Applied Water Science (2018) 8:74
https://doi.org/10.1007/s13201-018-0698-6

REVIEW ARTICLE

Removal of pathogenic bacteria from sewage-treated effluent and biosolids for agricultural purposes

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Received: 10 July 2014 / Accepted: 4 April 2018 / Published online: 4 May 2018

Abstract

The reuse of treated sewage for irrigation is considered as an important alternative water source in the new water management strategy of the countries that face a severe deficiency of water resources such as the Middle East countries. The organic material and fertilizing elements contained in biosolids are essential for maintaining soil fertility. However, both treated sewage and biosolids contain a large diversity of pathogens that would be transmitted to the environment and infect human directly or indirectly. Therefore, those pathogens should be reduced from the treated sewage and biosolids before the reuse in the agriculture. This paper reviews the considerations for reuse of treated sewage and biosolids in agriculture and further treatments used for reduction of pathogenic bacteria. The treatment methods used for the reduction of pathogens in these wastes have reviewed. It appeared that the main concern associated with the reduction of pathogenic bacteria lies in their ability to regrow in the treated sewage and biosolids. Therefore, the effective treatment method is that it has the potential to destruct pathogens cells and remove the nutrients to prevent the regrowth or recontamination from the surrounded environment. The removal of nutrients might be applicable in the sewage but not in the biosolids due to high nutrient contents. However, the reduction of health risk in the biosolids might be carried out by regulating the biosolid utilization and selecting the plant species grown in the fertilized soil with biosolids.

Keywords Treated sewage · Biosolids · Pathogenic bacteria · Reuse · Treatment technology · Pathogen growth potential

Introduction

Sewage effluent is defined as treated or untreated wastewater generated from a treatment plant (US EPA 2009). The treated sewage is classified based on its origin in domestic sewage, hospital sewage and industrial wastewaters. Domestic sewage is a complex mixture containing water together with organic and inorganic constituents and large numbers of pathogenic bacteria as well as viruses and parasites (US EPA 2003). Hospital sewage is that coming from the hospitals and medical centres and includes sewage and wastewater resulting from the cleaning of laboratories and other facilities. Antibiotics, disinfectants and antibiotic-resistant bacteria are the major constituents in these wastes (due to their major use in hospital practice) (Pauwels and Verstraete 2006; Jury et al. 2010).

Industrial wastewaters are unwanted wastewater from the industrial operation such as chemical, electrochemical, electronic, petro-chemical and food-processing industries (US EPA 2009). These wastewaters are associated with high concentrations of dissolved metal salts (heavy metals) and

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may include some domestic sewage, but the domestic sewage is not the main component (Rao et al. 2012; Yachigo and Sato 2013).

The sewage sludge is the solid, semisolid or liquid residue generated during the sewage treatment processes (US EPA 1993). The term sewage sludge has been replaced recently by the term biosolids. Biosolids represent sewage sludge that has been treated by advanced processes which included aerobic and anaerobic, heat or lime treatment and has met standards required for beneficial use. The particular characteristics of the biosolids vary depending on their origin (human, vegetable or animal) and the treatment process they have gone through (physical, chemical or biological, anaerobic or aerobic treatment, alkaline treatment by lime, etc.). The organic and inorganic contents of biosolids are essential for soil and plants (N'Dayegamiye et al. 2002 and Nowak 2007).

Solids recovered from industrial processes are also called sludge and the term is often associated with potentially hazardous industrial wastes. Industrial sludge may have little or no agronomic value. Hence, it is important to distinguish those solids produced from sewage that have value as a fertilizer or soil amendment (John 2005).

Treated sewage and biosolids contain high concentrations of nutrients, which improve plant growth and soil properties. However, it has pathogenic microorganisms such as bacteria, protozoa, viruses and parasites that can cause several diseases. Land application of treated sewage and biosolids creates a potential for human exposure to these organisms through direct and indirect contact. Therefore, to protect public health from these organisms, many countries have regulated the use and disposal of treated sewage and biosolids (Al-Gheethi et al. 2015).

In the field of biosolids, there are some technologies that have been developed which aimed to produce safe biosolids that had to be used as fertilizer in agriculture. The developments in this area are less than that in the field of sewage effluent treatment. However, some of the advanced technologies including temperature-phased anaerobic digestion and autothermal aerobic digestion processes have emerged (Wen et al. 2009; Jin et al. 2013). Further, the membrane filtration, ionised irradiation and oxidation processes by chemical disinfectants exhibited high treatment efficacy in the improvement of the quality of biosolids (Dutta et al. 1999; Aksu and Tunc 2005; Gomez et al. 2006; Dungeni et al. 2010; Tripathi et al. 2011).

In the present review, the main pathogenic bacteria that exist in treated sewage and biosolids are discussed. The treatment processes most common in the reduction of pathogenic bacteria in treated sewage and biosolids are revealed. Treatment processes that would produce treated sewage and biosolids of higher quality for reuse in agriculture, as well as their applicability in Middle East countries and other developing countries, are highlighted.

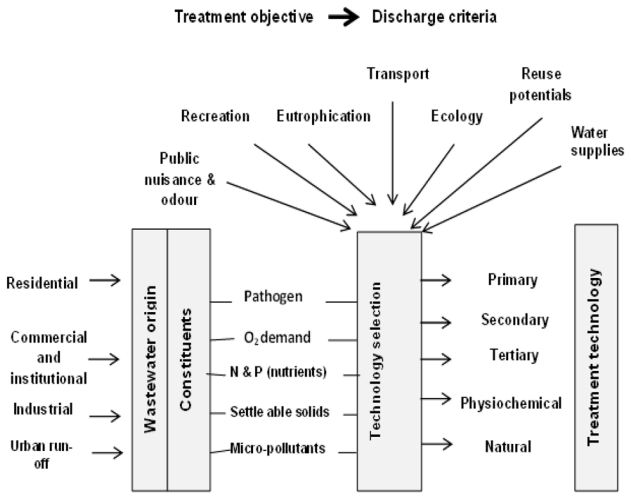

Fig. 1 Treatment technology selection in relation to the origin of the sewage (Veenstra et al. 1997)

Importance of sewage treatment

Human waste has increased tremendously with concomitant rapid growth of communities and cities. Large numbers of pathogenic microorganisms such as bacteria, viruses and protozoa parasites have originated from the sewage (US EPA 2003). Poor sanitation and contamination of drinking water have led to the death of more than 2000 children every day under the age five worldwide (UNICEF 2013). Hence, there is a need for sewage management to advert the magnitude of ecological degradation due to untreated sewage in the environment. Therefore, the selection of an appropriate technology for the treatment and disposal of treated sewage requires an analysis of the effects that the effluents would have on both agricultural and the environment (Zhou and Smith 2002).

Biosolid production has been estimated to be 20×10^9 tons annually worldwide (Markosyan et al. 2002). Hence, the management of sewage sludge has been a vital environmental issue worldwide. However, in the Middle East countries, adoption of a practical, economic and acceptable approach in managing and disposing sewage sludge is not applicable. The present practice is either to reuse for agricultural purpose or direct disposal in sea (UN 2003).

Sewage treatment system is a multistage process to remove organic matter, heavy metals, causative agents of diseases and other pollutants before its disposal or reuse for the agriculture (Al-Rekabi et al. 2007; Wani et al. 2013). The degree of treatment range from basic process such as individual septic tanks (ISTs), oxidation ponds, stabilization ponds, primary and secondary processes for heavily polluted waste to polishing process (advanced or tertiary process) for removing the trace concentrations,

which remain after the main treatment (Gupta et al. 2000; Heritage et al. 2003). The final selection of sewage treatment technology depends on the source of sewage and its applicability (Fig. 1). The common sewage treatment processes can be presented as follows.

Disposal and reuse of treated sewage and biosolids

In the planning and designing of sewage treatment facilities, the disposal of treated sewage and biosolids is an integral part of this structure. After the treatment, both treated sewage and biosolids are discharged into the environment or reused for agricultural purposes (UN 2003). In Middle East countries, the common practices of treated sewage depend on the country's economic structure. The discharge of treated sewage into the natural water is common in the coastal cities which takes advantage of the self-purification capacity of water bodies for the further treatment of treated sewage (UN 2003; Fine et al. 2006). However, these practices are becoming unacceptable due to increase of the smells generated and the volume of the wastes involved as well as risks to health (Heritage et al. 2003). The extreme quantities of organic compounds may cause a reduction of the dissolved oxygen resources of the natural waters and rapid bacterial growth. The changes in heavy metal concentrations and pH values are harmful to organisms in these water bodies (UN 2003).

Recently, many water sources (e.g. seas, rivers, oceans and surface water) started to represent health risks for humans due to the disposal of treated sewage contaminated with pathogenic bacteria (Wen et al. 2009; Garcia-Armisen et al. 2011). A wide variety of microbial pathogens that may pose a risk to human health are known to be abundant in the treated sewage and considered the major source of faecal contamination in aquatic ecosystems (Scott et al. 2003; Tyagi et al. 2006).

Transmission of infectious pathogenic organisms into rivers and water bodies from sewage effluent discharge increases the contamination by pathogenic bacteria such as *Salmonella* spp., *Shigella* spp., *P. aeruginosa*, *S. aureus*, *E. coli*, *V. cholerae*, *Y. enterocolitica* and *C. jejuni* (US EPA 1988; WHO 1993; Santhiya et al. 2011). Discharge of polluted effluent frequently contaminated sea life, particularly fish, cockles and prawns; therefore, people who eat these contaminated seafood could become seriously ill (WHO 2001).

Santhiya et al. (2011) revealed that the seawater and sediments polluted with discharged treated sewage in Morocco were heavily contaminated with *Vibrio* sp., *Pseudomonas* sp., *Salmonella* spp., *Shigella* sp. and coliforms group. Al-Sabahi et al. (2009) stated that the concentration of *E. coli* in three surface water bodies located at the downstream of treated sewage generated from STP in Ibb-Yemen was recorded in an average of 2200 CFU/100 mL. *E. coli* increased from zero/100 mL in boreholes located before the plant to 1100 CFU/100 mL boreholes located after the plant. The study revealed the role of the treated sewage in the distribution of pathogenic bacteria into the natural waster system.

