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Electrochemical disinfection of toilet wastewater using wastewater electrolysis cell

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ABSTRACT

The paucity of proper sanitation facilities has contributed to the spread of waterborne diseases in many developing countries. The primary goal of this study was to demonstrate the feasibility of using a wastewater electrolysis cell (WEC) for toilet wastewater disinfection. The treated wastewater was designed to reuse for toilet flushing and agricultural irrigation. Laboratory-scale electrochemical (EC) disinfection experiments were performed to investigate the disinfection efficiency of the WEC with four seeded microorganisms (Escherichia coli, Enterococcus, recombinant adenovirus serotype 5, and bacteriophage MS2). In addition, the formation of organic disinfection byproducts (DBPs) trihalomethanes (THMs) and haloacetic acids (HAA5) at the end of the EC treatment was also investigated. The results showed that at an applied cell voltage of +4 V, the WEC achieved 5-log10 reductions of all four seeded microorganisms in real toilet wastewater within 60 min. In contrast, chemical chlorination (CC) disinfection using hypochlorite [NaClO] was only effective for the inactivation of bacteria. Due to the rapid formation of chloramines, less than 0.5-log10 reduction of MS2 was observed in toilet wastewater even at the highest [NaClO] dosage (36 mg/L, as Cl2) over a 1 h reaction. Experiments using laboratory model waters showed that free reactive chlorine generated in situ during EC disinfection process was the main disinfectant responsible for the inactivation of microorganisms. However, the production of hydroxyl radicals [•OH], and other reactive oxygen species by the active bismuth-doped TiO2 anode were negligible under the same electrolytic conditions. The formation of THMs and HAA5 were found to increase with higher applied cell voltage. Based on the energy consumption estimates, the WEC system can be operated using solar energy stored in a DC battery as the sole power source.

1. Introduction

In highly developed countries, flush toilets and well-managed sanitation systems have been used for more than 80 years. Wastewater is collected in massive sewer systems and subsequently treated at large-scale centralized wastewater treatment plants before discharged into receiving waters or land. Advanced, tertiary wastewater treatment plants are now capable of producing high quality reclaimed water for indirect or even direct potable reuse (Wetterau et al., 2013). In contrast, approximately 2.4 billion people in the developing world still lack the access to adequate sanitation facilities (WHO, 2015). The discharge of untreated or partially treated domestic wastewater to the aquatic environment severely threatens public health and socio-economic development. It is abundantly clear that, in many parts of the world, the infrastructure required for conventional centralized wastewater treatment systems is prohibitively expensive. The development of cost-effective, decentralized wastewater treatment systems is an important step toward the eradication of waterborne diseases and to ensure water sustainability in the developing world (Massoud et al., 2009).

Conventional flush toilet wastewater (i.e., a mixture of urine, feces and flushing water) is characterized by high levels of microbial contaminants (e.g., pathogenic viruses, bacteria and protozoa) derived from human excrement. Untreated toilet wastewater is considered as health hazard and potential vector of infectious waterborne diseases. On the other hand, toilet wastewater also...
contains high concentrations of macro- and micro-nutrients essential for plant growth. A large portion of these nutrients (e.g., nitrogen and phosphate) are contained in the liquid phase (Karak and Bhattacharyya, 2011). With proper treatment, it can be used as safe and cheap liquid fertilizer, which helps reduce the use of synthetic chemical fertilizer in developing countries (Drechsel et al., 2009). Therefore, the primary challenge of toilet wastewater reuse lies in how to effectively remove or inactivate microbial contaminants. Many disinfection technologies (e.g., chlorination, UV and ozonation) have been utilized in large-scale wastewater treatment plants. However, the adaptation of conventional disinfection systems to smaller decentralized plants is often difficult due to the financial constraints or technological barriers (Schmalz et al., 2009). In recent years, electrochemical (EC) disinfection has been considered as a viable alternative for decentralized wastewater treatment. EC disinfection has been reported to be capable of disinfecting a wide spectrum of microbial pathogens in various water matrices (Canou et al., 2012; Li et al., 2002). The on-site generation of reactive chlorine species (RCS) such as free chlorine ([Cl2], [HOCl], [ClO\textsuperscript{2−}], and chlorine radical species ([Cl], [Cl\textsuperscript{−}]) are generated and recognized as primary disinfectants. Human urine could serve as an excellent electrolyte as it contains high concentration of chloride (50–150 mM) (Kim et al., 2013; Putnam, 1971). In addition, reactive oxygen species (ROS) including hydroxyl radicals (•OH), hydrogen peroxide ([H2O2]), ozone ([O3]), and superoxide anion radicals ([O2•−]) generated during electrochemical water splitting can enhance the overall disinfection efficiency (Bergmann et al., 2008; Jeong et al., 2006). Another major factor governing the generation of oxidants is the composition of the anode (Jeong et al., 2009). Anodes made from antimony-doped tin oxide (ATO), PbO2 and boron-doped diamond (BDD) electrodes, are known to favor the formation of free •OH. In contrast, Pt-, IrO2-, RuO2- anodes, known as dimensionally stable anodes, form surface bound •OH, which mediates the facile formation of RCS (Chen, 2004). Operational parameters, such as cell voltage, current density, reaction time, temperature, and pH are also important in the optimization of EC disinfection systems. In many previous studies, bacteria (usually Escherichia coli or total fecal coliform) were exclusively used as primary disinfection pathways for microbial inactivation were investigated along with the formation of THMs and HAA5 under typical operational conditions.

