

## Research Paper

# Air-drying bed as an alternative treatment for UASB sludge under tropical conditions

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### ABSTRACT

This study investigated the behaviour of pathogens and microbial indicators during dewatering of sludge from an upflow anaerobic sludge blanket (UASB) reactor treating real domestic wastewater under tropical conditions. The sludge was dewatered in air-drying beds during wet and dry seasons and was monitored for 90 days. Culture-based methods, direct microscopy identification and quantitative polymerase chain reaction assays, were used to evaluate pathogenic and microbiological indicator microorganisms in the sludge. Thermotolerant coliforms and coliphages (somatic and male F-specific) were monitored as bacterial and viral indicators, respectively. Pathogenic organisms monitored included *Salmonella* spp., *Salmonella* Typhimurium, *Salmonella* Enteritidis, pathogenic *Escherichia coli* strains (intimin-coding genes), *Campylobacter jejuni*, *Cryptosporidium* spp., adenovirus, and viable helminths eggs. Results revealed that microbial indicators did not show a significant variation between the dry and wet seasons, as it was verified for some pathogens. During the 90 days of sludge dewatering, the air-drying bed was able to remove microbial indicators and the pathogenic organisms *E. coli*, *S. Typhimurium*, and *Cryptosporidium* spp. (when present). Pathogenic *C. jejuni*, *S. Enteritidis*, and adenovirus decreased 0.5–1.7, 0.3–0.4, and 2.6–4.8 log units, respectively. These results highlight the potential of air-drying beds as a simple and low-cost process for sludge dewatering and hygienisation.

**Key words** | air-drying bed, microbial indicators, pathogens, sewage sludge, tropical climate, UASB reactor

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### HIGHLIGHTS

- The air-drying bed tested as a sustainable process to reduce pathogens in sewage sludge.
- Pathogen indicators decreased to below detection limits after 90 days of dewatering.
- Correlation not found among pathogens and microbial indicators.
- Rainfall promoted the regrowth of *Salmonella* spp. and thermotolerant coliforms during the dewatering.

## GRAPHICAL ABSTRACT



## INTRODUCTION

'Sewage sludge' or 'biosolids' are solid by-products generated during the biological treatment of wastewater, and, following stabilisation procedures, such as aerobic or anaerobic digestion, are widely used as an alternative fertiliser to improve crop production. Recycling to agricultural land is an essential outlet for sewage sludge. As such, treated sewage sludge can often be a valuable resource to improve crop production by increasing the nitrogen and organic matter contents in soil. Sewage sludge contains high amounts of solids (stabilised or otherwise), organic matter, nutrients (nitrogen, phosphorus, and others), and pathogenic organisms. Its composition varies according to different geographical locations and individual treatment facilities (Arthurson 2008). Some pathogens of concern in sludge are *Salmonella*, enteropathogenic *Escherichia coli*, *Campylobacter jejuni*, adenovirus, and *Cryptosporidium* spp., as they have been associated with several outbreaks worldwide (U.S. EPA 2003). Therefore, sewage sludge often requires treatment before final disposal to minimise risks to the environment and public health.

In 2006, the Brazilian Government adopted a series of specific policies, mostly based on the Control of Pathogens and Vector Attraction in Sewage Sludge (U.S. EPA 2003), to ensure the safe use of sewage sludge in agriculture. That regulatory approach classifies the sludge into two categories, Class A and Class B, according to pathogens and microbial indicator levels. Brazilian requirements for Class A sludge are less than 1,000 most probable number (MPN) of thermotolerant coliforms (TCs) per gram, less than 0.25 viable helminth eggs per gram, *Salmonella* absence in 10 g, and less than 0.25 enteric virus plaque-forming units

(PFUs) per gram. For Class B biosolids, TC requirements are less than one million MPN per gram and less than ten viable helminth eggs per gram, with site restrictions designed to protect public health and the environment. The purpose of this classification is to place barriers in exposure pathways by reducing the number of pathogens in the treated sewage sludge (biosolids).

Different processes can reduce pathogens and microbial indicators present in sewage sludge, such as anaerobic or aerobic digestion, composting, lime stabilisation, and air drying (U.S. EPA 2003). How sludge is treated and managed has a substantial impact on total treatment costs. Sludge management costs are estimated at 20–60% of total operating costs in wastewater treatment plants (WWTPs) (Andreoli et al. 2007). Most low-income countries have enormous deficits in sanitation infrastructure. As they plan to invest in building their sanitation infrastructure, special consideration should be given to sustainable, ecological solutions in the interest of reducing financial and environmental costs of sewage and sludge treatment and disposal, while maximising benefits to the environment and public health.

Nature-based and non-mechanized alternatives, such as air drying, could be a sustainable alternative for sludge hygienisation by natural environmental attenuation, especially in tropical regions. Air-drying beds have been the most commonly used alternative for dewatering of sludge from the upflow anaerobic sludge blanket (UASB) reactor in Brazil (Chernicharo et al. 2015). The sewage sludge is left to drain and dry by evaporation, which depends on the local climate. More research is needed to understand if air drying is a suitable process to reduce pathogens in

tropical climates. According to the Koppen–Geiger climate classification, tropical weather is characterised by distinct wet and dry seasons, with most of the precipitation occurring in the summer.

During the drying period, sewage sludge undergoes physical, chemical, and biological changes. Biological changes are relevant to public health research and can be evaluated by monitoring microbial indicators and pathogens (bacteria, viruses, protozoa, and helminths). Some bacteria have the potential to regrow to high numbers in sludge after dewatering in air-drying beds, such as *Salmonella* and TCs (Zaleski *et al.* 2005). Although microbial indicators are widely used to evaluate air-drying beds (Rouch *et al.* 2011; Santos *et al.* 2017), there are relevant data gaps on the persistence of pathogens, such as viruses, protozoa, and helminths. Pathogen survival can be described by quantitative decay kinetics to determine minimum process conditions and retention times for the effective removal of important pathogens. These data are essential for predicting human health risk, which can lead to treatment decisions.

