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Reduction and partitioning of viral and bacterial indicators in a UASB reactor followed by high rate algal ponds treating domestic sewage

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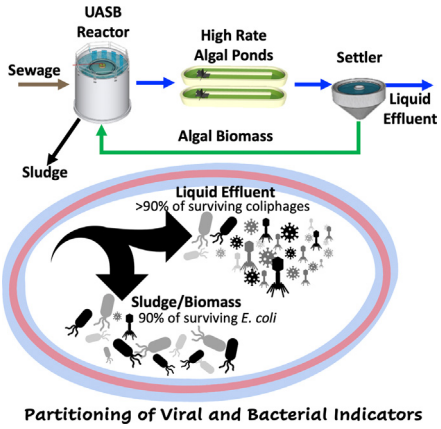
HIGHLIGHTS

- Virus and bacteria removal and solids partitioning evaluated in UASB-HRAP system.
- 90% of surviving *E. coli* left the system in the UASB sludge.
- >90% of surviving coliphages left the system in the liquid effluent.
- Coliphages did not show any affinity for associating with sludge or biomass.

GRAPHICAL ABSTRACT



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ABSTRACT

Human enteric pathogens are a major global concern, as they are responsible for thousands of preventable deaths every year. New pathogens in wastewater are constantly emerging. For example, SARS-CoV-2 has been recently detected in domestic sewage and primary sludge. Knowledge about the reduction of viruses in wastewater treatment and their partitioning between the treated liquid effluent versus the sludge or biosolids is still very scarce, especially in countries with emerging economies and tropical climates. Upflow anaerobic sludge blanket (UASB) reactors are among the top three most commonly used technologies for the treatment of sewage in Latin America and the Caribbean, and their use has become increasingly common in many other low- and middle-income countries. High-rate algal ponds (HRAP) are regarded as a sustainable technology for the post-treatment of UASB effluent. This study evaluated the overall reduction and the liquid-solid partitioning of somatic coliphages, F-specific coliphages, and *E. coli* in a pilot-scale system comprised of a UASB reactor followed by HRAPs treating real wastewater. Average log removal for somatic and F-specific coliphages were 0.40 and 0.56 for the UASB reactor, and 1.15 and 1.70 for HRAPs, respectively. The overall removal of both phages in the system was 2.06-log. Removal of *E. coli* was consistently higher. The number of viruses leaving the system in the UASB solids and algal biomass was less than 10% of the number leaving in the clarified liquid effluent. The number of *E. coli* leaving the system in solids residuals was estimated to be approximately one order of magnitude higher than the number of *E. coli* leaving in the liquid effluent. Results from this study demonstrate the suitability of UASB-HRAP systems to reduce viral and bacterial indicators from domestic sewage and the importance of adequately treating sludge for pathogen reduction before they are used as biosolids.

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1. Introduction

Viral and bacterial water pathogens are a major cause of diseases globally, causing a variety of illnesses, including gastroenteritis, diarrhea, respiratory illness, dysentery, hepatitis, cholera, and typhoid fever (García-Aljaro et al., 2018). Annually, more than 800,000 people die due to the lack of safe drinking water and basic sanitation facilities and more than 400,000 people die due to diarrhea related to poor sanitation (WHO, 2019). Diarrhea is the second leading cause of death in children under 5 years old, as it kills approximately 525,000 children in a year (WHO, 2017). Sewage is one of the main sources of pathogen contamination to surface waters, since it contains pathogenic microorganisms excreted by infected individuals (García-Aljaro et al., 2018). In their Guidelines on Sanitation and Health, the World Health Organization (WHO, 2018) emphasized a non-comprehensive list of 16 different bacterial pathogen groups, nine viral pathogen groups, four protozoan groups, and 11 helminth groups that are particularly important for sanitation. New pathogens are constantly emerging or being discovered in sewage. For example, the novel coronavirus SARS-CoV-2 has been recently detected in domestic sewage (Chernicharo et al., 2020; Heller et al., 2020; Medema et al., 2020; Wu et al., 2020) and primary sludge (Peccia et al., 2020). Knowledge about the presence and removal of enteric viruses in wastewater treatment plants, especially in countries with emerging economies and tropical climates, is still very scarce (Verbyla et al., 2017). Fecal indicator bacteria such as total coliforms and *E. coli* are commonly used as indicators of fecal contamination to surface waters, but they are not appropriate indicators of the presence of viral pathogens in wastewater, treated effluent, and sewage sludge (Harwood et al., 2017; Rodriguez-Manzano et al., 2012). Coliforms are less resistant than viruses to most treatment processes and are not adequate indicators for the reduction of enteric viruses in wastewater treatment systems (Harwood et al., 2017; Rodriguez-Manzano et al., 2012; WHO, 2001). For this reason, coliphages have been proposed as more suitable microbial process indicators of microbiological quality of sewage, due to their similar structure, persistence and resistance to treatment processes compared to human enteric viruses (Grabow, 2001; Jofre et al., 2016; Momba et al., 2019). Coliphages are viruses that infect coliform bacteria. They are found in the gut and are excreted in the feces of humans and other warm-blooded animals (Grabow, 2001).

In Latin America and the Caribbean, 65.5% of the population is covered by sewerage but only 15% of the collected wastewater is treated (Noyola et al., 2012; WHO/UNICEF, 2019). Specifically, in Brazil, 52% of the population is served by a sewage network and only 45% of the generated sewage receives any type of treatment (SNIS, 2018). Sewage treatment plants in Brazil as well as in many other places throughout the world are primarily designed to remove suspended solids and organic matter (von Sperling, 2016). Therefore, the systems are not always optimized to remove pathogens. Anaerobic bioreactors, such as the upflow anaerobic sludge blanket (UASB) reactor, are among the top three most commonly-used technologies for the treatment of sewage in Latin America and the Caribbean (Noyola et al., 2012), and their use has become increasingly common in many other low- and middle-income countries with large populations throughout the world, including India and Egypt (Khalil et al., 2018; Lettinga, 2011; Sato et al., 2007). The main advantages of UASB reactors, compared to other technologies, are their lower cost of operation (as they do not demand electricity), their smaller area footprint, and their ability to generate energy from biogas while removing suspended and dissolved organic matter (Chernicharo, 2007). However, the effluent from UASB reactors may not meet some legislation standards for discharge or reuse and, therefore requires post-treatment to further reduce the concentrations of organic matter, nutrients, or pathogens (Daud et al., 2018). Numerous technologies have already been studied and proven efficient for the post-treatment of UASB domestic effluent, including trickling filters, polishing ponds and constructed wetlands (Mungray et al., 2012). Algal wastewater treatment technologies (e.g. waste stabilization ponds and high-rate algal ponds) are one of the most common treatment technology used throughout the world, not necessarily in terms of flow rate, but in terms of the total number of facilities (Kumar and Asolekar, 2016; Noyola et al., 2012; Verbyla, 2015). High rate algal ponds (HRAP) have more recently been considered as an efficient option for treating the effluent of UASB reactors, and they offer multiple advantages over conventional pond systems, including lower construction and operation costs (compared to activated sludge systems), low demand for electricity, and potential to remove nutrients, micro pollutants, and pathogens (Buchanan et al., 2018; Fallowfield et al., 2018; Vassalle et al., 2020a, 2020b; Young et al., 2016). Bacterial reduction in UASB reactors and HRAPs has been studied separately (Araki et al., 2000; El Hamouri et al., 1994; Young et al., 2016). However, little is known about the reduction of viruses in these systems (Davies-Colley et al., 2005; Oakley et al., 2017).