The reuse of treated sewage for irrigation is considered as an important alternative water source in the new water management strategy of Middle East countries due to the severe scarcity of water (UN 2012). In Jordan, 13.8% of all water available for irrigation is sewage effluent, this percentage is predicted to increase to 25% in 2020 (UN 2003; Al-Enezi et al. 2004). In Kuwait, approximately 43.9% of the sewage effluents are reused for agricultural irrigation. In Yemen, the farmers use sewage effluents directly from stabilization ponds for irrigation of a wide range of vegetables and crops, especially the Qat farming which represents about 22.3% of irrigated area (Haidar 2005; Al-Asbahi 2005; ACWUA 2010; Ministry of Agriculture and Irrigation 2012; UN 2012). However, in Tunisia, Morocco and United Arab Emirates (UAE), the treated sewage has been used only for irrigation of gardens in urban centres and tourist facilities (ACWUA 2010). It can be concluded that the reuse of treated sewage for the irrigation in the Middle East countries depends mainly on the geographic area and the development level for each country. Yemen, Jordan, Kuwait and UAE are located in the same geographical area with arid and semi-arid environment; however, UAE is more developed than others. UEA has advanced technologies to the desalination of sea water, while Yemen is the least developed in the field of sewage treatment due to the economic status. Al-Sharabee (2009) reported that 95% of cultivated area in the zone around SSTP at Yemen (1.2–6.0 km) depends upon the sewage effluents. Animals in that zone have suffered due to intestinal diseases such as liver calcification, swelling of stomach, intestinal worms, diarrhoea, changes the taste of milk and mouth blister and farmers are infected by many of pathogenic bacteria because they did not wear plant gloves during the irrigation process (Haidar 2005).

The highly treated sewage from STPs is not necessarily a pollutant, rather it represents a nutrient resource for use in crop production (Gopakumar et al. 2000). The application of treated sewage and biosolids at a controlled rate can improve the physical and chemical properties of soils (Katterman and Day 1989). However, the incessant use of sewage effluents and biosolids may produce detrimental effects on soil and crops (US EPA 2004a; Al-Sa`ed 2007).

According to ACWUA (2010), two planning approaches for sewage reuse are applied. First, the intended reuse option determines the water quality and, therefore, the required treatment technology. This approach allows structured planning within a broader wastewater management master plan and gives the greatest flexibility for reuse. Second, the

available effluent qualities of existing treatment plants define possible reuse option. This approach is widespread in Middle East countries, but considerably limits the reuse of sewage and the development of new irrigation options.

Biosolids contain water, sand, organic matter, microorganisms, trace metals and other chemicals. Their moisture content, humus-like characteristics and essential nutrients for plants make biosolids beneficial and safe to use as a soil conditioner and fertilizer for agriculture purpose (County 2005). Nevertheless, the concerns in the reuse of treated sewage and biosolids in agriculture lie in the transfer of pathogenic microorganisms to humans directly or indirectly, with the direct transmission of microbial pathogens taking place via the consumption of effluent-irrigated vegetables (Heyman 2004). The irrigation of fresh vegetables by sewage effluents represents the main source of pathogenic bacteria. Some of those pathogens can survive even in washed vegetables (Ronner and Wong 1993). Indirect transmission of infection occurs when sewage effluents are discharged to the rivers, reservoirs and canals that supply irrigation water to farmlands (Cotruvo et al. 2004). Contamination of food, water and feed transmission may also occur through pathogens in biosolids that are spread on land field areas (Carlander 2006; Sahlstrom et al. 2006). The indirect pathway concerns a larger proportion of the human and animal population than the direct infection pathway. Pathogenic bacteria may be taken up by plants and enter into the food chain. Movement through the soil and contamination of groundwater with potential contamination of drinking water, runoff and erosion containing pathogens and contaminating surface water has been reported (Sahlstrom et al. 2006).

The potential of pathogenic bacteria to cause infection in human depends on the ability of bacteria to survive in the environment, which is highly dependent on numbers of environmental factors such as temperature, sunlight, moisture, the availability of organic matter, soil pH, soil particles and the presence of toxic substances as well as competitive organisms that influence bacterial survival in soils and sewage (Ibenyassine et al. 2007). AL-Jaboobi et al. (2013) evaluated the quality and suitability of canal sewage, shallow wells and ponds, in Bani Al-Harth area of Sana'a Yemen, when used to irrigate vegetable production. The results revealed high counts of total coliforms, faecal coliforms, *E. coli*, *E. amnigenus*, *E. intermedius*, *E. aerogenes*, *Klebsiella* sp., *Citrobacter* sp., *Serratia* sp., *Proteus* spp. *Staphylococcus* spp., *Vibrio* spp., *Salmonella* spp., yeasts and moulds.

On the other hand, the treatment processes of sewage are insufficient to remove antibiotics. Many antibiotics have been detected in large quantities in treated sewage and in surface water receiving effluents (Spongberg and Witter 2008). Antibiotics represent an emerging environmental problem due to their disposal into the aquatic ecosystem, even at minimum inhibitory concentrations (MICs) and increasing of microbial resistance that has occurred as one of the eminent public health concerns of the twenty-first century (Klavarioti et al. 2009: Velickovic-Radovanovic et al. 2009). Irrigation with treated sewage can lead to accumulation of pharmaceutical residues like antibiotics in the irrigated soil (Dalkmann et al. 2012).

Sewage treatment plants are responsible for spreading antibiotic resistance to the natural environment (Laroche et al. 2009; Servais and Passerat 2009; Garcia-Armisen et al. 2011). Therefore, the persistence of antibiotic-resistant strains in the treated sewage should be considered if they are used for land disposal or for water utilisation (Vilanova and Blanch 2005). Al-Gheethi et al. (2013c) investigated the prevalence of antibiotic resistance phenotypes among TC, *E. coli*, *E. faecalis* and *Salmonella* spp. in the treated sewage generated from three sewage treatment plants in Penang Malaysia. The study found that TC and *E. coli* exhibited high resistance for cephalexin, ampicillin and ciprofloxacin compared to *E. faecalis* and *Salmonella* spp., respectively. All *E. coli* strains, 76.18% of TC, 66.66% of *E. faecalis* and 35% of *Salmonella* spp. had multi-resistance for antibiotics. Al-Gheethi and Ismail (2014) investigated the antimicrobial resistance among total bacterial counts from sewage-treated effluents. The report revealed that about 83.82% of the bacterial isolates were multi-resistant (resistant to three antibiotics or more). Gram-positive bacteria exhibited more multi-resistance to antibiotics (cephalexin, amoxicillin, ampicillin and cefuroxime) than Gram-negative bacteria. Based on these studies it can be indicated that the sewage effluents represent a rich source of antimicrobial resistance bacteria, perhaps due to the high nutrients contents.

Frequently occurred pathogenic bacteria in the treated sewage and biosolids

Treated sewage and biosolids contain many pathogenic microorganisms, the most important are those transmitted by the faecal–oral route, which includes bacteria, viruses and parasites (US EPA 2003; Wen et al. 2009). However, bacteria represent then concern due to their ability to increase in the environment because it does not require a host cell for replication (Ceustermans et al. 2007). The actual species and density of pathogenic bacteria in sewage depend on public health, the size of the local community and the presence of hospitals, factories, as well as on sewage treatment processes (Harrison et al. 1999; US EPA 2003; Bitton 2005a).

There is a wide spectrum of pathogenic bacteria that has been detected in the treated sewage and biosolids, many of which are enteric in nature (Toze 1997). *V. cholera*, *Leptospira* spp., *Salmonella* spp., *C. jejuni*, *E. coli* O157:H7, *Y. enterocolitica* and *Shigella* sp. are considered as a major

concern which could result in disease to the general population, while *B. cereus, Enterobacter* spp., *Klebsiella* spp., *C. perfringens*, *L. monocytogenes*, *P. aeruginosa*, *S. aureus* and *Streptococcus* spp. are the minor concerns which are considered opportunistic pathogens that cause disease only in debilitated or immunologically compromised individuals (Kowal 1983; US EPA 1988; Synnott et al. 2009; Dungeni et al. 2010; Ellafi et al. 2010; Coronel-Olivares et al. 2011).

Markosyan et al. (2002) detected different genera of *Klebsiella*, *Enterobacter*, *Hafnia*, *Serratia*, *Proteus*, *Providencia* and *Escherichia* in the biosolids. Younis et al. (2003) isolated *Salmonella* spp., *Shigella* spp., *Vibrio* spp., *Staphylococcus* spp. and *Listeria* spp. from STP located in Aswan, Egypt. Lisle et al. (2004) observed that the untreated sewage at Memurdo station, Antarctica, contained relatively high concentrations of total coliforms, faecal coliforms, *E. coli*, enterococci and *C. perfringens.* Al-Zubeiry (2005) detected *S. aureus*, *S. pneumonia*, *E. coli*, *Salmonella* spp. and *P. aeruginosa* in raw sewage and secondary effluent generated from STP in Ibb, Yemen. El-Lathy et al. (2009) isolated *Salmonella* spp., *Listeria* spp and *Vibrio* spp. (*V. vulnificus*, *V. parahaemolyticus* and *V. cholera*) from sewage samples from oxidation pond in El-Sadat, Egypt, and biosolids from Zenin sewage treatment plant at Giza, Egypt. Ye and Zhang (2011) studied the presence of pathogenic bacteria in biosolids from 14 STPs in China, USA, Canada and Singapore. The study detected *Aeromonas veronii*, *A. hydrophila*, *C. perfringens* and *C. Diphtheria* as most common. Bala et al. (2012) investigated pathogenic bacteria in pharmaceutical wastewater in Nigeria. They noted that *E. coli*, *Salmonella* sp., *Klebsiella* sp., *P. aeruginosa*, *S. aureus*, *P. vulgaris*, *Clostridium* sp. and *E. faecalis* were predominant.

Al-Gheethi et al. (2013a) reported that *K. pneumonia*, *E. coli*, *Shigella* sp., *Salmonella* spp., *S. aureus*, *E. faecalis* and *P. aeruginosa* are abundant in three STPs in Malaysia. Al-Gheethi et al. (2014) also investigated the bacterial diversity in treated sewage and biosolid samples generated from five STPs in Yemen. The authors isolated hundred and sixty bacterial strains. Among those, *E. coli* was the most common, followed by *S. faecalis*, *K. pneumonia*, *E. aerogenes*, *S. typhi*, *S. typhimurium*, *S. sonni* and *Y. pestis*. The most concerned bacteria are discussed below;

Salmonella spp.