Due to the relatively low availability of local and regional studies concerning the safe, sanitary use of sewage sludge for agriculture purposes, low-income countries are adopting regulations similar to those of high-income regions and countries, such as the European Union and the USA. Nevertheless, most low-income countries are situated in warm climate regions, and it is urgent to create a fair and nuanced view concerning the reality of these countries where simple, economical, and sustainable solutions are strongly demanded.

To the best of our knowledge, this is the first study to investigate the use of air-drying beds to inactivate pathogens and microbial indicators in UASB sludge under tropical conditions. In this study, we quantified pathogens and microbial indicators by quantitative polymerase chain reaction (qPCR), culture-based assays, and direct microscopy methods. Advances in molecular techniques have led to improved detection of non-cultivable pathogens and have become extremely valuable tools for public health. Understanding the behaviour of pathogens during dewatering in tropical conditions could optimise design and operational conditions, resulting in cost reductions and meeting the discharge standards established by adequate environmental legislation.

## MATERIAL AND METHODS

### Study site, sample collection, and environmental parameters

The experiment was conducted in Belo Horizonte city, capital of Minas Gerais state in Brazil. Some socioeconomic aspects of the geographic area include 2.5 million inhabitants, a gross domestic product estimated at USD\$ 6,777 per capita, and human development index of 0.81, according to the Brazilian Institute of Geography and Statistics (IBGE 2017). Inside one of the city's major WWTPs is located the Centre for Research and Training in Sanitation (CePTS) of the Department of Sanitary and Environmental Engineering, Federal University of Minas Gerais (UFMG). CePTS contains several demonstration and pilot-scale wastewater treatment units, including a UASB reactor that is used to treat raw sewage. Sludge from such reactors was dewatered and hygienised using air-drying beds (a drying period of 30–45 days). Table 1 shows more information on the UASB reactor and the air-drying bed used in this study. The UASB reactor was fed with domestic wastewater taken from a chamber upstream of the WWTP's primary clarifiers that had undergone preliminary treatment for solids and grit removal.

The amount of sludge dewatered in the air-drying bed was 3–4 m<sup>3</sup>. Following dewatering, the sampling campaign was conducted over 90 days for dry and wet seasons. For each sampling campaign, an amount of 500 g of UASB

**Table 1** | Detail information on design and operational parameters of the UASB reactor and air-drying beds

Characteristic	UASB reactor	Air-drying bed
Material	Fibreglass	Concrete
Diameter (m)	2.5	NA
Height (m)	4.5	0.60
Length (m)	–	5.00
Width (m)	NA	2.00
Useful volume (m <sup>3</sup> )	22.0	4.00
Hydraulic retention time (h)	7	NA
Organic loading rate (kg BOD day <sup>-1</sup> )	24	NA
Sewage temperature range (°C)	20–25	NA
Population equivalent (inhabitant)	640	640

NA, not applicable.

sludge was collected in a previously sterilised bottle, in six different points randomly selected in the air-drying bed. All six aliquots were combined and thoroughly mixed to provide a representative composite sample. Composite samples were transported to the laboratory and refrigerated at 4 °C until further analysis. They were combined and mixed to provide a representative composite sample.

To evaluate the effect of climate conditions in sludge air drying, temperature (in Celsius) and rainfall (mm) were measured hourly at the meteorological station by using a Davis Instruments® probe placed onsite for continuous weather monitoring.

### Microbial enumeration by culture, direct microscopy identification, and molecular methods

#### Detection and quantification of microbial indicators and *Salmonella* spp.

Culture methods are widely used for quantifying microbial indicators, such as TCs and coliphages, and a small number of pathogens, such as *Salmonella* spp. TC and *Salmonella* spp., are described as a microbial parameter for classifying sludges according to EPA Methods 1680 and 1682, respectively (U.S. EPA 2006, 2010). Coliphages are used in enteric virus analysis, and, for that reason, somatic and male F-specific coliphages were analysed by standard double-overlay agar methods described by Method 1602 (U.S. EPA 2001a) with some modifications (Guzmán et al. 2007). All stock cultures used for coliphage analysis were cultured, stored, and checked according to the following instructions given in the respective EPA guidelines (U.S. EPA 2001a). The host bacteria used for assaying the sludge for somatic coliphages were American Type Culture Collection (ATCC 700609) and male F-specific coliphages (ATCC 700891). The determination of total solids (% dry weight) was performed according to Method 1604 (U.S. EPA 2001b). A total of 30 samples in each season were analysed using culture-based methods for quantifying microbial indicators and *Salmonella* spp. totalised.

#### Microscopy identification analyses

Viable helminth egg microscopy identification was performed according to Meyer et al. (1978). Briefly, 25 g of

sewage sludge was treated with 50% sodium hypochlorite solution to clarify the sample. Then, the sample was washed several times with 0.1% Tween 80 solution to segregate the eggs from other solid particles. After washing, ZnSO<sub>4</sub> solution (density 1.18) was added to float the eggs, and the supernatant was recovered. The supernatant was filtered through a 0.45 mm porosity Millipore membrane to retain the eggs. In the sequence, the filter was scraped and placed in a Petri dish containing H<sub>2</sub>SO<sub>4</sub> solution (0.1 N). The Petri dish was incubated in the dark at 28 °C for 28 days. After this period, the Sedgwick-Rafter chamber was used to count viable helminth eggs, using a 1 mL aliquot of the sample contained in the plate. Because it is a time-consuming and laborious analysis, viable helminth eggs were analysed only at days 0 and 90.