In most previous studies of pathogens in wastewater treatment facilities, reduction has been calculated only by considering pathogen or microbial indicator concentrations in the liquid fraction (raw sewage and treated effluent), effectively de-emphasizing (or in the worst cases, completely ignoring) the sludge, which is often highly contaminated with pathogens (Oakley et al., 2017; von Sperling et al., 2018). Given that all wastewater treatment processes produce sludge and that sludge is often land-applied or reused in agriculture, it is important to quantify pathogen loadings in sewage sludge and to understand how they differ for different types of treatment technologies. This will help better inform the design of sludge treatment and biosolids management systems that can effectively reduce risks associated with pathogens emitted to the environment. In addition, wastewater treatment systems often become overloaded or fail over time, especially in low- and middle-income countries (Davis et al., 2019). Deferred maintenance activities in particular, especially desludging, has been reported to impact the hydraulic performance of wastewater treatment systems (especially algal ponds) (Coggins et al., 2017) and cause reduced pathogen removal efficiency (Verbyla et al., 2013, 2016).

This study assessed the reduction of viral and bacterial process indicators in a pilot-scale system consisting of a UASB reactor followed by HRAPs, treating real domestic wastewater. In addition, this study evaluated the partitioning of viral and bacterial indicators between liquid and solid (sludge) effluents. Understanding virus partitioning has important implications, not only for understanding the performance of treatment technologies and the risks associated with the reuse of treated biosolids from wastewater facilities, but also for wastewater epidemiology. A recent study reported that concentrations of SARS-CoV-2 RNA in primary municipal sewage sludge was a good indicator of COVID-19 outbreak dynamics (Peccia et al., 2020), and it has been hypothesized that some enveloped viruses may have a higher affinity for solids compared to non-enveloped viruses and microbial indicators (Ye et al., 2016).

2. Materials and methods

2.1. Experimental set-up and operation

The pilot-scale UASB-HRAP system has been described in detail by Vassalle et al. (2020a, 2020b). Briefly, the system is located at the Research and Training Center for Sanitation (CePTS) in Belo Horizonte, Brazil. It treated 1176 L/day of raw sewage from the city of Belo Horizonte using a UASB reactor (343 L) followed by two HRAPs (operated in parallel) and a sedimentation chamber (30 L) that separated microalgal biomass from clarified liquid effluent. The harvested microalgal biomass was sent to the UASB reactor, using a centrifuge pump (BCR 2000 – Schneider® - Germany), where it was co-digested with raw sewage. The UASB reactor was operated with a hydraulic retention time of 7 h, a mean solids retention time of 21.3 days, and an organic loading rate of 0.71 g VS/L/day. Each HRAP had a working volume of 205 L, a depth of 0.30 m, and a surface area of 0.41 m^2. The ponds

received the effluent from the UASB reactor and were each operated at a flow rate of 25.5 L/day, with a mean theoretical hydraulic retention time of 8 days. The excess 1129 L/day of effluent from the UASB reactor was discharged to a separate experimental treatment system which was not a part of this study. Fig. 1 shows a diagram of the system, with boundaries drawn around the UASB reactor, the HRAPs, and the overall system, for the purpose of the mass balance.

2.2. Sample collection and analysis

Sampling and monitoring of fecal indicator bacteria (*E. coli*) and viruses (somatic and F-specific coliphages) took place between June 2019 and February 2020. Raw sewage, effluent from the UASB reactor, and liquid effluent and biomass from the settler were sampled once per month. Sample collection locations are shown on Fig. 1. Sludge

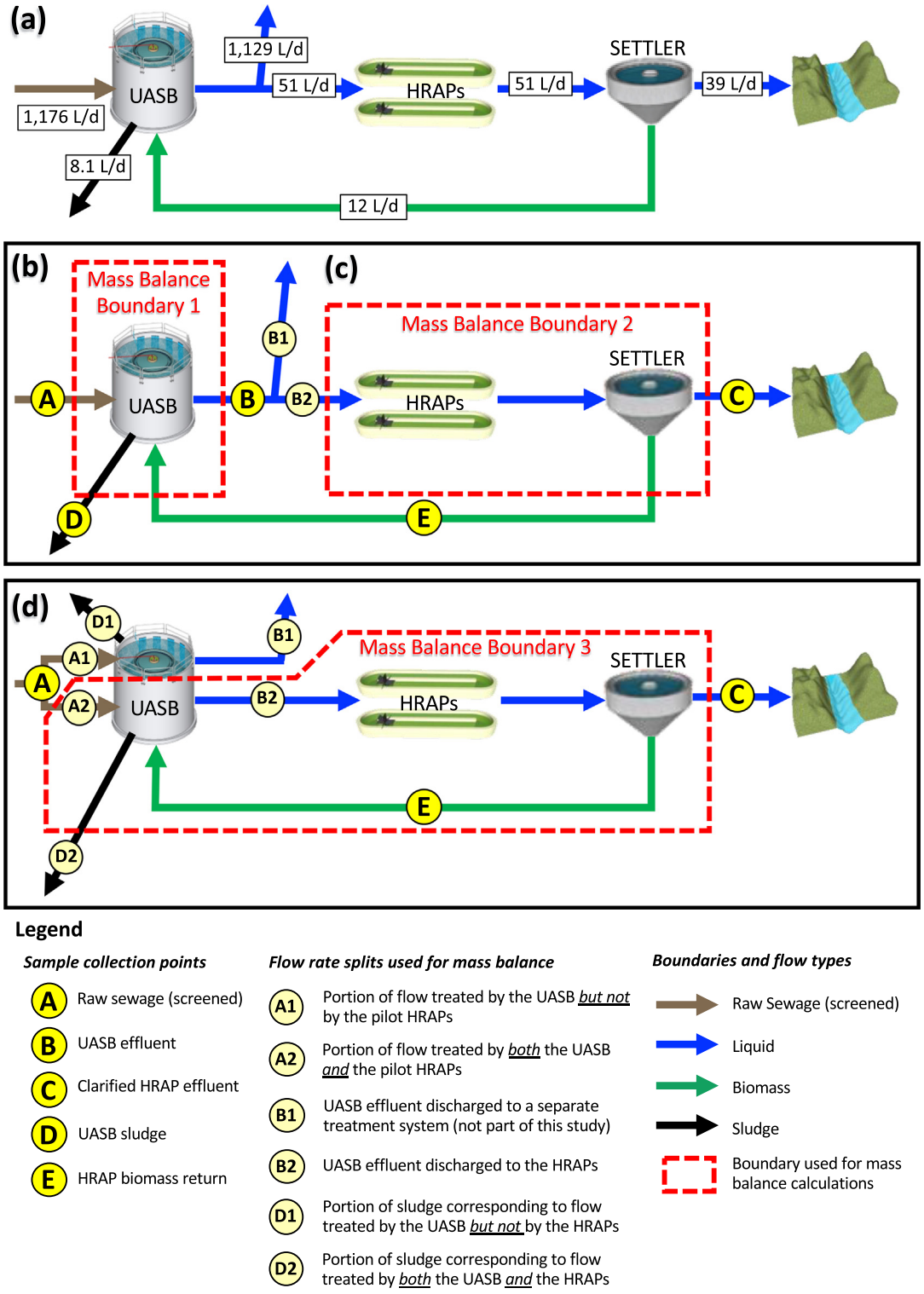

Fig. 1. Diagram of the pilot-scale upflow anaerobic sludge blanket (UASB) reactor followed by twin high rate algal ponds (HRAPs) with return of algal biomass, showing a) the measured flow rates, sample collection points and mass balance boundaries established to analyze virus and bacteria partitioning in b) the UASB reactor; c) the HRAPs; and d) the overall system.

samples from the UASB reactor were only analyzed for *E. coli* and coliphage in the samples collected in July and August 2019. All samples were stored at 4 °C and taken to the microbiology laboratory of the Sanitary and Environmental Engineering Department at UFMG (Federal University of Minas Gerais, Belo Horizonte, Brazil), where the analyses were conducted within 24 h of sample collection. To analyze the microbial loading out of the system in the UASB sludge and microalgal biomass, total solids (TS) and volatile solids (VS) were carried out according to Standard Methods for the Examination of Water and Wastewater 2540-G (APHA-AWWA-WEF, 2017). To analyze the units and global system performance, water quality parameters such as pH, temperature, dissolved oxygen (DO), total suspended solids (TSS), volatile suspended solids (VSS), total solids (TS) and volatile solids (VS) and chemical oxygen demand (COD) were evaluated on the same dates. COD was analyzed using a Hach kit (high range), DO was analyzed using a Hach (HQ30D) probe, solids (TS, VS, TSS and VSS) were analyzed according to Standard Methods for the Examination of Water and Wastewater 2540-G, 2540-D and 2450-E (APHA-AWWA-WEF, 2017). The COD load applied to the UASB reactor and the HRAP was used to calculate the ratio of production of solids in the system. The calculation was done by dividing the influent COD load, or the mass of COD removed, by the sludge solids load evacuated from the UASB reactor or the microalgal biomass removed from the clarifier of the HRAPs. The final ratios were calculated as the ratio of average values found (not as the average of the ratios).

