Coagulation–floculation pre-treatment of surface water used on dairy farms and evaluation of bacterial viability and gene transfer in treatment sludge
Éric Pariseau, Daniel I. Massé, Lucie Masse, Ed Topp, Vincent Burrus and François Malouin

ABSTRACT
On many dairy farms, the water used to wash milking equipment is contaminated with bacteria and has to be disinfected. Often, the water requires a coagulation–floculation (CF) pre-treatment to reduce turbidity and remove dissolved organics prior to disinfection. This paper examines the effect of temperature and water characteristics on the efficiency of an on-farm CF treatment using polyaluminum chloride (PACl) as coagulant. Since the CF process concentrates suspended solids and bacteria in a sludge that will be land-applied, *Escherichia coli* survival and gene transfer occurrences in the sludge were also determined. Coagulant dose was highly correlated to water UVA$_{254}$ nm, but not turbidity. For water with variable UVA$_{254}$ nm exceeding 0.85 cm$^{-1}$, the coagulant dose could be adjusted using a simple online UVA$_{254}$ nm sensor, while settling time should be increased when water temperature drops below 10°C. *E. coli* survived a 2-h PACl exposure at a dose of 0.05 mL ClearPAC/L. There was no difference in conjugative transfer of a multi-drug resistance conferring plasmid in water without PACl and in the PACl-derived sludge over a 2-day period. However, since bacteria remained viable in sludge and genetic conjugation may occur, sludge residues should be stored in the manure tank prior to land application.

Key words | bacteria survival, coagulation–floculation, dairy farm, gene transfer

INTRODUCTION
In some regions where groundwater is not appropriate for livestock, dairy farmers must rely on surface water sources to provide drinking water to the animals and to wash milking equipment. Surface water sources are easily contaminated by undesirable microorganisms (coliforms, viruses, protozoa) because of surface runoff, defective septic installations, improper manure handling and storage, manure spreading and wildlife intrusion. In 2007–2008, six surface sources supplying water to dairy farms in Eastern Ontario were sampled 23 times over a 16-month period (Masse et al. 2010). Total coliforms, fecal coliforms and *Escherichia coli* were detected in 91, 89 and 75% of the samples, respectively, at median concentrations of 66, 40 and 12 CFU/100 mL, respectively. Maximum concentrations were one to two orders of magnitude higher than median values.

The presence of microorganisms in the water used to wash milking equipment can contaminate raw milk (Oliver et al. 2005; Perkins et al. 2009). The ability of raw milk to retain its quality during storage has been related to its bacterial content, and in many countries, bacterial content is one of the factors considered in milk payment (Saran 1995). To maintain milk quality and ensure consumer safety, the Canadian Quality Milk (CQM) Program requires that the water used for milking equipment sanitation meet the provincial potability standards for

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bacteria. To comply with the CQM Program, some dairy farms will need to be equipped with on-site disinfection treatment systems.

The efficiency of most disinfection technologies is affected by the high suspended solids (SS) and dissolved organic matter (DOM) contents found in surface water (Masse et al. 2010). Particulates can shield bacteria from UV radiation and contribute to membrane fouling. High turbidity, a surrogate measure for SS, is also problematic for oxidizing technologies, such as ozonation, because it increases required doses and thus cost, and favors the formation of carcinogenic trihalomethanes (THM) if chlorination is used. High UV absorbance at 254 nm (UVA$_{254}$ nm), a parameter used as an approximation of DOM in water (Annadurai et al. 2004), reduces UV lamp efficiency and increases membrane fouling.

Coagulation–floculation (CF) is typically used in municipal water treatment plants to reduce SS and DOM prior to disinfection (Annadurai et al. 2003, 2004; Zouboulis & Traskas 2005). The coagulant polyaluminum chloride (PACl) was found to consume less alkalinity, accelerate floc formation, increase floc strength, generate lower sludge volume and produce less residual aluminum than other coagulants such as alum or ferric chloride (Duan & Gregory 2003). It is also less affected by changes in temperatures (Zouboulis & Traskas 2005).