Salmonella spp. are rod-shaped, Gram-negative, non-spore-forming, facultatively anaerobic bacteria, discovered by Salmon in 1880. This group consists of a range of very closely related bacteria that belongs to the genus *Salmonella* and the family Enterobacteriaceae. *Salmonella* spp. exhibit psychrotrophic properties and actively grow within a wide temperature range (10–54 °C) (Woteki and Kineman 2003).

Salmonella spp. are resistant microorganisms that are readily adapt to extreme environmental conditions and have the ability to survive under hostile environmental conditions (Espigares et al. 2006; Alvarez-Ordonez et al. 2011). These characteristics make them the indicator of choice for monitoring the effectiveness of biosolid pathogen reduction (US EPA 1995). US EPA (2003) also demonstrated that *Salmonella* spp. are bacteria of great concern as well as good representatives of the reduction of other bacterial pathogens because they are typically present in higher densities than other bacterial pathogens and have the ability to survive for a long time in the environment.

Salmonella spp. are the most prevalent bacterial pathogens of public healthcare concern that are frequently found in sewage (Dumontet et al. 2001; Espigares et al. 2006). *Salmonella* spp. can cause diseases to all organisms from insects to mammals (Bohm 2004). Enteric fever is a collective term given to the invasive infections caused by *S. typhi* and *S. paratyphi* causes paratyphoid fever. *S. typhi* is a pathogen that only has humans as its natural host (Heritage et al. 2003).

Burtscher and Wuertz (2003) reported that about 48% of the 46 biosolids samples tested were positive for *Salmonell*a spp. which could be detected in both untreated and treated waste samples during intermediate stages of treatment. However, *S. stanley* was the only pathogen isolated after the thermophilic anaerobic digestion (Sahlstrom et al. 2004).

Shigella sp.

This is a genus of gamma-Proteobacteria in the family Enterobacteriaceae (Brenner et al. 2005), discovered by Kiyoshi Shiga in 1896. They are rod-shaped, Gram-negative, non-spore-forming, non-motile bacteria that are very closely related to *E. coli* and only humans are always the host (Geldreich 1996). Members of the *Shigella* genus (*S. dysenteriae*, *S. boydii*, *S. flexneri* and *S. sonnei*) are the major cause of dysentery, diarrhoea, fever, vomiting and cramps, which frequently occurred in countries that lack potable drinkable water such as India (Niyogi 2005). The dysentery bacilli are the most common infectious diseases in third world countries and among travellers to tropical countries (Vila et al. 1994). *Shigella* spp. are the second pathogenic bacteria that cause intestinal diseases in China (Peng et al. 2002).

The infectious dose for *Shigella* sp. was determined to be as few as 10–100 organisms (Fratamico et al. 2005) and the waterborne transmission of shigellosis was documented epidemiologically (Alamanos et al. 2000). *Shigella* sp. was isolated from pharmaceuticals wastewater, sewage effluents and biosolids (Bala et al. 2012; Al-Gheethi et al. 2013a). Chen et al. (2012) found that *Shigella* sp. survived more than *Salmonella* sp. and *E. coli* during mesophilic anaerobic digestion of sludge. However, it has been reported previously

that *Shigella* sp. does not appear to survive long in the environment (Gebra 1996).

Escherichia coli O157:H7

Escherichia. coli is a rod-shaped Gram-negative bacterium, which belongs to the family *Enterobacteriaceae*. They are facultative, oxidase-negative anaerobes and produce gas from glucose. *E. coli* is a member of the physiological gastrointestinal flora bacterium species for human and warm-blooded animals. Additionally, it belongs to the normal intestinal flora and a facultative pathogen for human being (Kaper et al. 2004). However, some *E. coli* serotypes are pathogenic, among them enterohaemorrhagic strain *E. coli* O157:H7 which causes gastrointestinal disorders such as bloody diarrhoea, cramping and abdominal pain and the infectious "hemolytic uremic syndrome" (Fijalkowski et al. 2014). *E. coli* O157:H7 was first reported as a gastrointestinal pathogen in 1982 (Riley et al. 1983), they are able to survive in environment for long time without a host (Qing et al. 2010). Isolation of *E. coli* from surface water, treated sewage and biosolids has been reported by many investigators (Al-Zubeiry 2005; Jokinen et al. 2010; Al-Gheethi et al. 2013a; Al-Gheethi et al. 2014).

Clostridium perfringens

Clostridium. perfringens is an obligate anaerobic Gram-positive bacterium, bacilli-shaped and endospore forming. It is a member of the Sulfite-Reducing Clostridia (SRC) group. *C. perfringens* has been isolated from sewage by many researchers (Lisle et al. 2004; Ye and Zhang 2011), because it represents 0.5% of the faecal microflora (Leeming et al. 1998; Payment et al. 2002). Payment and Franco (1993) recommended this bacterium as an indicator for the presence of *Giardia* cysts in water treatment plants as well as to evaluate the quality of recreational waters (Fujioka 1997). Suresh et al. (1996) confirmed that the use of *C. perfringens* as well as faecal coliforms and faecal streptococci could serve as an excellent approach for identifying the presence of airborne pathogens and determining their origins or sources associated with the treatment and disposal of wastewater and biosolids.

Lepeuple et al. (2004) reported that the *C. perfringens* is common in raw sludge and resistant to heat, and hence their removal can be related to removal of spore-forming species such as *Bacillus* sp. *C. perfringens* has been reported as resistant to oxidizing agents and to UV disinfection (Alonso et al. 2004). Rouch et al. (2011) indicated that *C. perfringens* appeared to be a conservative indicator during their study on the air drying of sludge generated from two STPs in Victoria, Australia. However, some authors stated that the hardy spores of this bacterium make it too resistant to be useful as an indicator organism. Thus, it could be useful as an indicator of past pollution and as a trace to follow the fate of pathogens (Bitton 2005b). Vierheilig et al. (2013) investigated *C. perfringens* in different faecal sources at Austria for 3 years. They stated that *C. perfringens* was not suitable as indicator of faecal pollution but they suggested that it could be used as a tracer for excreta from human sewage.

Faecal indicator bacteria

Analytical techniques for the direct detection and identification of many types of pathogenic bacteria in the sewage effluents require well-trained technicians. These techniques are usually unpredictable, difficult, costly and time consuming. The faecal organisms which are used as pathogenic indicator can determine the relative risk of biosolids (Toze 1997; Wen et al. 2009).

The indicator bacteria are adapted to living in the gastrointestinal tract and can be harboured in other different habitats, such as a septic system or sewage collection system (Gordon et al. 2002). Indicator organisms are used as models for the behaviour of pathogens, for example, to determine the efficiency of treatment processes, where their growth characteristics (temperature and pH) are similar to those of numerous pathogens for which detection and quantification are difficult or sometimes impossible (Lepeuple et al. 2004).

Many bacteria have been studied for their suitability as faecal indicators (Ashbolt et al. 2001; US EPA 2007). The first bacterial species, which had been used as faecal indicators, were *K. pneumoniae* and *K. rhinoscleromatis,* which was suggested by Von Fritsch in 1880 (Geldreich 1978). However, many bacterial species were also suggested as indicators. Dancer (2004) has proposed *S. aureus* as an indicator of hospital hygiene for microbiological standards. Jin et al. (2013) used *S. aureus* as indicator to evaluate the hydrothermal treatment process in achievement of the hygienic safety of food waste.

Al-Gheethi et al. (2013a) studied the correlation between the frequencies and the numbers of faecal indicators and pathogenic bacteria. They found that *E. coli* has been correlated significantly with all pathogenic bacteria investigated (*K. pneumonia*, *P. aeruginosa*, *Shigella* sp. and *Salmonella* spp.), *E. faecalis* has correlated by 75% while total coliforms and faecal coliforms correlated by 50% with pathogens under study.

In general, the criteria for selection of faecal indicator organisms have been documented as follows (Cooper and Olivieri 1998; Dumontet et al. 1999; Bitton 2005a; Myers et al. 2007):

1. Should be a member of the *Enterobacteriaceae* family.
2. The presence of faecal indicators should be associated with the presence of pathogens.

3. Numbers should be greater than that of the pathogens.
4. It should be resistant to the disinfection processes as the pathogens.
5. It should not increase in the environment.
6. Should be easily detected by simple techniques.
7. Should be non-pathogenic.

Faecal indicators organisms are discussed below;

Total coliforms are groups belonging to *Enterobacteriaceae* and include the aerobic and facultative anaerobic, rod-shaped, non-spore-forming, Gram-negative bacteria that ferment lactose with gas production within 48 h at 35 °C (APHA 1989). These characteristics are suitable for the identification of this group and no confirmatory tests are required (Edberg et al. 1990).

Total coliforms include four genera, *Escherichia* sp., *Enterobacter* sp., *Klebsiella* sp. and *Citrobacter* sp. Some members of this group, e.g., *Klebsiella* sp. may grow in industrial waste. They are the historic indicators of faecal contamination since 100 years ago (Cooper and Olivieri 1998). Total coliforms are one of the best indicators for treatment efficiency of sewage treatment plants (Bitton 2005b).

Faecal coliforms (FC) are classified under the group of total coliforms and are more faecal specific in origin and include *E. coli* and other faecal (or thermotolerant) coliforms that can ferment lactose at 44.5 °C (Kimberly et al. 2005). The presence of these organisms more accurately correlates with warm-blooded animal faecal discharges. However, even this group contains a genus, *Klebsiella* with species that are not necessarily faecal in origin. *Klebsiella* sp. is commonly associated with textile, pulp and paper mill wastes (US EPA 1986a).

According to the US EPA (1992), FC is faecal bacteria that are used as indicators to show probable presence of pathogenic bacteria, because they are easily detected and their densities decline in the same proportion as pathogens during the treatment process. The EPA Part 503 regulations "Standards for the use or disposal of sewage sludge" have established pathogen reduction requirements for FC (US EPA 2003).

For recreational waters, FC was the primary bacterial indicator until 1986, when the EPA began recommending *E. coli* and enterococci as better indicators of health risk from water contact (US EPA 1986a). FC is still being used in many states of USA as the indicator bacteria. However, Byappanahalli and Fujioka (1998) reported that tropical soil environments such as in Hawaii provide sufficient means to support the growth of FC and *E. coli*. Polo et al. (1998) have shown poor to no correlation between *E. coli* and *Salmonella* spp. to FC. Hörman et al. (2004) reported that there was no correlation between *E. coli* and *Campylobacter* spp.