E. coli was quantified using the Colilert and Quanti-Tray 2000 most probable number (MPN) method (IDEXX, Maine, EUA) and results were given as MPN per 100 mL. Coliphages were quantified using a double agar plaque assay based on the protocols described in 9224B and 9224C of Standard Methods (APHA SMWW, 2017). Briefly, molten agar (5 mL) containing 50 μL of antibiotics was mixed with 500 μL of 0.22-μm filtered samples or diluted samples (samples were filtered to remove suspended solids and unwanted bacteria) and 100 μL of the antibiotic-resistant strains of the host bacteria, *E. coli* Famp (ATCC#700891) for F-specific coliphages and CN13 (ATCC#700609) for somatic coliphages. This mixture was then poured over a hardened bottom agar layer (15 mL) in a petri dish. After the top agar layer hardened, the plate was incubated at 37 °C overnight. Circular zones formed by the lysis of the bacterial cells were counted as plaques and the concentration of coliphages was calculated as plaque forming units (PFU) per unit volume of sample, after correcting for the dilutions made. Results of coliphages were given as PFU per 100 mL. Viruses were extracted from sludge samples following the protocol described by Guzmán et al. (2007). Briefly, 25 mL of sludge samples at a 1:10 ratio (v/v) is added to a 10% beef extract solution. To prepare the 10% beef extract solution, 225 mL of distilled water is mixed with 22.5 g of beef extract, pH is adjusted to 7.2 and the solution is sterilized before mixing with sample. After 20 min of homogenization by magnetic stirring at room temperature (500–900 rpm), samples were centrifuged at 4000 ×*g* for 30 min and the supernatant was filtered through low protein binding 0.22-μm pore size membranes. This filtrate was then analyzed using the double agar layer method, as mentioned before. Results of coliphages in sludge samples were calculated in PFU/mL and then were adjusted and reported as PFU/g of dry matter.

2.3. Removal versus reduction of microbial indicators

In wastewater treatment systems, the removal of pathogens or microbial indicators is commonly reported based on the log difference between concentrations detected in the wastewater influent and the liquid effluent (von Sperling et al., 2018). However, when concentration data from multiple dates are available, the central tendency for log difference can be calculated either as the arithmetic mean of the calculated log difference of each paired concentration, or as the calculated log difference of the geometric mean of all influent concentrations and the geometric mean of all effluent concentrations (von Sperling et al., 2020) as shown in Eqs. (1) and (2), respectively. The two approaches produce different results only when datasets of the concentration are not the same for influent and effluent samples. For processes with short retention times and highly variable influent, Eq. (1) is preferred. For processes with longer retention time (where the influent cannot be matched with the effluent sample), Eq. (2) is generally preferred. For this study, only Eq. (2) was used.

$$\text{mean of } log_{10} \text{ removal values} = \frac{1}{N_{liq}} \sum_{i=1}^{N_{liq}} \left(\log_{10} \left(\frac{C_{o,liq,i}}{C_{e,liq,i}} \right) \right) \quad (1)$$

$$log_{10} \text{ removal of geometric means} = \log_{10} \left(\frac{N_{e,liq} \sum_{i=1}^{N_{o,liq}} \log_{10}(C_{o,liq,i})}{N_{o,liq} \sum_{i=1}^{N_{e,liq}} \log_{10}(C_{e,liq,i})} \right) \quad (2)$$

where $C_{o,liq}$ is the microbial concentration in the liquid influent.$C_{e,liq}$ is the microbial concentration in the liquid effluent.$N_{o,liq}$ is the number of samples at the influent point (liquid matrix).$N_{e,liq}$ is the number of samples at the effluent point (liquid matrix).N_{liq} is equal to $N_{o,liq}$ and $N_{e,liq}$ (only possible when $N_{o,liq} = N_{e,liq}$).

Eqs. (1) and (2) are the most commonly used in the literature, however neither of these equations accounts for pathogens discharged in the sludge or pathogens that may come into the system from recycled sludge, fecal sludge, or other biomass that is mixed in with the sewage. From a systems perspective, both liquid and solid matrices should be accounted for when reporting pathogen reductions. It is important to distinguish pathogen *removal* (Eqs. (1) and (2)) from pathogen *reduction* (Eqs. (3) and (4)), given that the former indicates removal from the liquid fraction only, while the latter represents overall reduction by the treatment system (accounting for both solid and liquid fractions). Using the concept of mass balance, the log reductions of coliphage and *E. coli* were calculated based on their loadings. For this study, Eq. (4) was used instead of Eq. (3) (for the same reason Eq. (2) was chosen over Eq. (1)).

$$\text{mean of } log_{10} \text{ reduction values} = \frac{1}{N} \sum_{i=1}^{N} \left(\log_{10} \left(\frac{L_{o,liq,i} + L_{o,sol,i}}{L_{e,liq,i} + L_{e,sol,i}} \right) \right) \quad (3)$$

$$\begin{aligned} log_{10} \text{ reduction of geometric means} &= \log_{10} \left(\frac{10^{\frac{1}{N_{o,liq}} \sum_{i=1}^{N_{o,liq}} \log_{10}(L_{o,liq,i})} + 10^{\frac{1}{N_{o,sol}} \sum_{i=1}^{N_{o,sol}} \log_{10}(L_{o,sol,i})}}{10^{\frac{1}{N_{e,liq}} \sum_{i=1}^{N_{e,liq}} \log_{10}(L_{e,liq,i})} + 10^{\frac{1}{N_{e,sol}} \sum_{i=1}^{N_{e,sol}} \log_{10}(L_{e,sol,i})}} \right) \\ &= \log_{10} \left(\frac{\text{geomean}(L_{o,liq,i}) + \text{geomean}(L_{o,sol,i})}{\text{geomean}(L_{e,liq,i}) + \text{geomean}(L_{e,sol,i})} \right) \end{aligned} \quad (4)$$

where $L_{o,liq}$ is the microbial loading into the system in the liquid influent (per day).$L_{o,sol}$ is the microbial loading into the system from solids added to the influent (per day).$L_{e,liq}$ is the microbial loading out of the system in the liquid effluent (per day).$L_{e,sol}$ is the microbial loading out of the system in the sludge or biosolids (per day).$N_{o,liq}$ is the number of samples at the liquid influent point.$N_{e,liq}$ is the number of samples at the liquid effluent point.$N_{o,sol}$ is the number of samples at the influent point (solids matrix).$N_{e,sol}$ is the number of samples at the effluent point (solids matrix).N is equal to $N_{o,liq}$, $N_{e,liq}$, $N_{o,sol}$, and $N_{e,sol}$ (when $N_{o,liq} = N_{e,liq} = N_{o,sol} = N_{e,sol}$).