In recent years, small flocculators have been designed for commercial operations processing relatively small volumes of water compared with municipalities. However, surface water presents large variations in parameters such as turbidity, UVA$_{254}$ nm and temperature, which could affect required coagulant doses. Models have been developed to calculate optimum coagulant doses based on pH, turbidity, temperature, conductivity and UVA$_{254}$ nm (Bazer-Bache et al. 1990; Gagnon et al. 1997). Wu & Lo (2008) evaluated various models to predict optimum PACl dose for a municipal drinking water treatment plant. Input parameters included doses used in previous days, temperature, turbidity, color and pH. The most accurate model was based on the dose used the day before and turbidity of raw water. For on-farm systems, the dose should be adjusted using a minimum number of parameters that can be measured with low-cost, online monitoring devices.

During the CF sludge settling process, bacteria and SS are concentrated in a small volume. At the farm, the sludge would likely be stored in the manure storage structure and land-applied. Pathogenic and drug-resistant bacteria contained in agricultural effluents were found to survive in manure sludges and reach surface water sources (Finch & Smith 1986; Fremaux et al. 2008). The sludge may also represent an ideal environment for bacterial gene transfer through conjugation. Conjugation, an important gene transfer mechanism for bacteria, requires cell-to-cell contact for successful transfer. It is responsible for the rapid spread of drug resistance among bacteria (Burrus & Waldor 2004; Chen et al. 2005).

This project examined the effect of temperature and physicochemical characteristics of four surface water sources on PACl dose and CF pre-treatment efficiency. One objective was to determine whether a simple monitoring device could be used to establish optimum dose for an on-farm CF system. A second objective was to study the survival of E. coli in a PACl-derived sludge and determine whether gene transfer can occur in such a water-treatment residue that could be land-applied for disposal.

### MATERIALS AND METHODS

#### Experimental water

In January and July 2008, about 100 L of water were collected from four surface sources supplying water to dairy farms in Eastern Ontario: a pond dug in a peat bog (Pond), a river flowing through agricultural areas (River), and two creeks also flowing through agricultural areas (Creek 1 and Creek 2). Untreated water samples were analyzed for dry matter (DM), SS, turbidity, conductivity, hardness, UVA$_{254}$ nm, pH and NO$_3$-N, according to Standard Methods (2005). Aluminum and iron concentrations were determined by the Institut de Recherche et de Développement en Agroenvironnement (IRDA, Québec, QC, Canada) with an inductively coupled plasma (ICP) argon spectrophotometer (Optima 4300, Perkin Elmer). The water was kept at 4 °C and was transferred to a temperature-controlled room 12 h before the CF experiments.
Jar-test procedures

The coagulant, PACl, was obtained from Clear Tech Industries (Saskatoon, SK). The commercial liquid product (ClearPAC) contained on average 56 g Al/L and had a basicity of 50–58%. It was diluted 10-fold in deionized water before use. Doses are reported as milliliters of concentrated ClearPAC product by liters of untreated water.

Square beakers were filled with 2 L of water and placed on a standard jar-test apparatus (Phipps & Bird, Richmond, VA) in a temperature-controlled room. CF experiments were conducted at 8, 12, 15 and 28 °C, in order to cover most of the surface water temperature range in milk houses in Canada. The jars were mixed at 160 rpm (G of 170 s⁻¹ at 20 °C) during PACl injection. Rapid mixing was maintained for 105 s, and then reduced to 45 rpm (G of 36 s⁻¹ at 20 °C) for 10 min. During floc settling, samples were collected from a valve situated at the bottom one third (4.6 cm) of total water height (14.2 cm), each 30 min up to 120 min. Between 6 and 11 PACl doses, ranging from 0.0125 to 0.45 mL/L, were tested with each water. Most doses were tested once at each temperature, but experimental variability due to jar test procedures was evaluated by testing 24 doses in triplicate. An average coefficient of variation (CV) of 7% ± 2% was obtained for water turbidity after 120 min of settling.