#### DNA extraction and qPCR assay conditions

DNA extraction from sludge samples was performed using the FastDNA® SPIN Kit for Soil (MP Biomedicals, USA) following the manufacturer's instructions. For each sample, genomic DNA was extracted from 0.3 to 0.5 g of the sludge, and the quality and quantity of extracted DNA confirmed by Nanodrop®, ND-1000 (Thermo Fisher Scientific, USA) and the final purified DNA stored at -20 °C for further analysis.

PCR conditions, such as the annealing temperature, were tested and optimised for the primers used by performing PCR gradient analysis for each primer pair. Primer sequences, annealing temperature, and amplicon size are listed in Table 2. All primers were synthesised by Integrated DNA Technologies (Coralville, USA).

All qPCR amplifications were performed using the ABI 7500 Instrument (Applied Biosystems). The qPCR reactions (20 µL) were performed in triplicate using 10 µL SYBR Green qPCR Master Mix (Applied Biosystems Invitrogen, Carlsbad, CA), 250 nM of each primer, 7.5 µL of DNase and RNase free deionised water, and 2 µL of template DNA or 1 µL of PCR product in the case of the adenovirus nested reaction. The relative standard curve method was used to determine relative target quantities in samples, and the correct amplification products for these assays were chosen based on melting curve analysis. Due to high reagent

**Table 2** | Primers used for qPCR assays

Target organism	Primer sequence (5'-3')	Annealing temperature	Amplicon size (bp)	Reference
<i>S. Typhimurium</i>	Fli15- CGGTGTTGCCAGGTTGGTAAT Typ04- ACTCTTGCTGGCGGTGCGACTT	52	620	Zappellini et al. (2017)
<i>S. Enteritidis</i>	S1- GCCGTACACGAGCTTATAGA S4- ACCTACAGGGGCACAATAAC	52	250	
<i>C. jejuni</i>	Campj-F CATCTTCCCTAGTCAAGCCT Campj-R AAGATATGGCACTAGCAAGAC	54	773	BRASIL (2011)
Intimin-coding gene (EHEC/EPEC)	eae-F ATGCTTAGTGCTGGTTTAGG eae R GCCTTCATCATTTCGCTTTC	58	248	Fukushima et al. (2003)
<i>Cryptosporidium</i> spp.	P702-F CAAATTGATACCGTTTGTCCCTCTG P702-R GGCATGTCGATTCTAATTCAGCT	60	142	Guy et al. (2003)
Adenovirus	hexAA1885 GCCGCAGTGGTCTTACATGCACATC hexAA1913 -CAGCACGCCGCGGATGTCAAAG nehexAA1893 -GCCACCGAGACGTACTTCAGCCTG nehexAA1905 GTACGAGTACGCGGTATCCTCGCGGT	55	300 142	Allard et al. (1992)

qPCR analysis costs, the samples analysed corresponded to day 0, 20, 40, 60 and 90 days of dewatering.

### Data management and calculations

Nonparametric analyses were applied by Statistic 12 Software (StatSoft, OK, USA) with a critical  $p$ -value for all tests  $p < 0.05$ . The Mann-Whitney U test was used to evaluate the impact of different seasons on microbial indicators and pathogens. Additionally, Spearman's rank correlation was used for identifying any association between them.

To determine the microbial indicators and pathogen-specific persistence patterns during sludge dewatering, the first-order exponential one-parameter model was applied (Equation (1)).

$$C = Co. e^{-Kb.t} \quad (1)$$

where  $C$  is the number of microorganisms per g TS at treatment time,  $t$ , in days;  $Co$  is the initial number of microorganisms per g TS; and  $Kb$  is the decay coefficient. Correlation coefficients  $R$  values above 0.5 were considered to represent strong correlations, and T90 values were calculated only for these samples.

## RESULTS AND DISCUSSION

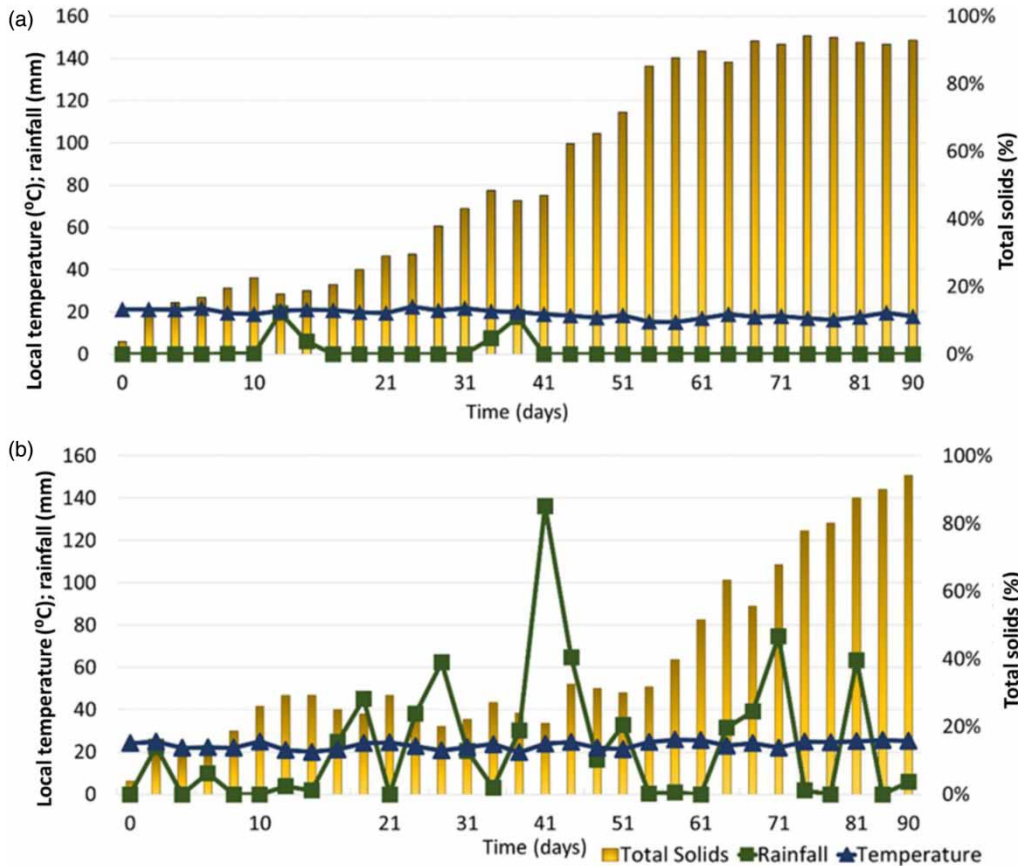
### Climate variables affecting sludge dewatering

