

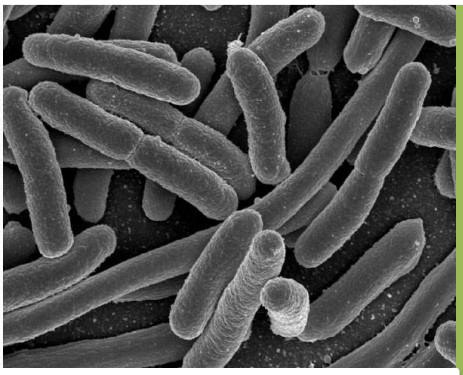

‘Evaluation of the Contemporary Guidelines and Practices of Pathogen Identification, Screening and Treatment in Sewage Sludge to obtain Biosolid Products which are safe for Land Application in Western Australia’

‘Evaluation of the Contemporary Guidelines and Practices of Pathogen Identification, Screening and Treatment in Sewage Sludge to obtain Biosolid Products which are safe for Land Application in Western Australia’

Final Report

Dissertation submitted in partial fulfilment of the requirements for the Degree of Master of Public Health

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ii. Abstract

Sewage sludge largely comprised of waste products generated by humans or their activities often contain various nutrients and minerals that may be used to stimulate plant growth.(1, 2) Humans have used sewage as soil fertilizer for over centuries without an understanding of scientific basis of its benefits. A prominent example of this is the traditional use of 'Night Soil'. Lofrano & Brown (2010) document the use of 'night soil' in London as early as 1300s.(3) The Victorian public health reformer, Sir Edwin Chadwick, reported on the agricultural and economic benefits of using sludge on crops.(4) The concept of sludge from wastewater came to existence in 1870 when Edward Frankland established the tricking filter method for wastewater treatment.(3) However, it was soon established that in addition to nutrients and critical minerals, sludge also contained various pathogens and chemical contaminants which may adversely impact on human and animal health.(5, 6) In developed countries, the management and application of sludge thus became a focus of political and public health regulation. This movement was marked by the United States Environmental Protection Agency's (US-EPA) 503 Rule introduction in 1990s.(3, 5) The 503 rule established standards for treatment and classification of biosolids (refined sewage sludge) that would be beneficially applicable for land use.(3)

Currently, various methods have been established for the treatment of sewage sludge to provide products beneficial for commercial use as fertilisers. These 'treated' products are often termed biosolids. Land application of biosolids has been commercialised over the past few decades. The use of biosolids has now expanded from traditional farming to land reclamation, horticulture, landscaping, forestry, industrial processes and resource/energy recovery.(7) Moreover, with growing human population, economic/financial upheavals and challenges of food security, biosolids are amongst a few cost-effective sustainable options for the production of crops. The expansion of this formidable industry has warranted establishment of guidelines to protect human health. Most of the developed countries have produced guidelines specific to treatment and application of biosolids. In Australia, the use of biosolids is somewhat restricted to agriculture and forestry, with other limited applications.(6, 8) This report examines the current Australian and International guidelines on management of pathogenic content and reduction of pathogens in biosolids.

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1. Introduction

Sewage wastewater has been found to have several nutrients and minerals which can act to stimulate plant growth. However, the application of sewage wastewater directly to vegetation soil poses a range of potential health risks. The presence of heavy metals and pathogens in wastewater can lead to adverse health outcomes.(9) Therefore treatment of wastewater to achieve safer products is inevitably important. The treatment of wastewater to achieve safer pathogen and metallic contaminant levels results in formation of stable organic solid products.(10) Traditionally these products have been termed sewage sludge or sludge. However, advancements in treatment of wastewater distinguish sewage sludge or sludge from a more refined product termed 'biosolids'. Despite significant differences in the properties of these products he term *biosolids* is sometimes confused or used interchangeably with sewage sludge or sludge.(9, 10) The principal contrast between the two products lies in the relative concentrations of pathogens and metallic contaminants. Biosolids are produced following several heating and stabilisation treatment processes, usually resulting in pathogen and metallic concentrations that are relatively harmless to human health.(10) Sewage sludge on the other hand, do not necessarily go through such treatments, and often have significantly high pathogen and metallic concentrations.(10)

Application of biosolids in agricultural practices across the world has shown great potential as an alternative to fertilizers as well as environmental management of wastewater sludge.(4-6, 8) Research suggests that biosolids often include essential organic and inorganic nutrients which adequately support plant growth.(5, 6) Some of these essential nutrients such as nitrogen, phosphorus, sulphur, calcium, magnesium and potassium are abundantly found in sludge. Currie et al. (2002) monitored growth of soybeans over winter and summer for four years and noted that application of biosolids increased yields consistently every year.(11) A recent Australian study by Nash et al. (2011) also observed higher yield of pasture and grape vines following application of biosolids in South-Eastern Australia.(12) These researchers also noted an increased amount of ammonia, phosphorus, nitrogen and carbon in soil.(12) Furthermore, existing research also suggests that biosolids application can positively influence the physical, chemical and biological characteristics of the soil.(13) Biosolids can improve soil structure by increasing water-holding capacity, soil aggregation, and water infiltration. Research also suggests reduction in soil erosion, and decreased bulk density of soil following biosolids application.(12, 13) These improvements in soil structure have a cumulative positive effect on plant growth.

However, the pathogenic and metallic content in biosolids has been a point of concern for national wastewater management authorities. Currently there is no acceptance to global standards of pathogen concentrations in biosolids. Specific

national guidelines however, exist in countries for the management, treatment and application of biosolids, including which the processing of sewage sludge to obtain safer pathogenic concentrations. Most of the countries have either adopted or modified the United States' Environmental Protection Agency (EPA) 503 rule as part of their national biosolids management guidelines. The majority of these guidelines quantify entire sludge pathogenic profile on the basis of a few indicator pathogens.(5-7, 14-16) However, there are certain discrepancies in guidelines for monitoring and quantifying indicator pathogens and metallic contaminants.(5)

This research aims to consolidate the existing literature on management of biosolids and review its relevance of land application to the Western Australian context. Particular emphasis is on the pathogenic quantification of graded biosolids. Despite being important to consider in land application of biosolids, metallic contaminants are out of scope of this research and are therefore not discussed in detail. This document aims to identify the inherent discrepancies in pathogenic quantification in guidelines within Australia and Internationally. This report will also consolidate best evidence on indicator pathogens and review treatment processes to formulate future recommendations for the management of biosolids for the Water Corporation of Western Australia.

1.1. An Overview of the Western Australian (WA) Guidelines for Biosolids Management

Australia is one of the leading producers of biosolids globally.(7, 17) Australian wastewater treatment plants produce approximately 300,000 tonnes of dry biosolids annually.(17) A document published by the United Nations (UN) on consolidated research on biosolids has appraised the quality of Western Australian biosolids in comparison to United States (US) and Canada.(7) In Western Australia (WA), Water Corporation solely controls the waste water treatment plants (WWTPs) currently producing biosolids.(7) The plants in WA produce over 25,800 tonnes of dry biosolids every year.(17) With promising research of the benefits of biosolids applications in WA, the production and use of biosolids is likely to increase.

In Western Australia (WA), biosolids management is a responsibility of the Department of Environment and Conservation (DEC). In February 2011, the DEC published a draft WA Guideline for Biosolids Management.(6) These guidelines identify different types of biosolid products and roles and responsibilities of the supplier and private industry in regards to application of biosolids. This guideline classifies biosolids by constituents based on contaminant and pathogen grading. Contaminant grading for chemical and metallic contaminants is calculated using descriptive statistics. There are three classifications to contaminant grading; C1, C2 and C3, based on the

concentration and number of metallic contaminants. Pathogen grading on the other hand, is based on numerical quantity or population of pathogens in the biosolids and are classified as P1, P2 and P3. These classifications are discussed in detail in a later section. The guideline also suggests routine assessment of the products to monitor pathogen regrowth potential which impacts on the eligibility of biosolids application. Soil quality, type of soil, proximity to water resources, depth of groundwater and slope of land are to be considered as essential environmental determinants in application of biosolids. The monitoring frequency is dependent on the amount of sewage sludge produced and treated over a single year.