2. Materials and methods

2.1. Toilet wastewater and model waters

Fig. 1 shows the design of the pilot scale PV-powered self-contained mobile toilet system located at California Institute of Technology (Caltech). The EC reactor works inline, treating 20 L of wastewater each time in a batch model. The reactor is fed by the supernatant of the wastewater storage/sedimentation tank, which was added to PBS buffer to provide [NH4\textsuperscript{+}], which ensured the formation of oxidants other than ROS during EC reaction. MW2 was phosphate buffered saline (PBS) solution containing only an inert electrolyte monopotassium phosphate ([KH2PO4]) in order to minimize the formation of oxidation products. Aliquots of wastewater were stored at 4°C refrigerator before use. In addition to raw toilet wastewater, three laboratory model waters (MW1 and MW2) were prepared and tested in order to characterize the role of RCS and ROS in EC disinfection. MW1 was phosphate buffered saline (PBS) solution containing only an inert electrolyte monopotassium phosphate ([KH2PO4]) in order to minimize the formation of oxidants other than ROS during EC reaction. MW2 was PBS buffer amended with 15 mM sodium chloride ([NaCl]). Free chlorine would equal to total chlorine in MW2 because of the absence of [NH4\textsuperscript{+}]. In MW3, 15 mM of ammonium chloride ([NH4Cl]) was added to PBS buffer to provide [NH4\textsuperscript{+}] and [Cl\textsuperscript{−}], which ensured that free ammonia was readily available for reaction with [HOCI] during the first 30 min of EC reaction. All model waters were free of organic and other reducing agents. The general characteristics of the toilet wastewater and model waters are summarized in Table 1.
Before each experiment, a freshly prepared 0.1 M bacterial suspension was re-suspended in 1 mL PBS, which were used as bacterial stock. The seeding level of MS2 was 105 to 106 PFU/mL. The concentration of MS2 in water samples was titrated by the double agar filter (GE Whatman, Pittsburgh, PA). The filtrate was diluted 1,000x with 1 × PBS and stored in −80 °C freezer, which was used as MS2 stock. The seeding level of MS2 was 105 to 106 PFU/mL. The concentration of MS2 in water samples was titrated by the double agar filter (Clokie, 2009).

Recombinant adenovirus serotype 5 (rAd5) with the E1A gene replaced by the green fluorescent protein (GFP) gene was also employed as model virus in the seeding study. A human embryonic kidney cell line (HEK-293) was used as rAd5 host for the propagation and the quantification of rAd5. Viral infectious units were quantified using a flow cytometer by detecting the GFP positive cells as described in detail by Li et al. (2010). The seeding level of rAd5 was 105 to 106 PFU/mL due to the relatively low concentration of rAd5 stock.

