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Ozone inactivation of infectious prions in rendering plant and municipal wastewaters

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HIGHLIGHTS

- Infectious prions are resistant to conventional wastewater treatment technologies.
- Prions partitioned into different phases by gravity separation in rendering plant wastewater.
- Organic removal improved prion inactivation by ozone in rendering plant wastewater.
- Constituents in municipal final effluent affected prion inactivation by ozone.

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ABSTRACT

Disposal of tissues and organs associated with prion accumulation and infectivity in infected animals (designated as Specified Risk Materials [SRM]) is strictly regulated by the Canadian Food Inspection Agency (CFIA); however, the contamination of wastewater from slaughterhouses that handle SRM still poses public concern. In this study, we examined for the first time the partitioning of infectious prions in rendering plant wastewater and found that a large proportion of infectious prions were partitioned into the scum layer formed at the top after gravity separation, while quite a few infectious prions still remained in the wastewater. Subsequently, we assessed the ozone inactivation of infectious prions in the raw, natural gravity-separated and dissolved air flotation (DAF)-treated (i.e., primary-treated) rendering plant wastewater, and in a municipal final effluent (i.e., secondary-treated municipal wastewater). At applied ozone doses of 43.4–44.6 mg/L, ozone was instantaneously depleted in the raw rendering plant wastewater, while a greater than 4-$\log_{10}$ inactivation was achieved at a 5 min exposure in the DAF-treated rendering plant wastewater. Prion inactivation in the municipal final effluent was conducted with two levels of applied ozone doses of 13.4 and 22.5 mg/L, and a greater than 4-$\log_{10}$ inactivation was achieved at a 5 min exposure with the higher ozone dose. Efficiency factor Hom (EFH) models were used to model (i.e., fit) the experimental data. The *CT* (disinfectant concentration multiplied by contact time) values were determined for 2- and 3-$\log_{10}$ inactivation in the municipal final effluent treated with an ozone dose of 13.4 mg/L. Our results indicate that ozone could serve as a final barrier for prion inactivation in primary- and/or secondary-treated wastewaters.

1. Introduction

Transmissible spongiform encephalopathies (TSEs) are a group of fatal neurodegenerative diseases that are associated with the presence and accumulation of a misfolded form (PrP^{Sc}) of normal prion protein (PrP^{C}), especially in neuronal tissues (Maddison et al., 2007). TSEs include scrapie in sheep and goats, Chronic Wasting Diseases (CWD) in deer, moose, and elk, bovine spongiform encephalopathy (BSE) in cows, and Kuru, Creutzfeldt–Jakob disease (CJD) and Gerstmann–Straussler–Scheinker syndrome (GSS) in humans (Prusiner, 1998). Familial CJD and GSS are genetic disorders in humans, whereas Kuru results from ritualistic cannibalism (Prusiner, 1991). The emergence of a new strain of CJD designated as vCJD in humans originated from the ingestion of BSE infected beef, during a BSE outbreak in cattle in Great Britain, and has raised great public concerns over the last two decades (Bruce et al., 1997; Will et al., 1999). Stringent regulations have been

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established that prohibit using potentially BSE laden tissues, designated as Specified Risks Materials (SRM), in food, animal feed, pet food and fertilizers by the industry (Canadian Food Inspection Agency, 2011; Womach, 2005). However, liquid waste generated from the disposal of SRM, as well as the rendering plants or slaughterhouses that illegally or unknowingly process BSE infected meat, still pose concerns due to the possible release of infectious prions to the environment. In Canada, some slaughterhouses have complete on-site wastewater treatment systems, while others discharge wastewater to municipal wastewater treatment plants after only primary treatment (i.e., primarily solids–liquid separation process) that results in partial removal of total suspended solids (TSS) and 5-day biochemical oxygen demand (BOD_5) (Mittal, 2006), thus increasing the possibility of releasing infectious prions to the environment.

Infectious prions are very resistant in the environment (Brown and Gajdusek, 1991; Georgsson et al., 2006; Miller et al., 2004). Scrapie and CWD are horizontally transmissible in the environment (Hadlow et al., 1982; Miller et al., 2004). It has been reported that scrapie can persist in the environment for 16 years (Georgsson et al., 2006), and survive in soil for at least 3 years (Brown and Gajdusek, 1991). CWD can remain infectious in pasture for at least 2 years (Miller et al., 2004). Low concentrations of CWD may have been detected in surface water samples from one river in a CWD epidemic area, as well as in the nearby municipal water treatment facility (Giles et al., 2008), strongly indicating that water may act as a transport agent for prions. In addition, the fate of infectious prions in various water matrices has been investigated in several studies, which suggested that infectious prions could also survive in water and wastewater for a long time (de Motes et al., 2008a, 2008b; Maluquer de Motes et al., 2012; Miles et al., 2011). Both scrapie and BSE have shown limited degradation (1–2 $\log_{10}$) while being incubated in phosphate buffered saline (PBS), distilled (DI) water, and tap water at 20–25 °C for 56–265 days (de Motes et al., 2008a; Miles et al., 2011). As evaluated by western blot detection of the presence of PrP^{Sc}, higher degradation efficiency of BSE and scrapie was initially observed when they were incubated in a raw municipal wastewater and in a slaughterhouse wastewater (de Motes et al., 2008a,b). Nevertheless, further studies using the "gold standard" animal infectivity test and an alternative cell culture assay have demonstrated minute degradation of BSE and scrapie in the raw municipal wastewater (Maluquer de Motes et al., 2012; Miles et al., 2011), verifying that western blot detection of PrP^{Sc} does not always correlate with the infectivity (Maluquer de Motes et al., 2012; McLeod et al., 2004). Prions can also survive in water and wastewater after treatment with UV irradiation (Alper et al., 1967), chlorine disinfection (Miles et al., 2011; Taylor et al., 1994), and mesophilic anaerobic digestion (Hinckley et al., 2008; Kirchmayr et al., 2006). The inactivation by UV radiation was under 1-$\log_{10}$ for UV dose as high as 7×10^3 J/m^2 at 280 nm (Alper et al., 1967). Approximately 1-$\log_{10}$ inactivation was achieved by residual chlorine in tap water (Miles et al., 2011). Similarly, no significant reduction of infectivity was observed after mesophilic anaerobic digestion (Hinckley et al., 2008; Kirchmayr et al., 2006).