Figure 1 shows average daily temperature, rainfall, and total solids monitored during 90 days in dry (Figure 1(a)) and wet seasons (Figure 1(b)). The average daily temperature of the dry season ranged from 15 to 23 °C and an accumulated rainfall of 56.4 mm during the period evaluated. The wet season exhibited similar average daily temperatures (20–26 °C) and an accumulated rainfall of 803.8 mm during the 90 days.

Classical mechanical sludge dewatering techniques (belt filters, press filters, and centrifuges) have difficulty in achieving greater than 20–30% total solid content. In this study, air-drying beds achieved this solid content range in 30 days in the dry season and 57 days in the wet season. These results highlight the potential of the air-drying bed as a sustainable, low-cost process for reducing moisture content in UASB sludge under tropical conditions.

Another relevant aspect observed in this study is that extreme rainfall events during the wet season contributed to a delay in the free water loss in the sludge until the 61st day of dewatering when the sludge achieved 52% of dry solids. According to Tao et al. (2005), when the sludge





**Figure 1** | Local temperature, rainfall, and total solid content of the sludge during the (a) dry season and (b) wet season 90 days after dewatering in the air-drying bed.

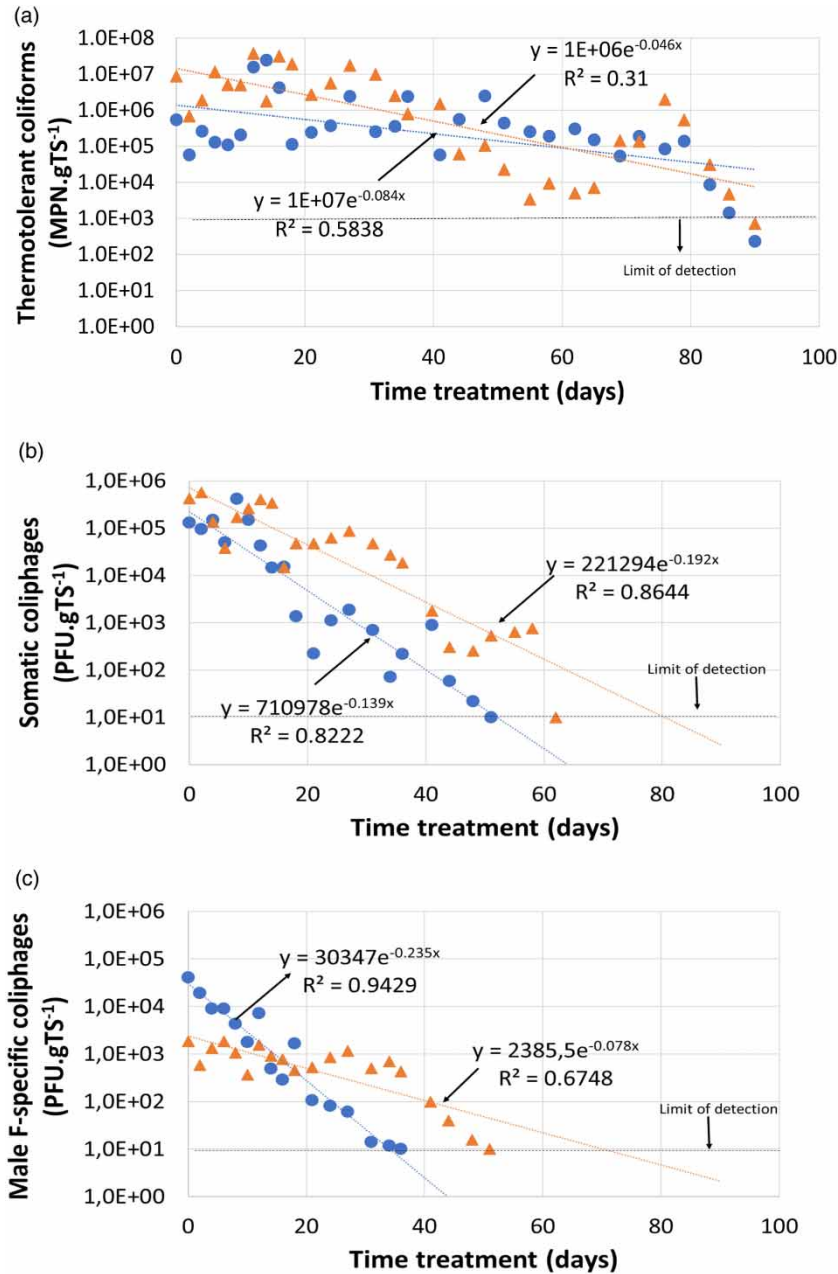
achieves 50–60% of dry solids during the dewatering process, the rapid moisture loss volume leads to drastic changes in characteristic dimensions of sludge. This phenomenon creates preferential pathways formed by shrinkage and cracking and allowed the rainwater to percolate rapidly through the sludge material and did not affect it.

