Risk assessment of Giardia from a full scale MBR sewage treatment plant caused by membrane integrity failure

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ABSTRACT

Membrane bioreactors (MBR) are highly efficient at intercepting particles and microbes and have become an important technology for wastewater reclamation. However, many pathogens can accumulate in activated sludge due to the long residence time usually adopted in MBR, and thus may pose health risks when membrane integrity problems occur. This study presents data from a survey on the occurrence of water-borne Giardia pathogens in reclaimed water from a full-scale wastewater treatment plant with MBR experiencing membrane integrity failure, and assessed the associated risk for green space irrigation. Due to membrane integrity failure, the MBR effluent turbidity varied between 0.23 and 1.90 NTU over a period of eight months. Though this turbidity level still met reclaimed water quality standards (<5 NTU), Giardia were detected at concentrations of 0.3 to 95 cysts/10 L, with a close correlation between effluent turbidity and Giardia concentration. All β-giardin gene sequences of Giardia in the WWTP influents were genotyped as Assemblages A and B, both of which are known to infect humans. An exponential dose–response model was applied to assess the risk of infection by Giardia. The risk in the MBR effluent with chlorination was 9.83 × 10⁻³, higher than the acceptable annual risk of 1.0 × 10⁻⁴. This study suggested that membrane integrity is very important for keeping a low pathogen level, and multiple barriers are needed to ensure the biological safety of MBR effluent.

Introduction

Reclaimed water from sewage has become increasingly important in solving water shortage problems in the world. In northern China, a growing number of sewage reclamation facilities have been constructed to produce water for irrigation, toilet flushing, and surface water supplementation (Asano et al., 2013). These efforts, however, are compromised by the water quality problems of reclaimed water, particularly the transport of pathogens through the use of reclaimed water (Rodriguez-Manzano et al., 2012).

Among the frequently encountered water-borne pathogens, protozoan parasites like Giardia sp. have caused particular concerns because the conventional disinfectants like chlorine are ineffective in inactivation of these parasites (Clark and Regli, 1993). Giardia duodenalis (syn. G. intestinalis, G. lamblia) is the only Giardia species found in human gastrointestinal tracts (Monis et al., 2009). It

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produces robust cysts, which are voided in the feces and transmitted directly through fecal/oral contact or by ingestion of contaminated water and food (An et al., 2012). Giardia sp. has been detected in sewage (Gassman and Schwartzbrod, 1991; Nasser et al., 2012) as well as in tertiary treatment effluents after chlorination and chloramination (Nasser et al., 2012; Quintero-Betancourt et al., 2003; Ryu et al., 2007).

Both UV and ozonation are efficient in parasite disinfection (Nasser et al., 2012; Linden et al., 2002; Widmer et al., 2009). On the other hand, physical disinfection using membrane filtration has recently been a focus because theoretically it could intercept almost all of the particles larger than its pore size, including pathogenic microbes (Lonigro et al., 2006). The combination of biological wastewater treatment and membrane separation, known as the membrane bioreactor (MBR) process, has attracted particular interest because of its advantages in achieving nutrient removal and disinfection in only one step (Jefferson et al., 2000). However, Jong et al. (2010) reported that microfiltration membranes (pore size 0.45 μm) in MBR did not perfectly remove microorganisms such as Escherichia coli, Salmonella sp., Staphylococcus aureus and Coliform. This could cause particular concerns over the release of pathogens when membrane integrity failure occurs, as pathogens may be concentrated in activated sludge in an MBR system. Lonigro et al. (2006) also detected Giardia and Cryptosporidium in filtered water from an MBR during occasional failure of the ultrafiltration system for treating secondary effluent. Therefore, it will be of importance to assess the risk of Giardia in MBR effluents when a membrane loses its integrity.

In the present study, we surveyed the occurrence of Giardia in reclaimed water from a full-scale wastewater treatment plant (WWTP) with MBR experiencing membrane integrity failure in an industrial park in China, and assessed the associated health risk (WWTP) with MBR experiencing membrane integrity failure in an industrial park in China, and assessed the associated health risk. Therefore, it will be of importance to assess the risk of Giardia in MBR effluents when a membrane loses its integrity.

In the present study, we surveyed the occurrence of Giardia in reclaimed water from a full-scale wastewater treatment plant (WWTP) with MBR experiencing membrane integrity failure in an industrial park in China, and assessed the associated health risk (WWTP) with MBR experiencing membrane integrity failure in an industrial park in China, and assessed the associated health risk. Therefore, it will be of importance to assess the risk of Giardia in MBR effluents when a membrane loses its integrity.
where, \( P_o \) is the overall probability of infection and \( n \) is the number of exposures (\( n = 52 \) per year, once a week).