To use these equations in a modeling framework, it is necessary to predict the fraction of pathogens exiting the system per day in the sludge relative to the liquid effluent (i.e., the *fraction emitted in sludge or biosolids*). Hence, we propose a model for estimating the fraction of pathogens that end up in the solids fraction of the effluent from wastewater treatment processes, as show Eqs. (5) and (6). This fraction can be calculated directly using the loadings. In this case, Eq. (5) (mean fraction of loadings in the solids) and 6 (fraction of geometric mean loadings in the solids) show different results, even when datasets are complete, so it is recommended to use and report both calculations. We will report

here only results of Eq. (6), and results from Eq. (5) can be found in the Supplementary Material (Table S4).

$$\text{mean of } \textit{fraction} \text{ of loadings } \textit{emitted in sludge or biosolids} = \frac{1}{N_e}\sum_{i=1}^{N_e}\left(\frac{L_{e,\mathrm{sol},i}}{L_{e,\mathrm{liq},i}+L_{e,\mathrm{sol},i}}\right) \quad (5)$$

$$\textit{fraction} \text{ of geometric mean loadings } \textit{emitted in sludge or biosolids} = \frac{10^{\left(\frac{1}{N_{e,\mathrm{sol}}}\sum_{i=1}^{N_{e,\mathrm{sol}}}\left(\log_{10}\left(L_{e,\mathrm{sol},i}\right)\right)\right)}}{10^{\left(\frac{1}{N_{e,\mathrm{liq}}}\sum_{i=1}^{N_{e,\mathrm{liq}}}\left(\log_{10}\left(L_{e,\mathrm{liq},i}\right)\right)\right)}+10^{\left(\frac{1}{N_{e,\mathrm{sol}}}\sum_{i=1}^{N_{e,\mathrm{sol}}}\left(\log_{10}\left(L_{e,\mathrm{sol},i}\right)\right)\right)}} = \frac{\mathrm{geomean}\left(L_{e,\mathrm{sol},i}\right)}{\mathrm{geomean}\left(L_{e,\mathrm{liq},i}\right)+\mathrm{geomean}\left(L_{e,\mathrm{sol},i}\right)} \quad (6)$$

Alternatively, this fraction can be indirectly expressed using the concentrations in the liquid effluent ($C_{e,\mathrm{liq}}$) and in the sludge ($C_{e,\mathrm{sol}}$), along with the liquid flow rate (Q) and sludge accumulation rate, also known as the total solids yield (Y_{sol}):

$$\textit{fraction emitted in sludge or biosolids} = \frac{C_{e,sol}Y_{sol}}{C_{e,liq}Q+C_{e,sol}Y_{sol}} \quad (7)$$

Pathogen or indicator concentrations in the sludge from wastewater treatment systems are seldom reported in the literature, and when they are, sludge accumulation rates (solids yields) are often not provided. However, the total or volatile solids yield for wastewater treatment processes can be estimated using stoichiometry and thermodynamics (McCarty, 2007), and typical yields for different aerobic and anaerobic wastewater treatment processes have been well characterized (Andreoli et al., 2007). To compare our results to other findings in the literature, we expressed the total solids yields for the system studied with respect to the mass of organic matter oxidized (i.e., kg TS produced per kg COD treated), and compared our calculated total solids yields for the UASB-HRAP system with values reported in the literature.

2.4. Influence of desludging on virus and bacteria removal

Throughout the duration of the experiment, ~240 L of sludge from the UASB reactor (~70% of its effective volume) was removed every 34 days (SD = 12.7) on average. However, the number of days between sludge removals ranged from a minimum of 14 days to a maximum of 61 days (Table S3). To investigate the presence or absence of a relationship for the amount of time between sludge removal and the efficiency of coliphage and *E. coli* removal from the wastewater, Pearson correlation coefficients were calculated between the number of days since desludging and the overall reduction of viral and bacterial indicators. All statistical computations were performed using MS Excel or R v3.4.2.

3. Results and discussion

3.1. Wastewater quality and solids yield

Results from physical-chemical analyses for raw sewage, UASB reactor and HRAP are summarized in Table 1. The mean COD in the UASB reactor effluent and in the HRAP effluent were 175.4 mg L^{-1} and 116.5 mg L^{-1}, with average removals of 55% and 70% in the UASB reactor and UASB + HRAP, respectively. COD removals between 55 and 65% have been reported for UASB reactors and between 65 and 80% for UASB followed by polishing ponds (von Sperling and Chernicharo, 2005) or HRAPs (Villar-Navarro et al., 2018).

TSS and VSS concentrations found in the effluent samples were comparable with the typical values from domestic effluents (120-360 mg TSS L^{-1} and 90–280 mg VSS L^{-1}) (Metcalf and Eddy, 2014), from UASB reactor effluents (50–160 mg TSS L^{-1} and 30 mg VSS L^{-1}) (Chernicharo, 2007) and from HRAPs effluents used as UASB post-treatment units (145 mg TSS L^{-1} and 124 VSS mg L^{-1}) (Santiago et al., 2013). Global removal (UASB + HRAP) for TSS and VSS were approximately 36% and 45% respectively. However, there were increases in solids concentrations from UASB to HRAP, due to the production of microalgal biomass in the HRAP system. This is frequently reported for systems that use microalgae-based systems as post-treatment of UASB reactors (Santiago et al., 2013; Vassalle et al., 2020a; Villar-Navarro et al., 2018).

Considering the current Brazilian and Minas Gerais (where this study was performed) legislation on urban wastewater CONAMA Directive 430/2011 (2011) and COPAM directive 01/2008 (2008), maximum effluent discharge concentrations for COD and TSS are set at 180 mg L^{-1} and 150 mg L^{-1} (Morais and dos Santos, 2019). The effluent from the tested system met all the required limits for COD and TSS. On the other hand, considering the comparatively more restrictive European urban wastewater Directive Council Directive 91/271/EEC (1991) with COD and TSS discharge limits in effluents set at 125 mg L^{-1} and 35 mg L^{-1} respectively, the quality of the final effluent would not be compliant. Improvement in the removal of microalgal biomass from the final effluent would be an alternative to increase compliance with stricter discharge regulations.

For the UASB reactor, an average of 0.44 kg TS was generated per kg of influent COD, and an average of 0.78 kg TS was generated per kg of COD removed. These values are slightly higher than those previously reported in the literature. For example, a range of 0.12 to 0.18 kg TS per kg COD loading at the influent of UASB reactors has been reported (Andreoli et al., 2007). Agrawal et al. (1997) reported a range of 0.27 to 0.65 kg TS per kg COD removed for UASB reactors treating domestic sewage. Gonçalves et al. (2002) reported 0.4 kg TS per kg COD removed in a UASB reactor treating sewage, with a similar HRT (8 h). The higher values reported in this system, relative to previously reported values in the literature, are likely due to the microalgal biomass injection produced in the HRAP to the UASB reactor, which is a unique aspect of this system (Vassalle et al., 2020a). In the HRAPs, an average of 6.52 kg TS of algal biomass were generated per kg COD loaded, and

Table 1
Physical-chemical characterization of raw sewage, UASB effluent and final effluent from the treatment system (total number of samples = 9).