### Die-off of indicator organisms

The concentration of microbial indicators decreased over the 90 days of UASB sludge dewatering (Figure 2). TC (Figure 2(a)) initial concentrations ranged from  $5.42 \times 10^5$  to  $8.71 \times 10^6$  MPN gTS<sup>-1</sup>. After 90 days of treatment, TC decreased 3–4 log units achieving the required limit ( $<3 \log_{10}$  MPN gTS<sup>-1</sup>) to classify the sludge as Class A according to the EPA (U.S. EPA 2003). These results are in agreement with a previous study (Santos et al. 2017), which identified TC reductions of 1.8–2.8 orders of magnitude in

UASB sludge after dewatering for 90 days in an air-drying bed. Although TC values showed a significant reduction, the first-order exponential model showed relatively low values of  $R^2$  (Figure 2(a)). The low  $R^2$  values observed for TC were caused by regrowth events detected during the study, mainly in the wet season due to rainfall events.

TC has been used as a suitable microbial indicator in low-income countries as a parameter to classify sludge safety for agriculture use. After 90 days of dehydration in uncovered air-drying beds in tropical conditions, the UASB sludge achieved a TC concentration below the detection limits ( $<3.0 \log$  MPN gTS<sup>-1</sup>) producing a Class A quality sludge. This result showed that Brazilian regulations could be conservative for TC once the regulation adopts the pre-set 90 days of air drying to produce Class B sludge. Concerning regrowth events, some authors (Zaleski et al. 2005; Schwarz et al. 2019) report that it is not unusual in biosolids storage for extended periods, and it is considered an overly



**Figure 2** | Effect of the air-drying bed on bacterial and virus indicators survival in UASB sludge over the wet season (▲) and the dry season (●).

conservative indicator (U.S. EPA 2003). According to NRC (2004), some bacteria can coordinate changes in gene expression in response to environmental challenges such as desiccation and high temperatures.

In this study, initial concentrations of somatic coliphages (Figure 2(b)) ranged from  $1.31 \times 10^5$  to  $4.21 \times 10^5$  PFU gTS<sup>-1</sup> (day 0) and decreased to undetectable levels after 51 days in

the dry season and 65 days in the wet season. Additionally, the coliphages survival showed a good fit in the exponential model, with  $R^2$  values above 0.8 for both seasons. These results permitted the prediction of T90 values of 16 days for the dry season and 12 days for the wet season.

Compared to somatic coliphages, male F-specific (Figure 2(c)) showed initial concentrations (day 0) ranging

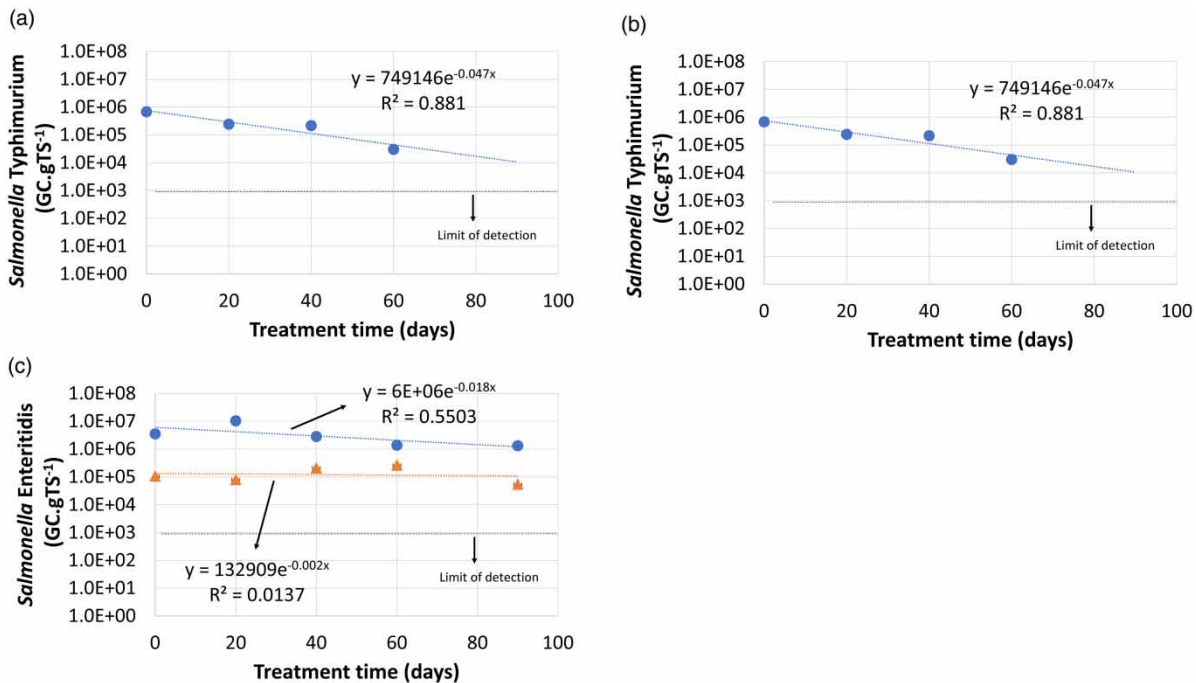
from  $1.85 \times 10^3$  to  $4.1 \times 10^4$  PFU gTS<sup>-1</sup> corresponding to the wet and dry season, respectively. During the 90 days of treatment, male F-specific decay occurred more rapidly compared to that of somatic coliphages and was reduced to undetectable levels in 34 days in the dry season and 48 days in the wet season. Additionally, the exponential model showed high  $R^2$  values in dry and wet seasons, which represent T90 values of 10 and 29 days, respectively, thereby showing that different seasons had an essential effect in the decay of this indicator.