### 2.2. Model microorganisms

Microorganisms are naturally present in toilet wastewater. However, as shown in Table S1, the concentrations of culturable bacteria in the raw toilet wastewater collected from the pilot mobile toilet at Caltech were relatively low compared with those found in domestic wastewater. The low concentrations of microorganisms were likely due to the low usage of the toilet for defecation, the reuse of EC treated water for toilet flush (which contains chlorine residual) and the long water retention time (>20 days) in the storage/sedimentation tank (anaerobic digestion). The concentrations of indigenous microorganisms become even lower after 4 °C refrigerator storage (data not shown). To maintain consistent initial levels of target microorganisms and keep other physiochemical parameters (e.g., pH, chloride, and ammonia) comparable, we chose to seed pure-cultured model bacteria or viruses to aliquots of the single batch of 40 L wastewater sample before each trial. E. coli K12 (ATCC 10798) and Enterococcus faecalis (ATCC 29212) were employed as model bacteria in the disinfection experiments. Both bacteria were cultured in Luria–Bertani broth at 37 °C for 18 ± 2 h. The bacterial suspensions were centrifuged at 3000 × g for 15 min and re-suspended in 1 × PBS, which were used as bacterial stock. Before each experiment, a freshly prepared 0.1–1 mL bacterial stock suspension was seeded to water samples to give an approximate final concentration of 105–106 CFU/mL. E. coli and Enterococcus concentrations in the water samples were quantified using membrane filtration via US EPA method 1063 and method 1600, respectively. Part of the samples were also analyzed using a flow cytometer (FCM, Accuri Cytometers, Ann Arbor, MI, USA) to assess the bacterial nucleic acid injuries induced by EC disinfection. The indigenous bacterial cells in the toilet wastewater samples were negligible in FCM analysis, because the concentrations of seeded bacteria were more than 100 times higher than those remaining in the 4 °C stored toilet wastewater sample. The samples were fixed with 2% glutaraldehyde (final concentration) and stained with 0.5x SYBR-Gold (Invitrogen Corp., Carlsbad, CA). The FCM instrument setting and data acquisition procedure were described in detail in our previous study except the threshold was set at 10,000 in fluorescence channel 1 (FL1) (Huang et al., 2015). Green fluorescence and side scatter (SSC) light were collected in the FL1 channel (533 ± 30 nm) and the SSC channel on a logarithmic scale, respectively. Data analysis was carried out using the BD CFlow® software. FL1 vs. SSC density plots were used to differentiate different bacterial populations as well as the background noise.

Coliphage MS2 (ATCC 15597-B1) was propagated using E. coli – 3000 (ATCC 15597) as host. Briefly, 0.1 mL (10⁶ pfu/mL) MS2 was inoculated into 20 mL actively growing E. coli host suspension. The infected bacteria were continuously aerated at 37 °C for 36 h. The host-associated MS2 suspension was then centrifuged at 3000 × g for 20 min to remove the bacterial cells and debris. The supernatant containing the MS2 phages was further purified by 0.2 μm syringe filter (GE Whatman, Pittsburgh, PA). The filtrate was diluted 1,000x with 1 × PBS and stored in −80 °C freezer, which was used as MS2 stock. The seeding level of MS2 was 10⁵ to 10⁶ PFU/mL. The concentration of MS2 in water samples was titrated by the double agar plate method (Clokie, 2009).

Table 1

<table>
<thead>
<tr>
<th>Chemical parameters of toilet wastewater and model waters.</th>
<th>pH</th>
<th>Electrical conductivity (mS/cm)</th>
<th>Cl⁻ (mM)</th>
<th>NH₄⁺(mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Toilet water</td>
<td>6.7–8.3</td>
<td>3.2–3.4</td>
<td>12–20</td>
<td>4.6–4.7</td>
</tr>
<tr>
<td>MW1</td>
<td>7.4–7.5</td>
<td>3.2–3.4</td>
<td>n.d. a</td>
<td>n.d.</td>
</tr>
<tr>
<td>MW2</td>
<td>7.4–7.5</td>
<td>3.2–3.4</td>
<td>15</td>
<td>n.d.</td>
</tr>
<tr>
<td>MW3</td>
<td>7.4–7.5</td>
<td>3.2–3.4</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

a. n. d. – non-detectable.