Permitted use of biosolids is restricted to its pathogen grading or classification. Table 1 shows the restrictive uses of biosolids based on the pathogen grading as documented in the WA Guideline. Since it is impossible and economically unviable to screen for all pathogens, biosolids are graded based on the amount of indicator pathogens. The WA guidelines only identify *Salmonella* and *E.coli* as sufficient indicators for pathogenic quantity in biosolids. Several treatment protocols and methods are specified to obtain products from sewage sludge. These are discussed in detail in a later section. Different treatment methods are considered to achieve the desired pathogen and contaminant grading. In WA, urban wastewater treatment plants (such as Subiaco) utilise mesophilic anaerobic digestion and lime addition treatment methodology to achieve P1 and P2 grade biosolids whereas the rural towns (such as Busselton, Kemerton and Northam) treat sludge with alum to achieve P2 and P3 grade biosolids.(8, 18) In addition to this, biosolids need a safety assessment which requires fulfilment of a criterion to assess eligibility of application to the area of interest. Items in this criterion include vector reduction, odour reduction, routine monitoring, testing for pathogen regrowth and storage viability.(6) Table 1 shows the relationship between pathogens grades and indicator pathogens in the WA guidelines.

2. Methods

This research aimed to consolidate white and grey literature available on the management of biosolids in terms of the pathogenic content. The search strategies employed a number of free-text keywords as well as controlled vocabulary terms (See Appendix A). These keywords were used to search both white and grey literature. There were three phases to the research strategy which involved sourcing of information from the literature.

The initial strategy involved researching for evidence relating to pathogenic presence in sludge and biosolids. Combinations of key topic specific terms were used; one each from Category A and Category B in this stage. Following this approach, the intermediate strategy involved sourcing of information in relation to guidelines available for management of biosolids. Combinations of key topic specific terms were used from Category A, B and C along with non-specific terms; 'guidelines', 'prevalence' and 'epidemiology'. The final stage of this research involved searching of treatment strategies for sludge and biosolids. Treatment-specific terms were used in combination with Category A, B and C. In all the stages above, the terms were used interchangeably to research with different combinations, without repetition, until the options were exhausted.

White literature research was broadly aimed at obtaining international guidelines on biosolids management. External webpages of reputable government organisations were resourced to obtain creditable documents and information were also extracted using Google and Yahoo search facilities. Websites which were not run by government organisations were excluded from the analysis due to the lack of clarity of authenticity of information. National guidelines were obtained for United States, Canada, New Zealand. However due to language barriers and the general unavailability of resources, guidelines from European countries could not be obtained. Other non-government websites were resourced in this case to obtain an understanding of regulatory mechanisms for wastewater treatment in the European countries. Twenty-nine resources from white literature were included in this research. Australian government reports were provided by the Environmental Health Directorate at the Department of Health in Western Australia during orientation to this research project which was also included in the analysis. Non-literature sources of information involved visits to biosolids application sites (Myalup Pine Plantation) in Western Australia.

Peer-reviewed articles were extracted through The University of Western Australia's 'One Search' facility. Since the focus of this research was on scientific knowledge, popular science databases such as JSTOR, Wiley, MEDLINE, PROQUEST and Science Direct were also specifically researched. A total of 120 articles were extracted throughout the time of this research. Abstracts and results of all articles were further read to screen for inclusion and exclusion of articles. Articles were included if they satisfied coverage of pathogen related information in sludge or biosolids. Articles which did not include information on pathogens were excluded. Most commonly these

excluded articles analysed the metallic and other contaminants in biosolids which were out of scope of this research. Moreover, some other articles which focussed on pathogenic concentration in wastewater only were also excluded. Only studies which were available as full-text options through the search facility and were peer-reviewed were included in the analysis. Initial screening of the articles resulted in exclusion of 59 articles. Of the 61 remaining, 56 journal articles were used in this research.

3. Results

3.1. The Diversity of Human Pathogens in Sewage Sludge and Biosolids

Sewage sludge can contain excessive amounts of pathogens which can be deleterious to human and animal health.(15, 19) The profile, number and diversity of these pathogens vary with population's health, presence of sources (such as industrial and agricultural sites) in the catchment.(19) It is imperative that pathogenic content and activity be closely monitored and reviewed for safety of biosolid applications.(19) Four main types of pathogens can be found in sewage sludge and biosolids; bacteria, viruses, protozoa and helminths. These pathogens can be transferred into humans through air (inhalation), skin or mucous membranes, through vector-borne transmission (through flying insects), or via food, water or other vehicles.(7, 20-22) The pathogens that were found to be present in sewage sludge and biosolids and which pose a potential threat to human health are discussed below.

3.1.1. Bacteria

Surveillance data from the United States indicate that *Campylobacter spp.,* pathogenic forms of *E.coli, Salmonella spp.* and *Shigella spp.* are the most commonly reported bacterial causes of gastroenteritis.(16) A number of these bacterial pathogens have been isolated from raw sludge and biosolids.(19) Recent research has also raised interest in screening for emerging and re-emerging bacteria in biosolids, such as *E.coli 0157, H.pylori* and *L.monocytogenes*.(19) The most important bacterial pathogens present in raw sludge and biosolids are discussed in detail below.

Escherichia coli (*E.coli*) numbers vary significantly in sewage sludge and biosolids (See Table 1). The results from literature review (Table 1) show that *E.coli* can exist in low to significantly high numbers in raw sludge (3.2×10^2 – 7.3×10^7 g^{-1}).(23) The pathogenic forms of *E.coli* are of particular concern given they have a lower infectious dose and high severity of infection. For example, enterohaemorrhagic *E.coli* strain 0157:H7, at low doses (less than 100 viable organisms) has been found to cause severe illness and mortality in children from haemolytic uremic syndrome.(20, 22) The more virulent form 0157:H7 also exists in high numbers in sewage sludge (1.4×10^4 g^{-1}).(23)

E.coli is also known to survive for longer term periods in pasture and animal manure with prolonged survival in winter.(19, 24, 25) The longer survival pattern has implications for the treatment and storage of biosolids. Moreover, a recent Australian study has also shown presence and persistence of variety of *E.coli* strains in sewage sludge and biosolids at different wastewater treatment plants

(WWTPs).(26) Research into regrowth of *E.coli* following application of biosolids to land has also shown significant increase in numbers.(23, 27) Estrada et al. (23) studied survival patterns of *E.coli*, in soil treated with aerobic and anaerobic digested treated sludge from three different WWTPs. Growth was observed in both open air and laboratory controlled environments.(23) Initial regrowth from 0.01 x $10^4 g^{-1}$ to 1.5 x 10^4 g^{-1} was observed within the first 10 days of open air application.(23) The regrowth potential was also found to be higher for heat-dried sludge as compared to mechanical dehydration.(23) By the 80^{th} day following application, however, the number of *E.coli* decreased significantly below 0.01 x 10^4 g^{-1}. Seasonal variation in E.coli growth has also been demonstrated in literature research. A large study by Pillai et al. (24) reported some seasonal variation in occurrence of *E.coli* in treated sludge. Summer and early fall were found to be the peak seasons for *E.coli* numbers.(24)

Salmonella is frequently found in sludge and biosolids. *Salmonella* presence in biosolids has been linked to human gastroenteritis outbreaks in the past. (20, 28) *Salmonella* numbers have been reported to range from 11 and 5900 g^{-1} of dry biosolids (See Table 1). A large Swedish study which investigated eight sludge treatment plants found only a 12% reduction in *Salmonella* presence in different samples of biosolids compared to sewage sludge concentrations.(28) More than half the treated samples still contained *Salmonella* arising from resistance to treatment methods.(28) The most common *Salmonella* species found in this study were *S.hadar, S.newport, S.bardo, S.otmarschen, S.stanley, S.enteritidis phagetype 1, S.panama, S.erta and S.blockley*.(28) Like *E.coli, Salmonella* can also persist and survive in soil treated with biosolids. Zaleski et al. (27) observed that the *Salmonella* numbers increased to five times the limit set by the United States (US) guidelines during storage following treatment.(27) On one of the two sites of biosolids application in this study, the *Salmonella* counts remained above the limit for the duration of the study (27 weeks).(27) No significant seasonal variation has been observed for *Salmonella* in sludge or biosolids.

