Verification of an alternative sludge treatment process for pathogen reduction at two wastewater treatment plants in Victoria, Australia

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ABSTRACT

At South East Water wastewater treatment plants (WwTPs) in Victoria, Australia, biosolids are stockpiled for three years in compliance with the State guidelines to achieve the highest pathogen reduction grade (T1), suitable for unrestricted use in agriculture and landscaping. However, extended stockpiling is costly, may increase odour nuisance and greenhouse gas emissions, and reduces the fertiliser value of the biosolids. A verification programme of sampling and analysis for enteric pathogens was conducted at two WwTPs where sludge is treated by aerobic and anaerobic digestion, air drying (in drying pans or solar drying sheds) and stockpiling, to enumerate and, if present, monitor the decay of a range of enteric pathogens and parasites. The sludge treatment processes at both WwTPs achieved T1 grade biosolids with respect to prescribed pathogenic bacterial numbers (<1 *Salmonella* spp. 50 g^{-1} dry solids (DS) and <100 *Escherichia coli* g^{-1} DS) and >3 $\log_{10}$ enteric virus reduction after a storage period of one year. No *Ascaris* eggs were detected in the influent to the WwTPs, confirming previous studies that the presence of helminth infections in Victoria is extremely low and that *Ascaris* is not applicable as a control criterion for the microbiological quality of biosolids in the region.

Key words | *Adenovirus*, agriculture, *E. coli*, enteric pathogens, *Salmonella*, stockpiling

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INTRODUCTION

Sewage sludge, the organic residue from wastewater treatment, may contain a range of pathogenic microorganisms, including bacteria, viruses, protozoa and parasites, depending on the pathogenic load of the resident population in the catchment area (Grant *et al.* 2012; Karkashan *et al.* 2015). Before sewage sludge can be recycled for beneficial purposes, for example, as an agricultural soil improver and fertiliser, it must be stabilised to reduce odour and pathogens so that it presents minimal nuisance and risk to public health. Cropping and harvest restrictions are implemented to provide further barriers to pathogen transmission from the soil to food when biosolids (treated sewage sludge) are land applied, depending on the degree of treatment and pathogen reduction achieved (Karkashan *et al.* 2015). Thus, biosolids may be treated to eliminate or reduce the pathogen content to background residual values and, under these circumstances, where a single barrier to pathogen transmission is provided, the biosolids may be utilised without restriction (US EPA 2003).

As part of the sludge treatment process, biosolids are typically dewatered to reduce volume, improve ease of handling, and minimise the cost of further management (Rouch *et al.* 2011a). Sludge drying pans or solar drying beds provide an economic method of sludge drying, and are frequently used in small- to medium-sized wastewater treatment plants (WwTPs) in warmer climatic regions when sufficient land is

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available (Mondal *et al.* 2015). In Victoria, Australia, sludge dewatering generally involves air drying, typically in outdoor, clay-lined drying pans, followed by stockpiling of dried sludge (Rouch *et al.* 2011a; Mondal *et al.* 2015).

Biosolids produced at the WwTPs operated by South East Water (SEW) in Victoria are managed in accordance with the Environmental Protection Authority (EPA) Publication 943 (EPA Victoria 2004). Biosolids in the Victorian guidelines are classified by contaminant grades (C1 and C2) and treatment (pathogen reduction) grades (T1, T2 and T3). The contaminant grades ensure that the concentrations of potentially toxic chemicals are within safe limits, and the treatment grades are based on the process used to stabilise and dry the sludge and seek to protect human health and the environment from a microbiological contamination perspective.

The treatment grades are determined according to three main criteria: (i) adoption of a prescribed treatment process with minimum performance criteria (e.g. temperature/time); (ii) microbiological limits to demonstrate that the treatment processes are operating effectively; and (iii) measures for controlling bacterial regrowth, vector attraction and generation of nuisance odours. Grade T1 represents the highest quality grade and from a microbiological perspective is suitable for unrestricted use, whereas restrictions on end use apply to T2 and T3 biosolids (EPA Victoria 2004).

The guidelines set out a number of prescribed treatment processes for the different microbiological treatment grades. However, alternative treatment methods may be included as prescribed T1 processes if they undergo verification to demonstrate significant log reductions for a range of pathogenic microorganisms. The nominated performance objectives under realistic worst-case process conditions include:

- maximum *Escherichia coli* and *Salmonella* criteria (<100 *E. coli* g^{-1} dry solids (DS); <1 *Salmonella* spp. 50 g^{-1} DS);
- >3 $\log_{10}$ reduction in enteric viruses;
- >2 $\log_{10}$ reduction in *Ascaris* ova.

The prescribed treatment process adopted to produce T1 grade biosolids at SEW WwTPs, as well as at other treatment plants in Victoria, includes drying followed by three years of storage of sludge (EPA Victoria 2004). However, sludge production is increasing due to population growth across the operational area of SEW. Accommodating the extra sludge requires considerable capital investment to provide additional facilities and land suitable for processing, handling and storage. Achieving the T1 treatment grade is necessary to market the biosolids as the outlets for lower grade products are very limited in Victoria. However, constructing new stockpile areas to accommodate at least three years' storage and drying treatment required by the EPA guideline would be difficult at most WwTPs. Hence, SEW has sought to adopt a shorter storage period of one year as an alternative treatment process to produce biosolids compliant with the T1 grade. One year stockpiling is not a prescribed treatment process to produce T1 biosolids, therefore verification of this alternative procedure is necessary (EPA Victoria 2004).

