Quantification of the bacterial pathogens in municipal wastewater treatment plants

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INTRODUCTION

Wastewater is a huge reservoir of different pathogens that are excreted by disease carrying humans and animals. Selection of treatment system for understanding the potential to reuse recycled water has become imperative for minimal risk to human health and other life forms. From retrospect, the capability of removing pathogenic microorganisms from the most popular conventional activated sludge (CAS) has been extensively investigated. However, this process is associated with high operational and capital costs. As an alternative, there have been studies documenting the advantage of using DHS system over CAS (Tandukar et al., 2007). Literature reviews have already addressed the indicator organisms i.e. Escherichia coli, total coliforms (TC), fecal coliforms (FC), protozoans and other microbial communities existing in DHS system. The DHS reactor is a trickling filter that uses a polyurethane-sponge media to carry biomass (Onodera et al., 2014). Wastewater trickles from the top of the reactor and is oxidized by the prokaryotes within and on the surface of the sponge medium as it flows down through the reactor by gravity. Previous studies also have highlighted the advantages of Downflow Hanging Sponge (DHS) system for the removal of microbial indicator bacteria and virus from the effluent discharges. However, little attention was given to the individual bacterial pathogens and their removal performance in the biological conventional activated sludge (CAS) process as well as DHS. The purpose of the present study is to employ cultural and qPCR methodologies to examine the presence and removal rate of pathogenic bacteria in municipal wastewater by CAS and DHS processes.

MATERIALS AND METHODS

This study was performed in Nagaoka Sewage Treatment Facility in Nagaoka, Japan. A pilot scale DHS of 857 L volume with 3.2 HRT was selected which is in operation for a decade now. The wastewater samples were collected in sterile bottles. The samples were collected in the sterile plastic bottles of sewage (200 ml), Settling Tank (200ml), DHS (500 ml) and CAS tank effluent (1L). The samples were concentrated by centrifugation at 10000 × g for 15 min at 4^{o}C and the pellet was resuspended in phosphate buffer solution (PBS). The washing step was repeated three times. The harvested and washed cells were resuspended in 1 ml of PBS and stored in -80^{o}C till DNA extraction. In cultural method, indicator micro-organisms i.e. TC and *E. coli* were measured. For molecular analysis, qPCR was employed for the eight target organisms adapted from the study by Ishii et al., (2013) as shown in Table 1.

Table 1. Detection of the positive and negative bacteria stains in the sewage samples

Target organism	Target gene	Samples			
		Sewage	Settling Tank	DHS	CAS
General *E. coli*	*ftsZ*	+	+	+	+
	uidA	+	+	+	+
	stx1	-	-	-	-
	stx2	-	-	-	-
EHEC	*eaeA*	+	+	+	+
Shigella spp.	*ipaH7, 8*	-	-	-	-
	ipaH all	-	-	-	-
	virA	-	-	-	-
Salmonella spp.	*invA*	-	-	-	-
	urC	-	-	-	-
Campylobacter jejuni	*cadF*	-	-	-	-
	ciaB	-	-	-	
Clostridium perfringens	*cpe*	+	+	+	+
	plc	+	+	+	+
Legionella pneumophila	*mip*	-	-	-	-
Listeria monocytogenes	*iap*	-	-	-	-
	hlyA	-	-	-	-

+: Positive -: Negative

RESULTS AND DISCUSSION

In colilert method, TC and *E. coli* were enumerated. The concentration of TC and *E. coli* are expressed in MPN/100ml for sewage, settling tank, DHS system and CAS as shown in Table 2. The concentration of TC was $4.5 \times 10^7 \pm 2.6 \times 10^7$ MPN / 100 ml in the sewage, $2.1 \times 10^7 \pm 7.7 \times 10^6$ MPN / 100 ml in the settling tank effluent, $2.6 \times 10^5 \pm 4.3 \times 10^5$ MPN / 100 mL in the DHS effluent and $5.9 \times 10^5 \pm 6.1 \times 10^5$ MPN / 100 mL in CAS effluent.

Out of 8 strains with 18 targeted genes tested, the targeted genes fstZ, uidA of *E. coli*, eaeA of *EHEC* strain, cpe and plc of *C. perfringens* were confirmed positive (Table1). The other bacteria species such as *Shigella spp., Salmonella spp., Campylobacter jejuni, Legionella pneumophila* and *Listeria monocytogenes* were not detected in the sample. In order words, only 5 targeted genes of 3 strains were detected positive. The overall removal performance of each treatment units was evaluated by comparing the effluent and influent concentrations. For qPCR analysis, the results showed that $6.8\times10^6 \pm 8.6\times10^6$ copies/L of fstZ in sewage was reduced to $5.4\times10^6\pm8.6\times10^6$ copies/L by Settling tank, $1.8\times10^4\pm1.7\times10^5$ copies/L by DHS and $8.7\times10^4\pm1.7\times10^5$ copies/L by CAS.

Table 2: The average concentrations and removal rates of the detected pathogens in DHS and CAS systems

Target Gene /(Indicator microorganism)	Sewage	Settling Tank eff. (S.T)	DHS eff.	CAS eff.	Removal percentage	
					DHS	CAS
E.coli (MPN/100 mL)	8.90E+06	4.50E+06	5.80E+04	1.10E+05	99.35	98.76
Total Coliform (MPN/100	4.50E+07	2.10E+07	2.60E+05	5.90E+05	99.42	98.69
ftsZ (copies/L)	6.80E+06	5.40E+06	1.80E+04	8.70E+04	99.74	98.72
uidA (copies/L)	9.70E+06	4.50E+06	1.70E+04	6.70E+03	99.82	99.93
eaeA (copies/L)	7.90E+05	1.30E+06	6.80E+03	1.90E+04	99.14	97.59
plc (copies/L)	9.00E+05	3.30E+05	2.10E+04	7.80E+03	97.67	99.13
cpe (copies/L)	4.10E+05	1.60E+05	1.10E+04	7.20E+03	97.32	98.24

Settling tank showed poor removal efficiency. The removal rate obtained by DHS system for *E. coli* genes were 99.74%(*fstZ*), 99.82%(*uidA*), for *EHEC* was 99.14% (*eaeA*) and for *C. perfringens* were 97.67% (*plc*) and 97.32 % (*cpe*). Similarly, CAS system also delivered good removal of 98.72%(*fstZ*), 98.93% (*uidA*), 97.59% (*eaeA*), 99.13% (*plc*) and 98.24% (*cpe*). In this study, concentrations of *E. coli* and *EHEC* showed difference of 10-fold in DHS system. However, the removal rate of *E. coli* and *EHEC* by DHS and CAS were almost similar. Regarding *C. perfringens,* CAS process showcased better removal than DHS. The reason for this difference was assumed to be the larger size of *C. perfringens* which resisted it to straining and adsorption in DHS sponges. Furthermore, DHS showed better removal performance for other genes owing to its adsorption and protozoa predation over CAS process.

CONCLUSION

In this study, we examined two municipal wastewater treatment systems, CAS and DHS. Colilert method and qPCR were utilized to monitor the removal of pathogenic species in the treatment systems. *General E. coli, Clostridium perfringens* and *EHEC* were detected and quantified in samples from two treatment systems. The results show a high level of *E. coli* concentrations in the raw water. Results showed that specific removal rates vary widely regardless of the system employed. There was no significant difference in the removal of *E. coli* by CAS and DHS processes. Data on pathogen detection in treated effluents confirmed the potential for environmental contamination by bacteria and could be useful to establish standards for policies on wastewater management.

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