	Raw sewage		UASB effluent		HRAP effluent	
	Mean (SD)	Min/Max	Mean (SD)	Min/Max	Mean (SD)	Min/Max
TSS (mg L^{-1})	245.0 (91.8)	163.3/387.5	53.1(39.8)	25.0/130.0	146.9(118.0)	8.3/335.0
VSS (mg L^{-1})	195.4 (165.0)	115.0/322.5	42.8(32.1)	17.5/101.7	108.0(57.2)	37.5/180.0
TS (mg L^{-1})	550.8 (323.1)	236.3/1130.0	493.6(277.4)	151.5/1030.0	684.0(483.6)	36.1/1456.5
VS (mg L^{-1})	509.7 (325.5)	98.7/1052.0	186.2(110.4)	47.8/380.0	245.4(129.9)	85.2/409.1
COD (mg L^{-1})	393.1 (85.2)	275.0/535.0	175.4(78.8)	90.0/336.0	116.5(60.4)	54.0/249.0
pH	7.65 (0.1)	7.5/7.8	7.6(0.1)	7.3/7.9	7.7(0.4)	7.1/8.3
DO (mg L^{-1})	0.6 (0.4)	0.2/2.2	0.4(0.1)	0.2/0.7	8.5(1.7)	5.3/11.7
Temp. (°C)	24.5 (1.9)	21.4/27.5	23.6(2.3)	20.1/25.7	23.3(3.0)	17.7/27.9

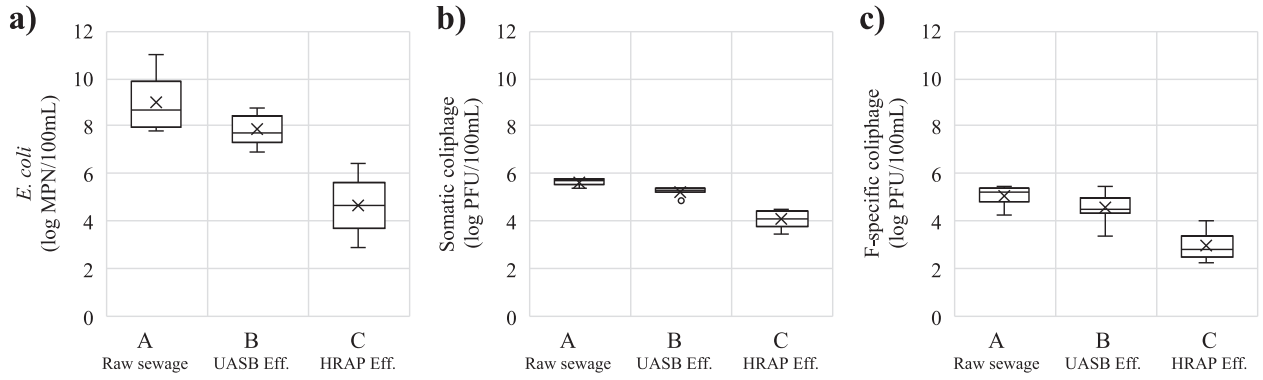

Fig. 2. Boxplots of the concentrations in the liquid phase for: a) *E. coli*; b) somatic coliphage; and c) F-specific coliphage at the different stages from the pilot-scale system UASB reactor followed by twin HRAPs. The lower and upper bars denote minimum and maximum values, respectively. The lower and upper boxes represent the 25th and 75th percentiles, respectively. Mean values are represented by an "x". The line inside the box denotes the median value.

9.14 kg TS of algal biomass were generated per kg COD removed (based on filtered COD measurements in the effluent). Little is known about typical solids yields in UASB + HRAP systems, as few studies are available in the literature. Santiago et al. (2013) reported solids yield for HRAP treating UASB effluent in the order of 2.02 kg TS of algal biomass per kg COD loaded and 7.70 kg TS of algal biomass per kg COD removed.

3.2. Concentrations and removal of E. coli and coliphage

Fig. 2 shows boxplots of the concentrations of *E. coli*, somatic and F-specific coliphages throughout the different stages of the pilot-scale wastewater treatment system. The geometric mean concentrations of *E. coli* in the influent (raw sewage) and liquid effluent from the UASB reactor were 8.98 and 7.84-log MPN/100 mL, respectively, and the calculated removal (using Eq. (2)) was 1.09-log units, similar to one order of magnitude removal reported in the literature for UASB reactor (Dias et al., 2014; Khan et al., 2012; Oakley et al., 2017). Comparing with the findings in the UASB reactor of Lucena et al. (2004), where the log removals of indicators ranged between 0.3 and 0.7, our results for the fecal indicator bacteria (*E. coli*) were higher. For somatic and F-specific coliphages, the geometric mean concentrations in raw sewage were 5.66 and 5.10-log PFU/100 mL with mean removal values (using Eq. (2)) of 0.40 and 0.56-log units, respectively. Our results for coliphages are similar to the mean 0.5-log removal of adenoviruses reported by El-Senousy and Abou-Elela (2017) and the 0.5-log removal of culturable enteroviruses reported by Symonds et al. (2014) for UASB reactors. However, Symonds et al. (2014) also reported negligible removal of norovirus, rotavirus and pepper mild mottle virus in the same UASB reactor. Recent reviews indicated that typical removal of viruses from wastewater treated by UASB reactors ranges from zero to 0.7-log (Oakley et al., 2017). Therefore, our results are in accordance with values previously reported in the literature for virus removal in UASB reactors. UASB reactors are not optimized for pathogen removal and require post treatment to produce an effluent of high enough water quality for reuse or discharge back to the environment (Mungray et al., 2012; Van Der Steen et al., 1999). In this study, HRAPs were used for the post treatment of UASB reactor effluent (Fig. 1).

The geometric mean concentrations of *E. coli* in the influent and effluent of the HRAPs were 7.84 and 4.69-log MPN/100 mL, and the removal (using Eq. (2)) was 4.06-log units. This is considerably higher than values previously reported in the literature, which ranged from 1.76 to 2.19-log units (Buchanan et al., 2018; Chambonniere et al., 2020; Fallowfield et al., 2018; Young et al., 2016). El Hamouri et al. (1994) operated a pilot-scale HRAP system treating wastewater and achieved a removal of fecal coliforms of 3.19-log units in the summer, which is also lower than the removals reported in the current study. Geometric mean concentrations of somatic and F-specific coliphages, respectively, were 5.26 and 4.58-log PFU/100 mL in the HRAP influent (i.e., UASB reactor effluent) and 4.08 and 2.96-log PFU/100 mL in the final HRAP liquid effluent. The calculated removals in the HRAP (Eq. (2)) were 1.15 and 1.70-log for somatic and F-specific coliphages, respectively. Verbyla et al. (2017) reported removals ranging from 1.2 to 1.6-log for bacteria and zero to 1.7-log for viruses in HRAPs, so the values reported in the present study are high for bacteria, but within range for viruses.

The mean overall (UASB + HRAP) removal of *E. coli* (Eq. (2)) was 5.16-log. Limited information is available in the literature on the removal of bacterial indicators in combined UASB + HRAP systems, for that reason the discussion in this part used a comparison with similar treatment systems. In a meta-analysis of full-scale wastewater treatment facilities consisting of anaerobic reactors followed by maturation ponds, an overall geometric mean removal of thermotolerant coliforms of 1.95-log units was reported (Espinosa et al., 2017) which is more than three times lower than the value obtained in this study. *E. coli* removals of 2-log units have also been reported for two similar systems, a UASB followed by an HRAP (Santiago et al., 2013) and a septic tank with post treatment using HRAPs (Young et al., 2016). Our findings show removals that are more than twice as high as these results.

The mean overall (UASB + HRAPs) removal of viral indicators (Eq. (2)) was 1.54-log for somatic coliphages and 2.26-log for F-specific coliphages. We compared our results with other similar systems consisting of anaerobic reactors followed by algal-based treatment technologies. For example, in an anaerobic digester followed by an HRAP, a 1-log overall removal of somatic coliphages was reported (Davies-Colley et al., 2005). In a septic tank followed by an HRAP, a 1.81-log overall removal of F-specific coliphages was reported (Young et al., 2016). In a UASB reactor followed by two polishing ponds, a 0.8-log overall removal of culturable enteroviruses was reported (Symonds et al., 2014). These similar systems presented lower removal values compared with our findings. Table 2 shows the concentrations of the

Table 2
Microbial influent concentrations (PFU/100 mL or MPN/100 mL) throughout the different stages of the pilot-scale wastewater treatment system.