Enterococci bacteria have been considered useful as secondary indicators of faecal contamination (APHA 1998). However, Bitton (2005b) reported that enterococci are good indicators of faecal pollution like faecal coliforms. Enterococcus species (*E. faecium*, *E. faecalis*, *E. avium*, *E. gallinarum* and *E. durans*) have the ability to grow at both 10 and 45 °C, at high pH 9.6 and in medium containing 6.5% NaCl (Cooper and Olivieri 1998; Carvalho and Teixeira 2002). This group has been suggested as useful for indicating the presence of viruses, particularly in sludge, sea water and biosolids (Bitton 2005b), because these organisms are relatively easy to enumerate and survive longer than faecal coliforms (Mote et al. 2012).

Enterococci along with faecal coliforms have been used to differentiate human faecal contamination from that of other warm-blooded animals based on FC/FS ratio, if the ratio less than 1.0 this means that the source of faecal contamination are all warm-blooded animals other than man, if the ratio was 4.0, this means the source of faecal contamination is human. Intermediate ratios indicate contamination from both man and animals (Young and Thackston 1999; Baudišová 2009). Enterococci are a more reliable indicator than faecal coliforms for the detection of microbial pollution as they are more resistant to the environment than faecal coliforms (Celico et al. 2004). It has been frequently considered as reference microorganism for thermal treatments to be applied in pasteurized foods (Smith et al. 1990; Ghazala et al. 1991). However, US EPA (US 2004a) and WHO (1989) did not regulate standards limits of enterococci in treated sewage if the effluents is to be reused for irrigation or groundwater recharge.

Regulations of treated sewage and biosolids

To maintain safe reuse of sewage treated effluents, WHO (1989) reported that the geometric mean of FC should be less than 1000 cells/100 mL (Table 1), these standards are also used in Jordan and Palestine. However, US EPA (2004b) recommended more stringent standards for sewage-treated effluents. FC should not exceed 14 cells/100 mL (Table 2). In Saudi Arabia, the standards for sewage effluents reuse in agricultural irrigation are issued by the Ministry of Municipal and Rural Affairs (MMRA). According to those standards, FC should be less than 2.2 cells/100 mL of unrestricted irrigation and less than 1000 cells/100 mL of restricted irrigation (Al-Jasser 2011). The EPA part 503 regulations for the reuse of biosolids (CFR 1995) divided stabilization biosolids into "Class A" and ''Class B''. Class A biosolids must meet either FC limit of less than 1000 MPN g-1 TS or less than 3 *Salmonella*/4 g TS. Class B biosolids should be meet a FC limit of less than 10^6 MPN g^{-1} TS. The standards for reuse of

Table 1 Guidelines for using treated wastewater in agriculture (WHO 1989)

Category	Reuse conditions	Exposed group	Intestinal nematode (arithmetic mean no eggs/L)[b]	Coliforms (geometric mean/100 mL)[b]	Wastewater treatment expected to achieve the required microbiological guideline
A	Irrigation of crops likely to be eaten uncooked, sports fields, public parks[c]	Workers, consumers, public	≤ 1	≤ 1000	A series of stabilization ponds designed to achieve the microbiological quality indicated, or equivalent treatment
B	Irrigation of cereal crops, industrial crops, fodder crops, pasture and trees[d]	Workers	≤ 1	No standard recommended	Retention in stabilization ponds for 8–10 days or equivalent helminthic and FC removal
C	Localized irrigation of crops in category B if exposure to workers and the public does not occur	None	Not applicable	Not applicable	Pre-treatment as required by irrigation technology, but not less than primary sedimentation

[a]Ascaris and Trichuris species and hookworms

[b]During the irrigation period

[c]A more stringent guideline (200 FC/100 mL) is appropriate for public lawns, such as hotel lawns, with which the public may come into direct contact

[d]In the case of fruit trees, irrigation should cease 2 weeks before fruit is picket, and no fruit should be picked off the ground. Sprinkler irrigation should be used

Table 2 Microbiological requirements for reclaimed water (US EPA 2004b)

Type of use	Treatment	Reclaimed water quality
Urban uses, food crops eaten raw, recreational impoundments	Secondary, filtration, disinfection	pH = 6–9 no detectable FC/100 mL[a]
Restricted access area irrigation, processed food crops, non-food crops, aesthetic impoundments, construction uses, industrial cooling, environmental reuse	Secondary, disinfection	pH = 6–9 200 FC/100 mL[b]
Groundwater recharge of potable aquifers by spreading	Site-specific secondary and disinfection (minimum)	Site-specific meet drinking water standards after percolation through vadose zone
Groundwater recharge of potable aquifers by injection, augmentation of surface supplies	Includes: secondary, filtration, disinfection, advanced wastewater treatment	Includes: pH = 6–8.5 no detectable FC/100 mL[a]

[a]Based on 7-day median value. Should not exceed 14 FC/100 mL in any sample

[b]Based on 7-day median value. Should not exceed 800 FC/100 mL in any sample

sewage effluents and biosolids issued out by the Yemen agricultural sector have focused on the basic parameters of sewage such as COD, BOD, TS and TSS. However, these standards are neither met nor controlled, because most of STPs are overloading, and Yemeni laboratories are not equipped to measure all mentioned parameters (ACWUA 2010).

Further treatment of treated sewage

Recent studies revealed that detectable amounts of pollutants remain in sewage effluents, even after secondary sewage treatment processes are performed. These pollutants could be transmitted into rivers and other environment during the final disposal or reuse of the effluent (Spongberg and Witter 2008). Rizzo et al. (2013) reported that conventional disinfection processes might not be effective in the inactivation of antibiotic-resistant bacteria. Therefore, to achieve safe reuse of sewage-treated effluents, advanced treatment technologies have been applied to reduce various potentially harmful compounds that could not be effectively removed by conventional treatment processes. Advanced treatment technologies have high potential to produce an effluent of higher quality than normally achieved by secondary treatment processes (Zhou and Smith 2002; Jin et al. 2013). The techniques used for this

purpose include reduction of pathogens by disinfection process (Al-Rekabi et al. 2007).

Disinfection processes of sewage-treated effluents

The concentrations of faecal indicator bacteria in untreated sewage are 8 $\log_{10}$ CFU/100 mL for TC, 7.48 $\log_{10}$ CFU/100 mL for FC and 6.6 $\log_{10}$ CFU/100 mL for enterococci (Wilén et al. 2012). The conventional treatment processes for sewage (primary and secondary processes) remove 95–99% of most microorganisms (Koivunen et al. 2003). However, their numbers in the sewage effluent usually remain higher than 4 $\log_{10}$ CFU/100 mL (Luczkiewicz et al. 2010). The stabilization pond was reported to reduce 84% of total coliform, 96% of faecal coliform and 89% of enterococci (Reinosoa et al. 2008). The reduction efficacies of faecal indicator bacteria by the septic tank and oxidation pond were reported to be 15 and 38% for FC and 11 and 16% for FS, respectively (Samhan et al. 2007).

Many studies have found that the concentrations of faecal indicators in the treated sewage and biosolids are still more than the standard limits of US EPA and WHO guidelines. Heng et al. (2006) found that the concentrations of TC in the treated sewage generated from two-oxidation ponds at Kajang and UKM-Malaysia were 8.95 and 8.58 $\log_{10}$ CFU/100 mL. Siti Khadijah et al. (2013) reported that the concentration of FC in the treated sewage from the oxidation ponds in USM Engineering Campus, Malaysia, were also more than WHO guidelines, where FC concentrations ranged from 2.36 to 5.57 $\log_{10}$ CFU/100 mL. Al-Gheethi et al. (2013a) revealed that the concentrations of FC at the three STPs in Penang, Malaysia were greater than the standard limits recommended by the WHO guideline for use of treated wastewater in agriculture and US EPA microbiological requirements for wastewater reuse.

According to those studies, it can be concluded that the treated sewage still contains high concentrations of pathogenic bacteria even after sewage treatment processes. The high bacteria levels in the natural water that received sewage-treated effluent lead to increase in BOD, resulting in depletion of oxygen levels required for the various types of living organisms supported by the estuary. Therefore, treated sewage needs to undergo further treatment to reduce the density of pathogenic bacteria to achieve a favourable sanitary effluent quality (Koivunen et al. 2003; Jin et al. 2013). Disinfection process of effluents increases the reduction of pathogens for high-quality reuse (Crook 1998; Neis and Blume 2002). The tertiary treatment is able to achieve the guidelines of WHO and US EPA standards for pathogen inactivation. The development of a tertiary treatment process includes all techniques that offer significantly higher removal of pathogenic bacteria. The most common disinfection processes of treated sewage are discussed below.

Chlorination

Chlorination is the most common method used for disinfection of treated sewage in Middle East countries because it is easily applied, readily available and cheaper than other oxidising agents. It can also be used to inhibit bacteria growth in treated sewage (Gomez et al. 2006). Tree et al. (2003) found that chlorination has significant effect in the reduction of *E. coli* and *E. faecalis* in sewage-treated effluents. However, the occurrence of pathogenic bacteria in treated sewage after chlorination has been observed. Dungeni et al. (2010) stated that despite high free chlorine residual concentrations in sewage-treated effluents, the survival of *E. coli*, *S. typhimurium* and *V. cholerae* was significantly high and they suggested an upgrading of the STPs by other processes to increase the inactivation of pathogenic bacteria. CDPH (2009) reported that the chlorination disinfection system is the primary disinfection process, and the whole disinfection of reclaimed water should be performed by the pasteurisation system.

Moreover, one main disadvantage for utilization of chlorine disinfection is the presence of free and combined chlorine residues which is being toxic to aquatic organisms. Therefore, the requirement to de-chlorinate or to remove chlorine residues from the treated sewage before it is discharged into the environment has increased in recent years due to the potential health hazards of nitrosodimethylamine (NDMA) which is reported as a probable human carcinogen (Pehlivanoglu-Mantas et al. 2006).

Ozonation

One of the most effective disinfectants used in water disinfection is ozone. This is because ozone has high ability to destroy pathogenic cells through an irreversible physiochemical action. Ozonation destroys the cell wall of the bacteria as well as semi-permeable membrane. The destruction in the cell wall and membrane leads to the bacterial cell death (Facile et al. 2000). Tripathi et al. (2011) claimed that 5 min of exposure at a concentration of 10 mg ozone L^{-1} was suitable for the inactivation of pathogenic bacteria by 95–98%. Previous studies reported that ozone effectively removes TC and FC from sewage-treated effluents. Battaler et al. (2005) found that the ozone disinfection of secondary effluents at concentration 4.7 mg L^{-1} for 5 min had eliminated TC and FC. At 21.4 mg L^{-1} the bacteria that resisted for chlorine such as *P. aeruginosa* decreased by 2 log reduction after 5 min of disinfection process by ozone.