### 2.3. Experimental procedures

EC disinfection experiments were carried out in a bench scale WEC with a working volume of 250 mL. The electrode module used...
for this study consists of a semiconductor anode (Nanopac, Korea, 13.5 × 6.7 cm) and a stainless steel cathode (Hastello C-22, 13.5 × 6.7 cm). The details about the preparation and characteristics of the BiOX/TiO2 anode can be found in our previous reports (Cho et al., 2014a, 2014b). The electrode pairs were installed in the reactor with a separation between anode and cathode of 0.5 cm. The setup was connected to a direct current power supply (HP-6236B Triple Output Power Supply, Palo Alto, CA) and operated in potentiostatic mode at 3 V, 4 V and 5.5 V providing current density values of 0.39, 1.2 and 2.4 mA/cm². All the experiments were carried out at room temperature (21 ± 0.5 °C). Before water electrolysis, target concentration of model microorganisms were seeded into 250 mL toilet wastewater or laboratory MWs and stabilized for 30 min. In a sub-set of the experimental trials using MWs, an excessive amount of tert-Butyl alcohol (t-BuOH) (30 mM), a well-known [•OH] scavenger (k•OH = t-BuOH = 5 × 10² M⁻¹s⁻¹), was added to raw water to assess the presence and role of [•OH] in EC disinfection (Enami et al., 2014; Jeong et al., 2006). During the EC reaction, the electrolyte was well mixed by a magnetic stir bar. Samples were withdrawn at time intervals to measure the concentrations of oxidants or to count the number of viable microorganisms. The change of the volume and wetted electrode surface area due to the sample withdraw from the reactor was negligible.

For microorganism enumeration, samples were quenched immediately with excess [Na₂S₂O₃] (10 mM) to eliminate the residual disinfectants (Jeong et al., 2009). The level of inactivation was expressed as a log reduction of the microbe survival ratio (N/N₀) measured by an EPA certified laboratory using US EPA method SM 6251 B by gas chromatography (Eurofins Eaton Analytical, Inc., Monrovia, CA). Brieﬂy, unseeded toilet wastewater was treated with WEC under applied cell voltage of 4 V and 5.5 V for 60 min. At the end of the reaction, the treated water was divided into two portions. The first portion was immediately transferred to sampling bottles with quencher [Na₂S₂O₃] (for THMs) or [NH₄Cl] (for HAA5) to stop the reaction (0 h samples). The other portion was incubated in amber glass bottles capped with Teflon-faced septa at room temperature (21 ± 0.5 °C) in the dark for 24 h before transferred to sampling bottles (24 h samples). For comparison, the formation of DBPs during CC disinfection were also tested with [NaClO] addition at the total chlorine concentration of 5 mg/L (as Cl₂), which was at equivalent to the chlorine concentration at the end of 60 min EC disinfection under 4 V. Similarly, 0 h and 24 h samples were collected and tested in the same way as in EC disinfection.

3. Results

3.1. Electrolysis production of oxidants in toilet wastewater and model waters

The influence of applied cell voltage on oxidants generation in toilet wastewater is shown in Fig. 2a. Very limited oxidants production was observed for model water during CC disinfection.
(<0.5 mg/L) were detected under the applied cell voltage of 3 V. At cell voltage of 4 V, the concentration of oxidants increased to 4 mg/L within 10 min and stabilized at 5–6 mg/L during rest of reaction time. When the applied cell voltage was raised to 5.5 V, the oxidants generation curve showed resemblance to the direct chlorination process (breakpoint chlorination). At both 4 V and 5.5 V, free chlorine (>1 mg/L) was detected by the DPD method after 5 min of reaction (data not shown). Due to the interference of chloramines, DPD method cannot precisely quantify the free chlorine level in the grab samples (the magenta color increased with time resulting in the overestimation of free chlorine) (Spon, 2008). However, the existence of free chlorine before breakpoint (with the present of free ammonia in the system) during the EC reaction was confirmed in our 20 L prototype WEC by an online free chlorine probe (Chemtrac, Norcross, GA). The kinetics of free chlorine evolution and pH changes in the prototype WEC are shown in Fig. S1.