System	Microbial	Geo. mean	Maximum	Minimum
UASB	Somatic coliphages	$4.56 \cdot 10^5$	$2.69 \cdot 10^5$	$7.10 \cdot 10^4$
	F-specific coliphage	$1.25 \cdot 10^5$	$2.80 \cdot 10^5$	$2.65 \cdot 10^3$
	E. coli	$9.45 \cdot 10^8$	$5.61 \cdot 10^8$	$8.74 \cdot 10^6$
HRAP	Somatic coliphages	$1.83 \cdot 10^5$	$3.34 \cdot 10^4$	$2.90 \cdot 10^3$
	F-specific coliphage	$3.81 \cdot 10^4$	$1.02 \cdot 10^4$	$2.00 \cdot 10^2$
	E. coli	$6.93 \cdot 10^7$	$2.72 \cdot 10^6$	$7.40 \cdot 10^2$
UASB + HRAP	Somatic coliphages	$4.56 \cdot 10^5$	$3.34 \cdot 10^4$	$2.90 \cdot 10^3$
	F-specific coliphage	$1.25 \cdot 10^5$	$1.02 \cdot 10^4$	$2.00 \cdot 10^2$
	E. coli	$9.45 \cdot 10^8$	$2.72 \cdot 10^6$	$7.40 \cdot 10^2$

three microbial groups (geometric means, maximum and minimum) at the influent points in each of the reactors, and Table 3 shows the concentrations in the effluent points, as well as the log removal values (Eq. (2)) throughout the different stages of the pilot-scale wastewater treatment system. The concentrations of indicator microorganisms in liquid and solid influent and effluent are summarized in the supplementary material (Table S1). The geometric mean of the influent and effluent concentration with the respective ranges are summarized in Table S2.

As mentioned before, fecal indicator bacteria (FIB) such as *E. coli* are generally used to warn about the possible contamination of a water source with fecal matter (Harwood et al., 2017). However, *E. coli* and another FIB are less resistant to treatment than many viruses (Momba et al., 2019), which is confirmed by our observations here. We observed that *E. coli* removal was almost twice that of the coliphages, and the final concentration was >1E+03 MPN/100 mL (4.94E+04 MPN/100 mL, Table 3). Higher removal or reduction of *E. coli* in a treatment system could give some practitioners the false indication that other pathogens are also removed or reduced to the same extent, which may not be the case. Even if *E. coli* removal is high, the treated effluent may not be suitable for reuse, based on the WHO (2006) standards, which suggest several scenarios where pathogen reduction of 3 to 6-log at wastewater treatment plants could adequately protect the public health.

3.3. Influence of desludging on coliphage and E. coli removals

Correlation analysis was carried out using data on the amount of time between desludging events and removals of indicator organisms. There were weak to moderate, but non-significant, negative correlations between the number of days since desludging and the removal of coliphages from wastewater in the UASB reactor, with Pearson r coefficients of -0.31 ($p = 0.42$) and -0.49 ($p = 0.18$), respectively, for somatic and F-specific coliphages. No correlation was observed for the number of days since desludging and the removal of *E. coli* from wastewater in the UASB reactor (Pearson's $r = 0.15$, $p = 0.70$). The lack of a significant correlation between virus removal and desludging in this study does not necessarily mean that the length of desludging intervals does not have an impact on pathogen removal. Future studies should extend the range of desludging intervals to determine if longer periods between desludging events might affect pathogen removal efficiency.

3.4. Overall reduction, loadings, and partitioning to liquid vs. solids

Tables 4, 5, and 6 show the flow rates, the calculated microbial loadings, and the log differences between the overall log reduction of the geometric means (Eq. (4)) for the UASB reactor, the pilot-scale HRAPs, and the overall UASB + HRAP system, respectively. For the mass balance around the UASB reactor alone (Fig. 1b, Mass Balance Boundary 1), the majority of coliphages and *E. coli* coming into the system came from the raw sewage. The microalgal biomass from the HRAP contributed negligible microbial loadings into the UASB reactor. The majority of coliphages and *E. coli* leaving the system were discharged in the liquid effluent, with less than 1% discharged in the UASB sludge. Using the loadings with a mass balance approach (Eq. (4) and Fig. 1b), the overall log reduction of the geometric means for the UASB reactor were 1.60-log for *E. coli*, 0.41-log for somatic coliphages, and 0.62-log for F-specific coliphages. Table 4 shows the microbial mass balance for the UASB reactor with the geometric means of the loadings and the overall reduction using Eq. (4).

Table 4
Microbial mass balance for the UASB reactor.

Point location	Flow rate (L/d)	*E. coli* loading (MPN/d)	Somatic coliphage loading (PFU/d)	F-specific coliphage loading (PFU/d)
A Raw sewage	1176	$1.11 \cdot 10^{13}$	$5.36 \cdot 10^{9}$	$1.47 \cdot 10^{9}$
E HRAP algal biomass (returned to UASB)	12	$3.25 \cdot 10^{6}$	$1.13 \cdot 10^{6}$	$8.08 \cdot 10^{4}$
Total in	1188	$1.11 \cdot 10^{13}$	$5.36 \cdot 10^{9}$	$1.47 \cdot 10^{9}$
B UASB liquid effluent	1180	$8.18 \cdot 10^{11}$	$2.16 \cdot 10^{9}$	$4.50 \cdot 10^{8}$
D UASB sludge	8	$5.13 \cdot 10^{9}$	$7.10 \cdot 10^{6}$	$9.72 \cdot 10^{5}$
Total out	1188	$8.23 \cdot 10^{11}$	$2.16 \cdot 10^{9}$	$4.50 \cdot 10^{8}$
Total inactivated	–	$1.03 \cdot 10^{13}$	$3.20 \cdot 10^{9}$	$1.02 \cdot 10^{9}$
log reduction of geometric means (Eq. (4))	–	1.60	0.41	0.62

For the mass balance around the pilot-scale HRAPs (Fig. 1c, Mass Balance Boundary 2), the only inlet to the system was the liquid effluent from the UASB reactor. The majority (81–86%) of coliphages and *E. coli* left the system in the clarified liquid effluent, with 14–19% leaving in the algal biomass. This partitioning was very similar to the volumetric partitioning for the clarifier. The clarifier received a total flow rate of 51 L/d from the HRAPs, of which 12 L/d (24%) of settled microalgal biomass were sent to the UASB reactor, and 39 L/d (76%) of clarified liquid supernatant were discharged as liquid effluent. A t-test analysis was performed and showed that there was no significant difference between the volumetric partitioning and the pathogen partitioning in algal biomass (p values for *E. coli* and coliphages were <0.05).

Using the loadings with a mass balance approach, the overall log reduction values (Eq. (4)) for the HRAPs were 3.14-log for *E. coli*, 1.21-log for somatic coliphages, and 1.64-log for F-specific coliphages. Table 5 shows the microbial mass balance for the HRAPs with the geometric means of the loadings and the overall reduction using Eq. (4). For microbial partitioning in the HRAPs, 85.6% of *E. coli* left the unit in the liquid effluent and 14.4% in the algal biomass. Both coliphages showed similar partitioning in the HRAPs, 81% were removed in the liquid effluent and 19% left in the biomass.

A mass balance was performed considering Boundaries 1 and 2 (previous paragraphs), and subsequently a mass balance was performed using Boundary 3, which represents a closed UASB + HRAP in terms of flow (inlets = outlets). For the overall UASB + HRAP system (Fig. 1d, Boundary 3), raw sewage was the only inlet to the system,

Table 3
Microbial effluent concentrations (PFU/100 mL or MPN/100 mL) and log removal throughout the different stages of the pilot-scale wastewater treatment system.