Listeria monocytogenes is a food borne bacteria that can cause haemolytic disease in immune-compromised individuals and can be lethal for foetus if the mother is infected.(22) Research has shown that *L.monocytogenes* can survive for longer than *Salmonella* on sludge application.(29, 30) Typically, *L.monocytogenes* is found in small numbers in raw sludge (3.8 – 380 g^{-1}). Since *L.monocytogenes* is present in low numbers in untreated sludge, treated biosolids are expected to be virtually free of this pathogen. Garrec et al.(30) observed *Listeria spp*. and *L.monocytogenes* numbers in raw and treated sludge.(30) In their study, removal of *L.monocytogenes* was achieved through simple lime addition and prolonged storage.(30) Garrec et al. (30) also found a marginal seasonal variation in *L.monocytogenes* numbers with peaks during summer and winter.(30)

Campylobacter has been reported in high numbers in sludge (10^3-10^5 g^{-1}). Of particular interest is *C.jejuni* which has been isolated from composted

biosolids.(19) Sahlstrom et al. (28) analysed sludge samples from eight different WWTPs in Sweden. Although numbers were not reported, *C. coli* was found in 9% of the samples and *C.jejuni* was found in 20% of the samples.(28) Research suggests that the current methods for detection of *Campylobacter* in treatment plants may be inadequate(28) and there may be potential limitations in the use of *Campylobacter spp.* as indicators.(28, 31)

L.pneumophila is of particular interest because of its potential transmission through aerosols of contaminated sludge and biosolids.(20) *Legionella spp.* has been found in wastewater in large numbers in several countries (See Table 1). This bacteria is associated with life threatening illness in the immune-compromised individuals.(19) The transmission of the bacteria through garden compost has also been documented.(20) Pillai et al. have reported extremely high numbers of *Legionella* presence in sludge and biosolids (8.6×10^8 g^{-1}). Sidhu and Toze (19) also report on longer survival of *Legionella* following land application; up to seven months in potting mix (19). Viau et al. (31) also observed high numbers of *Legionella* in biosolids, especially *L. pneumophila,* in their research.(31)

Shigella spp. are a well-recognised cause of diarrhoeal outbreaks globally.(19, 20, 24) Scientific evidence on *Shigella* in sludge and biosolids is limited as the bacteria is traditionally found in low numbers.(19) However, monitoring is important given that the infectious dose of most *Shigella spp.* is also relatively low (10-100),

Several other pathogens have been found in sewage sludge, including *Pseudomonas, Aeromonas, Klebsiella, Flavobacterium, Enterobacteria, Helicobacter pylori* and *Mycobacteria*. Many of these pathogens are associated with opportunistic infections, such as *Helicobacter pylori*; a prominent cause of stomach ulcers and cancers in humans.(19, 20). Wery et al. (2008) have reported relatively large numbers of *Pseudomonas aeruginosa, Klebsiella pneumonia* and *Bacillus cereus* in comparison to *E.coli* in wastewater.(32) *Aeromonas* can also be found in high numbers in sewage sludge.(24) Nsabimana et al. report high numbers (up to 1×10^{10} g^{-1}) and wide diversity of *aeromonas* in sewage sludge and wastewater in France.(33) There is limited research on the number of *H.pylori* in sludge and biosolids particularly due to the lack of culture methods available.(20, 22)

Presence and persistence of *enterococcus spp.* in sludge and biosolids has also been reported in some studies.(32, 34-36) Numbers of the *enterococci* have been reported to range from 8.5×10^5 to 8.5×10^6. Bonjoch and Blanch (2009) report numbers of *enterococci* and *faecal coliforms* to be similar in sludge obtained from 2 urban wastewater treatment plants in Barcelona, Spain.(35) They also report diversity and consistence in prevalence of *enterococcus spp.* in their research.

Another issue of concern is the presence of antibiotic resistant bacteria in wastewater. Currently the research into emerging resistant bacteria is relatively limited and therefore conclusions on relationship between bacterial numbers and sewage sludge cannot be drawn.(19, 20) Martins da Costa and colleagues (2006) document increased numbers of resistant *Enterococcus spp*. (on average 2.76 x 10^6 counts) in sludge found in wastewater treatment plants of Portugal.(37) Similarly, Reinthaler and his colleagues (38) have reported multi-drug resistant *E.coli* in Austrian sludge in three different wastewater treatment plants.(38)

3.1.2. Viruses

Several groups of viruses have been isolated from sewage sludge and biosolids. These include enterovirus, norovirus, rotavirus, adenovirus, astrovirus, hepatitis A virus and polyomavirus.(19, 20, 22) Adenovirus, norovirus and enterovirus are most commonly found in high numbers in raw sludge (See Table 1). Few studies, however, have adequately reported on viral diversity and numbers in sewage sludge and biosolids, and therefore it is difficult to generalise on the range of viruses in sludge and biosolids from a global perspective (See Table 1).

Adenoviruses are a common cause of gastrointestinal diarrhoea, respiratory diseases in children and immune-compromised individuals.(22, 25) High numbers of adenoviruses can be present in raw sewage (1.9 x 10^5 – 2.1 x 10^7 PCR units g^{-1}).(39) Significant numbers of enteroviruses (100-1000 PCR units g^{-1}) have also been reported in several studies. Numbers tend to be higher in winter seasons, however, enteroviruses have also been reported to be present all seasons in wastewater in Tunisia.(40, 41) Bofill-Mas (39) and her colleagues conducted a study on urban sewage virus concentrations in four countries. Of the total 28 sewage samples they collected, 96 per cent were positive for adenovirus and polyomavirus (10^4 – 10^8 viruses per 4 ml).(39) Polyomaviruses can cause nephropathy, leukoencephalopathy and are associated with increased risk of colon cancer.(20, 39) Several other viruses such as coronavirus and picobirnavirus, have been found in low numbers in sewage sludge.(20, 22)

3.1.3. Protozoa and Helminths

Cryptosporidium and *Giardia spp.* are frequently isolated from wastewater and sewage sludge.(19) Both these types of protozoa can cause severe diarrhoea. Helminth ova are also frequently isolated from sewage sludge.(19, 20) Helminths are expected to be concentrated in sludge and have been reported at around 0 – 9 ova (viable eggs) on average.(19) *Cryptosporidium* oocysts have been reported between 0.74 – 6.7 ova (viable eggs), which is below the

infectious dosage. *Giardia* cysts, however have been reported in high numbers (on average 3.5 x 10^5 ova) in a Water Environment Research Foundation (WERF) report from England, UK.(24) Levels of *Cryptosporidium* oocysts tend to vary seasonally, while *Giardia* oocysts are commonly isolated all seasons.(19) Rapid and cost-effective identification methods of protozoa from sludge and biosolids are limited, therefore helminth ova are more commonly used.(19)

Table 1. Summary of Pathogen quantities in (Raw Sludge and Treated Sludge for Virus, and Raw Sludge and Minimal Infectious Dose for Bacteria and Protozoa) with Log Reduction with Effective Treatments

Virus	Raw Sludge (g^{-1})[A]	Treated Sludge (g^{-1})[A]	Log Reduction with Effective Treatments			
			AS	MAD	TAD	Lime with High Temperature
Enterovirus	(19) $1x10^{2} – 1x10^{4}$ (L^{-1}) (24) $7.1 x 10^{1}$	$4.5 x 10^{2}$ (19) 30 – $1.61x10^{3}$ (L^{-1})	Anaerobic Digestion			
Norovirus	(42) 10^{3}-10^{6} (L^{-1}) (43) $1 x 10^{6}$			4.45(44)		None detected (43)
Rotavirus						
Adenovirus	(19) $1.9 x 10^{5}$ (PCR units) (24) $2.1 x 10^{7}$	(19) $1 x 10^{4}$ (PCR units)				
Astrovirus						
HAV		(19) $1.9 x 10^{5}$ (PCR units)				
Polyoma-virus		(19) $1.2 x 10^{4}$ (PCR units)	Aerobic Digestion			