Disinfection of treated sewage by ozone is applied because the use of ozone is cheap and low energy is needed. Nonetheless, the effectiveness of disinfection using ozone depends on the ozone dose, the ozone demand, the quality

of the effluent and the transfer efficiency of the ozone system (Paraskeva and Graham 2002). The COD and total suspended solids (TSS) of treated sewage might affect the efficiency of disinfection process by ozone (Janex et al. 2000). The properties of the treated sewage might induce the microbial resistance for the ozone as noted for *Enterococcus* sp., *Clostridium* sp. and *Salmonella* spp. which exhibit resistance to ozonation (Xu et al. 2002).

In USA, the disinfection of drinking water by ozonation process is more attractive disinfection method due to the promulgation of the EPA's restrictions of disinfection by-products (DBPs) permitted in America's drinking water attributable to chlorination. Nevertheless, ozonation can also lead to the formation of potentially harmful by-products inclusive of bromate ions (BrO_3), aldehydes and peroxides. Vital et al. (2010) reported that the major concern associated with the ozone application lies in the increase in microbial regrowth due to the oxidation process which generates the assimilable organic carbon (AOC).

UV irradiation

Disinfection by ultraviolet irradiation has been reported as a suitable technology for inactivating coliforms and *Salmonella* spp. (Keller et al. 2003). The principle of a UV disinfection system is to destroy the genetic material of the bacterial cell and thus retard its ability to reproduce (US EPA 1986b). The effectiveness of a UV disinfection system depends on the characteristics of the effluents including the concentration of the sewage effluent, the intensity of the UV radiation and the time of the treatment (Kollu and Ormeci 2012).

Nasser et al. (2006) revealed that the treated sewage disinfected by UV is suitable for the unrestricted irrigation of food crops (FC < 1000 CFU/100 mL). Dungeni et al. (2010) suggested UV disinfection as an additional treatment process of effluents to increase the effective inactivation and removal of pathogenic bacteria and viruses. However, Alonso et al. (2004) found that *Clostridium* spp., showed a greatest degree of resistance to UV treatment. Munir et al. (2010) indicated that the disinfection of effluents by UV process did not contribute to the significant reduction of antibiotic-resistant bacteria. Ting et al. (2011) observed a re-growth and repair potential of *E. coli,* FC and *B. subtilis* in reclaimed water after UV disinfection. Wang et al. (2012) suggested that the higher number of particles in the treated sewage might have protected the bacteria against UV damage. Based on those studies, it can be indicated that UV technology might have a limitation for the sewage disinfection. But it has to be motioned that the disinfection processes of the treated sewage might be more effective as two methods have been used simultaneously such as the solar disinfection which depends on the UV radiation, photo-oxidation and temperature (Sect. "Solar disinfection (SODIS)").

Filtration technology

Filtration systems are designed to remove very small particulate or "suspended" solids from the treated sewage. Onnis-Hayden et al. (2011) evaluated sand filtration technology in the disinfection of sewage generated by STPs and found that the *E. coli* concentration reduced to below 2 logs in the filtered sewage-treated effluents. Despite its high efficiency in the removal of pathogenic bacteria and producing effluents with good microbiological quality, the effluents would not be considered as sterile, since contamination of permeation zones gave rise to the presence of pathogenic bacteria (Gomez et al. 2006). At the same time, the membrane filtration systems are expensive with regard to construction and maintenance (Neis and Blume 2002). Different filtration systems have been developed which depend on the utilization of raw and low-cost materials such as sands and ceramic (Mohamed et al. 2016). These systema have exhibited high efficiency in the reduction of the main parameters of wastewater such as COD, BOD and TSS, but their efficiency in the reduction of pathogenic bacteria still needs more investigation.

Storage of treated sewage

Storage systems are used typically for accumulating wastewater before its ultimate disposal, or for temporarily holding batch streams before treatment (US EPA 1989). A storage basin was also used for treating effluent storage, especially during winter in temperate country. The stored effluents will be released to environment normally during the rainy season where the dilution rate is higher (Al-Gheethi et al. 2017).

Storage of sewage has become the option selected in European and Mediterranean countries because of the advantages they present in comparison with other treatment alternatives. This includes the coupling of two purposes: stabilization and seasonal regulation to regulate between sewage production and demand of treated wastewater for irrigation (Barbagallo et al. 2003).

Al-Gheethi et al. (2013a) studied the effect of storage of treated sewage on survival of faecal indicators and *Salmonella* spp. and the susceptibility of these bacteria to antibiotics during the storage period. The treated sewage was stored at room temperature for 28 days. The study noted that FC density in stored effluents dropped from 5.182 $\log_{10}$ CFU/100 mL to below 2 $\log_{10}$ CFU/100 mL after 28 days and the treated sewage met WHO guidelines. *E. faecalis* decreased to less than detection limits during the storage period of 1 week. However, they observed that TC and *Salmonella* spp. which have survived during the storage

period acquired more resistance to antibiotics. At the end of the storage period, TC and *Salmonella* spp. were resistant to cephalexin, cefuroxime, ampicillin and amoxicillin. The study concluded that the storage system of treated sewage would increase the distribution of antimicrobial resistance among bacterial population after the disposal or reuse of treated sewage for agricultural purposes. Indeed, the efficiency of the storage system on the reduction of pathogenic bacteria depends mainly on the storage temperature, the storage system at the room temperature might be effective for the reduction of pathogenic bacteria but it needs long time to achieve high reduction of pathogens and then to meet the international standards required for the sewage disposal or reuse of the sewage for the irrigations (Al-Gheethi et al. 2017). The mechanism which takes place in the storage system and leads to the reduction of pathogens might be the competition process between microorganisms due to the deficiency in the nutrients contents (Al-Gheethi et al. 2016a).

Heat pasteurization

Heat disinfection is a proven technology in Europe that requires skills such as boiler operation and the understanding of high-temperature and -pressure processes. The EPA Part 503 regulations (US EPA 2003) consider pasteurisation as a process to further reduce pathogen (PFRP). CDPH (2009) reported that the use of pasteurisation is recognised as an acceptable disinfection process for meeting the inactivation criteria of coliform bacteria. It is a known fact that pathogenic bacteria are inactivated during exposure to heat, especially when the temperature of the treatment is above the optimum temperature of growth (Himathongkham and Riemann 1999). Lucero-Ramirez (2000) reported that pathogenic bacteria are reduced to less than detectable levels in properly operated heat-drying systems. Alcalde et al. (2003) indicated that the retention time and temperature are the most important factors for the removal of pathogenic bacteria.

Bacteria can be classified into different groups based on optimum temperature. Most human pathogenic bacteria are mesophilic (10–40 °C) with an optimum temperature of 37 °C. Metabolic enzymes of bacteria denature and inactive when exposed to temperature above its optimum. This eventually led to the death of the bacterial cell. Removal of faecal indicators (enterococci and *E. coli*) in piggery effluents to achieve hygiene standards could easily be met by treatment at 60 °C (Cunault et al. 2011). Al-Gheethi et al. (2013a) investigated the heat treatment of treated sewage at different temperatures (45, 55 and 65 °C). They found that the sewage effluents disinfected at 45 °C for 192 h, 55 °C for 24 h and 65 °C for 30 min have met standard limits regulated by the US EPA. The sewage effluents can be reused for irrigation purpose.

Solar disinfection (SODIS)

SODIS-based technologies are an efficient approach for the reduction of pathogenic microorganism in the water due to high availability of solar radiation and sustainable nature of these water treatment methods (Gomez-Couso et al. 2009). According to WHO (2002), SODIS depends on using transparent polyethylene terephthalate (PET) bottles and then exposing to the sun for a period between 4 and 8 h. Meierhofer and Wegelin (2002) recommended that PET bottles containing untreated raw water should be exposed to direct sunlight for at least 6 h. Bacteria, viruses, *Giardia* and *Cryptosporidium* cysts, and parasite eggs could be inactivated through the combination of ultraviolet radiation and elevated water temperature. Large field tests of SODIS were conducted in a number of countries in South America, Africa, and Asia in the 90s (Acra 1990).

The destruction of the bacterial cells takes place due to the combination of UV radiation and high temperature which has high potential to destroy the cell membrane (Al-Gheethi et al. 2015). However, SODIS has no efficiency for the reduction of chemical pollutants in the water. The sunlight has been reported as the single most important disinfection factor in the stabilization pond (Leduc and Gehr 1990; Maynard et al. 1999). Three main mechanisms are involved during the SODIS simultaneously, included the absorption of solar UV-B by microorganism DNA which causes direct damage by pyrimidine dimer formation. The process is independent of oxygen and other pond conditions. The second mechanism depends on the absorption of UV-B and some shorter wavelength UV-A by cell constituents including DNA (called endogenous photo-sensitisers). The activated constituents react with oxygen to form highly reactive photo-oxidising species that damage genetic material within the cell or viral particle. The third mechanism involves absorption of a wide range of UV and visible wavelengths in sunlight by extra-cellular constituents of the pond medium (exogenous photo-sensitisers—notably humic material) (Jagger 1985).

Al-Gheethi et al. (2013b) investigated the effect SODIS on the survival of FC, *E. faecalis*, *Salmonella* spp. and *S. aureus* in treated sewage inside PET bottles. The study revealed high reduction of FC, *Salmonella* spp. and *S. aureus* by more than 4 $\log_{10}$ CFU/100 mL after 6 h, while these pathogens were below the detection limits after 8 h as detected by using the enrichment medium. The treated sewage met US EPA (2004a) standards after 6 h, where FC counts were less than 14 CFU/100 mL. SODIS has several advantages to the reduction of faecal indicator in treated sewage compared to the others techniques such as

chlorination, UV irradiation and ozonation. SODIS is compatible to meet the standards of the disinfection processes of sewage treated effluents. This is because SODIS is effective, more efficient, easily implementable and lower cost. SODIS is a natural process that produces no toxic by-product (Al-Gheethi 2014).