A further driver to reduce the storage period for biosolids is to maximise the agronomic value of the biosolids and minimise the environmental impact of the process. Long-term open storage of sludge in stockpiles significantly reduces the nutrient and carbon contents of the biosolids, reducing the fertiliser value and benefits to soil microbiology (Rouch *et al.* 2011b; Majumder *et al.* 2015). Furthermore, stockpiled sludge is susceptible to contamination by weeds and weed seeds, requiring the application of agrochemicals to control weed populations. Shortening the storage time would also reduce overall site odour potential, improve site aesthetics as well as reducing the total area of land required for stockpiling. Additionally, biosolids stockpiles are potentially a significant point source of greenhouse gas emissions, although these decline with stockpile age (Majumder *et al.* 2015).

Two SEW WwTPs were selected for this study, at Boneo and Somers. Boneo WwTP operates two sludge treatment lines, comprising: (a) aerobic digestion, anaerobic lagoon storage, polymer dosing followed by belt press dewatering, air drying in solar drying sheds and stockpiling; and (b) aerobic digestion, anaerobic lagoon storage, air drying in a sludge drying pan and stockpiling. At Somers WwTP, sludge is treated by anaerobic lagoon storage, air drying in sludge drying pans and stockpiling. The Boneo plant faces increasing demand from population growth and the regional sewer connections programme and SEW is unable to obtain suitable land to extend the solids handling

facilities. Similarly, at Somers, the existing stockpile area is at capacity and the available land is unlikely to be suitable to construct a new dedicated stockpile area.

A comprehensive microbiological sampling and verification programme of raw wastewater, various sludges and biosolids stockpiles was carried out at Boneo and Somers WwTPs. The aim was to investigate whether a shorter stockpiling period could produce T1 quality biosolids, with equivalent inactivation of pathogenic bacteria, viruses and helminths to that achieved by stockpiling sludge for three years.

METHODS

Site descriptions

The Boneo WwTP is located 83 km south-east of Melbourne, in the south-eastern corner of the Mornington Peninsula, Victoria. The plant accepts domestic wastewater and tankered waste, including leachate, from the local area, and serves a population equivalent to approximately 47,800 persons. The annual median inflow of domestic wastewater to the plant is 10 ML day^{-1}; however, there is significant seasonal variation due to the holiday homes in the area. Wastewater treatment is by a twin stream activated sludge process. Waste activated sludge (WAS) from the bioreactors is drawn from the returned activated sludge (RAS) underflow from the clarifiers and treated by aerobic digestion for approximately 10 days. The treated sludge is transferred to an anaerobic storage lagoon for further digestion to reduce volatile solids (VS). Most of the sludge from the anaerobic lagoon is pumped to a continuously mixed storage tank of approximately 10 m^3 capacity that supplies feed sludge to a belt press for dewatering. Dewatered sludge from the belt press is conveyed to one of three enclosed solar drying sheds where it is distributed over the drying floor by a mechanical tiller, and dried to >50% DS. After a predetermined period of time, equivalent to three months in summer and ten months during the winter season, the sludge is harvested and transported to the biosolids stockpile area. Surplus sludge from the anaerobic lagoon that exceeds the capacity of the belt press/solar drying shed route is pumped to an open-air sludge drying pan. After approximately one year in the drying pan the sludge is removed, usually at the end of summer when the sludge is at its driest, and transported to the biosolids stockpile area. The dried sludge from the sludge drying pan and the solar dryers is stockpiled until classified as T1 grade biosolids (currently after storing for three years).

Somers WwTP is located 72 km south-east of Melbourne on the east coast of the Mornington Peninsula, Victoria. The plant treats the wastewater collected from the towns of Tyabb, Hastings, Bittern, Balnarring, Cerberus, Somers and Flinders, serving a combined population equivalent to approximately 29,000 persons. The annual median inflow of domestic wastewater to the plant is 4 ML day^{-1}. Wastewater treatment is by a twin stream activated sludge process using sequencing batch reactors (SBRs). WAS is pumped intermittently from the two SBRs to four open-air sludge drying pans. After approximately one year the sludge is removed from the drying pans, usually at the end of summer, and transported to the biosolids stockpile area for the required period of storage (three years) for the biosolids to be classified as T1 grade.

The mean local climatic conditions on the Mornington Peninsula (as recorded at the closest three weather stations: Cerberus, Phillip Island and Mornington) are minimum and maximum temperatures of 10.0 and 18.9 °C, respectively, annual average rainfall of 728 mm, and 9 a.m. and 3 p.m. wind speeds of 16.4 and 18.9 km h^{-1}, respectively (BOM 2016).

Sampling methodology

An external professional sampling services provider conducted sampling of influent (raw wastewater) and various sludge streams (e.g. WAS; sludge feed to the belt press and solar dryer sludge at Boneo WwTP). SEW operational staff conducted drying pan sludge sampling following hazard analysis and critical control point (HACCP) sampling protocols. The sampling programme at Boneo and Somers WwTPs was performed over the period November 2014–December 2015. Comprehensive sampling of all stages of sludge and biosolids treatment was conducted as summarised in Table 1.