Bacteria	Raw Sludge (g^{-1})[A]	Minimal Infectious Dose	Log Reduction with Effective Treatments [B]			
			AS	MAD	TAD	Lime with High Temperature
Faecal Coliforms	$7 \times 10^1 – 1.1 \times 10^{10}$ (19) $7.2 \times 10^5 – 1.2 \times 10^8$ (45) 1.8×10^7 (24) $5.1 \times 10^5 – 8.3 \times 10^5$ (29) $3.7 \times 10^7 – 3.1 \times 10^8$ (35)		0.79-4.18(19)	1.41-2.2(19) 6.20(28)	3.02-5.6(19) 6.65(28)	6.8(45)
Enterococci	$7.2 \times 10^5 – 2.6 \times 10^6$ (19) 8.4×10^5 (24) $5.3 \times 10^6 – 8.5 \times 10^6$ (35)		0.5(19)	1.1-3(19) 4.5(28) 0.88-2.68(35)	4-5(19) 4.5(28)	
Aeromonas	1.0×10^8 (24) up to 10^{10} (33)					
Campylo-bacter	10^3-10^5 (19) 1×10^3 (31)	500	5.0; 50 days(19)	0.34 (19) 0.32 – 0.36 (31)		
Salmonella	$1.1 \times 10^1 – 5.9 \times 10^3$ (19) $3.8 \times 10^1 – 3.3 \times 10^3$ (45) 1.1×10^1 (24) 10-100 (46) $1.3 – 4.2 \times 10^4$ (32)	10^4 - 10^7	0.12-0.96(19)	0.86-2.26(19) 2.23 (31)		2.41(45)
E.coli	3.2×10^2 - 6.0×10^4 (19) $9.5 \times 10^5 – 7.3 \times 10^7$ (45) 1.1×10^7 (24)	10^6 - 10^8	0.9(19)	0.9-2.1(19) 5.54(28)	2.6-4.4(19) 6.0(28)	6.7 (45)

	10^4 - 10^5 (46) 5.0 x 10^5 – 1.3 x 10^6 (31) 2.2 x10^5 – 6.1 x 10^5 (29) 2.6 x 10^6 – 1.5 x 10^8 (32)			3.36(31) 1.66	2.2(47)	
E.coli 0157:H7	1.4 x 10^4 (24)	<10^2				
Shigella	4.6 x 10^1 (24)	10^1 - 10^2				
Legionella	8.6 x 10^8 (24)					
Listeria mono-cytogenes	3.8 – 3.8x10^2 (19) 60 – 80 (31) 4 - 190 (30)			2.23 (31)		

Protozoa	Raw Sludge (g^{-1})[A]	Minimum Infectious Dose	Achievement of less than one viable (oocysts or cyst)			
			AS	MAD	TAD	Lime with High Temperature
Giardia	3.5 x 10^5 cysts (24)	10^1 – 10^2 cysts	1.40(19)			30 mins at 50^0C(48)
Helminths	3.8 x 10^{-1} (24) 1.7 (49) 0 – 9 eggs (19)					
Cryptosporidium	0.74 – 6.7 oocysts (49)	10^1 oocysts	2.96(19)			

Aerobic Stabilization (AS); Mesophilic Anaerobic Digestion (MAD); Thermophilic Anaerobic Digestion (TAD)

[A] Numbers of pathogens are in per gram of dry weight, unless otherwise specified.

[B] Time taken to achieve the log reduction has been included where it was reported in the study.

3.2. Sewage Sludge Treatment Processes

Significant research has been conducted on the treatment of sewage sludge to achieve biosolids products. This report discusses the sewage sludge treatment from conservative to advanced methodologies. Sewage treatment occurs in two different stages: primary and secondary.(10) The primary treatment stage involves sedimentation of inflowing wastewater to remove the amount of suspended solids.(10) Secondary treatment of the inflowing sludge is usually customized to achieve the desired level of pathogenic and contaminant content suitable for application. Most treatments use a combination of treatment processes to satisfy the regulatory requirements of environmental protection agencies and authorities.(6, 7, 13-17, 50-55) These can include clarification, stabilization, conditioning, thickening, dewatering and drying. The processes most relevant to treatment of pathogenic content in sewage sludge are outlined below.

3.2.1. Clarification

Clarification involves separation of suspended particles from the inflowing wastewater that has gone through primary treatment. This inflowing wastewater is also termed effluent or liquid sludge.(10) This process can be achieved in three ways: sedimentation, flotation and membrane clarification.(10) The clarification process is not specifically targeted at removal of pathogens from wastewater, however, it can remove a marginal percentage of inflowing cysts or protozoa. This is exemplified in the research conducted by Gomez et al. (56) where the researchers measured pathogen quantities following clarification processes. They observed that although smaller pathogens such as *E.coli* numbers were only marginally reduced, more than 99.85% of pathogenic nematode eggs were successfully removed during the process.(56)

3.2.2. Stabilization

Stabilization process involves chemical and physical applications to liquid sludge mainly to reduce the pathogen quantity and to eliminate odours in order to reduce vector attraction.(10, 57) This process is widely used by WWTPs following the US EPA 503 rule. The final sludge product can be acquired by several stabilization processes: aerobic stabilization, alkaline stabilization, anaerobic digestion, composting and pasteurization.(10, 57)

3.2.2.1. Aerobic Stabilization

This process involves heating of the effluent or liquid sludge in the presence of oxygen to oxidize the organic content in sludge. This process results in a

decrease in the overall pH of the sludge which assists in removal of pathogens.(10, 57) Several adjustments have been adapted to this technique for commercial and financial viability in production of biosolids. The more commonly known adjustments include thermophilic aerobic digestion, anoxic/aerobic digestion and simultaneous sludge digestion and metal leaching (SSDML).(10)

3.2.2.2. Alkaline Stabilization

This process involves addition of lime or alkaline solution to the effluent to raise its pH greater than 12. This environment is unfavourable for growth of pathogens and also eliminates odour related issues. Most common methods of alkaline stabilization is addition of lime.(10) Lime addition is usually accompanied by heating which has been found to be most effective in treatment of most pathogens in sewage sludge.(48)

3.2.2.3. Anaerobic Digestion

This process involves heating of the effluent in the absence of oxygen or air which results in production of methane (biogas) and carbon dioxide. This treatment method involves a balance between bacteria, absence of oxygen, neutral pH and sufficient nutrients such as nitrogen and phosphorus.(10, 57) This treatment can also be adapted to achieve the desired level of biosolid products. Most commonly these adjustments include two-stage digesters, anaerobic-baffled reactor, Columbus biosolids flow-through thermophilic treatment, high rate plug flow and temperature-phased anaerobic digestion.(10) The two stage digester involves heating in two digesters and is primarily used to concentrate biosolid and generate biogas.(57) This process and the anaerobic-baffled reactor, which aims to treat sludge with less emphasis on yield of biosolids, are commonly used in developing or low income countries. The most important adjustment, however, is the temperature-phased anaerobic digestion, which involves thermophilic and mesophilic anaerobic digestion.(57) This process combines thermophilic and mesophilic anaerobic digestion at moderate temperatures (55^0C and 35^0C respectively) to achieve higher quality of biosolid products.(10) This process can be further adjusted to include fermentation which provides biosolid products as suitable substitutes to fertilizers.(10) Thermophilic Anaerobic Digestion (TAD) is most commonly adapted by WWTPs and is a model of comparison for research studies.

3.2.3. Composting

Composting is a method of degrading organic content of biosolids under controlled aerobic conditions.(10) It is generally assumed that the end product through this process is virtually free of pathogens, which makes these products suitable to be marketed to the public. This method utilizes high temperatures

(50^0C – 70^0C) initially to eradicate pathogens from the effluent.(10) Further thermophilic and mesophilic treatment of the product achieves the desired profile of the biosolid product. Finally bulking agents are used to increase the porosity of the treated sludge.(10) Four common methods of composting the treated product include aerated static pile, windrow, in-vessel and vermin-composting.

3.2.4. Pasteurisation

Pasteurisation is another conservative method that involves heating the effluent at temperatures more than 70^0C.(10, 57) This method is less preferred in treatment of sewage sludge to achieve biosolid products. Pasteurisation does not place as much emphasis on the nutrient content of biosolids.