SODIS is more appropriate to be applied in the developing countries that do not have the facilities to build typical sewage treatment plants. The climate of many Middle East countries such as Yemen, Saudi Arabia, Oman, Jordan and Egypt is semi-arid to arid and the temperatures during summer season vary from 27 to 50 °C (FAO 2008). These countries also have a large space of the deserts. The desert temperatures range from 45 to 60 °C in summer and from 7 to 35 °C in the winter and the sunlight intensity ranges from 5.2 to 6.8 kWh/m^2/day (Al-Ashwal and Basalah 2012). Therefore, one of the proposals to reduce the environment pollution by sewage generated from STPs is by extending the sewerage network to the desert. The desert provides many of the features of SODIS necessary to treat the sewage effluents such as the large areas and the temperature and the treated sewage could be used in the land reclamation.

Reduction of pathogenic bacteria in biosolids

Sewage treatment processes can be classified as primary and secondary processes. In primary treatment, solids are mainly removed mechanically from untreated sewage. Secondary treatment is a biological process in which decomposers are utilized to remove biodegradable pollutants. Decomposers are organisms such bacteria and fungi that get energy and nutrients by digesting waste matter in the sewage. In the activated sludge process, sewage is pumped into a large tank where aerobic microorganisms decompose the organic matter (WHO 2002). Chemical treatment is sometimes used and it encourages small particles and dissolved substances to form large particles which facilitate separation. This is called chemical precipitation. Sludge is formed when these larger particles clump together during suitable separation methods (Casey 1997).

All the sludge that is separated during these treatment methods (mechanical, biological and chemical) is defined as a raw sludge, which has to undergo various kinds of further treatment for the improving the microbiological quality and then for safe disposal or reuse. Physical, chemical and biological processes accomplish the stabilization and disinfection of sewage sludge. Stabilization refers to those processes that reduce the volatile solids content, pathogen levels and odour. The biosolids generated from sewage treatment processes must be subject to further treatment to reduce pathogenic bacteria which are still high in concentrations even after the treatment processes (Al-Gheethi et al. 2014). Disinfection processes emphasize the reduction of pathogenic bacteria below detectable limits. Major stabilization methods include anaerobic digestion, aerobic digestion, composting, alkaline (lime) stabilization and air-drying. Disinfection includes pasteurization, long-term storage, irradiation, heat drying and heat treatment (US EPA 2003).

According to US EPA, there are two processes are used to the reduction of pathogenic bacteria to comply the standards limits of biosolids (Class A and B). These processes named Processes to Further Reduce Pathogens (PFRPs, Table 3) and Processes to Significantly Reduce Pathogens (PSRPs, Table 4). The details of the most common treatment processes are described below.

Table 3 Processes to Further Reduce Pathogens (PFRPs). Source; US EPA (2003)

Treatment process	Procedure of the treatment process
Composting	Using either the within-vessel composting method or the static aerated pile composting method, the temperature of sewage sludge is maintained at 55 °C or higher for 3 consecutive days. Using the windrow composting method, the temperature of the sewage sludge is maintained at 55 °C or higher for 15 consecutive days or longer. During the period when the compost is maintained at 55 °C or higher, there shall be a minimum of five turnings of the windrow
Heat drying	Sewage sludge is dried by direct or indirect contact with hot gases to reduce the moisture content of the sewage sludge to 10% or lower. Either the temperature of the sewage sludge particles exceeds 80 °C or the wet bulb temperature of the gas in contact with the sewage sludge as the sewage sludge leaves the dryer exceeds 80 °C
Heat treatment	Liquid sewage sludge is heated to a temperature of 180 °C or higher for 30 min
Thermophilic aerobic digestion	Liquid sewage sludge is agitated with air or oxygen to maintain aerobic conditions and the mean cell residence time (i.e., the solids retention time) of the sewage sludge is 10 days at 55–60 °C
Beta ray irradiation	Sewage sludge is irradiated with beta rays from an electron accelerator at dosages of at least 1.0 megarad at room temperature (ca. 20 °C)
Gamma-ray irradiation	Sewage sludge is irradiated with gamma rays from certain isotopes, such as Cobalt 60 and Cesium 137, at dosages of at least 1.0 megarad at room temperature (ca. 20 °C)
Pasteurization	The temperature of the sewage sludge is maintained at 70 °C or higher for 30 min or longer

Table 4 Processes to significantly reduce pathogens (PSRPs). Source; US EPA (2003)

Treatment process	Procedure of the treatment process
Composting	Using either the within-vessel, static aerated pile, or windrow composting methods, the temperature of the sewage sludge is raised to 40 °C (104 °F) or higher and remains at 40 °C or higher for 5 days. For 4 h during the 5 day period, the temperature in the compost pile exceeds 55 °C
Aerobic digestion	Sewage sludge is agitated with air or oxygen to maintain aerobic conditions for a specific mean cell residence time (i.e., solids retention time) at a specific temperature. Values for the mean cell residence time and temperature shall be between 40 days at 20 °C and 60 days at 15 °C
Air drying	Sewage sludge is dried on sand beds or on paved or unpaved basins. The sewage sludge dries for a minimum of 3 months. During 2 of the 3 months, the ambient average daily temperature is above 0 °C
Anaerobic digestion	Sewage sludge is treated in the absence of air for a specific mean cell residence time (i.e., solids retention time) at a specific temperature. Values for the mean cell residence time and temperature shall be between 15 days at 35–55 °C and 60 days at 20 °C
Lime stabilization	Sufficient lime is added to the sewage sludge to raise the pH of the sewage sludge to 12 for $\geq$ 2 h of contact

Anaerobic digestion

Anaerobic digestion is a biological process that uses bacteria that function in an oxygen-free environment to convert volatile solids into carbon dioxide, methane and ammonia. Those reactions take place in an enclosed tank that may or may not be heated, because the biological activity consumes most of the volatile solids needed for further bacterial growth (US EPA 2003). The mesophilic anaerobic digestion (MAD) of biosolids has been reported to produce biosolids Class B, this treatment process is common in USA (Wong et al. 2010). However, most Middle East countries used air-drying as will be presented below (Sect. "Air drying").

Telles et al. (2002) investigated the reduction of TC, FC, *P. aeruginosa* and FS by anaerobic digestion of sewage sludge generated from STP in Maringá-Paraná, Brazil. The study showed that 99.9% of FS, 96.3% of TC and 95% of *P. aeruginosa* were reduced at the end of the treatment process. These findings revealed the potential of anaerobic digestion in the reduction of pathogenic bacteria from the biosolids. Wakelin et al. (2003) showed that FC was reduced from 7.5 $\log_{10}$ CFU g^{-1} in the raw sewage to 6.3 $\log_{10}$ CFU g^{-1} in dewatered biosolids after mesophilic anaerobic digestion process. The main pathogen-reducing factor during thermophilic anaerobic digestion is temperature in relation to time, while the competition among microorganisms for nutrients is the limiting factor that reduces pathogen amounts in anaerobic mesophilic treatment of biosolids (Smith et al. 2005). It can be noted that the thermophilic treatment is more efficient than the mesophilic because the biosolids have high contents of nutrients; therefore, the competition between microorganisms in the biosolids is weak. Carrington (1998) also elucidated that temperature is not the main factor in mesophilic anaerobic digestion processes at 35 °C, but this process produces fatty acids and other products that are lethal to many pathogenic bacteria.

Ziemba and Peccia (2011) evaluated mesophilic, thermophilic, temperature-phase, and high-temperature (60 or 70 °C) batch pre-treatment digester configurations for *E. coli* and *E. faecalis* inactivation potential. The results revealed that the inactivation rates have increased dramatically at temperatures above 55 °C. In mesophilic treatment, 1–2 log inactivation was recorded, while was 2–5 log inactivation at 50–55 °C in thermophilic and temperature-phase treatments. Incorporating a 60 or 70 °C batch pre-treatment phase has achieved completed inactivation (over 100 log reductions) of *E. coli* and *E. faecalis*.

Chen et al. (2012) investigated inactivation of *Salmonella* sp. *E. coli* and *Shigella* sp. during mesophilic anaerobic digestion of biosolids. They found that the anaerobic digestion process efficiency reduces *Salmonella* sp. and *E. coli* during the retention time from 11 to 25 day. However, the reduction of *Shigella* sp. was insignificant. However, the long period of the treatment process (25 days) might lead to increase the overload of the STP and thus required high capacity to store the received sewage before the treatment process. Astals et al. (2012) studied the mesophilic anaerobic digestion, thermophilic anaerobic digestion and mesophilic anaerobic digestion followed by a 60 °C or by an 80 °C hygienization treatment of sewage sludge. They recorded that both thermophilic anaerobic digestion and mesophilic anaerobic digestion followed by a hygienization step reduced *E. coli* to meet the standards limits recommended by US EPA and the European legislation for land application.

According to aforementioned studies, the anaerobic digestion process of biosolids showed potential for effective reduction of pathogenic bacteria to meet the standards limits recommended by US EPA. However, Viau and Peccia (2009) detected *Legionella pneumophila*, *S. aureus* and *C. difficile* in biosolids generated from 29 STPs in USA which was treated with mesophilic, temperature-phase anaerobic digestion and composting process, which

indicates to the ability of some pathogenic bacteria to survive in the biosolids even after the thermophilic treatment. This might be due to the presence solids materials which might protect the bacterial cells form the temperature actions. Chen et al. (2011) indicated that the pathogenic bacteria in the treated biosolids could regrow again during the storage periods of biosolids. This is due to the microbial response to substrate release and environmental changes, such as oxygen, which favour the bacterial regrowth during 1–2 weeks of storage period.

Aerobic digestion

In aerobic digestion, biosolids are biochemically oxidized by bacteria in an open or enclosed vessel. Under proper operating conditions, the volatile solids in biosolids are converted to CO_2 and H_2O (US EPA 2003). The PSRPs described aerobic digestion as follows: Sewage sludge is agitated with oxygen to maintain aerobic condition for indigenous cell. Time and temperature shall be between 40 days at 20 °C and 60 days at 15 °C. Aerobic digestion carried out according to the part 503 requirement typically reduces pathogenic bacteria by 2 log (US EPA 2004a). Kabrick and Jewell (1982) found that *Salmonella* spp. was reduced to undetectable levels in an aerobic reactor at 35 °C in 24 h, while at 60 °C *Salmonella* spp. was eliminated in few hours.