Composite 24-hour screened influent raw wastewater samples were collected from the inlet works at both WwTPs using refrigerated autosamplers. Initially, 30 minute time-based samples were collected until the flow

Table 1 | Sludge and biosolids sampling programme at Boneo and Somers WwTPs, Victoria, Australia

Site/Type	Method	Frequency	Monitoring parameters			
			E. coli	*Salmonella*	*Adenovirus*	Helminths
BONEO WwTP						
Raw wastewater (influent)	24 hour composite	Fortnightly				X
WAS	10 L	Fortnightly			X	X[a,b]
Belt press feed sludge	10 L	Fortnightly			X	X[a]
Solar dryer sludge (sheds 1–3)	Composite	At harvest			X	X[a,b]
Drying pan sludge	Composite	At harvest	X	X	X	X[a,b]
Two 1-year biosolids stockpiles derived from drying pan sludge	Composite	Single sample	X	X	X	X
One 1-year biosolids stockpile derived from solar dryer sludge	Composite	Single sample	X	X	X	X
Two 2-year biosolids stockpiles derived from the drying pan sludge	Composite	Single sample	X	X	X	X
One 2-year stockpile derived from the solar dryer sludge	Composite	Single sample	X	X	X	X
SOMERS WwTP						
Raw wastewater (influent)	24 hour composite	Fortnightly				X
WAS	10 L	Fortnightly			X	X[a,c]
Drying pan sludge (1–4)	Composite	At harvest	X	X	X	X[a,c]
Three 1-year stockpiles derived from the drying pan sludge	Composite	Single sample	X	X	X	X
One 2-year stockpile derived from the drying pan sludge	Composite	Single sample	X	X	X	X

[a]Only if *Ascaris* was detected in the influent (raw wastewater).
[b]Single sampling events on 13 February 2015 (WAS), 8 April 2015 (solar drying sheds 1–3), 30 April 2015 and 7 December 2015 (drying pans).
[c]Single sampling events on 17 February 2015 (WAS) and 30 April 2015 (drying pans 1–4).

signal from the Supervisory Control and Data Acquisition (SCADA) system was established and thereafter flow-weighted sampling was used. Where a sampling event was not completed due to autosampler failure, it was immediately rescheduled and a sample was obtained to replace the missed collection. Influent samples were collected in a 20 L bucket, and mixed thoroughly using a 1.5 L plastic jug, before sub-sampling into sterile 1 L plastic containers provided by the external laboratory conducting the microbiological analysis.

Sludge samples were collected at various stages of the sludge treatment process streams as follows.

Boneo WwTP

- WAS samples were collected from the continuously flowing RAS pipeline from the bottom of the secondary clarifiers. The valve was flushed by running sludge to waste and a 20 L bucket was filled for sample collection, mixed and subsampled following the same procedure as for the influent.
- Samples of sludge feed to the belt press were collected from the pipeline downstream of the feed pump when the dewatering system was operating. The sample valve was flushed by running sludge to waste and a 20 L

bucket was filled for sample collection, mixed and subsampled following the same procedure as for the influent.

- Solar dried sludge samples were collected from the solar drying sheds at harvest. Ten subsamples of approximately 100 g each were collected manually from random positions on the shed floor, and thoroughly mixed in a 20 L plastic bucket to form a composite sample. The sludge was subsampled into 3×500 mL sterile plastic containers provided by the external laboratory.
- Four representative, replicate, 1 L sludge samples were collected separately from the sludge drying pan at harvest (after approximately eight months of storage) using a long-handled sample scoop. These were combined, mixed thoroughly in a 10 L bucket to produce a composite sample, and subsampled by transferring the sludge into sterile sample bottles provided by the external laboratory.

Somers WwTP

- WAS samples were collected from the pipeline that conveys WAS from the two SBRs to the drying pans. Activated sludge wastage occurs intermittently eight times per day and samples were collected when the sludge wastage system was operating to ensure collection of a representative sample. The sample valve on the side of the WAS discharge pipe was flushed by running sludge to waste prior to filling a 20 L bucket and the collected sludge was mixed and subsampled following the procedure used for the other liquid samples.
- Drying pan sludge was sampled from the four drying pans at harvest (after approximately one year of storage), following the procedure adopted at Boneo WwTP.

Sampling of the available one-year and two-year biosolids stockpiles at both sites (Table 1) was carried out by another external professional sampling services provider. All sampling was conducted in line with Victorian EPA Guidelines (EPA Victoria 2004, 2009). Five replicate sludge cores were taken from each of the stockpiles using either trailer mounted hydraulic and/or portable percussion sampling equipment. The surface material (100–150 mm) was removed prior to coring to avoid the inclusion of foreign biological material in the sample. Cores were taken to represent the entire depth of the stockpile (1.5–3 m) and from a range of angles. Once the sample had been extruded from the coring tool, the biosolids material was thoroughly mixed in an intermediary container before being subsampled into sealed sterile sample containers (3×500 mL plastic and 2×150 mL glass per core).

The plastic buckets used for collecting the influent and sludge samples and the other sampling equipment were cleaned using laboratory grade detergent, rinsed with potable water and air-dried prior to use on site. The buckets and jugs used for the collection of liquid samples were rinsed three times with an aliquot of the sample from the valve prior to filling for sample collection. Decontamination of stockpile sampling equipment, using the EPA approved triple wash procedure (Extran® solution followed by rinsing with tap water then de-ionised water), was conducted between each sample core to avoid cross-contamination. In addition, disposable gloves and boot covers were worn during the sampling of stockpiles and were replaced between each sample location within a stockpile.