3.3. Selective Inactivation of Pathogens

Almost all sludge treatments producing biosolids involve dewatering as an initial step. Dewatering reduces the water content in sludge, and research suggests that the pathogenic numbers in biosolids can potentially be higher than the sludge or wastewater it is derived from (19, 46). Wery et al. (2008) observed levels of *E.coli, Salmonella spp.*, and *Clostridium perfringes* throughout different stages of a wastewater treatment process.(32) The reported number of these pathogens decreased during the initial treatment processes of screening and settling. However, following centrifugation, dewatered sludge in their study observed an increase for all pathogens.(32)

Research suggests that time and temperature are key aspects to inactivation of bacteria in all treatment methods.(10, 57) Table 1 shows four major treatment methods and their effectiveness against the most commonly found pathogens in biosolids. *Faecal coliforms*, *enterococci*, *Salmonella* and *E.coli* have been shown to be reduced effectively by most anaerobic digestion methods. *Campylobacter* is the only exception, in this case, with highest log reduction through aerobic stabilization (See Table 1). Combination of lime and heating has shown even stronger log reductions for *faecal coliforms*, *Salmonella* spp. and *E.coli*. A growing concern in treatment of sewage sludge is the presence of resistant and newly emerging pathogens such as *E.coli* 0157:H7 and *Helicobacter pylori*.(19)

In general, anaerobic digestion and composting inactivate bacteria to a greater degree than aerobic stabilisation.(19, 21, 23-25, 27-29, 45, 46, 49, 58-61) In particular thermophilic anaerobic digestion (TAD) inactivates a high proportion of bacteria more than mesophilic anaerobic digestion (MAD) if the sludge is digested.(19) Sahlstrom et al. (28) report that *Salmonella*, *E.coli* and faecal

coliforms were less frequently isolated from thermophilic digested and composted biosolids compared to those that were treated with mesophilic digestion.(28) Their research shows highest log reduction through TAD maintained at 34^0C to 55^0C. However, their findings also raise concern in relation to *Salmonella*'s persistence in biosolid products and potential for regrowth in prolonged storage.(19, 28) These conditions included traditionally stored biosolids in warehouse, bagged biosolids, composted biosolids and soils treated with biosolids.(19) An Austrian study reported thermal treatment to be optimal for *E.coli* eradication whereas stabilization and dehydration only minimally reduced *E.coli* numbers.(47) Viau et al. (31) compared MAD, TAD with composting to observe presence of *Legionella* and adenoviruses.(31) Their observations were similar to that of the Swedish study for TAD's effectiveness in reducing bacterial pathogens in comparison to MAD.(28)

Composting of sludge can effectively reduce a majority of pathogens found in sewage sludge. A review of pathogen inactivation by MAD and composting was conducted by Viau et al. (62) which demonstrates that composting achieved reduced numbers of *Salmonella*, *E.coli*, *faecal coliforms*, *Enterococci, Listeria* and *Clostridium*.(62) However, this research also confirms the inability of composting to inactivate *Legionella* as previously demonstrated by Viau et al. (31). Wery et al. (2008) studied *E.coli, Salmonella spp., Clostridium perfringens,* and *enterococcus spp.* numbers during different stages of composting process.(32) Overall, the composting process observed 4 log reductions for *E.coli*, and 2 log reductions for *C.perfringens* and *enterococcus spp*.(32) Following completion of maturation and storage process, all pathogen numbers were either undetectable or less than 100 with the exception of *enterococcus spp.(32)*

Moreover, survival and regrowth of bacteria can vary in soil with different treatment methods. Estrada et al. (23) analysed application of biosolids from two WWTPs to compare survival of *E.coli*, faecal coliforms and *Enterobacteriaceae* in aerobically stabilized sludge and anaerobically digested sludge.(23, 29) Estrada et al. (29) report that the number of bacteria significantly increased up to 10^{th} day following application of biosolids on soil (*E.coli*: 1.5×10^4, faecal Coliforms: 1.9×10^4, and *Enterobacteriaceae* 4.3×10^4).(23) However, there was a decline in bacteria numbers beginning on the tenth day of application reaching to numbers below detection limits by 80^{th} day of application of biosolids. The numbers of bacteria were significantly lower for aerobically stabilized biosolids as compared to anaerobically digested biosolids.(23, 29)

It is generally established that *Campylobacter* is significantly inactivated through aeration as compared to anaerobic digestion.(19, 59) Sidhu and Toze report 99.63% inactivation or a 5 log reduction in the presence of oxygen. In contrast, mesophilic anaerobic digestion is ineffective in inactivating *Campylobacter*. Hence, in the absence of oxygen, *Campylobacter* may be able to survive in stockpiles.(19)

Lime stabilisation has also shown selective inhibition of most bacterial pathogens.(45) Meckes et al.(45) observed more than 3 log reductions for *E.coli*, *Salmonella* and faecal coliforms.(45) Proportionally, 98% of the bacterial pathogens in the sludge were removed following the stabilisation process. (45)

Anaerobic digestion can often be ineffective for eliminating viruses and protozoa from sewage sludge.(19) TAD and MAD treatments were found to be ineffective for adenovirus reduction, with 75% and 88% of positive samples respectively, following treatment.(31) In this same study, highest reduction in adenoviruses were observed for composted biosolids with 70% of positive samples.(31) Guzman et al. (49) reported significantly decreased numbers of faecal coliforms, coliphages, enteroviruses, *Cryptosporidium* and helminth ova in their analysis of a sludge composting facility.(49) Viau et al. (62) report significantly reduced numbers of enteroviruses and reovirus by both MAD and composting. Wei et al.(43) monitored the survival of norovirus and hepatitis A virus in lime-stabilized and alum inactivated biosolids. They report that no viral RNA for norovirus or hepatitis A virus was detected in the lime-stabilized biosolids.(43) However, alum inactivation had little or no effect on the reduction and inactivation these viruses.(43) Their research also suggests that composting is a better method for reducing viral content in biosolids.(62) Aerobic digestion has been found to be effective in reducing protozoa from sewage sludge.(19) At high temperatures, *Cryptosporidium* and G*iardia* have impaired cell viability with a loss of infectivity.(19)

Deactivation of protozoa can also be achieved through several methods. *Cryptosporidium* oocysts and *Giardia* cysts have been found to be inactivated through lime and heating at 50^0C.(48) Capizzi-Banas et al. (48) treated sewage sludge with lime and heat to observe inactivation of helminth ova.(48) Their aim was to observe less than one viable egg per 10g of the final product. When the sludge was treated with slaked lime (40% lime in sludge) and heating at 60^0C, the desired levels were achieved after 4-8 minutes.(48)

3.4. Comparison of National and International Guidelines on Management of Pathogen Concentrations in Biosolids

3.4.1. Australian Guidelines on Biosolids Management

At a global level, several national and state level guidelines have been formulated to regulate and manage production, treatment and application of biosolids.(5, 6, 15) The broad objectives of these guidelines are to promote responsible management, protect public health interests and promote consistent practices for the production and application of biosolids. However there are many inconsistencies in regards to provision of which pathogens to screen, their specific treatment and outcome requirements in biosolid products.

The Australian national guideline was developed under the National Water Quality Management Strategy (NWQMS). The WA guideline and the NWQMS guideline have many similarities, including the pathogenic grading and required number of indicator pathogens. The monitoring periods and protocols for pathogen regrowth and application of biosolids are also comparable.(6, 15) However, differences exist in the screening protocol of sewage sludge. The NWQMS guideline suggests screening for *Salmonella*, *Campylobacter, E.coli* and *Listeria* as indicator bacterial pathogens, *Giardia lamblia, Entamoeba histolytica* and *Cryptosporidium* as indicator protozoa, and roundworms and flatworms as indicator helminths. (15) In comparison, the WA guideline recommends screening for *Salmonella*, *E.coli*, *Cryptosporidium*, *Giardia,* adenoviruses, hepatitis viruses, and nematodes and hookworms only.(6)

In South Australia, the Environment Protection Authority (EPAu) formulated their own 'South Australian (SA) Biosolids Guidelines for the safe handling and reuse of biosolids' which is in final drafting stage as of 2009. The SA guideline adapts a different grading system for pathogens which includes measures for vector reduction and odour control.(54) This 'stabilisation grade' is adapted from the United States (US) Environmental Protection Agency (EPAg) guideline. The stabilisation grade has three categories, namely A, B and C. Table 1 shows the indicator pathogen requirements under the SA guideline. The A and B grade biosolids are most commonly used in South Australia with most application to forestry and landscaping.(54) The one added aspect in application is for home garden and retail sale of top grade biosolids (A grade). The greater emphasis in the SA guideline is on management of metallic contaminants and they do not mention specific pathogens other than *Salmonella* and *E.coli* for screening. (54)