System	Microbial	Geo. mean	Maximum	Minimum	Log. removal Eq. (2)
UASB	Somatic coliphages	$1.83 \cdot 10^{5}$	$2.69 \cdot 10^{5}$	$7.10 \cdot 10^{4}$	0.40
	F-specific coliphage	$3.81 \cdot 10^{4}$	$2.80 \cdot 10^{5}$	$2.65 \cdot 10^{3}$	0.56
	E. coli	$6.93 \cdot 10^{7}$	$5.61 \cdot 10^{8}$	$8.74 \cdot 10^{6}$	1.09
HRAP	Somatic coliphages	$1.20 \cdot 10^{4}$	$3.34 \cdot 10^{4}$	$2.90 \cdot 10^{3}$	1.15
	F-specific coliphage	$9.13 \cdot 10^{2}$	$1.02 \cdot 10^{4}$	$2.00 \cdot 10^{2}$	1.70
	E. coli	$4.94 \cdot 10^{4}$	$2.72 \cdot 10^{6}$	$7.40 \cdot 10^{2}$	4.06
UASB + HRAP	Somatic coliphages	$1.20 \cdot 10^{4}$	$3.34 \cdot 10^{4}$	$2.90 \cdot 10^{3}$	1.54
	F-specific coliphage	$9.13 \cdot 10^{2}$	$1.02 \cdot 10^{4}$	$2.00 \cdot 10^{2}$	2.26
	E. coli	$4.94 \cdot 10^{4}$	$2.72 \cdot 10^{6}$	$7.40 \cdot 10^{2}$	5.16

Table 5
Microbial mass balance for the HRAP reactor.

Point location		Flow rate (L/d)	*E. coli* loading (MPN/d)	Somatic coliphage loading (PFU/d)	F-specific coliphage loading (PFU/d)
B_2	UASB effluent (sent to pilot-scale HRAPs)	51	$3.53 \cdot 10^{10}$	$9.32 \cdot 10^{7}$	$1.94 \cdot 10^{7}$
Total in		51	$3.53 \cdot 10^{10}$	$9.32 \cdot 10^{7}$	$1.94 \cdot 10^{7}$
C	Clarified HRAP liquid effluent	39	$1.93 \cdot 10^{7}$	$4.69 \cdot 10^{6}$	$3.56 \cdot 10^{5}$
E	HRAP algal biomass (returned to UASB)	12	$3.25 \cdot 10^{6}$	$1.13 \cdot 10^{6}$	$8.08 \cdot 10^{4}$
Total out		51	$2.25 \cdot 10^{7}$	$5.82 \cdot 10^{6}$	$4.37 \cdot 10^{5}$
Total inactivated		–	$3.53 \cdot 10^{10}$	$8.74 \cdot 10^{7}$	$1.90 \cdot 10^{7}$
log reduction of geometric means (Eq. (4))		–	3.14	1.21	1.64

and there were two outlets: sludge from the UASB reactor and the final treated liquid effluent from the HRAP clarifier. Of the viable coliphages leaving the system, nearly 95% were discharged in the clarified liquid effluent, with the remainder discharged in the sludge from the UASB reactor. Based on the calculated daily loadings, the number of coliphages leaving the system in the liquid effluent was approximately an order of magnitude higher than the number of coliphages leaving the system in the UASB sludge. For *E. coli*, the opposite was true, as the number of *E. coli* leaving the system in the UASB sludge was approximately one order of magnitude greater than the number leaving the system in the treated liquid effluent. Using this mass balance approach with Eq. (4) the overall log reductions for the UASB + HRAP system were 2.71-log for *E. coli*, 1.68-log for somatic coliphages, and 1.81-log for F-specific coliphages. Table 6 shows the microbial mass balance for the overall UASB + HRAPs system. Fig. 3 shows a pathogen flow diagram indicating the fate and transport of the microbial indicators throughout the system.

When using Eq. 6 to analyze the fraction of viral and bacterial indicators in sludge and liquid effluent, only 10% of surviving *E. coli* but nearly 95% of viable coliphages were discharged in the clarified liquid effluent, with the remaining fractions discharged in the sludge. The mechanisms for the inactivation of pathogens in algae-based wastewater treatment processes include predation and direct or indirect sunlight-mediated inactivation, high pH, high DO, starvation and other factors (von Sperling, 2005). Pathogens can also be removed from the liquid fraction via sedimentation after adsorption to solid particles (Tyagi et al., 2008).

One mechanism that has been established for the removal of viruses from wastewater is the adsorption to solids and their subsequent removal via sedimentation or filtration (Armanious et al., 2016; Verbyla and Mihelcic, 2015; Yin et al., 2018). However, it is important to note that adsorption may vary considerably from one virus species to another (Verbyla, 2015). Characteristics of a virus, such as size, hydrophobicity, and isoelectric point (pI) play an important role in virus adsorption (Xagoraraki et al., 2014). Considering that wastewater and sludge matrices tend to have near-neutral pH levels (Vassalle et al., 2020a), most enteric viruses and coliphages would be expected to have overall negative surface charges and adsorb to positively charged surfaces (Xagoraraki et al., 2014). Therefore, the association of most enteric viruses and coliphages (with some exceptions) based on electrostatic interactions is anticipated to be minimal. This is reflected in our findings, which indicate that the proportion of coliphage loadings to sludge vs. liquid effluent of the UASB reactor and the HRAPs is very similar to the proportion of sludge volume discharged vs. liquid effluent discharged from the UASB reactor and the HRAP (e.g., Tables 4 and 5).

Due to their hydrophobicity, enveloped viruses such as murine hepatitis virus (MHV), *Pseudomonas* phage ϕ6, and the novel coronavirus (SARS-COV-2), have been reported to have a high affinity to wastewater solids (Balboa et al., 2020; Gundy et al., 2009; Ye et al., 2016), often appearing in higher concentrations in primary sludge than in wastewater (Peccia et al., 2020). As summarized by Yin et al. (2018), the sorption of viruses to wastewater solids is highly variable, even within different strains of the same virus group. Our results showed that nearly 99% of coliphages left the UASB reactor in the liquid effluent, but less than 0.5% left in the sludge (with the other fraction either being inactivated or retained within sludge granules that are not removed during desludging). This differs from results for human adenovirus presented in Verbyla (2015), which showed that adenovirus genome targets became volumetrically concentrated in the sludge from two different UASB reactors in Brazil.

Given that only 8 L/day of sludge left the reactor on average, compared to 1180 L/day of liquid effluent that left the reactor (on average), there is no evidence that somatic or F-specific coliphages here were preferentially associating with sludge particles. The same was true for the HRAP and settler. The volumetric fraction of algal biomass leaving the settler is 12/51 = 24%, and the fraction of virus loading in the algal biomass compared to the total loading leaving the reactor (calculated using Eq. 6) is equal to 1.13E+06/(469E+06 + 1.13E+06) = 19%, which is very similar to the volumetric fraction with a non-significant difference ($p < 0.05$). In this aspect, our findings are in contrast to other findings in the literature. For example, Young et al. (2016) found a significant relationship of the log removal between the F-specific coliphages and chlorophyll-*a* (used as indirect measurement of microalgae concentration) in the HRAP system (Park and Craggs, 2011). The authors reported that microalgae could influence pathogen removal not only by increasing the pH but also by increasing adsorption to biomass. Symonds et al. (2014) studied virus-particle partitioning using a cascade filter approach in samples from two wastewater treatment systems—one with a UASB reactor followed by polishing ponds, and the other with three waste stabilization ponds in series. They reported that the ratios of norovirus (genogroup I), rotavirus, and pepper mild mottle virus retained on 180-μm filters, relative to the total count, were generally below 5% in samples collected from all treatment unit processes. They likewise found that viruses retained on 0.45-μm filters (positively charged and neutral charge) were less than 50% of the total count for all samples but were greater for the UASB reactor liquid effluent than they were for any of the stabilization pond samples (Symonds et al., 2014).

Table 6
Microbial mass balance for the entire UASB + HRAP system.