Influent, sludge and biosolids samples for microbiological analysis were transported in cooler boxes with frozen ice packs and were delivered to the laboratory within 6 hours on the day of sampling, where they were immediately refrigerated on arrival. *Escherichia coli* analysis was conducted on the same day, and *Adenovirus* and *Salmonella* analyses were conducted within 48 hours.

Raw wastewater and sludge properties

The mean and range of total suspended solids (TSS) and volatile suspended solids (VSS) concentrations in raw wastewater influent at Boneo WwTP were 528 (170–1,300) mg L^{-1} and 450 (160–990) mg L^{-1}, respectively; the TSS and VSS of influent wastewater at Somers were lower and equivalent to 294 (260–330) mg L^{-1} and 247 (90–290) mg L^{-1}, respectively. Biochemical oxygen demand (BOD_5) and chemical oxygen demand (COD) of the raw wastewater at Boneo during the study period were 404 (290–690) mg L^{-1} and 894 (520–1,400) mg L^{-1}, respectively. At Somers, the BOD_5 and COD of the wastewater were similar to Boneo and equivalent to 398 (290–540) mg L^{-1} and 688 (560–960) mg L^{-1}, respectively. These properties were consistent with typical values of BOD_5 and COD reported for high-strength wastewater

equivalent to 350 mg L^{-1} and 800 mg L^{-1}, respectively (Tchobanoglous *et al.* 2015).

At Boneo WwTP, for the solar drying route, the mean DS and VS contents of the sludges after drying in the solar dryer sheds were 63% and 68%, respectively, with mean values of 42% DS and 50% VS for biosolids in the stockpiles (no significant difference was observed between one-year and two-year-old stockpiles). For the drying pan route, the sludge had a lower DS content of 21% at harvest from the drying pan, and the VS content was also lower at 50%, compared to the solar drying process; the mean DS and VS contents of biosolids in the stockpiles of drying pan sludge were 68% and 23%, respectively. At Somers WwTP, the mean DS and VS contents of the drying pan sludge were equivalent to 18% and 46%, respectively, and were similar to the values recorded at Boneo; the mean DS and VS in the stockpiled biosolids were 63% and 23%, respectively. The VS contents reported here were in a similar range to those measured by Rouch *et al.* (2011a) for biosolids following drying pan and stockpile treatment (26–59%).

Microbiological analysis

All samples were analysed by a National Association of Testing Authorities (NATA) accredited external laboratory using internationally recognised standard techniques and procedures.

Bacterial pathogens (*Escherichia coli* and *Salmonella*)

The Idexx Colilert-18 system was used to analyse *E. coli* in sludge and biosolids, based on a most probable number (MPN) procedure following Australian Standard Test Method AS4276.21 (Standards Australia 2005). The method detects *E. coli* based on the activity of the β-D-galactosidase and β-glucuronidase enzymes, which hydrolyse defined substrates to develop by-products that can be identified by colour and fluorescence, respectively. The Colilert-18 system can detect *E. coli* at 1 colony forming unit (CFU) g^{-1} DS after incubation for 18 to 22 hours. The results for stockpiles were reported as the average CFU g^{-1} DS from five replicate core samples; drying pan results were based on a composite sample of sludge collected from each pan.

The procedure used to detect *Salmonella* spp. was a modification of the method described in AS4276.14 (Standards Australia 2014). In brief, the presence of *Salmonella* in the selective broth enrichment culture was analysed using a VIDAS immunoanalyser (Biomerieux, Marcy l'Etoile, France). The presence of *Salmonella* was confirmed as described in AS4276.14 (Standards Australia 2014) only if the VIDAS immunoanalyser returned a positive result. The mean values were expressed as the maximum likelihood estimate (MLE) of the mean for the number of not detected (<1 *Salmonella* count) samples recorded multiplied by the mass of material analysed per sample. In the case of biosolids stockpiles, a total of 250 g DS (i.e. five composite samples, each of 50 g DS) of biosolids material was examined from each stockpile and thus the MLE of the mean *Salmonella* count was <1 *Salmonella* per 250 g DS, or <0.2 *Salmonella* per 50 g DS of stockpiled biosolids material. Therefore, the MLE varied with the number of composite samples collected and the total mass of material analysed.

Enteric viruses

Adenovirus was used as a conservative indicator to demonstrate the presence of enteric viruses (Jiang 2006). *Adenovirus* is the most abundant group of human enteric viruses in Australian raw wastewater that can be easily cultured and enumerated in the laboratory (NRMMC 2006) and are comparatively resistant to removal or disinfection (Gerba *et al.* 2002; WHO 2004). Preliminary investigations showed that *Enteroviruses* were below the limit of detection (LoD) and therefore *Enteroviruses* were not analysed. Viruses adsorbed to solid particles were eluted using the method described in APHA (2011) and were subsequently concentrated by precipitation with polyethylene glycol. *Adenovirus* was cultured by inoculating A549 cells with the final concentrated sample using a ten-tube MPN technique. The presence of *Adenovirus* in the cell cultures was confirmed by real time polymerase chain reaction as described by Heim *et al.* (2003). Results were reported as the MPN of infectious units (MPNIU).