We have demonstrated that ozone is extremely effective in the inactivation of prions (Ding et al., 2012, 2013). In wastewater matrices, however, suspended flocs of organic and/or inorganic matter may shield organisms, that are enmeshed inside these flocs, from the attack of disinfectants (Geldreich, 1996). They may also cause decrease in the disinfectant residual concentration available for prions, due to the disinfectant demand caused primarily by the presence of organics in the water or wastewater matrix to be treated (Hart et al., 1983; U.S. Environmental Protection Agency, 1999a).

The goal of this study was to provide further investigation on the effectiveness of ozone inactivation of prions in rendering plant wastewater and municipal final effluent. Solids–liquid gravity separation and dissolved air flotation (DAF) as primary treatments were applied on a raw rendering plant wastewater to evaluate the effect of organics and solids removal on prion inactivation by ozone. The specific objectives of this study were to: a) examine the partitioning of prions during a natural gravity separation process of a raw rendering plant wastewater; b) assess the effectiveness of ozone inactivation of prions in a raw rendering plant wastewater; c) determine the kinetics of ozone inactivation of prions in the rendering plant wastewater after organics removal by gravity separation and DAF treatment; and d) investigate the kinetics of ozone inactivation of prions in municipal final effluent (i.e., secondary-treated municipal wastewater).

2. Materials and methods

2.1. Animals and preparation of hamster brain homogenates

Scrapie 263K infectious brain homogenates (IBH) and normal brain homogenates (NBH) were prepared using 3- to 6-week old female Syrian golden hamsters (Charles River Laboratories International, Inc., Wilmington, MA, USA). The detailed procedure for the preparation of homogenates has been described previously by Ding et al. (2012).

2.2. Partitioning of prions during natural gravity separation of raw rendering plant wastewater

Raw rendering plant wastewater (after screening and settling of coarser solids) was collected from an undisclosed rendering plant in Canada in March 2012. A 100 μL aliquot of 10% IBH was added into 100 mL of the rendering plant wastewater in a 120-mL glass container. Samples were mixed by inverting and gently shaking the container. An aliquot (1 mL) of the sample was transferred into a 1.5-mL plastic tube immediately after mixing and kept static at a room temperature (20 ± 1 °C). Samples in the container were gravity separated after 1.5 h, of natural gravity separation, into three layers, with the scum at the top, supernatant wastewater in the middle, and a very thin layer of scattered precipitated solids at the bottom. After gravity separation, a 1 mL aliquot was withdrawn from the scum and from the supernatant wastewater, respectively. All three samples were subjected to protein misfolding cyclic amplification (PMCA) assay to assess any possible partitioning of prions. This prion partitioning experiment was performed in triplicate. All prion-related work was performed in the biocontainment facilities at the Center for Prion and Protein Folding Diseases (CPPFD) at the University of Alberta, Edmonton, Canada. Optimization experiments for natural gravity separation of raw rendering plant wastewater not containing prions were performed first in the wastewater treatment laboratories in the Department of Civil and Environmental Engineering at the University of Alberta to decide on the best conditions for performing the natural gravity separation of the raw rendering plant wastewater in the CPPFD.

2.3. Dissolved air flotation (DAF) treatment of raw rendering plant wastewater

A bench-scale DAF jar test unit (Aztec Environmental Control, UK) having a jar volume of 1400 mL was used to simulate the dissolved air flotation (DAF) process for treating raw rendering plant wastewater. The DAF experiment was started with the pressure of the DAF unit maintained at 70 psi and a temperature of 20 ± 1 °C. The jar was filled with 1000 mL of the raw rendering plant wastewater. DAF treatment was initiated by rapidly mixing (200 rpm) the wastewater for 30 s, followed by slow mixing at 20 rpm for 10 min. Air flow of microbubbles was introduced to the bottom of the jar for 40 s, with an air to solids ratio of 0.006, followed by a resting period for 1.5 h to allow for flocculation followed by solids–liquid separation to occur. Samples were separated into three layers including the scum, the supernatant wastewater, and the scattered solids precipitated at the bottom. Samples from the supernatant wastewater were taken from a sampling port and set aside for ozone inactivation experiments. The DAF treatment experiment was carried out in duplicate in the wastewater treatment laboratories in