The analysis of individual oxidants during electrolysis of toilet wastewater is extremely difficult due to the complexity of the water as well as the potentially fast reactions of oxidants with ammonia or other reducing substances (e.g., organic matter). The EC experiments using laboratory model waters provided additional evidence for explaining the role of different oxidants in disinfection (Fig. 2b). In the absence of [Cl−], less than 0.5 mg/L total oxidants were detected in MW1. In MW2 and MW3, total chlorine concentration steadily increased with the reaction time (Fig. 2b), although the chlorine evolution rate in MW3 was obviously slower than that in MW2. The addition of excess t-BuOH (30 mM) was intended to quench [·OH] in order to inhibit the chlorine production mediated by [·OH]. Unexpectedly, t-BuOH in fact promoted the chlorine evolution in both MW2 and MW3 (Fig. 2b). However, the similar stimulating effect was not observed in MW1, in which less than 0.5 mg/L total oxidants were detected.

3.2. Inactivation of microorganisms in toilet wastewater by EC and CC disinfection

The successful inactivation of all four seeded microorganisms was achieved within 60 min at the applied cell voltage of 4 V (Fig. 3a). E. coli were highly susceptible to EC disinfection. A 2-log10 reduction of E. coli was observed within the first 5 min and no viable E. coli was detected after 20 min of reaction. For Enterococcus, a clear lag phase (0–5 min) prior to the expected pseudo first-order kinetics was observed indicating their mild resistance to low level of EC produced oxidants. All Enterococcus were inactivated to below the detection limit after 30 min. The differences in resistance to EC disinfection between E. coli and Enterococcus were also reflected by the FCM results (Fig. S2). After 30 min of EC reaction, no significant change of the particle fluorescence intensity of Enterococcus cells was observed (R1, Fig. S2b), while about 40% of the E. coli cells shifted from high fluorescence region (R2, Fig. S2a) to lower fluorescence region (R3, Fig. S2a). For both types of bacteria, the fluorescence total cell counts decreased about 10–15% indicating they may have become ghost cells (lost DNA integrity) or been totally destroyed after EC disinfection. Due to the lower seeding level, only a 3-log10 reduction of rAd5 was shown in Fig. 3a, yet it demonstrated higher resistance than both types of bacteria. The inactivation of MS2 was the slowest taking about 60 min to reach a 5-log10 reduction.

In the comparative CC disinfection experiments, a 5-log10 reduction of Enterococcus was achieved within 10 min at the total chlorine dosing level of 1.5 mg/L (Fig. 3b). On the contrary, the inactivation rate of MS2 was significantly lower (<0.5-log10) even at the highest [HClO] dosage (36 mg/L, as Cl2). Considering the [NH4+] concentration in the toilet wastewater (Table 1), monochloramine was the main disinfectant under all [NaClO] dosing levels, although free chlorine may exist momentarily in the system at the beginning of [NaClO] dosing.

3.3. Inactivation of MS2 by EC and CC in model waters

The inactivation kinetics of MS2 by EC disinfection in MWs are shown in Fig. 4a. No MS2 reduction was observed in MW1 electrolysis, while a 5-log10 reduction of MS2 were achieved within 10 min and 20 min in MW2 and MW3, respectively. The addition of t-BuOH (30 mM) slightly enhanced the inactivation rate in MW2 and MW3, but it did not change the inactivation rate of MS2 in MW1. Fig. 4b shows the inactivation of MS2 in MWs by CC disinfection. Similar to the toilet wastewater (Fig. 3b), in MW3, less than 0.5-log10 reduction of MS2 was observed at the chlorine concentration as high as 40 mg/L (combined chlorine, as Cl2). In contrast, in the absence of [NH4+], a 5-log10 reduction of MS2 was achieved within 5 min in MW2 at the chlorine concentration of 1.5 mg/L (free chlorine, as Cl2) (Fig. 4b).
3.4. The formation of trihalomethanes (THMs) and haloacetic acids (HAA5)