Helminths

Helminth ova were recovered from raw wastewater, sludge and biosolids samples using a modification of the Tulane

method, as described by Bowman *et al.* (2003). Raw wastewater and sludge samples were settled overnight and the sediment was collected by decanting the liquid phase and mixed with anionic detergent. For biosolids, the samples were mixed in buffer containing the anionic detergent. Debris was removed by passing through a 250 μm sieve and the ova were further recovered by flotation in a solution of magnesium sulphate, specific gravity = 1.30, i.e. higher than that of the helminth ova. The final concentrate was analysed for helminth ova by microscopy. The detection limit was one ovum per volume analysed and for the purposes of statistical analysis, a not detected result in 1 L of raw wastewater was assumed to be equivalent to <1 ovum per litre.

RESULTS AND DISCUSSION

Bacterial pathogens (*Escherichia coli* and *Salmonella* spp.)

Escherichia coli was found in drying pan samples at low mean concentrations of 160 and 580 CFU g^{-1} DS for Boneo and Somers WwTPs, respectively (Table 2). *Escherichia coli* is typically found in raw wastewater in concentrations of 10^6–10^8 CFU 100 mL^{-1} and wastewater treatment processes typically provide a 1 to 2 $\log_{10}$ reduction in the indicator bacteria (Koivunen *et al.* 2003). Solar drying processes (such as the drying pan and solar drying shed used at Boneo, and drying pans at Somers WwTPs) are also effective at reducing numbers of faecal pathogens (Bennamoun *et al.* 2013). Rouch *et al.* (2011b) reported a >5 $\log_{10}$ reduction in *E. coli* during drying pan treatment for 8–15 months compared to the sludge entering the pan. The relatively low numbers of *E. coli* detected following drying pan treatment were consistent with these previous findings. The results demonstrated that the harvested drying pan sludge may typically comply with the T2 standard for *E. coli* of $<10^3$ CFU g^{-1} DS (EPA Victoria 2004). However, the Somers drying pan 2 sample exceeded this limit at 1,100 CFU g^{-1} DS (replicate data not shown).

Escherichia coli numbers in the December 2015 sample from the Boneo drying pan were reduced below the LoD (<17 CFU g^{-1} DS (replicate data not shown)). This may reflect the higher ambient temperatures and solar radiation during the summer period, which increase the inactivation of *E. coli* (Song *et al.* 2014). Consequently, this sample complied with the T1 standard for *E. coli* (<100 *E. coli* g^{-1} DS) (EPA Victoria 2004).

No *Salmonella* spp. were detected in biosolids collected from any of the drying pans (Table 2) and, thus, *Salmonella* spp. were either relatively uncommon in Boneo and Somers wastewater or the wastewater and/or sludge treatment

Table 2 | Bacterial pathogens in drying pans and one- and two-year biosolids stockpiles at Boneo and Somers WwTPs

Sample date	Stockpile core depth (m)	*E. coli* (CFU g^{-1} DS)[a] Mean	S.D.[a]	*Salmonella spp.* MLE
Boneo WwTP				
Drying pan[b]				
30-Apr-15/ 7-Dec-15	n/a	160	200	<0.50
Drying pan route – 1-year stockpiles[c]				
13-Mar-15	2.8	<14	n/a	0.20
11-Nov-15	2.0–2.5	<16	n/a	<0.20
Drying pan route – 2-year stockpiles[c]				
13-Mar-15	2.3	<14	n/a	<0.20
19-Mar-15	2.0	<14	n/a	0.20
Solar dryer route – 1-year stockpile[c]				
11-Nov-15	1.7–2.3	<25	n/a	<0.20
Solar dryer route – 2-year stockpile[c]				
13-Mar-15	2.7	<22	n/a	<0.20
Somers WwTP				
Drying pan[d]				
30-Apr-15	n/a	580	320	<0.30
1-year stockpiles[c]				
18-Mar-15	1.5	<15	n/a	<0.20
12-Nov-15	1.5	15	3.0	<0.20
12-Nov-15	1.5	<15	n/a	<0.20
2-year stockpile[c]				
18-Mar-15	2.8	<17	n/a	<0.20

DS = dry solids; S.D. = standard deviation; MLE = maximum likelihood estimate of the mean; n/a = not applicable.
[a]If no *E. coli* were detected the concentration was assumed to be equivalent to the LoD to calculate the mean and standard deviation where applicable.
[b]Means/MLEs of two composite samples of the same drying pan.
[c]Means/MLEs of five composite samples.
[d]Means/MLEs of four composite samples of four drying pans.

processes at the sites were efficient at inactivating these organisms. The results therefore suggested that the treated sludge complied with the EPA requirement for T1 biosolids of <1 *Salmonella* spp. 50 g^{-1} DS (EPA Victoria 2004) following approximately one year of treatment in a drying pan. These findings were consistent with previous reports showing the numbers of bacterial pathogens present in biosolids from industrialised countries are typically very small (Lang *et al.* 2007; Mondal *et al.* 2015). For example, Mondal *et al.* (2015) detected no *Salmonella* spp. in digested and air-dried biosolids from three WwTPs in Melbourne, Australia.