The EPAu in New South Wales (NSW) published their guideline 'The use and disposal of biosolids' in 2000.(63) NSW and Tasmanian guidelines follow a similar structure of pathogen grading to that of South Australia. However, the indicator pathogen requirements are different and incorporate faecal coliforms along with *Salmonella* and *E.coli* for NSW guideline and faecal coliforms and

Listeria along with *Salmonella* and *E.coli* for Tasmanian guideline (See Table 2).(55, 63) Other than these microbial indicators, there are no recommended pathogens that these guidelines suggest screening for in comparison to WA and NWQMS guidelines.(55, 63)

EPAu Victoria's biosolids land application guideline follows a similar pattern to that of the WA and NWQMS guidelines. Pathogen grading process under the Victorian guideline is rigid and incorporates elements of the NWQMS and US EPAu guideline. The term given to this is 'treatment grading' which incorporates treatment processes with indicator pathogen numbers, vector attraction and odour reduction (See Table 1).(14) The Victorian guideline recommends screening for viruses such as adenovirus, reovirus and enterovirus. In particular, *Taenia saginata* screening and reduction is required for when the biosolids are used for cattle grazing.(14) *Taenia saginata* is the main cause of taeniasis in humans and is directly transferred from consumption of meat from infested cattle.(14)

The main differences across the states are mainly in the application of biosolids and the indicator pathogen thresholds. Approved application of biosolids for the highest grade biosolids (graded P1, A or T_1) in all States corresponds to unrestricted use. In Western Australia and Victoria, the use of highest grade biosolids has not yet been certified for home use or retail in the guidelines. The indicator pathogen thresholds for this grade are consistent for all Australian States, with the exception of more stringent measurements for *Salmonella* in NSW and Tasmania (See Table 2).

The second highest grade biosolids (graded P2, B or T_2) are mainly approved for landscaping, foresting and restricted agricultural use. The indicator pathogen thresholds in this grade, are however inconsistent with *Salmonella*, viruses and helminths only screened for in WA and Victoria. SA guideline considers screening for *E.coli* in this grade, but Tasmania and NSW guidelines do not specify any required screening. Moreover, for the lower grade biosolids (graded P3, P4, C, T_3 or T_4) approved applications are inconsistent across States. Biosolids graded C in Tasmania, SA and NSW are only suitable for use in landfills or disposal, whereas in WA and Victoria, applications in forestry, agriculture and landscaping are allowed. This could be attributed to the lack of indicator pathogen screening, which are required under the WA and Victoria guidelines.

Table 2. Approved Pathogenic Grading and Application of Biosolids in Australia

Pathogen Grade	Indicator Pathogens [a]	Approved Applications
Western Australia [b]		
P1	*Salmonella* - less than 1 count per 50g *E.coli* - less than 100 counts per gram Virus – less than 1 per 50g Helminths Ova – less than 1 viable ova per 50g	Unrestricted Use, and Agricultural direct land application (crops that may be consumed raw and are in contact with biosolids)
P2	*Salmonella* - less than 10 counts per 50g *E.coli* - less than 1000 counts per gram Virus – less than 2 per 50g Helminths Ova – less than 1 viable ova per 50g	Urban Landscaping (non-household), horticulture, agricultural direct land application (crops that may be consumed raw but not in contact with biosolids)
P3	*E.coli* - less than 2,000,000 counts per gram Virus – less than 2 per 50g Helminths Ova – less than 1 viable ova per 50g	Forestry direct land application, Agricultural direct land application (pasture and crops that are cooked/processed before being consumed, but not root crops), Land rehabilitation
P4 or Ungraded	*E.coli* - greater than 2,000,000 counts per gram	Composting and Class II, III & IV landfill
South Australia		
A	*Salmonella* – less than 1 count per 50g *E.coli* – less than 100 counts per gram Virus – less than 1 per 50g Helminths Ova – less than 1 viable ova per 50g	Home Garden and Retail Sale, Urban Landscaping, Forestry, and Site Rehabilitation
B	*E.coli* – less than 1000 counts per gram	Urban Landscaping, Forestry and Site Rehabilitation
C	None Specified	Landfill or Disposal only

New South Wales		
A	*Salmonella* – None per 50g *E.coli* – less than 100 counts per gram Faecal Coliforms – less than 1000 counts per gram Virus – less than 1 per 4g Helminths Ova – less than 1 viable ova per 4g	Unrestricted use (home lawns and gardens, public contact sites, urban landscaping, agriculture, forestry, landfills, Soil and site rehabilitation, Surface land disposal)
B	Not necessary to sample and determine pathogen levels.	Restricted Use 2 and 3 (Agriculture, Forestry, Soil and site rehabilitation, landfill disposal, surface land disposal)
C	None Specified	Landfill or Disposal only
Tasmania		
A	*Salmonella* – None per 50g *E.coli* – less than 100 counts per gram Faecal Coliforms – less than 100 counts per gram Listeria – None per 50g Virus – less than 1 per 4g Helminths Ova – less than 1 viable ova per 4g	Home lawns and gardens, public contact sites, and urban landscaping
B	Not necessary to sample and determine pathogen levels	Agriculture, forestry, land rehabilitation
C	None Specified	Landfill or Disposal only
Victoria		
T_1	*Salmonella* – less than 1 count per 50g *E.coli* – less than 100 counts per gram Virus – less than 1 per 100g	Unrestricted use, and all uses including human food crops consumed raw in direct contact with biosolids, and Pasture/Fodder for cattle and poultry
T_2	*Salmonella* – less than 10 counts per 50g	Human food crops consumed raw but with harvested produce more

	E.coli – less than 1000 counts per gram Virus – less than 2 per 10g Taenia ova – Less than 1 viable ova per 10g	than or equal to 1 metre above soil surface, Pasture/Fodder for cattle and poultry, and Landscaping of public use land
T_3	*E.coli* – less than 2,000,000 counts per gram	Human food crops cooked or processed prior to sale to customers, landscaping of land with limited public use, and forestry and site rehabilitation
T_4	None Specified	Landfill or Disposal only

[a] Pathogen numbers presented in this table are in dry weight of the product.

[b] Helminths Ova screening only to be done above the 26th Parallel.

3.4.2. International Guidelines on Biosolids Categorization

At an international level, there is limited literature on guidelines for biosolids management. Developed countries currently producing large amounts of biosolids tend to have a more rigorous and systematic approach to the management of biosolids. (7)

The United States (US) guideline developed by the Environmental Protection Agency (EPAg), also known as the EPA 503 Rule, is the most recognized framework for biosolids management globally.(10) Most of the Australian guidelines, as mentioned earlier, are based on this framework. The US guideline incorporates two grading systems: 'contaminant' and 'stabilisation'.(16) Stabilisation grade for biosolids is based on three criteria: pathogen numbers, vector reduction and odour reduction. The stabilisation grade however, is based on two grades .(16) The A grade biosolids correspond to highest quality and unrestricted use. The B grade biosolids are of a lower quality and have restricted use (See Table 3). The prominent difference between WA guideline and EPA 503 rule is the land application requirements.(16) Most of the land application of biosolids in the US is in the agricultural industry, therefore the US guideline further breaks down the A grade pathogen into four different sub-categories based on concentration of metallic contaminants.(7, 16) In comparison, WA land application is predominantly in landfills and forestry, with limited commercial applications.

Moreover, most of the States in the US exercise their own regulations for management of biosolids. For instance, in California, the Biosolids Environmental Management Program System (EMS) runs under the National Biosolids Partnership (NBP) EMS program. The EMS enforces further restrictions and regulations to land application of biosolids applicable for Californian landscapes.(7, 64) Illinois, Colorado, Washington and Wisconsin also administer similar programs under the EPA 503 rule and NBP EMS.(7, 50, 51) Overall, regulations in these States have a higher emphasis on metallic contaminant control and application of biosolids. These regulations do not enforce further changes to the EPA 503 rule on pathogenic quantification and sampling.