Point location		Flow rate (L/d)	*E. coli* loading (MPN/d)	Somatic coliphage loading (PFU/d)	F-specific coliphage loading (PFU/d)
A	Raw sewage (partial flow)	39.3	$3.71 \cdot 10^{11}$	$1.79 \cdot 10^{8}$	$4.90 \cdot 10^{7}$
Total in		39.3	$3.71 \cdot 10^{11}$	$1.79 \cdot 10^{8}$	$4.90 \cdot 10^{7}$
C	Clarified HRAP liquid effluent	39	$1.93 \cdot 10^{7}$	$4.69 \cdot 10^{6}$	$3.56 \cdot 10^{5}$
D	UASB sludge (partial flow)	0.3	$1.72 \cdot 10^{8}$	$2.38 \cdot 10^{5}$	$3.27 \cdot 10^{4}$
Total out		39.3	$1.92 \cdot 10^{8}$	$4.93 \cdot 10^{6}$	$3.89 \cdot 10^{5}$
Total inactivated		–	$3.71 \cdot 10^{11}$	$1.74 \cdot 10^{8}$	$4.86 \cdot 10^{7}$
log reduction of geometric means (Eq. (4))		–	2.71	1.68	1.81

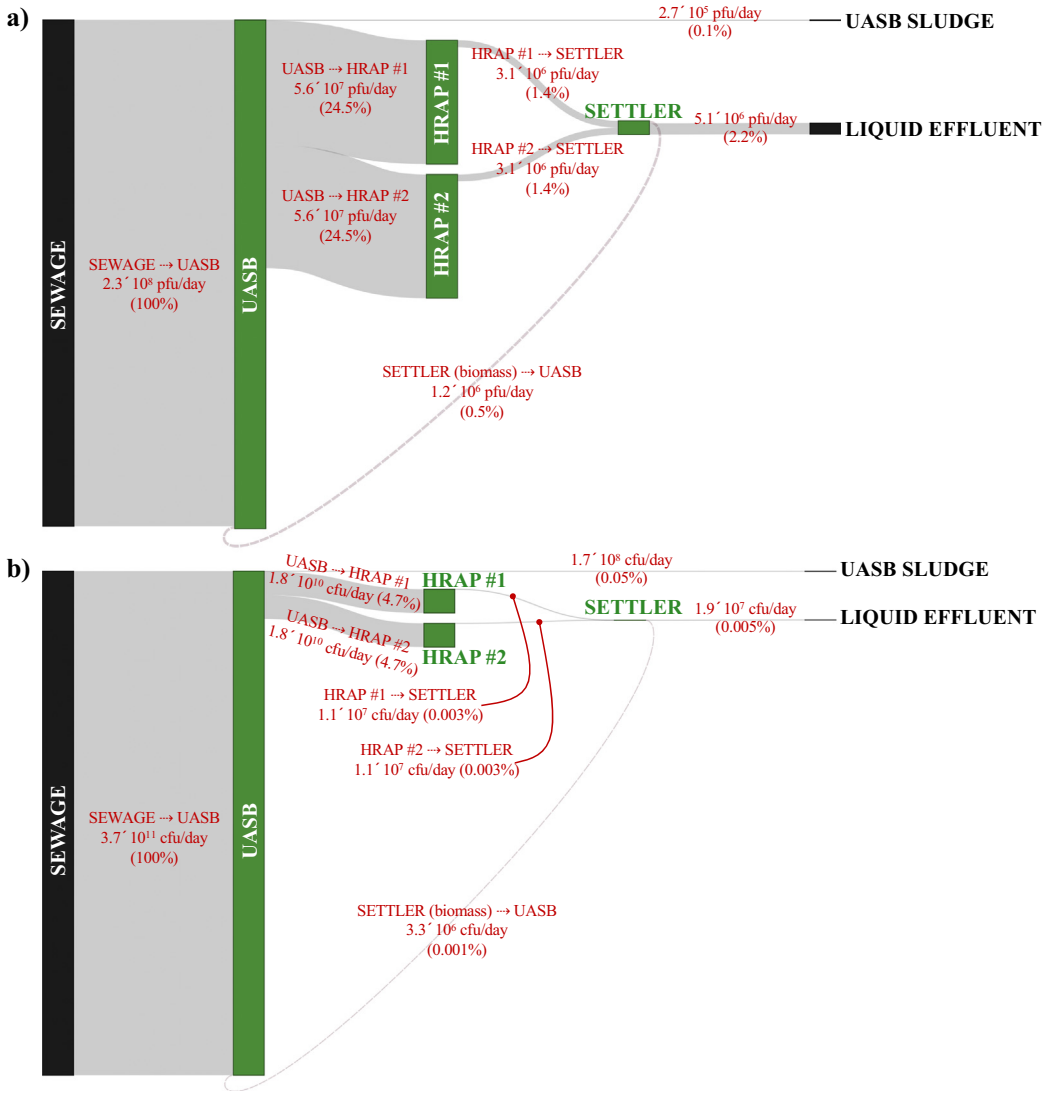

Fig. 3. Pathogen flow diagram showing the fate and transport of a) coliphages and b) *E. coli* throughout the overall system. The width of the bars is proportional to the percentage of viable microorganisms remaining in the system. Loadings shown are geometric mean loadings associated with Mass Balance Boundary #3 (Fig. 1d) for all samples analyzed, and percentage values are calculated from the geometric mean loadings (as opposed to the mean of the calculated percentages).

In contrast, our results indicated a low affinity of coliphages to solids. Thus, it is possible that coliphages may not flow through these systems the same way that other viruses do, especially when talking about partitioning and adsorption to solids. This requires further research, as coliphages are often regarded as good process indicators for the removal and reduction of enteric viruses in wastewater treatment systems.

In general, results from this study demonstrate the suitability of UASB-HRAP systems to reduce viral and bacterial indicators from domestic sewage and the importance of adequately treating sludge for pathogen reduction before they are used as biosolids. More studies are needed about the partitioning of viruses in liquid effluent and solids fraction.

4. Conclusions

The UASB-HRAP wastewater treatment system studied here showed efficient treatment of wastewater in terms of the reduction of solids, organic matter, and microbial indicators *E. coli*, somatic coliphages, and F-specific coliphages. The removal of the coliphages from wastewater, considering only the liquid fraction, was 2.06-log, and the overall reduction, considering both the liquid effluent and the sludge removed, was 1.75-log. Pathogen loadings in sludges should also be considered when reporting pathogen reduction efficiencies in wastewater treatment processes.

Partitioning analysis demonstrated that 90% of *E. coli* left the system in the UASB sludge and only 10% was removed in the liquid effluent. For viral indicators, 19% left the system in the algal biomass, less than 10% left in the UASB sludge and more than 90% was removed in the liquid effluent. The coliphage loadings in the sludge removed were small relative to the coliphage loadings in the liquid effluent and were proportional to the respective volume loadings, indicating a lack of evidence to support any preferential partitioning of coliphages to sludge particles. More research is needed to determine appropriate viral indicators in wastewater treatment systems, not only as proxy measurements for log virus reductions, but also as proxy indicators for virus partitioning between liquid and solid fractions of the waste.

CRediT authorship contribution statement

Maria Fernanda Espinosa: Conceptualization, Methodology, Validation, Data curation, Formal analysis, Investigation, Writing - original draft, Writing - review & editing, Visualization. **Matthew E. Verbyla:**

Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing - review & editing, Visualization, Funding acquisition, Resources, Supervision. **Lucas Vassalle:** Data curation, Formal analysis, Investigation, Writing - original draft. **Alcino Trindade Rosa-Machado:** Data curation, Formal analysis. **Fei Zhao:** Data curation. **Anaïs Gaunin:** Data curation. **César Rossas Mota:** Conceptualization, Validation, Formal analysis, Investigation, Writing - review & editing, Visualization, Funding acquisition, Resources, Supervision, Project administration.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2020.144309.

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