Han et al. (2011) studied the efficiency of anaerobic lagoon fermentation (ALF) and autothermal thermophilic aerobic digestion (ATAD) for removal of pathogenic bacteria in raw swine manure. The results revealed that in raw swine manure, *Dialister pneumosintes*, *Erysipelothrix rhusiopathiae*, *Succinivibrioan dextrinosolvens*, and *Schineria* sp. were detected. ATAD exhibited more efficiency to eliminate of these pathogens than ALT. In the mesophilic ALF-treated swine manure, *Schineria* sp. and *Succinivibrio dextrinosolvens* were still detected, while were undetected in ATAD. These findings support the superiority of ATAD in selectively reducing potential human and animal pathogens compared to ALF, which is a typical manure stabilization method used in livestock farms. In a comparison between aerobic and anaerobic treatment of biosolids, it can be indicated that the anaerobic process is more efficient than the aerobic process, but the limitations to apply the anaerobic treatment lie in the design and the maintenance in the developing countries.

Air-drying

Air-drying allows partially digested biosolids to dry naturally in the open air. Wet biosolids are usually applied to a depth of approximately 23 cm onto sand drying beds or even deeper (US EPA 2000). The sewage sludge is left to dry by the evaporation. The effectiveness of the air-drying process depends very much on the local climate. Drying occurs faster and more completely in warm and dry weather and slower and less completely in cold and wet weather. The density of pathogenic bacteria is reduced by approximately 2 log under these conditions of air drying. However, this reduction is not enough to produce high quality of biosolids for safe disposal. More disadvantage of air drying is the deficiency in the produce biosolids with only 38% volatile solids destruction (US EPA 2003).

Ward et al. (1981) studied the response of *Klebsiella*, *Enterobacter*, *Proteus mirabilis*, *S. typhimurium*, *E. coli* and *S. faecalis* to the moisture loss through evaporation at 21 °C. In raw sterilized sludge with 5% solids, seeded enteric bacteria initially increased in concentration with dewatering. This increase was always followed by a consistent decrease in numbers with further dewatering, especially below 50% moisture. A 1–2 log reduction was seen for all bacterial strains except *Proteus* sp. which showed a 4 log reduction within 7 days as biosolids percent solids increased to 95%. Rouch et al. (2011) examined the inactivation of *E. coli* and *C. perfringens* during the air-drying of anaerobically digested biosolids generated from two STPs in Victoria, Australia. The results found that *E. coli* were reduced to below 10^2 CFU g^{-1} dry solids after drying of 8–15 days and the biosolids met US EPA standards for Class A. *C. perfringens* appeared to be a better indicator.

Air-drying of biosolids is common in Middle East countries because the climate is semi-arid to arid and the temperatures range from 27 to 50 °C (FAO 2008). Besides, this process is not expensive and easily implementable. However, one of the major disadvantages of this process is the vector attractions, which increase as result to volatile solids in the biosolids. The pathogens are transmitted from air-drying basin by the vectors to animals and human (Palmgren 2002). STPs in Yemen overcame this problem by using pesticide but this method leads to accumulate of pesticide in the lands during land application of biosolids. To overcome the vector attraction, US EPA recommended that the reduction of VS % should be more than 38%. The lime stabilization was reported to achieve these standards as will be presented in section 10.6. Another way to prevent the vector attraction is by covering the air-drying basin by transparent polyethylene terephthalate (PET) as in the case of SODIS of water and wastewater. However, more research is needed in this regards.

Effect of storage

Biosolids are stored inside buildings and outside enclosed in steel or concrete tanks. The storage duration of biosolids could strongly affect the survival of pathogenic bacteria. During storage, the biosolids undergo biochemical changes, which depend on the storage temperature. These

changes effect on the survival of pathogenic bacteria. The storage of biosolids at low temperatures prolong pathogens survival, while at high storage temperatures free ammonia which would shorten the pathogens chances for survival is produced (Svoboda and Carcluie 2003).

Ahmed and Sorensen (1995) evaluated pathogen inactivation during storage of biosolids. The pathogens tested included *S. typhimurium*, *Y. enterocolitica*, *Campylobacter jejuni*. Biosolids samples seeded with the pathogens were incubated under both anaerobic and aerobic conditions in reactors at 5, 22, 38 and 49.5 °C for up to 62 days. They revealed that pathogenic bacteria decreased in stored biosolids at all temperatures and survival decreased as the temperature increased.

Liu (2000) observed that FC in biosolids was inactivated naturally by storing the sample in an airtight container at room temperature (20–23 °C). This natural inactivation started after 17–28 days of storage and FC were not detected after 100 days of storage. However, FC density in samples stored at − 22 and 4 °C had no significant reduction in 3 months. Nicholson et al. (2000) recorded that slight increases in temperature during summer destroy *E. coli* O157 and radically decrease number of *Salmonella* after a few months.

Placha et al. (2001) found that in pig slurry, *S. typhimurium* survived for 26 days in summer and 85 days in winter and FC were reduced by 90% in 35 and 233 days during summer and winter time, respectively. However, storage alone is not regarded as an effective way to inactivate pathogens in sludge (Carrington 1998). Avery et al. (2005) monitored the decline in *E. coli* O157 in different wastes over 64 days. They concluded that the storage decreases the amount of *E. coli* O157 but does not eliminate the pathogen. According to Sahlstrom et al. (2006), the sludge is stored usually for 6 months, in heaps outdoors on the ground or on a concrete surface, before being used. After 2 months of storage of sewage sludge, no *Salmonella* spp. could be isolated in the heaped material. Al-Gheethi et al. (2014) observed that the biosolids stored for 24 weeks at room temperature (25 ± 2 °C) met the standards limits recommended by US EPA, Class A, and suitable for reuse in the agriculture as fertilizers.

Heat treatments

Heat drying involves using active or passive dryers to destroy pathogens and remove water from biosolids. In this process, biosolids are dried with hot gases at temperatures greater than 80 °C to reduce the moisture content to 10% or lower. Pathogenic bacteria, viruses, and helminth ova are reduced to below detectable levels in properly operated heat-drying systems (Lucero-Ramirez 2000). Pathogenic bacteria are inactivated during exposure to heat, above their optimum growth temperature. The period of exposure is depended on the temperature as well as the bacterial species (Himathongkham and Riemann 1999).

The total inactivation of pathogenic bacteria in biosolids requires a holding time of 4.78 h at 60 °C compared to 30 min at 70 °C (US EPA 1994). Moce-Llivina et al. (2003) recorded significant reductions in indigenous *E. coli* to below detectable levels after 30 and 90 min at 80 °C. The indigenous FS was only reduced between 1.4 and 1.8 $\log_{10}$ units after 30 min but undetectable after 90 min. *S. choleraesuis* cells that were added to sludge at high counts were reduced by approximately 6 $\log_{10}$ after 30 min at 80 °C and by more than 8 $\log_{10}$ after 60 min at 80 °C. They concluded that temperature of 80 °C for 30–60 min would probably qualify the biosolids product as Class A requirements.

The EPA Part 503 regulations consider heat drying as PFRPs and suggest that, the temperature of the biosolids should exceed 80 °C but no time is given treatment period. In other instances, it seems clear that processes using higher than 60–70 °C can produce biosolids Class A (Springthorpe and Sattar 2004).

Abdel-Monem et al. (2008a) investigated the inactivation of TC, FC, FS and *Salmonella* spp. in biosolids by thermal treatment at 60 and 80 °C. The study revealed that the inactivation of *Salmonella* spp. and FS was significantly greater than the inactivation of TC at 80 °C for 90 min. After heat treatment at 80 °C for 120 min, TC reduced by 5.5 $\log_{10}$ while FC, FS and *Salmonella* were undetected and the biosolids met the US EPA, Class A standards for biosolids reuse.

Lime stabilization treatments

The principle objectives of alkaline stabilization are to reduce the activity of pathogenic bacteria and inhibit their regrowth and thus reduce the health hazard associated with the biosolids (WEF 1995). The most commonly used alkaline is the quick lime (calcium oxide, CaO) and its derivative hydrated lime or slaked lime (calcium hydroxide, $Ca(OH)_2$) which are used due to their low cost. Adding adequate volume of CaO to the biosolids leads to increase of pH to 12 (or higher) and temperature to be between 55 and 70 °C, and as results for these conditions the pathogenic bacteria are inactivated or destroyed (Hansen et al. 2007).

Plachy et al. (1996) demonstrated that *S. typhimurium* was eliminated from biosolids after 60 min of hydrated lime addition. Reimers (1997) found that the inactivation of pathogenic bacteria by lime was not only because of the hydroxide ions but also because of silicate components in lime. This conclusion was derived from studies by comparing pathogen inactivation effects of sodium hydroxide and calcium hydroxide. According to Schwartzbrod et al. (1997) the raising of the pH to at least pH 12 by the use of lime has

the effect to suspend microbiological activity and lime conditioning in specific conditions (pH of 12.5 for 2-4 months) can cause helminths reduction by 98.5% and a virus inactivation by 90%, while biosolids met the Class B requirements when the pH was 12 or above after 2 h of contact.

Amer (1997) studied the effect of quick lime (CaO) and cement dust additions to biosolids on the reduction of pathogenic bacteria. Rapid reduction in TC, *Salmonella* and *Shigella* counts were achieved after the addition of quick lime and cement dust. US EPA (2000) reported that biosolids Class A requirements can be achieved when the pH of the mixture is maintained at or above 12 for at least 72 h, with a temperature of 52 °C maintained for at least 12 h during the period.

Bina et al. (2004) investigated the effect of liming on the microbiological quality of urban liquid raw biosolids. The lime was added to increase the pH of biosolids to pH 11 and 12 for 2, 24, 72 and 120 h. *Salmonella* spp. were inactivated completely in treated biosolids after 2 h. 99% of FC reduction was obtained for two ranges of pH (pH 11 and 12). Biosolids treated with lime met US EPA standards for Class B and Class A after 2 and 24 h, respectively. At pH higher than pH 11 and 12 treated biosolids with lime met vector attraction reduction requirements after 2 h.

Sasdkovd et al. (2005) stored biosolids amended with zeolite and lime for 42 days at a temperature between 14.5 and 17.9 °C. Samples were taken at 5-day intervals for the determination of TC, FC and FS. The results revealed a decrease in plate counts of the observed bacteria in experimental substrates compared to the control biosolids. Yurtsever (2005) found that about 150–200 g CaO kg^{-1} TS is satisfactory for keeping pH on recommended levels and to reduce FC to less than detectable limits.