Canada follows a similar structure to that of the US, however, there is no regulatory guideline for the management of biosolids. Instead the Canadian Council of Ministers of the Environment (CCME) publishes strategies under the Canadian legislative framework for biosolids.(7) The requirements for the top quality biosolids under the legislation is absence of *Salmonella* and less than 1000 counts of faecal coliforms (per 4 gram of dry product).(65) However, this requirement is not consistent across Canada, as most of the provinces have adapted their own regulations on pathogen quantification (See Table 3). Most of the provinces have utilized the EPA 503 rule (categorizing biosolid products to class A or B), with the exception of Quebec which uses a treatment based

approach.(7, 65) The treatment based approach is also adapted by European countries which aims to reduce metallic contaminants in sludge.(7)

The Australia and New Zealand Biosolids Partnership (ANZBP) is based on the experience of NBP EMS in the US. The objective of the ANZBP is to support sustainable management and public engagement of biosolids in Australasia.(66) Presence of this partnership has resulted in similarities in the management of biosolids in New Zealand and Australia. The New Zealand guideline on management of biosolids also uses the EPA 503 rule for grading of biosolids.(53) Biosolids are either classified as grade A or B for contaminants and pathogens based on sampling (See Table 3). The difference between the WA and New Zealand guidelines is the addition of *Campylobacter* for high grade biosolids.(53) Moreover, there is no specific pathogen quantification required for grade B biosolids. Instead different applications of biosolids require different vector attraction reduction measures.(53)

Most of the European countries have limited treatment and application of sewage sludge and biosolids.(7) A number of European countries are also opposed to the use of biosolids, although they are used in Germany, France, United Kingdom, Portugal and Italy.(7) Obtaining guidelines and regulations through white literature search has been unsuccessful for the European countries which practice application of biosolids. However, in United Kingdom, the use of Safe Sludge Matrix provides an overview of pathogen quantification requirements. The Safe Sludge Matrix identifies two categories of sludge: namely, 'conventionally treated sludge', and 'enhanced treated sludge'.(67) The conventionally treated sludge contains only 1 per cent of the original number of pathogens, whereas the enhanced treated sludge is virtually free from *Salmonella* and is treated to eliminate 99.99% of pathogens.(67) The Safe Sludge Matrix itself originates from the 'Codes of Practice for Agricultural Use of Sewage Sludge' developed by the United Kingdom (UK) Department of Environment, Food and Rural Affairs (DEFRA) which itself fails to mention pathogen quantification thresholds.(52)

The most prominent countries with advanced treatment of sludge include Japan and China. Japan has one of the most advanced sewage sludge treatment systems, however most of their application is in landfills and landscaping.(7) This is also the case for China, although China seems to be far behind the rest of the world in relation to use of biosolids for non-agricultural purposes.(7) The emphasis on pathogen treatment and quantification is very limited in these regions, while having higher requirement of metallic screening.

Table 3. Approved Pathogenic Grading and Application of Biosolids Internationally

Pathogen Grade	Indicator Pathogens [a]	Approved Applications
United States		
A	*Salmonella* - less than 3 counts per 4g Faecal Coliform - less than 1000 counts per gram	Unrestricted use (some restrictions apply based on Vector Attraction Reduction scores)
B	Faecal Coliform - less than 2,000,000 counts per gram	Food crops with harvested parts above the soil/ground, animal grazing, turf, land filling, disposal etc.
Canada (Newfoundland and Labrador, Nova Scotia, Prince Edward Island, New Brunswick, British Columbia)		
A	*Salmonella* - less than 3 counts per 4g Faecal Coliform - less than 1000 counts per gram	Unrestricted use
B	Faecal Coliform - less than 2,000,000 counts per gram	Land filling
Canada (Ontario)		
A	*Salmonella* - less than 3 counts per 4g *E.coli* - less than 1000 counts per gram	Unrestricted use
B	*E.coli* - less than 2,000,000 counts per gram	Land filling, incineration and land reclamation
Canada (Quebec)		
P_1	*Salmonella* - None per 10g *E.coli* - less than 1000 counts per gram	Composting
P_2	*Salmonella* - None per 10g *E.coli* - less than 2,000,000 counts per gram	Land filling, incineration and land reclamation

New Zealand		
A	*Salmonella* – less than 1 count per 25g *Campylobacter* – less than 1 count per 25g *E.coli* – less than 100 counts per gram Virus – less than 1 per 4g Helminths Ova – less than 1 viable ova per 4g	Unrestricted use
B	None Specified	Restrictions apply to application (different applications require different vector attraction reduction treatments)

[a] Pathogen numbers presented in this table are in dry weight of the product.

4. Discussion

Several considerations need to be assessed prior to treatment of sewage sludge. Of particular importance is the target numbers of pathogen to be achieved, the infectious dose of those pathogens and their survival following inactivation.

The number of bacteria, viruses and protozoa can vary significantly in sewage sludge and biosolids. Current literature evidence is mostly centred around pathogens and wastewater, with limited emphasis on sewage sludge and biosolids. Studies investigating pathogens in sewage sludge mostly focus on bacteria such as *E.coli*, faecal coliforms, *Enterobacteriacaea*, *Salmonella*, *Clostridium* and *Staphylococcus* spp. There is limited published evidence on the levels of *Legionella, Listeria, Yersinia*, *Shigella*, viruses and protozoa in sewage sludge. It is imperative for future research to focus on these important aspects of sewage sludge treatment, which may pose potential hazards to the health of community. The results from the literature review analysis in this report show that *Salmonella*, *Escherichia coli,* enterococci, *Legionella,* enteroviruses, noroviruses, adenoviruses are found most commonly in sewage sludge. These pathogens are of particular concern due to their variable responses to treatment mechanisms and persistence in soil following application of biosolids. All aerobic, anaerobic, lime and composting treatment methods are effective in reducing the number of pathogens in sewage sludge. Anaerobic digestion, in particular thermophilic anaerobic digestion seems to be most effective against most of the bacterial pathogens in sewage sludge. However, a notable exception is *Campylobacter spp*. which have shown high inactivation rates in presence of oxygen, thereby favouring aerobic stabilisation methods. Lime-stabilization inclusive of heating has so far consistently shown high inactivation of most bacteria and some viruses. Moreover, composting with sludge digestion also seems to be a safe approach to significantly reduce the pathogen load in biosolids. Most of the guidelines, importantly those following the US EPA 503 rule prescribe and target achievement of treatment objectives.(7) Non-prescriptive guidelines, on the other hand, identify requirements for pathogenic quantification to be achieved following the treatment process. The practicability and suitability of these methods to WWTPs however depends on economic and practical factors, which are outside the scope of this research and therefore are not discussed.

Monitoring and testing of all pathogens in general can be difficult and time consuming as pathogens are heterogeneous in their nature of inactivation and survival.(19) Therefore, it is imperative to select organisms that are most suitable or representative of the pathogenic profile of sewage sludge. Traditionally, faecal coliforms and faecal streptococci have been used as indicators for biosolids.(19) However, other enteric viruses and *enterococci* have also been used as indicators.(19) Many international guidelines following the EPA Rule 503 identify *Salmonella* and *E.coli* as most suitable bacterial

indicators. However, some guidelines, such as those in Australia and some States in the US have made adjustments to include viruses and helminth ova.(19) Sahlstrom et al. (28) investigated presence of *Salmonella*, *E.coli* and *Campylobacter* through the use of coliforms as indicators. They report that average reduction of coliforms being comparable to that for *Salmonella* and *E.coli*. However, absence of an indicator cannot simply be translated to absence of other pathogens, such as the case of *Legionella* and *Campylobacter*.(43, 59) Wery et al. (2008) describes indicators *'be present notably: at a density that has some constant, direct relationship to the density of the pathogen; always be present when the pathogen is present, and have a survival rate in the environment or during sanitary treatments equal to or slightly higher than that of pathogen'*. Based on this description, the *E.coli*, *enterococci, Salmonella spp., L.monocytogenes,* and *Campylobacter spp.* would be the ideal indicator pathogens. However, none of the current guidelines include all these pathogens for screening purposes.

Furthermore, it can be established that the presence of pathogens in sewage sludge is dependent on the pathogenic profile of the wastewater. Therefore the indicators to assess the safety of biosolids should be adapted following screening of pathogens found in sewage sludge within a geographical area. This can be beneficial for identification of new and emerging pathogens. Increased numbers of *Aeromonas* spp.*, E.coli 0157:H7* and *Legionella* spp. have also been found in recent studies, thereby raising concerns over their likely health impact.