Currently, coliphages have been suggested as virus indicators in wastewater treatment performance due to physical similarity to human enteric viruses of concern, and they have similar persistence patterns (U.S. EPA 2016). Air drying under tropical conditions resulted in decay to below detection limits of somatic coliphages in 51–62 days and male F-specific in 36–55 days. A previous study (Martin-Diaz et al. 2016) evaluated the somatic coliphages as surrogates for enteroviruses in sludge hygienisation and verified that the relationship of  $4 \log_{10}$  PFU gTS<sup>-1</sup> orders of magnitude contained infectious enterovirus levels that were very low or below detection limits.

## Die-off of pathogens

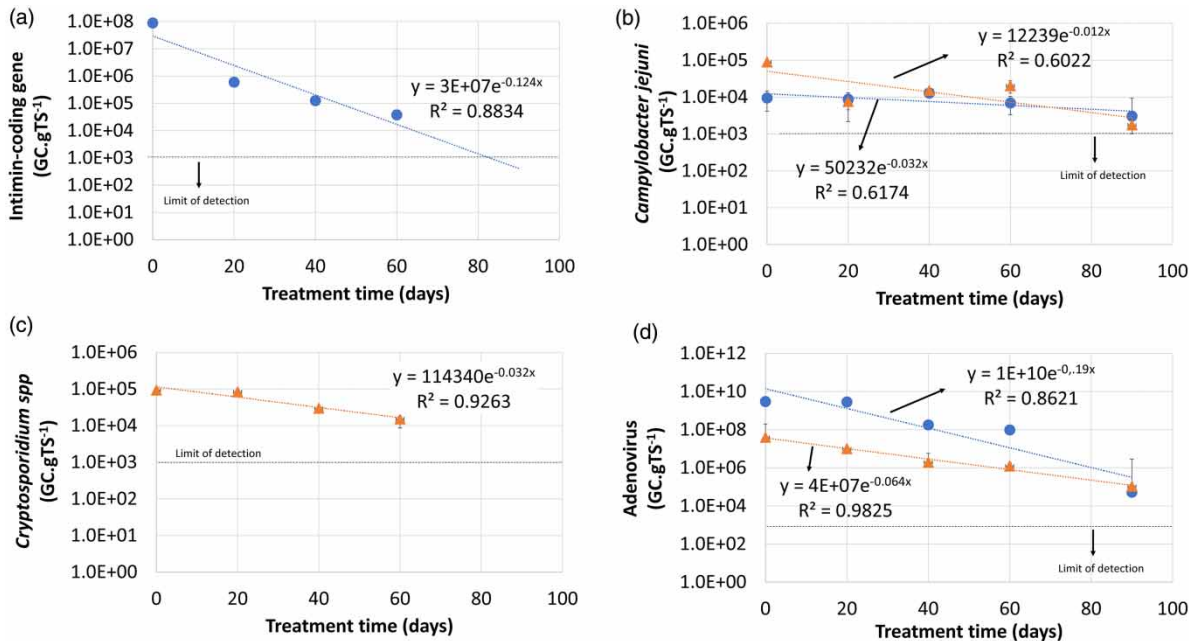
Pathogens are inherent to sludge produced in WWTP and vary spatially and temporally (NRC 2004). This study evaluated some important waterborne pathogens of public health concern. All pathogens evaluated in this study were detected in UASB sludge. The identification and quantification of pathogens by culture methods are limited because this technique can identify less than 2% of bacteria. To fill this gap, molecular techniques, such as qPCR, are valuable tools to understand the behaviour of the pathogen during the sludge dewatering process. However, it is pertinent to emphasise that qPCR quantifies the genomic material present in the sample, but it does not determine whether the pathogen is viable or not. qPCR results revealed that different seasons had a significant impact on the occurrence of the *E. coli* pathogenic strain (encoded by *intimin-coding genes*), *Salmonella* Typhimurium, *Salmonella* Enteritidis, and *Cryptosporidium* spp. (Figures 3 and 4).

*Salmonella* spp. quantified by a culture-based method (Figure 3(a)) showed a noticeable regrowth in the first 50 days of dewatering sludge. In this period, *Salmonella* spp.



**Figure 3** | Effect of the air-drying bed on the survival of (a) *Salmonella* spp. by the culture-based method, (b) *S. Enteritidis*, and (c) *S. Typhimurium* by qPCRs in UASB sludge over the wet season (▲) and the dry season (●).





**Figure 4** | The occurrence of pathogenic microorganisms during the 90 days of dewatering sludge treatment revealed by qPCR: (a) *Intimin-coding gene*, (b) *C. jejuni*, (c) *Cryptosporidium* spp., and (d) adenovirus in UASB sludge over the wet season ( $\blacktriangle$ ) and dry season ( $\bullet$ ).

reached the highest concentration on the 12th day, with values of  $21.3 \text{ MPN gTS}^{-1}$  in the dry season and  $18.2 \text{ MPN gTS}^{-1}$  in the wet season. Furthermore, in the dry season, *Salmonella* spp. remained *undetectable* or below the detection limit after 51 days. However, in the wet season, *Salmonella* spp. was detected throughout the experiment showing another regrowth event between 83 and 90 days. Due to regrowth events, the exponential model did not describe the best fit for *Salmonella* spp. data, as demonstrated by the low coefficient of determination ( $R^2$ ).