Fig. 5 shows the formation of DBPs in toilet wastewater after EC and CC disinfection. It is found that under both treatment processes, the composition of THMs and HAA5 appears to be quite similar. For example, trichloromethane [CHCl3] accounted for more than 90% of THMs, while trichloroacetic acid [C 2HCl3O2] and dichloroacetic acid [C2H2Cl2O2] were the most abundant compounds of HAA5. Only a small amount of brominated DPBs were detected in the treated water samples (less than 5%). However, the results also demonstrated that the formation of DPBs during EC disinfection was significantly higher than in CC disinfection, although the measured total chlorine concentrations in EC disinfection (4 V, 60 min) was equivalent to that in CC disinfection (5 mg/L, as Cl2). For EC disinfection, when the applied cell voltage was raised from 4 V to 5.5 V, the concentrations of THMs and HAA5 were increased (Fig. 5). The impact of incubation time on DBPs formation was limited. Most of the DPBs had been generated during the reaction (0 h samples), except for the THMs formed during CC disinfection, which increased about 45% after a 24 h-incubation.

4. Discussion

4.1. Oxidant generation and energy requirements

For effective RCS production, the actual anodic potential needs to be higher than the chlorine evolution overpotential \(E^0_{\text{Cl}_2}/[\text{Cl}^-] = 1.36 \text{ V}\). Based on the current data, the applied cell voltage of 4 V was necessary to overcome the ohmic resistance to have chlorine generation in the toilet wastewater (Fig. 2a). The \([\text{Cl}_2]\) produced by EC reaction quickly reaches equilibrium with [HOCl] and [ClO\(^-\)] in the bulk aqueous phase. At the same time, further oxidation of free chlorine ([HOCl] or [ClO\(^-\)]) to [ClO\(_2\)] and [ClO\(_3\)] can happen considering the relatively lower reducing potential for the redox couples \(E^0_{\text{ClO}_2}/[\text{ClO}^-] = 0.94 \text{ V}, E^0_{\text{ClO}_3}/[\text{ClO}_2] = 1.23 \text{ V}\). Cho et al. (2014b) reported that, [ClO\(_2\)] was the main inorganic chlorinated byproduct in the electrolysis of municipal wastewater. Trace-levels of [ClO\(_2\)] may also be produced during the reaction, which is depending on the pH. Acidic solutions were reported to favor the formation of [ClO\(_2\)], while alkaline conditions support the production of [ClO\(_3\)] (Gheraout et al., 2011). Although higher applied cell voltage can increase the [Cl\(_2\)] generation rate and maintain the total oxidants at a higher level in the bulk solution (Fig. 2a), it may also increase the formation of [ClO\(_2\)] and other highly oxidized oxidants. These oxidants are toxic to plants even at low concentration, which may limit the potential uses of the treated water. Therefore, higher applied voltage or extended reaction time should be avoided once the disinfection goal is achieved. The oxidants generated during the electrolysis of MW samples are consistent with the characteristics of active electrodes. [OH\(^-\)] and other ROS were not efficiently produced by the BiOx/TiO\(_2\) anode as indicated by the low level of oxidants detected during the electrolysis of inert sample, MW1 (Fig. 2b). As a consequence, no inactivation of MS2 was observed in MW1 (Fig. 4a). The result indicates [Cl\(^-\)] is an indispensable component for the current EC disinfection system. The increase of chlorine evolution rate in MW2 and MW3 with the addition of the [OH\(^-\)] scavenger t-BuOH conflicts with a previous report, which showed no significant changes in chlorine evolution rates were observed with active anode (Jeong et al., 2009). The possible reason could be the reaction of t-BuOH with the surface bounded [OH\(^-\)], which in return enhanced the oxidation of [Cl\(^-\)] at the interface due to electron transfer.