The results from the bacteriological examination of the ten biosolids stockpiles at the WwTPs (described in Table 1) are also presented in Table 2. The concentrations of *E. coli* in the one- and two-year stockpiles at both plants were below the LoD or were very small. *Escherichia coli* was not detected in any of the stockpiles of sludge at Boneo WwTP (Table 2), and was detected in only one of the stockpiles at Somers WwTP; a mean number of 15 CFU g^{-1} DS was recorded for one of the one-year stockpiles (Table 2). Hence, all the one-year and two-year biosolids stockpiles complied with the T1 performance standards required for faecal bacteria in dried and stockpiled biosolids of <100 *E. coli* g^{-1} DS (EPA Victoria 2004). Therefore, storing sludge in stockpiles for one year reduced the overall numbers of *E. coli* compared to the drying pan stage by up to 2 $\log_{10}$, to small or undetectable values (Table 2). Consequently, the enumeration results for the faecal indicator bacteria showed there was no advantage for the microbiological quality of biosolids of extending the stockpiling period beyond one year. Rouch *et al.* (2011b) also investigated pathogen inactivation during pan drying and stockpiling of sewage sludge and similarly found no *E. coli* in stockpiles of pan-dried sludge stored for six months to three years (LoD 20 CFU g^{-1} DS).

Salmonella spp. were detected in two stockpile samples from a total number of 50 samples collected across both sites. Thus, a mean concentration of *Salmonella* spp. of 0.2 MLE 50 g^{-1} DS (Table 2) was found in a one-year and a two-year stockpile prepared with sludge from the drying pan route at Boneo WwTP (Table 1). Serotyping of the isolates was not carried out and therefore it is not possible to confirm whether these were of human origin. However, *Salmonella* spp. were not detected in any of the drying pan samples at either WwTP (Table 2) and it is plausible that the small numbers of *Salmonella* spp. measured in these cases were potentially explained by the presence of animal faeces from wild animals and birds, which tend to be attracted to stockpiled biosolids (Zaleski *et al.* 2005). No *Salmonella* spp. were detected in the other samples of stockpiled biosolids and the overall MLE for the concentration of *Salmonella* spp. was therefore equivalent to <0.2 per 50 g DS.

Similarly, Rouch *et al.* (2011b) did not detect *Salmonella* in pan-dried and stockpiled biosolids. Hence, all the one-year and two-year biosolids stockpiles at both WwTPs achieved the T1 performance standard for *Salmonella* required for stockpiled biosolids of <1 *Salmonella* spp. 50 g^{-1} DS (EPA Victoria 2004).

Adenovirus

The *Adenovirus* results obtained for sludges sampled at various stages of treatment and biosolids in one- and two-year stockpiles at Boneo and Somers WwTPs are presented in Table 3.

At Boneo WwTP, the mean concentration of *Adenovirus* detected in WAS was 340 MPNIU g^{-1} DS (2.4 $\log_{10}$ MPNIU g^{-1} DS), and the mean concentration declined to 0.12 MPNIU g^{-1} DS (−0.86 $\log_{10}$ MPNIU g^{-1} DS) following solar drying and 1.2 MPNIU g^{-1} DS (−0.33 $\log_{10}$ MPNIU g^{-1} DS) with drying pan treatment (Table 3). Samples of WAS collected from Somers WwTP contained more *Adenovirus* than Boneo WWTP, with mean numbers equivalent to 950 MPNIU g^{-1} DS (2.8 $\log_{10}$ MPNIU g^{-1} DS); however, drying pan treatment reduced the mean MPNIU for the virus to a similar small value of 2.4 g^{-1} DS (0.19 $\log_{10}$ MPNIU g^{-1} DS). Hence, a significant inactivation of *Adenovirus* occurred across the sludge treatment processes prior to stockpiling.

The EPA Guidelines require new/alternative biosolids treatments to be verified for T1 classification by demonstrating that, among other criteria, enteric viruses are reduced by >3 $\log_{10}$ (EPA Victoria 2004). To demonstrate the viral inactivation achieved by the sludge treatment processes operated by SEW, the enumeration data for various sludge/biosolids streams were used to calculate the mean $\log_{10}$ reduction in *Adenovirus*, and these are presented in Figure 1.

Table 3 | *Adenovirus* concentrations at various stages of the sludge treatment process at Boneo and Somers WwTPs

Sludge source and stockpile sample date	*Adenovirus* (MPNIU g^{-1} DS) Mean/MLE	Standard deviation	Number of samples (n)
Boneo WwTP			
WAS[a]	340	430	26
Belt press feed sludge[a]	26	50	23
Solar dryer sludge[b,c]	0.12	0.01	5
Solar dryer shed 1[c]	0.48	0.81	7
Solar dryer shed 2[c]	0.18	0.12	7
Solar dryer shed 3[c]	0.12	0	6
Drying pan sludge[c]	1.2	1.5	2
Drying pan route – 1-year stockpiles			
13-Mar-2015	<0.02	n/a	5
12-Nov-2015	<0.02	n/a	5
Drying pan route – 2-year stockpiles			
13-Mar-2015	<0.02	n/a	5
19-Mar-2015	<0.02	n/a	5
Solar drying route – 1-year stockpile			
12-Nov-2015	<0.02	n/a	5
Solar drying route – 2-year stockpile			
13-Mar-2015	<0.02	n/a	5
Somers WwTP			
WAS[a]	950	1,100	24
Drying pan sludge[c]	2.4	1.6	4
1-year stockpiles			
18-Mar-2015	<0.02	n/a	5
12-Nov-2015	<0.02	n/a	5
12-Nov-2015	<0.02	n/a	5
2-year stockpile			
18-Mar-2015	<0.02	n/a	5

n/a = not applicable; MLE = maximum likelihood estimate of the mean.
[a]Sampling was conducted on an approximately fortnightly basis between 19 November 2014 and 19 November 2015 at Boneo WwTP and 19 November 2014 and 18 November 2015 at Somers WwTP.
[b]Composite samples of solar drying sheds 1–3.
[c]Sampling conducted at harvest.