The average exposure (\( d \)) to cysts was estimated using the following equation:

\[
d = C \times 10^{DR} \times V
\]

where, \( d \) is the number of cysts ingested daily by a person through reclaimed water from green space irrigation, \( C \) (cysts/L) is the concentration of pathogens, \( DR \) is the disinfection efficiency (0.5, 3.0, and 3.0 log-unit removal credits were considered for chlorination, UV irradiation and ozonation, respectively (Ryu et al., 2007; USEPA, 1989)) and \( V \) (L) is accidental consumption of a small amount of reclaimed water. The amount of reclaimed water ingested for green space irrigation was estimated at 0.005 L per outing (Ryu et al., 2007). The Poisson probability distribution model used to describe the count data of microbes (Consul and Famoye, 2006) was applied to describe the prevalence of Giardia cysts in reclaimed water (Eq. (1)). There was no provision for the potential die-off of Giardia cysts by desiccation, sunlight, predation or other reasons because of the extreme resistance of the thick-walled cysts to environmental factors. The applied level of acceptable annual risk was \( 1.00 \times 10^{-4} \). This is the annual risk established in the Surface Water Treatment Rule for domestic water supply (USEPA, 1989).

### 2. Results and discussion

#### 2.1. Occurrence of Giardia in reclaimed water samples

Table 1 presents the concentration levels of \( G. \) duodenalis in the MBR effluents. A total of 12 samples were examined by microscopy, with Giardia detected in all samples (100%). A Giardia concentration of 3 to 95 cysts/10 L was detected during the survey (April to November, 2010), which was high in comparison with that (0.046–3.65 cysts/10 L) in non-potable reclaimed water after tertiary treatment in the southwestern United States (Ryu et al., 2007). The prevalence of Giardia cysts in the samples was fitted by a Poisson distribution with \( P < 0.05 \) by Chi-Square test (\( \lambda = 60; \) R statistic software). This result indicated the prevalence of Giardia cysts in the studied water samples.
been reported for MBR effluents with pore size <0.4 and NTU present study satisfied wastewater reclamation standards integrity. Although effluent turbidity (0.23–1.90 NTU) as shown in Table 1 and Fig. 1. Theoretically, an intact MBR (average membrane pore size 0.01-0.4 μm) is a barrier to pathogens and suspended solids larger in size than the membrane pore size, and therefore can offer better pathogen removal than conventional treatment. As a result, total coliform reduction can reach an average of log7 (Hirani et al., 2010). However, our study showed that high concentrations of Giardia (>4 μm) could occur in the MBR effluent caused by membrane integrity failure. Lonigro et al. (2006) also found Giardia and Cryptosporidium in filtered water during occasional failure of the ultrafiltration system for treating secondary effluent.

The turbidity of reclaimed water has often been used as an indicator for biological safety and membrane integrity (Antony et al., 2012; Naismith, 2005). Theoretically, a MBR could achieve almost complete solid–liquid separation, and turbidity for a functional MBR is usually below 0.2 NTU or even lower. However, the turbidity of 0.23–1.90 NTU is abnormally high for MBR permeation. As shown in Fig. 1, the Giardia concentration was significantly correlated with turbidity in the collected water samples ($R^2 = 0.7666$, $P < 0.05$), which was consistent with previous research on drinking water (Carmena et al., 2007). Therefore, the turbidity of MBR effluent should be closely related to membrane module integrity. Although effluent turbidity (0.23–1.90 NTU) in the present study satisfied wastewater reclamation standards (NTU ≤ 5, GB/T 25499-2010, China), Giardia may leak from the MBR system at a relatively high concentration. Turbidities of 1.5 ± 0.4 and 0–6 (average 1.63) NTU in effluents have also been reported for MBR effluents with pore size <0.4 and 0.45 μm, respectively (Jong et al., 2010; Rodriguez-Hernández et al., 2013), suggesting that leakage of particles may be a frequently occurring problem in actual application. This is understandable since membranes are under stress (e.g., high shear stress, existence of sharp particles) in an MBR system. Antony et al. (2012) reported that membrane damage during membrane operation could generate virus breakthrough. They concluded that any anomaly in the membrane surface (e.g., abnormally big pores, compromised glue line, holes) and the filtration system (e.g., compromised O-rings, broken mechanical seals) results in microbial contamination risk for the product water. Therefore, monitoring membrane treatment system integrity is essential for the protection of public health from microbial risk. In particular, occasional failure of MBR systems may cause serious problems since many pathogens – mainly viruses and protozoa – can accumulate in activated sludge at high concentrations due to the long sludge retention times usually adopted in the process (Marti et al., 2011). Leakage of Giardia cysts could be reduced by maintaining membrane integrity (Lonigro et al., 2006).

On the other hand, the Giardia concentration in WWTP influent (after primary treatment) was 22,000 cysts/10 L in the present study. Nasser et al. (2012) reported that in 23 out of 30 studies, all tested raw wastewater samples were positive for Giardia, at concentrations ranging from 2.3 to 1,000,000 cysts/10 L, showing that Giardia pollution is quite universal and requires effective control to secure the biological safety of reclaimed water. Giardia concentrations in raw wastewater and primary, secondary and tertiary treatment effluents were 1300–36,000, 5330–20,330, 0–320 and 0–21 cysts/10 L, respectively, in three municipal wastewater treatment plants in Beijing, China (Fu et al., 2010). Thus, significant removals of Giardia were achieved through secondary and tertiary treatment, showing the importance of establishing multiple barriers for pathogen control. Therefore, a disinfection system is proposed as an additional barrier to ensure the safety of MBR effluent.