A review by Jury et al. (2011) found multi-drug resistant faecal coliforms, *E.coli*, enterococcus and *Pseudomonas* to be present in sewage treatment plant effluents.(68) Similarly, Sengupta et al. (69) also found multi-drug resistant strains of bacteria in soil and sewage samples in India.(69) Of particular concern, is the newly emerging Extended Spectrum Beta Lactamase (ESBL)-producing *E.coli* found in Austrian sewage sludge.(38, 47) In all, 44% of the samples found in the five plants studied by Reinthaler et al. (47) were positive for ESBL-*E.coli*.(47) However, more importantly, lime-stabilized plants in this study showed no incidence of ESBL-*E.coli*, while most of the positive samples represented stabilized and dehydrated sludge.(47) A study on five wastewater utilities in Michigan State of the US reported sulphonamide- and tetracycline-resistant bacteria consistently in raw sludge and biosolids.(70) This raises concerns for the guideline review of indicator bacteria and recommended treatment of sludge. Currently, the level of evidence on emerging bacteria is very limited and therefore recommendations cannot be drawn regarding treatment for biosolids management. This lack of best evidence was also identified in a recent waterlines report by the Australian National Water Commission.(71) This highlights the need for more research in to the identification and treatment of pathogens in sewage sludge and biosolids.

Moreover, the treatment of sewage sludge through treatment processes is mostly dependent on time and temperature of the treatments. Pathogen inactivation rates have been shown to vary independently with time and

temperature in most research studies (10, 12, 23, 26-29, 31, 44, 48, 62) A recent WERF report identifies the importance of these two factors in treatment of sewage sludge. *Salmonella* and faecal coliforms were found vulnerable to inactivation at 36^0C, while *E.coli* was found to be resistant to inactivation at the same temperature. Although increased inactivation was observed at 60^0C for *E.coli*, inactivation still took 120 minutes for complete eradication.(24) The report also notes that moderate temperatures (55^oC) are sufficient for most viral inactivation. Since most of the guidelines are not prescriptive of the treatment and temperature requirements, the findings from this research suggests adaptation of strategies that will ensure uniform treatment incorporating specific time and temperature.

Furthermore, the quantification methods used to isolate pathogens are also not consistent between guidelines and studies. Most studies have either used PCR or culturing techniques, therefore restricting the comparability of results which is a limitation of this report. Inconsistencies between the two of the above mentioned culturing techniques have been highlighted in previous studies. (32, 72) Wery et al. (2008) consistently found higher number of pathogens using qPCR method in comparison to culturing.(32) The differences observed between the two methods for *E.coli* ranged between 25 and 5011, for *Salmonella* between 40 and 1.26×10^5, and for *enterococci* between 5 and 1259.(32) Moreover, inconsistencies have also been reported inter-methods, for instance using qPCR versus ICC-PCR.(72) Despite identifying higher number of pathogens, as compared to culturing, PCR may be ineffective in quantifying pathogens for composting as demonstrated by Wery et al. (2008).(32) This issue is of particular importance as the choice of quantification method may underestimate the requirements set out in guidelines.(31, 71)

Pathogenic contamination in biosolids can lead to indirect and direct impact to human and animal health. There are three main pathways for crop contamination: the spray irrigation of surface crops, surface splash following application of excreta/biosolids to soil during rainfall, and sub-surface-drip irrigation or rain leached through biosolids to subterranean crops (e.g. carrots). Current evidence of transmission of pathogens from sludge applied soil to animals supports the safety of high grade biosolids.(46, 73, 74) Quantifying the level of pathogen contamination on food crops following irrigation with wastewater or biosolids application is also considered necessary for the risk model, however it has not been well characterised in Western Australia.

In Western Australia, Water Corporation mainly controls the WWTPs currently producing biosolids. Urban wastewater treatment plants utilize mesophilic anaerobic digestion and lime addition treatment methodology whereas the rural towns treat sludge with alum.(8) Lime stabilization treatment adds quicklime to dewatered sludge cake to increase the pH of the mixture and significantly reduce pathogens.(8) The land applications of Lime Adjusted Biosolids (LAB) is influenced by soil profile for the prospective land application.(8) Alum inactivation involves dosing with alum (Al2(SO4)3) to reduce the concentration

of phosphorus in effluent, a regulatory requirement to minimise the pollution of inland waterways.(8) Lime stabilization treatment has shown to be effective against most bacterial pathogens in research. However, the published evidence is limited to evaluate the effectiveness of lime stabilization on viruses and protozoa. A large study conducted by the Curtin University of Technology for the Water Corporation also raised concerns over the use of Alum treated sludge.(18) Alum treatment, in this review, was not effective in reducing pathogen from sludge.(18) Researchers highlight the need for more research into alum treated sludge and their suitability for land application.

Production of biosolids must also undertake identification and assessment of potential environmental, human/animal, food and legal risks. These factors have been well covered under the Western Australian guidelines. However, this research identifies the need for review of sludge treatment in rural treatment plants in Western Australia, specifically for Alum. Collective data from this research also identified the need for profiling pathogens to identify indicator pathogens in sewage sludge within the geographical area of the WWTPs.

4.1. Recommendations

Based on the findings of this report, the following recommendations are suggested for future research and development of guidelines;

- Further developments in guidelines for biosolids management should incorporate screening of wastewater in a given geographical location to establish screening indicators for biosolids.
- Profiling of wastewater can also be used to direct most suitable methods of treatment of sludge to obtain safer biosolids.
- More research needs to be conducted to quantify pathogens using PCR and culture methods to establish criteria for quantification methods. This may require a review of the thresholds of pathogens in biosolid products.
- The concern with resistant bacteria in sewage sludge is relatively new and therefore more research is required to evaluate current treatment methods.
- Lime stabilization and Composting is ideal for removal of pathogens and should be further researched if it is to be adapted into the guidelines.

5. Limitations

The main limitation of this research is the lack of literature and evidence on pathogen quantification in sewage sludge and biosolids, especially for protozoa and viruses. Moreover, recent developments have also taken place in this field of research such as those discussed above. The area of resistant bacteria in sewage sludge is relatively new and therefore more research is required to evaluate current treatment methods and indicator pathogen requirements. Moreover, data available for investigation in this field are often restricted to private and commercial interests which can only be purchased (such as the WERF reports) and were not covered in this report. The specifics of the quantification methods have also not been explored in this report, as the topic was out of scope of the project. Quantification methods can have a significant impact on the monitoring of indicator pathogens and the reliability/transferability of statistical data across studies for comparison.

6. Conclusion

Biosolids are a by-product of sewage sludge treatment. Biosolids can contain several nutrients and therefore have a number of potential applications in agriculture and forestry. Use of biosolids has increased significantly over the past few decades. Much research has been focussed on improving the quality of nutrients in biosolids for their application as substitutes or co-substitute to fertilizers. However, biosolids can contain several pathogens that can be deleterious to human and animal health. Current literature suggests that biosolids can contain a diverse combination of bacteria, viruses and protozoa. Australian and International guidelines on management of biosolids categorize biosolid products based on quantities of indicator pathogens. This review has shown that the current choice of indicator pathogens may not be sufficient given existence of resistant and newly emerging bacteria. Although most of the treatment processes are successful at inactivating pathogens, the challenges posed by organisms such as *Campylobacter* and *Legionella* have important implications for a prescriptive approach of guidelines for the treatment of sewage sludge. Lime-stabilization and composting seem to be the most effective treatments for sewage sludge, however, there is insufficient evidence to make firm conclusions regarding these treatment options.

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8. Appendix A

Key Research Terms

Topic Specific

Category A	Category B	Category C
Pathogens	Sewage Sludge	Australia
Bacteria	Sludge	Europe
Virus/Virology	Biosolids	Canada
Helminths	Wastewater	America
Protozoa	Wastewater treatment plants	International
Microbes		Sweden
		Germany
		India
		China
		South East Asia

Treatment Specific

Treatment

Stabilization

Mesophilic

Thermophilic

Composting

Digestion

Non-Topic Specific

Prevalence

Guidelines

Epidemiology