrations of fecal coliform indicators, pathogen-free Class A biosolids can hardly be confirmed, and fecal coliform inactivation is insufficient to assure safe pathogen inactivation (Viau et al., 2011). In addition, the regrowth of pathogenic microorganisms in treated biosolids tended to occur in the case of improper storage (Sidhu et al., 2001; Zaleski et al., 2005). After being treated by thermophilic anaerobic digestion, the density of fecal coliforms in biosolids may meet the Class A biosolid requirement. However, the density may unfortunately increase to as high as 10^7 MPN/g dry weight after the application of biosolids to farmland, which indicates the regrowth of fecal coliforms during transportation (Iranpour and Cox, 2006). Upon the application of biosolids to land, the regrowth of *Salmonella* was observed to be on the time scale of 10 to 39 weeks, whereas that of *E. coli* was between 19 and 25 weeks (Eamens et al., 2006). However, the regrowth potential of different pathogen species has not been carefully evaluated, and the quantitative standard to rediscover these pathogens and assess their risks may be of crucial importance.

4.3 Potential application of quantitative microbial risk assessment in sludge

To achieve a reliable evaluation of risks, a better understanding of the migration, transmission, exposure and regrowth of pathogens in sludge treatment and biosolid land application is valuable. Quantitative microbial risk assessment (QMRA) has been widely employed to assess health risks in different environments and the safety of food (Lammerding, 2006; Elliott et al., 2019; Chen et al., 2021), and the development of standard methods such as QMRA is expected to improve the reliability of risk

assessments on sludge land applications. QMRA by mathematical methods may quantitatively evaluate the probability of infection, disease, and death caused by pathogenic microorganisms and integrates information on the occurrence, infection, exposure assessment, and dose–response model of pathogens (Eisenberg et al., 2008; Hamilton and Haas, 2016). QMRA has been applied to assess the risk of SARS-CoV-2 in WWTPs in South Brazil; the viable virus concentrations were determined to be in the range from 0.04 to 5.23 PFU·mL^{-1} at the WWTP entrance, and the risk in aggressive and extreme situations was higher than the tolerable value (Zaneti et al., 2021). QMRA has also been applied to assess the aerosol exposure risk in different units of WWTPs, including sludge treatment units; the hazard index of exposure was higher than 1, and *L. pneumophila* exhibited a higher risk of infection and disease in men than in women (Xu et al., 2020). This may be ascribed to the higher exposure dose considering that men have a higher average respiratory volume (Yan et al., 2021). Regarding the exposure risk of *S. aureus* aerosols, staff at sludge storage yards have higher risks than field engineers (Yan et al., 2021). QMRA may also be applied to assess the exposure risk involved in the land application of sludge. After applying Class B biosolids to land, enteroviruses and *Campylobacter jejuni* caused the greatest risk in a short time among these pathogenic microorganisms, and the direct consumption of soil was the greatest one-time risk, with a value above 10^{-1} (Brooks et al., 2012). In addition, the exposure risk of viruses was mainly derived from biosolids, and the land application of biosolids showed a higher risk than manure due to the high infectiousness of viruses (Tanner et al., 2008; Brooks et al., 2012).

However, most risk studies of sludge treatment and disposal processes only focused on one or a few pathogenic microorganisms, and the representativeness of the pathogenic microorganisms used requires further verification (Yang et al., 2019; Xu et al., 2020; Yan et al., 2021). Selecting more pathogenic microorganisms with a wide distribution, high abundance and high toxicity is recommended for risk assessment after a more comprehensive test of sludge and environmental samples is performed. Because the values of the parameters in the model vary with pathogenic microorganisms (Armstrong and Haas, 2007; Van Abel et al., 2017; Hamilton et al., 2019), the dose–response model is very important for newly discovered pathogenic microorganisms. For high-risk pathogens without regulations and standards in sludge, the corresponding dose–response model needs more research to increase the reliability of risk assessment. When analyzing exposure pathways, all the possibilities of the three media, solid, liquid and gas, as well as the influence of the regeneration of pathogenic microorganisms at different time nodes, should be fully considered (Eisenberg et al., 2008). To control the exposure of pathogens in sludge, a hazard analysis and critical control point (HACCP) system can be adopted to control exposure risks at key points (Tsitsifli and Tsoukalas, 2021). More representative pathogens, more comprehensive exposure pathways and more accurate dose–response models can make risk assessments during sludge treatment and disposal more reliable.

5   Conclusions and perspectives

The risk control of pathogenic microorganisms in sludge is crucially important to achieve resource and energy recovery, minimize adverse environmental effects, and ensure public health safety. This review clarifies the diversity and detection methods of pathogenic micro-organisms in sludge, including pathogenic bacteria, viruses, and protozoans. The control performance and inactivation mechanism of various sludge treatment processes on pathogenic microorganisms are analyzed and compared with emphasis. And the health risks involved in sludge treatment and land application are discussed. These results may be valuable for pathogen risk control with regard to sludge management.

Unfortunately, there is still a lack of sufficient research on the detection methods, occurrence, survival, transfer, and infection of unconventional pathogens in sludge, such as some nonenveloped viruses, which restricts us from proposing an effective strategy to avoid infection in this pandemic period. The control performance of different sludge treatment processes on pathogens can provide us with some references. Although most sludge treatment processes can inactivate indicator pathogens through different mechanisms, such as high temperature, extreme pH, and competition of microorganisms, combined treatment processes are still suggested as a strategy to enhance control performance. Finally, it is important to update the guidelines and standards to regulate the treatment and disposal of sludge based on fundamental research, field investigation and theoretical modeling. For a more comprehensive and reliable quantitative assessment model, it is important to combine diverse species and concentrations of pathogens, exposure pathways, and dose–response data in the future.

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