*Salmonella* spp. detected by culture-based methods (Figure 3(a)) is generally used as a biosolid parameter to ensure the sludge is safe to use. The results indicate that *Salmonella*, even when initial concentrations (day 0) were below the detection limits, experienced regrowth and was detected until 40–51 days of dewatering in both seasons. This transient regrowth was also observed in a previous study (Zaleski *et al.* 2005) in treated sludges dewatered in air-drying beds and storage.

The serovars *S. Enteritidis* (Figure 3(b)) and *S. Typhimurium* (Figure 3(c)) concentrations were determined by the qPCR technique. *S. Enteritidis* persisted throughout the 90 days with concentrations ranging from  $3.54 \times 10^6 \text{ GC}$

$\text{gTS}^{-1}$  (day 0) to  $1.33 \times 10^6 \text{ GC gTS}^{-1}$  (day 90) in the dry season and from  $1.07 \times 10^5 \text{ GC gTS}^{-1}$  (day 0) to  $5.49 \times 10^4 \text{ GC gTS}^{-1}$  (day 90) in the wet season. The exponential model was not adequate to describe the *S. Enteritidis* decay in the air-drying bed. *S. Typhimurium* was detected only in the dry season, which suggested that this variability could be associated with different seasons. Also, the exponential model showed high coefficient determination values ( $R^2 > 0.8$ ), thereby allowing the inference of T90 at 49 days. These results suggest that *S. Typhimurium* is naturally attenuated by the air-drying bed after 90 days and did not show regrowth in this period. To the best of our knowledge, this is the first study that investigated the behaviour of *S. Typhimurium* and *S. Enteritidis* in sludge dehydration at air-drying beds.

Among *Salmonella* spp. serotypes, the non-typhoidal *S. Enteritidis* and *S. Typhimurium* are considered the most isolated in 43 countries in global research. In this study, the incidence of these two serovars in UASB sludge was affected by different seasons because *S. Typhimurium* was only detected in the dry season. According to Tavechio *et al.* (2002), *S. Enteritidis* was the most prevalent serotype (32.5%) and *S. Typhimurium* (2.4%) the least one isolated

in foodstuffs in Brazil from 1996 through 2000. This outcome could explain the higher incidence of *S. Enteritidis* compared to *S. Typhimurium* in UASB sludges.

For *E. coli* pathogenic strains, the intimin-coding gene (Figure 4(a)) is responsible for codifying a protein with the ability to produce lesions in the intestines of infected humans by attaching and effacing cells, and it is present in both enteropathogenic *E. coli* (EPEC) and enterohemorrhagic *E. coli* (EHEC) (Jerse & Kaper 1991). The intimin-coding gene was detected in samples from the dry season (Figure 4(a)). The initial concentration decreased from  $8.79 \times 10^7$  GC gTS<sup>-1</sup> (day 0) to  $3.77 \times 10^4$  GC gTS<sup>-1</sup> (day 60) and remained below detection levels after this period. The results showed high coefficient of determination ( $R^2 > 0.8$ ), and, according to this exponential model, T90 calculated for the intimin-coding gene in the dry season was 19 days.

*C. jejuni* is one of the pathogens of concern in domestic sewage and sewage sludge (U.S. EPA 2003), which is considered one possible means of *Campylobacter* entry into the human food chain through the use of sewage sludge in agricultural cropland. In the present study, *C. jejuni* (Figure 4(b)) was not affected by different seasons and persisted through all treatment periods. During the dry season, the initial concentration ranged from  $9.42 \times 10^5$  GC gTS<sup>-1</sup> (day 0) to  $3.03 \times 10^5$  GC gTS<sup>-1</sup> (day 90), and during the wet season, it ranged from  $8.97 \times 10^4$  GC gTS<sup>-1</sup> (day 0) to  $1.76 \times 10^3$  GC gTS<sup>-1</sup> (day 90). According to these results, the T90 decay of *C. jejuni* genes under this natural condition will occur in 191 days in the wet season and 72 days in the dry season.

Wastewater treatment transfers and concentrates the oocysts of the *Cryptosporidium* spp. into the sewage sludge. In Brazil, there is no systematic monitoring of the occurrence of *Cryptosporidium* in sanitary sludges, thereby resulting in few available data. In the present study, *Cryptosporidium* spp. (Figure 4(c)) was detected only in the wet season with initial concentrations that ranged from  $9.28 \times 10^4$  GC gTS<sup>-1</sup> (day 0) to  $1.51 \times 10^4$  GC gTS<sup>-1</sup> (day 60), remaining below detection limits after this period. The exponential model showed a high coefficient of determination values, which allows us to infer that *Cryptosporidium* will reduce by 90% (T90) over 72 days.

Adenovirus and *C. jejuni* were detected in both seasons. Adenoviruses are among the most common human pathogens, causing a variety of infection symptoms, and it is

prevalent (100%) in Brazilian raw domestic sewage (Schlindwein et al. 2010). After being excreted into wastewater collection systems, adenoviruses are concentrated in sewage sludge during wastewater treatment, where they are often considered to be the most abundant human pathogen. Most studies indicate that the occurrence of *C. jejuni* in sewage sludge is low (Pepper et al. 2010), although it is a persistent pathogen that could survive for extended periods (10 months) in compost (Inglis et al. 2010).

Contaminated sewage effluents are recognised as potential sources of waterborne protozoa, such as *Cryptosporidium* spp. In this study, this pathogen was detected in UASB sludge only in the wet season. To our knowledge, only one study (Valdez 2016) investigating *Cryptosporidium* in the UASB reactor was conducted in Brazil, and it is suggested that oocysts concentrate in sludge. However, no studies were found about the different season effect for the presence of this pathogen.