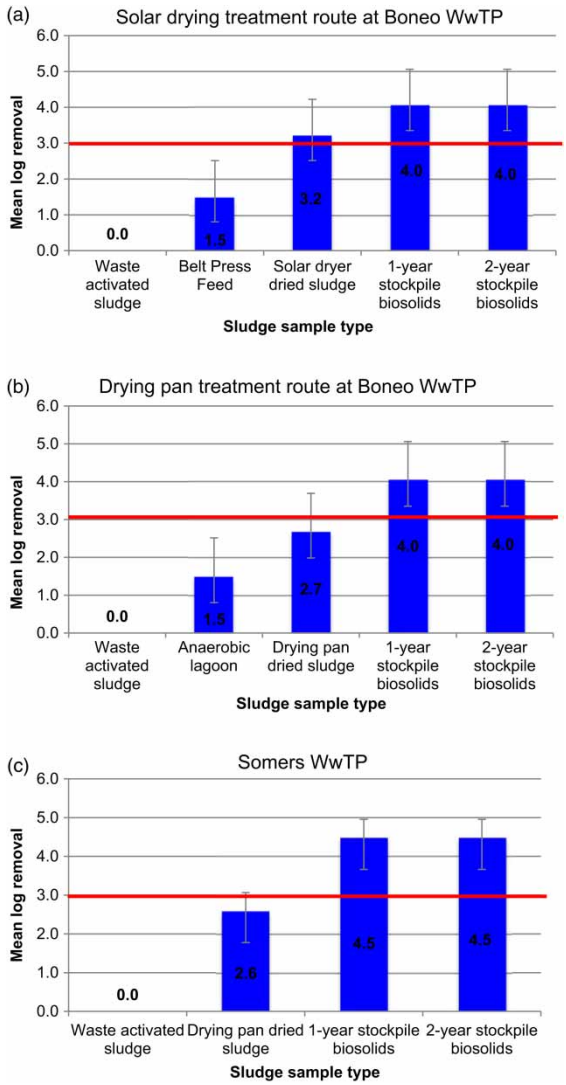

Figure 1 | Mean $\log_{10}$ reduction in *Adenovirus* concentration by (a) solar drying and (b) drying pan treatment, and stockpile storage of sludge at Boneo WwTP, and (c) drying pan sludge treatment and stockpile storage at Somers WwTP. Thick horizontal line = EPA $\log_{10}$ removal requirement for verification of an alternative biosolids treatment as a T1 process (EPA Victoria 2004). The error bars represent the difference between the mean $\log_{10}$ concentration at each stage of the process compared to the minimum and maximum influent sludge values.

The results demonstrated that sludge treatment was effective at reducing the survival of *Adenovirus*. For example, the mean $\log_{10}$ removal of *Adenovirus* obtained by the solar dryer process at Boneo WwTP was equivalent to 3.2. A significant reduction in *Adenovirus* numbers was also measured in drying pan sludge at both WwTPs, compared to the feed sludge (mean $\log_{10}$ reduction = 2.7 and 2.6 at Boneo and Somers WwTPs, respectively), although in these cases, the rates of reduction were smaller compared to the solar dryer. The increased rate of inactivation observed in the solar drying sheds may be due to the degree of desiccation and elevated temperatures that develop in the sludge during the solar drying process. This may present a more challenging environment for the survival of virus particles compared to dehydrating sludge

under ambient conditions in drying pans. Indeed, the DS content was significantly higher following drying shed treatment, at 63% compared to sludge from the drying pan, which had a mean DS content of 21%.

Storing sludge in stockpiles for one year reduced the concentration of *Adenovirus* further to below the LoD at both sites (Table 3). Overall $\log_{10}$ reductions of *Adenovirus* in stored biosolids were equivalent to approximately 4.0–4.5 compared to the input numbers measured entering the sludge treatment processes in the WAS feed sludge. Furthermore, the $\log_{10}$ reductions reported here were based on MLE values and therefore represent a conservative estimate of the $\log_{10}$ removal rate.

The results demonstrated that the EPA performance standard to reduce enteric viruses by >3 $\log_{10}$ (EPA Victoria 2004), for verification of an alternative T1 process, was readily achieved by the sewage sludge treatment processes operating at Boneo and Somers WwTPs, followed by stockpiling biosolids for a maximum period of one year. *Adenovirus* was reduced to an undetectable value within a one-year period of biosolids storage, therefore no additional improvement in the microbiological quality of biosolids, with respect to enteric viruses, was gained by extending the duration of the stockpiling period further.

The rate of *Adenovirus* inactivation obtained in the solar drying sheds suggested that the EPA performance standard (>3 $\log_{10}$ reduction, Figure 1(a)) for T1 grade biosolids could be achieved by the solar dryer treatment stage, potentially eliminating the requirement for sludge storage altogether. This remains to be verified as an alternative treatment method to produce T1 quality biosolids with equivalent inactivation of pathogenic bacteria, viruses and helminths to that achieved by stockpiling for one year.