### 2.2. Exposure assessment of Giardia

The average Giardia concentration in reclaimed water was 60.7 cysts/10 L during the survey. The daily and annual risks of infection associated with Giardia were calculated using the exponential dose–response model (Table 2). The annual acceptable risk of infection of 1.00 × 10⁻⁴ from water-borne exposure through potable water was applied for performing risk characterizations (Rose and Gerba, 1991; USEPA, 1989). Annual risk with chlorination (9.83 × 10⁻³) was high in comparison with the suggested annual acceptable microbial risk of infection. The annual risks could be reduced to acceptable levels by combining ozonation or UV (3.13 × 10⁻³) (Table 2). Despite the fact that turbidity met the standard of reclaimed water for green space irrigation, the risk of infection by Giardia in the MBR effluents (chlorine) was still at an unacceptable level. Adding a barrier like UV irradiation or ozonation to the MBR system helped ensure the biological safety of reclaimed water.

### 2.3. Molecular identification and genotyping of Giardia

To identify the genotypes and possible transmission patterns of Giardia, PCR amplification was performed for Giardia from influent samples after primary treatment. Twenty positive
clone sequences were obtained by sequence analysis of the nested PCR products of the \( \beta \)-giardin gene. According to sequence analysis (Fig. 2), eighteen sequences were genotyped as Assemblage A and two as Assemblage B, which were in accordance with previous wastewater studies (Sulaiman et al., 2004). All clones exhibited more than 99% identity with known \( \beta \)-giardin gene sequences in NCBI (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Nine clone sequences exhibited 100% identity with the representative sequence for Assemblage A (FN386482). The two clones belonging to Assemblage B (Clone 1 and Clone 12) had two bases different from the representative sequence (EU637579).

Considerable data have shown that \( G. \) duodenalis should be considered as a species complex, whose members, although morphologically identical, can be assigned to at least eight distinct Assemblages (A–H) based on genetic analyses (genotypes) (Monis et al., 2009; Xiao and Fayer, 2008). Assemblages A and B can infect humans and a variety of mammals, whereas Assemblages C to H are considered to be nonhuman host-specific (Monis et al., 2009; Sulaiman et al., 2004; Xiao and Fayer, 2008). On the other hand, it is unlikely that all \( Giardia \) detected in the water samples were human-pathogenic species. The probability of infection may be overestimated (Ryu et al., 2007) since microscopic detection for risk assessment of \( Giardia \) does not differentiate among \( Giardia \) species. Thus, molecular genotyping of the detected \( Giardia \) in the water samples

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**Table 2 – Annual risks of \( Giardia \) infection from reclaimed water used in green space irrigation during the survey.**

| Sample          | Average cysts/L | Disinfection efficiency | Daily risk | Annual risk |
|-----------------|-----------------|-------------------------|------------|-------------|
| MBR effluent (chlorine) | 6.07           | 0.5                     | 1.90 \times 10^{-4} | 9.83 \times 10^{-3} |
| MBR effluent (ozonation/chlorine) | 6.07           | 3                       | 6.01 \times 10^{-7} | 3.13 \times 10^{-5} |
| MBR effluent (UV/chlorine)     | 6.07           | 3                       | 6.01 \times 10^{-7} | 3.13 \times 10^{-5} |

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**Fig. 2 – Phylogenetic analysis of \( Giardia \) inferred by neighbor-joining analysis of partial \( \beta \)-giardin gene sequences.** Evolutionary distances between sequences were calculated by the Kimura two-parameter model. Values on branches are bootstrapping value percentages (>50%) using 1000 replicates.
will help reduce uncertainty in Giardia risk assessment. The predominance of G. duodenalis Assemblages A and B in the present study showed that the contaminant source in this study may have originated from humans, stressing the potential risk associated with the use of reclaimed water.

3. Conclusions

The results of this study showed that the risk of infection by Giardia for green space irrigation exceeded the annual acceptable risk in the studied MBR effluents with chlorine disinfection when the membrane lost its integrity. Genotype analysis based on the β-giardin gene revealed that the human-infective A and B assemblages were the main genotypes in wastewater, stressing the potential risk associated with the use of the MBR effluents with a high level of membrane integrity failure. Ozonation or UV was required to ensure the biological safety of the reclaimed MBR effluent. The above results showed that the monitoring of membrane integrity in MBR is very important for the biological safety of the MBR effluent, and establishing multiple barriers in treatment of wastewater is necessary for MBR.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found in online version at http://dx.doi.org/10.1016/j.jes.2014.09.033.

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