In alkaline treatment study conducted by Abdel-Monem et al. (2008b), the results revealed that 19.12 g of quicklime kg^{-1} TS reduced FC to meet the requirements of US EPA standards for Class B after 2 h and Class A criteria after 24 h. *Salmonella* spp. reduced to meet Class A criteria after 120 h. At 29.41 g kg^{-1} TS, FC and *Salmonella* spp. dropped to below US EPA Class A limit after 24 h. At 42.65 g kg^{-1} TS, FC has reduced to below detection level after 24 h and *Salmonella* after 2 h. US EPA requirements for Class B met after 2 h, while the requirements for Class A met for *Salmonella* spp. after 2 h and FC after 24 h. Finally, 133.52 g kg^{-1} TS of lime was found to be very effective, *Salmonella* spp. and FC were inactivated completely after 2 h. To reduce vector attraction, US EPA recommended that the reduction of VS % should be more than 38% (US EPA 2003). In the study conducted by Al-Gheethi (2008), the mass of volatile solids in the biosolids has reduced by 63.47% with 42.65 g kg^{-1} TS and 91.41% with 133.52 g kg^{-1} after 120 h.

Toth et al. (2012) investigated the alkaline treatment of animal manures by hydrated lime ($CaOH_2$) to eliminate *S. enterica* and *E. coli* O157:H7. They found that both pathogens were killed when pH was more than 11.0 within 2 weeks. Farzadkia and Bazrafshan (2014) studied the lime stabilization of biosolids on the inactivation of FC. The study was conducted in a reactor for 6 weeks, the biosolids stabilization with hydrated lime reduced FC more than 99.99% and the stabilized biosolids met US EPA standards limits of class B.

Pathogen growth potential (PGP)

PGP is defined as the ability of inactivated bacteria to regrow again in the disinfected sample. The effectiveness of disinfection process for treated sewage and biosolids depends on inactivation of pathogenic bacteria. However, it is well known that under unfavourable conditions, bacteria may transfer into a dormant state called viable but non-culturable (VBNC), which mean the microorganism has slow metabolic processes and no cell division (Oliver 2005). Therefore, the development of the detection, enumeration and viability assessment of pathogenic bacteria in biosolids is required (Sidhu and Toze 2009). For microbial cell, death is the irreversible loss of microbial cell to growth and reproduction. To verify this fact the isolation on solid culture medium is the suitable method. The microbial cell is considered dead if they fail to form colonies. However, the cultural based technique depends on culture medium used. Bacteria may fail to grow on solid media and need to be cultured first in enrichment media (Block 2001; Efaq et al. 2015).

The condition of storage for disinfected sample affect the ability of bacteria to regrow, for example, bacterial inactivation by UV irradiation may reach to 99.99%, but if the disinfected sample stored at optimal conditions the regrowth of inactivated bacteria may reach to 90%. This means the bacteria can repair damage caused by UV. Al-Gheethi et al. (2013b) found that *Salmonella* spp. *S. aureus* and *E. faecalis* grow again in treated sewage exposed to SODIS for 6 h, when the samples were incubated at 37 °C for 4 days. The bacterial regrowth may occur if the treatment process did not lead to physical damage of bacterial cell and PGP bioassay should be carried out (Al-Gheethi et al. 2016a, b; Efaq et al. 2017). PGP has been used to assay bacterial growth in drinking water disinfected by ozonation and different bio-filter systems (Berney et al. 2006; Vital et al. 2010).

Choi et al. (1999) stated that the main factors, which convert dormant bacteria cell to be active, the factors

included the incubation and substrate addition such as glucose, amino acids or yeast extract. Luna et al. (2002) found that after nutrient enrichment, a large fraction of dormant bacteria (6–11% of the inactivated bacterial number) was reactivated.

The factors, which might induce bacterial cell to be VBNC, are less nutrients, nucleotide, inactive cell membrane, size of bacterial cells and presence of antibiotics, which inhibit DNA synthesis without affecting other cellular metabolic activities (Gasoll et al. 1995; Joux and LeBaron 1997; Luna et al. 2002; Kim et al. 2009). Therefore, some authors recommended using molecular techniques such as PCR to detect the presence of VBNC bacteria after disinfection process (Straub et al. 1993; Choi et al. 1996). Chen et al. (2012) determined log reduction of *Salmonella* sp., *E. coli* and *Shigella* sp. during mesophilic anaerobic digestion of sludge by MPN and PCR. They found that log reduction determined by MPN was much higher than the data recorded by quantitative PCR. They explained their findings due to the presence of viable but non-culturable pathogen cells.

However, other investigators reported that PCR technique is not suitable after disinfection process as PCR can still recognize or detect the DNA fragments of the bacterial cells that have been killed during disinfection processes, thus, yielding false positive results (Baloda and Krovacek 1994). Fijalkowski et al. (2014) detected the presence of *E. coli* O157:H7 in wastewater and sewage sludge in Poland by quantitation method of (EMA) real time-PCR. They found that *E. coli* O157:H7 gene copies were detected in primary influents and final effluents in winter from municipal wastewater treatment plant. However, the ethidium monoazide bromide (EMA) application revealed false-positive detection of this bacterium in final effluents. They also detected large amount of "free DNA" derived from dead cells that gave false-positive results.

In the last decades, authors have detected the viability of bacterial cells based on metabolic activity, such as detection of RNA transcripts, a positive energy status and responsiveness. The detected RNA transcripts were used to detect viable cells in combination with DNA amplification techniques. The technology depends on the treatment of bacterial cells with photoactivatable, and cell membrane impermeant with nucleic acid intercalating dyes propidium monoazide (PMA) or ethidium monoazide (EMA) followed by light exposure prior to extraction of DNA and amplification. Light activation of DNA-bound dye molecules results in irreversible DNA modification and subsequent inhibition of its amplification. Sample pre-treatment with viability dyes has been used in combination with PCR (leading to the term viability PCR,v-PCR), and increasingly with isothermal amplification method (Fittipaldi et al. 2012). This technique still has some limitations especially when applied to environmental samples.

Some researchers used the culture method and the molecular analysis to investigate the presence of pathogenic bacteria in the VBNC state. Jiang et al. (2013) combined the standards culture method (MPN technique) with the reverse transcription quantitative PCR (RT-qPCR) assay for the quantification of *S. typhimurium*, *E. coli* and *S, flexneri* in the VBNC state during anaerobic digestion of biosolids. They revealed that the cycle threshold (C_T) values from RT-qPCR assays have significant correlation to the counts of *E. coli* ($R^2 = 0.9964$), *S. typhimurium* ($R^2 = 0.9938$) and *S. flexneri* ($R^2 = 0.997$). In addition, they stated that the quantification results of VBNC pathogens using RT-qPCR could provide an improved evaluation of pathogen inactivation efficiency and biological safety in the biosolids.

Real-time quantitative polymerase chain reaction (qPCR) is fast, sensitive, and specific molecular tool for enumeration of pathogens in biosolids. However, the main limitation is that it amplifies all target DNAs, including that from non-viable cells. Therefore, to overcome this limitation the authors suggested that the couple qPCR with PMA (van Frankenhuyzen et al. 2011) or EMA (Fijalkowski et al. 2014) can be used to monitor the presence of viable pathogens in several different matrices.

Current status of sewage treatment in Middle East countries

In the Middle East countries, there is no plan to build tertiary treatment systems (except some STPs in Riyadh, Saudi Arabia which use sand filters as a tertiary treatment process, Al-Jasser 2011). They focus on the basic standard of primary and secondary treatment. Most sewage is reused for agricultural irrigation purpose. This is due to increase in population growth rate, besides present expansion of water networks in these countries without parallel construction of new sewage network, or rehabilitation of the existing ones (Abdel-Raouf et al. 2012).

Most treated sewage and biosolids generated from STPs in Middle East countries have not met the standards limits required by WHO and US EPA. Saleem et al. (2001) investigated the counts FC during summer and winter seasons in the drying biosolids generated from Al-Khobar sewage treatment plant in Saudi Arabia. They found that FC was more than standards limits required for US EPA, Class B. El-Lathy et al. (2009) reported that the concentrations of *Salmonella* spp., in biosolids generated from Zenin sewage treatment plant at Giza Governorate, Egypt were 330 CFU/mL, which means the biosolids, has not met the standards limits recommended by US EPA for biosolids Class A. AL-Jaboobi et al. (2013) stated that FC in the treated sewage canal generated

from STP (primary and secondary processes) located in Sana'a Yemen was more than WHO guidelines. Al-Gheethi et al. (2014) evaluated the efficiency of five STPs in Yemen for reduction of faecal indicators and pathogenic bacteria in the secondary effluents and biosolids. They also found that FC was more than WHO guidelines for sewage effluents as well as US EPA Class A, and B for biosolids.

In comparison to developed countries, Rose et al. (2004) investigated the efficiency of six STPs in USA for reduction of FC. All STPs used primary treatment, four STPs used the biological treatment (activated sludge), one STP used nitrification and one STP used biological nutrient removal. Filtration process is carried out by fabric, sand and anthracite. Disinfection process is carried out by chlorine, sodium hypochlorite and UV irradiation. However, the concentrations of FC in treated sewage at six STPs were more than the WHO guidelines. In the light of the aforementioned, Enerhall and Stenmark (2012) also reported that TC and enterococci were 5.8 and 5.08 $\log_{10}$ CFU/100 mL, respectively in treated sewage generated from STP in Sweden, which used filtration system during the disinfection process. Viau and Peccia (2009) stated that the concentrations of FC in biosolids generated from 29 STPs in USA, which use mesophilic anaerobic digestion (MAD), temperature-phased anaerobic digestion (TPAD), composting the biosolids after anaerobic digestion (COM) corresponded to US EPA regulatory limits for each biosolids Class A and Class B.

Conclusion

The reuse of sewage-treated effluent and biosolids for agricultural purpose has increased extensively in the last few decades in the Middle East countries. Those countries face a severe shortage of water resources and tertiary sewage treatment plants are not available. Treated sewage and biosolids are rich with nitrogen and phosphate that would improve plant growth and soil properties. However, it has also a large diversity of pathogenic bacteria, which represent a potential risk for human and animal. Most of these pathogens could survive for a long time in the environment, because these pathogens produce endospores and others can survive in VBNC state. Therefore, further treatment of sewage effluents and biosolids generated from secondary treatment is necessary to reduce the pathogenic bacteria before reuse for agricultural purpose. Further treatment using technologies such as SODIS, air-drying and lime treatment appears to be more suitable to the nature and climate of Middle East countries and can vouch for their efficiencies. These processes have the potential to reduce pathogenic bacteria in treated sewage and biosolids. Economically, the processes are efficient, easily implementable, natural, with no toxic by-products and most of all are of low cost.

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