Helminths

The EPA Guidelines require alternative T1 processes for biosolids treatment to demonstrate >2 $\log_{10}$ reduction in *Ascaris* ova (EPA Victoria 2004). However, theoretical estimates of the parasite transfer to sludge during wastewater treatment based on predicted infection and excretion rates in the human population show that there is a minimal risk of microbiological contamination of biosolids with *Ascaris* in the Australian State of Victoria (Grant *et al.* 2012). Consequently, in this study we focused on sampling the raw wastewater influent to the WwTPs to determine whether *Ascaris* ova were present in SEW sewerage catchments. However, no helminth ova were detected in raw wastewater samples (51 samples were collected from Boneo and Somers WwTP between 18 November 2014 and 18 November 2015) and therefore it was not possible to determine a log removal rate for this organism. In addition to the influent raw wastewater sampling programme, sampling of the various sludge streams and biosolids stockpiles was undertaken (Table 1). No helminths were detected in any of the samples. The absence of *Ascaris* ova in raw wastewater, sludge and biosolids was consistent with the rare frequency of *Ascaris* infections in the human population and the negligible occurrence of ova in wastewater and sludge in Victoria (Grant *et al.* 2012). Indeed, it is recognised that adopting *Ascaris* as an indicator in Victoria is problematic, since low levels in raw sludge mean that demonstrating the required reductions is difficult (EPA Victoria 2004).

The sampling programme for *Ascaris* ova tested 51×1 L samples of raw wastewater collected from both WwTPs. For a dataset of n samples of raw wastewater, each of 1 L, with x ova detected, and assuming a laboratory enumeration recovery rate (r) of 50%, a conservative estimate based on internal laboratory testing, the mean density of ova (M) in the wastewater is given by:

$$M = \frac{(x/r)}{n}$$

Therefore, as <1 ova was detected in each sample:

$$\text{Mean density} = \frac{(<1/0.5)}{51} = <0.0392\ \text{ova/L}^{-1}$$

Increasing the sample size raises the upper estimate of the mean. Thus, the occurrence of helminth ova in raw wastewater from the two catchments was <1 per 20 L.

The recommended WHO limit for the protection of human health is <1 *Ascaris* ovum g^{-1} DS of raw sludge (WHO 2006). Assuming that the raw wastewater contains approximately 350 mg L^{-1} of suspended solids (Tchobanoglous *et al.* 2015) and that all the ova are associated with the solids fraction, the mean density of *Ascaris* ova in raw

sludge entering the treatment plant was:

$$\text{Mean density} = \frac{<0.0392}{0.35} = <0.11 \text{ ova g}^{-1} \text{ sludge } DS$$

This was consistent with the results obtained from the parasitological examination of samples of WAS collected from Boneo and Somers WwTPs where no ova were detected in samples of 5 g DS of sludge.

CONCLUSIONS

The EPA performance standards for faecal bacteria in stockpiled biosolids to produce a T1 product are <100 *E. coli* g^{-1} DS and <1 *Salmonella* spp. 50 g^{-1} DS. Mean *E. coli* in one- and two-year stockpiles of biosolids examined at Boneo and Somers WwTPs ranged between 14 and 22 *E. coli* g^{-1} DS. In general, *Salmonella* spp. were not detected in biosolids stored in one- and two-year stockpiles. Small numbers of *Salmonella* spp. were measured in two biosolids samples, one each from one-year and two-year stockpiles of biosolids at Boneo WwTP; however, in these cases, the presence of non-human sources of contamination with *Salmonella* spp. was suspected.

The EPA virus performance standard for verification of alternative treatment processes to produce T1 grade biosolids is >3.0 $\log_{10}$ reduction in enteric viruses. *Adenovirus* is regarded as a relatively conservative indicator of enteric virus survival in sludge. The virus was not detected in biosolids sampled from one- and two-year stockpiles at either WwTP and, overall, the concentration of *Adenovirus* decreased by >4.0 $\log_{10}$ in one-year biosolids stockpiles relative to untreated WAS entering the sludge treatment process stream.

No helminth ova were detected in samples of raw wastewater, sludge or biosolids collected from the WwTPs. Thus, a $\log_{10}$ reduction for *Ascaris* could not be provided based on operational microbiological monitoring criteria. However, the mean density of *Ascaris* ova in raw wastewater was estimated based on the number and volume of the samples examined and was equivalent to <0.0392 ova L^{-1}.

The MLE of the concentration of *Ascaris* ova in biosolids from one- and two-year stockpiles at Boneo and Somers WwTPs was <0.11 ova g^{-1} DS, and was therefore significantly below the WHO guideline value for unrestricted use of biosolids in agriculture of <1 helminth ovum g^{-1} DS (WHO 2006) and below the US EPA Class A criteria for viable helminth ova for the unrestricted use of biosolids in agricultural and domestic applications of <0.25 helminth ovum g^{-1} (US EPA 2003).

Overall, the data demonstrated the following:

- The sludge treatment and management processes operating at Boneo and Somers WwTPs exceeded the verification requirements for alternative treatment processes to produce T1 grade biosolids with respect to prescribed faecal bacterial numbers and enteric virus reduction after stockpile storage for one year.
- The enteric microbiological properties of biosolids from one- and two-year stockpiles were equivalent and, consequently, no additional improvement in the microbiological quality was gained by extending the storage period beyond one year.
- There was no evidence of a significant *Ascaris* challenge present in the raw wastewater entering the treatment plants; consequently, demonstrating the $\log_{10}$ reduction of this parasite by sewage sludge treatment processes is not applicable to managing the microbiological safety of biosolids for agricultural use in Victoria, Australia. In this situation, the routine monitoring of *Ascaris* ova in raw wastewater is recommended to verify that the concentration remains below the LoD.

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