Based on the current setting for microbial inactivation, the energy requirement of the WEC can be calculated using equation \(e = E_{\text{cell}} \int i(t) dt\), where \(E_{\text{cell}}\) is the applied cell voltage, \(i\) is the current and \(V\) is the volume of reactor. Under the optimal condition \((E_{\text{cell}} = 4 \text{ V}, i = 0.125 \text{ A})\), to achieve a 5-log\(_{10}\) reduction of the MS2 (conservative model microorganism), the reaction time was about 1 h and thus the energy consumption was estimated to be 2 Wh/L. Considering the ohmic losses and the power required for the circulation pumps and electronic controller, the actual energy consumption of the Caltech 20 L prototype WEC was estimated to be 13–15 Wh/L (260–300 Wh/reaction for 20 L). The energy requirement indicates that the WEC can use inexpensive commercial PV-panels as the sole power source (e.g., Sonali 300W, Miami Gardens, FL). As there is no need for external power supply and supporting chemicals, the system is suitable for decentralized toilet wastewater disinfection.

4.2. Disinfection mechanisms

The inactivation of bacteria during disinfection process can be generally explained by two types of damages to bacterial cells. First,
disinfectants can react with cell surface components causing cell membrane permeability changes or the malfunction of enzymatic transport systems. Second, impairments to the intracellular components, especially the loss of DNA integrity, could be introduced with or without obvious cell surface damages (Cho et al., 2010). Certain disinfectants cause more significant damages to either the cell surface or internal components, but these two types of damages are not exclusive, which would also strongly depend on the Ct-value (disinfectant concentration × reaction time) as well as the type of bacterial cells. During EC disinfection, *E. coli* and *Enterococcus* behaved quite differently, especially in the beginning of the reaction when the concentration of oxidants was below 2 mg/L (0–5 min, 4 V). Similar results were found in the study on traditional chlorination disinfection process (Tree et al., 2003). The different inactivation kinetics between the two indicator bacteria are likely related to their cell surface structure differences (Gram-negative vs. Gram-positive bacteria), since at low chlorine concentration (<0.5 mg/L as Cl₂), damages of chlorine were reported mainly to the cell surfaces (Phe et al., 2005). When the chlorine concentration exceed the threshold (free chlorine, between 1.5 and 3 mg/L as Cl₂), severe damages to bacterial genomes could happen (Phe et al., 2005; Ramseier et al., 2011). This effect was indicated by the bacterial clusters shift (from the high fluorescence region to the low fluorescence region) and particle count loss in the FCM analysis, because damaged bacterial genomic DNA cannot be effectively stained by fluorescent dyes. In the current study, although both *E. coli* and *Enterococcus* became non-culturable after 30 min of EC reaction, significant fluorescent intensity decrease was only observed for *E. coli* cells (Fig. S2, R2 to R3). No such changes were observed for *Enterococcus* cells (Fig. S2, R1). This result indicates that most of the *Enterococcus* cells still maintained their genomic DNA integrity. Therefore, a longer treatment time would be necessary to ensure all the non-culturable bacteria were actually dead as injured bacterial cells have been known to be able to repair certain damages when the environmental forcing factors are removed (Li et al., 2013). Also, the maintenance of a chlorine residual (e.g., 2 mg/L) in the clean water storage tank (Fig.1) is suggested, which will restrict the regrowth of bacteria after EC treatment.

In addition to bacteria, pathogenic viruses raise even greater concerns in toilet wastewater reuse. Current results confirmed that neither *E. coli* nor *Enterococcus* is an adequate indicator for virus inactivation during toilet wastewater disinfection. The fate of human adenovirus (a double stranded DNA virus) was for the first time studied in an EC disinfection system. The rAd5 demonstrated higher resistance compared to both bacterial indicators (Fig. 3a). Virus inactivation is more complicated than bacteria by the fact that highly related viruses can exhibit different disinfection kinetics when treated by the same disinfection procedure. For example, human adenovirus serotype 40 (HADV40) and HADV41 were reported to be more susceptible to monochloramine disinfection than HADV2 (Cromeans et al., 2010). These variable responses suggest that even minor variations in structural or genomic components can have a remarkable impact on virus resistance to inactivation. Studies have also shown that seeded laboratory-cultured viruses are less resistant to disinfection processes compared to their indigenous counterpart, because indigenous strains are often embedded in biofilm or attached to suspended solids, which may shield the virus against disinfectants (Tree et al., 2003). All these factors highlighted the importance of choosing a conservative virus surrogate in evaluating the disinfection capability of EC systems. In this context, MS2 should be an ideal candidate as it demonstrated higher resistance than rAd5 in the current study and had been reported to be more resistant than poliovirus (Tree et al., 2003), coxsackieviruses (Tree et al., 2003), and hepatitis A virus (Casteel et al., 2008) to a variety of disinfectants. When a 5-log₁₀ reduction of MS2 is achieved after EC disinfection, other bacteria and viruses in the toilet wastewater should also be effectively inactivated and the health risks associated with non-potable water reuse would be significantly reduced.