Positive intimin-coding gene *E. coli* isolated from wastewater are released by slaughterhouses connected to the sewage network. A previous study (Tozzoli et al. 2017) isolated and identified *E. coli* colonies from WWTPs and suggested that intimin-coding genes are more abundant in sludge (33%) compared to effluent samples (7.6%), which suggests that this *E. coli* group should be the subject of greater focus in future research.

During UASB sludge dewatering, adenovirus persisted for 90 days in both periods evaluated. The persistence of adenovirus showed a high coefficient of determination values ( $R^2 > 0.8$ ), which inferred T90 of 19 days in the dry season and 36 days in the wet season. In this scenario, the initial concentration decreased from  $2.69 \times 10^9$  GC gTS<sup>-1</sup> (day 0) to  $5.23 \times 10^4$  GC gTS<sup>-1</sup> (day 90) during the dry season and decreased from  $3.93 \times 10^7$  GC gTS<sup>-1</sup> (day 0) to  $1.06 \times 10^5$  GC gTS<sup>-1</sup> (day 90) during the wet season. These results are consistent with a previous study (Schlindwein et al. 2010), which reported 100% of adenovirus prevalence in sewage sludge ranging from  $4.6 \times 10^4$  to  $1.2 \times 10^6$  GC mL<sup>-1</sup>. Although the presence of adenovirus was not impacted by the different seasons, it presented a marked decrease during the 90 days of dewatering sludge.

Viable helminth eggs, evaluated at day 0 and day 90, in both seasons, were not detected in this study. Probably, this absence is associated with the high socioeconomic

**Table 3** | Correlation coefficient values of the Spearman test to determine significant correlations between culture methods and qPCR pathogens analysis

Variable	TCs	<i>Salmonella</i> spp.	Somatic coliphage	Male F-specific coliphage	Intimin-coding gene	<i>S. Typhimurium</i>	<i>S. Enteritidis</i>	<i>C. jejuni</i>	<i>Cryptosporidium</i> spp.	AdV
TCs	1.000									
<i>Salmonella</i> spp.	<b>0.396</b>	1.000								
Somatic coliphage	<b>0.391</b>	0.110	1.000							
Male F-specific coliphage	0.121	– 0.060	<b>0.713</b>	1.000						
Intimin-coding gene	0.400	0.105	0.500		1.000					
<i>S. Typhimurium</i>	0.400	0.105	0.500		1.000	1.000				
<i>S. Enteritidis</i>	– 0.151	– 0.034	– 0.429	– 0.429	0.800	0.800	1.000			
<i>C. jejuni</i>	0.503	– 0.4035	0.071	0.071	0.4000	0.400	0.079	1.000		
<i>Cryptosporidium</i> spp.	1.000	– 0.447	1.000	1.000			0.100	0.600	1.000	
AdV	0.454	0.136	0.321	0.700	1.000	1.000	<b>0.697</b>	0.273	1.000	1.000

Statistically significant correlation coefficients are in bold.

indicators of the population served by the sewage collection network, as described in the ‘Study site, sample collection, and environmental parameters’ section.

### Correlation between microbial indicators and pathogens

Correlation analysis was conducted to determine which pathogens correlated with microbial indicator organisms (quantified by qPCR and enumerated with culture methods) in sludge samples (Table 3). Spearman (nonparametric) correlation analysis was conducted for microorganisms enumerated with culture methods (TC, *Salmonella* spp., and coliphages) and pathogens (adenovirus, *C. jejuni*, intimin-coding gene, *S. Typhimurium*, and *S. Enteritidis*) quantified by qPCR.

In general, the correlation analysis for the microbial indicators versus pathogens enumerated with culture methods, showed significant positive correlations for TC versus *Salmonella* spp. ( $r = 0.396$ ). Another significant positive correlation was observed between indicators, such as TC versus somatic coliphages ( $r = 0.391$ ) and somatic coliphages versus male F-specific coliphages ( $r = 0.713$ ). Moreover, a significant positive correlation was observed between the pathogens adenovirus versus *S. Enteritidis* ( $r = 0.697$ ). Concerning the presence/absence of pathogens

and microbial indicators, the persistence of TCs during 90 days of sludge dewatering was higher comparing to pathogens, such as *S. Typhimurium*, intimin-coding genes, and *Cryptosporidium* spp. but not for adenovirus, *S. Enteritidis*, and *C. jejuni*. These results are corroborated by another study (Pepper *et al.* 2010) that reported the absence of the correlation between microbial indicators and pathogens in sludge/biosolids.

### CONCLUSIONS

This study highlights the potential of air-drying beds as a sustainable and low-cost option for sludge treatment under tropical conditions. Microbial indicators decreased to below the detection levels after 90 days of dewatering, fitting the requirements for a sludge Class A quality. Despite the regrowth of *Salmonella* spp. in both seasons, 90 days of sludge dewatering in the air-drying bed could produce a sludge Class A in the dry season and Class B in the wet season. This difference could be related to intense rainfall events during the wet season. Molecular methods revealed that *S. Typhimurium*, pathogenic *E. coli* strain (intimin-coding genes), and *Cryptosporidium* spp. decreased to below the detection limits after 60 days of treatment.

Meanwhile, *S. Enteritidis*, *C. jejuni*, and adenovirus remained detectable after 90 days. It is important to emphasize that the molecular methods used in this study cannot distinguish whether the detected pathogens were viable or not. These results indicate that air-drying beds can decrease microbial indicators and most of the pathogens evaluated in this study to below detection limits in 90 days under tropical conditions.

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## DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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