The comparative experiments between EC and CC disinfection clearly demonstrated the advantage of EC disinfection for virus disinfection in toilet wastewater. In [NH₄] free MW1, a 5-log₁₀ reduction of MS2 was achieved at free chlorine level of 1.5 mg/L within 5 min. The result showed that MS2 was in fact very sensitive to free chlorine. Wigginton et al. (2012) studied the damages of free chlorine to MS2 and found it is a non-discriminative oxidant causing the losses of functions such as genome-mediated replication and protein-mediated injection. The current results indicate that the chlorine species (free or combined chlorine) were the decisive factor controlling the virus disinfection efficacy. Compared to free chlorine, monochloramine is a much weaker...
disinfectant, which is only effective for bacterial inactivation, but not effective for viruses (Fig. 3b). When free [Cl2] to [NH4+] ratio (weight ratio Cl2: NH3–N) was less than 5, the conversion of free chlorine to monochloramine can be completed within seconds under optimal conditions (pH = 8.4; 25 °C) (Kirmeyer et al., 2004). This explained the limited virus reduction observed during CC disinfection in the toilet wastewater and MW3. For CC disinfection, free chlorine residual can be created by adding extra amount of [NaClO] to bring the system past the breakpoint (weight ratio Cl2: NH3–N = 9:1). However, this may not be practical for raw toilet wastewater disinfection, as the total chlorine residual would be too high for any reuse applications (>500 mg/L as Cl2). In contrast, free chlorine was always present during toilet wastewater electrolysis even with the presence of free ammonia, because it was continuously produced on the surface of anode. Free chlorine may react with viruses before it is converted to chloramines. In addition, a local pH decrease at the anode surface occurs due to the production of [H+] through [O2] evolution. Lower pH can significantly reduce the chlorine-ammonia reaction rate. Acidic conditions also favor the formation of neutral [HClO], a more effective disinfectant than the negatively charged [ClO−]. Lastly, ammonia was converted to [NO3−] and [N2] during EC reaction, and thus the [Cl2] to [NH4+] ratio was increasing with reaction time. Besides free chlorine, [ClO2] may also contribute to the MS2 inactivation in EC disinfection. [ClO2] have been shown to cause the degradation of viral proteins and thus inhibit the host-cell recognition and binding (Wigginton et al., 2012). However, considering the low concentration of [ClO2] during the reaction, its virucidal effect may be limited.

5. Conclusions

- EC disinfection using WEC can effectively inactivate both viruses and bacteria in toilet wastewater without adding any supporting electrolytes. The system can be developed into a commercial viable self-sufficient solar-powered mobile toilet for decentralized wastewater treatment.
- Viruses were more resistant than bacteria in both EC and CC disinfection. A 5-log10 reduction of MS2 (the conservative model microorganism) in toilet wastewater can be achieved by EC disinfection at applied cell voltage of 4 V in 1 h, while CC disinfection is not effective for virus inactivation in toilet wastewater.
- RCS are the main disinfectants produced by the active bismuth-doped TiO2 anode. Ammonia can significantly reduce the disinfection efficiency by converting free chlorine to chloramines. The high inactivation rate of viruses with EC disinfection can be explained by the coexistence of free chlorine and free ammonia during EC reaction.
- Higher applied cell voltage and longer reaction time will generate more organic DBPs (THMs and HAA5). Most of the DBPs are formed during the EC reaction rather than the after treatment incubation period.
- The WEC treated toilet wastewater is safe for non-potable reuse, such as toilet flushing and agricultural irrigation.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.watres.2016.01.040.

References

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