Optimum dose was defined as the dose required to reach a turbidity of 1.0 ± 0.1 NTU after 120 min of settling. The coefficient of determination (R²) and significance (p < 0.05) of the correlation between water parameters and optimum dose were evaluated using the linear regression subroutine in SPSS (SPSS 9.0 for Windows).

Microbiological analyses

Microbial analyses of the untreated water samples are fully described in Masse et al. (2010). Briefly, 100-mL samples were filtered using a sterile GN-6 membrane (Pall-Gelman, VWR, Mississauga, ON) and deposited on the desired medium. Total coliforms were counted on agar mEndo-LES medium (Difco, Mississauga, ON) and incubated at 37 °C for 18–20 h. Fecal coliforms were counted on mFC agar (Difco) and incubated at 44.5 °C for 18–20 h. Escherichia coli was counted on basal medium mFC agar (Difco) with 100 mg/L of 3-bromo-4-chloro-5-indolyl-b-D-glucuronide (Med-OX Diagnoses, Ottawa, ON) and incubated between 18 and 24 h at 44.5 °C. Enterococci spp. were counted on mEnterococcus agar (Difco) followed by 48 h of incubation at 37 °C. Yersinia enterocolitica was counted on CIN agar (Difco) and incubated for 18 h at 30 °C. Reported values are average of duplicate analyses.

Bacterial strains

The plasmid donor MG1655RifR and recipient MG1655NalR E. coli strains resistant to rifampicin (Rif) and nalidixic acid (Nal), respectively, were used in this study (Ceccarelli et al. 2008). The strain MG1655RifR bears a self-transmissible conjugative plasmid, pVCR94, which was originally isolated from a multi-drug resistant clinical isolate of Vibrio cholerae O1 El Tor. Plasmid pVCR94 was identified as belonging to the A/C incompatibility group based on amplification and sequencing of the repA gene (Burrus, unpublished results), which is nearly identical to that of pIP1202, a conjugative plasmid from a Madagascar multiple-drug resistant (MDR) isolate of Yersinia pestis (Welch et al. 2007; Pan et al. 2008). Plasmid pVCR94 mediates resistance to at least six antibiotics, including sulfamethoxazole, trimethoprim, chloramphenicol, streptomycin, spectinomycin and tetracycline (Tet). The Rif and Nal resistance determinants were chromosomal and non-transferable. Antibiotics were routinely used at the following concentrations: tetracycline, 12 μg/mL; nalidixic acid, 40 μg/mL; and rifampicin, 50 μg/mL.

Bactericidal effect of PACl

The bactericidal effect of PACl was tested on the two studied E. coli strains (MG1655RifR and MG1655NalR) and Creek 2 was used as the water source. Ten milliliters of water from Creek 2 was transferred to 15-mL test tubes. The tubes were injected with four PACl doses (0, 0.005, 0.05, and 0.5 mL of ClearPAC/L) and various bacterial concentrations ranging from 10³ to 10⁷ CFU/mL. Bacterial suspensions were regularly mixed by vortex to prevent settling and maximize exposure of bacteria to PACl. After 2 and 8 h of incubation at room temperature (22–25 °C), 10-fold dilutions were plated on MacConkey agar medium (Difco) with the appropriate antibiotic supplements. Plates
were incubated at 35 °C and the detection limit was 100 CFU/mL.

**Survival of bacteria in CF sludge**

Beakers were filled with 1 L of water from Creek 2 and strain MG1655RifR was added at 10⁴ CFU/mL. This concentration is at the upper limit of values reported for *E. coli* in dairy farm waters (Ibekwe et al. 2002). The water was stirred and injected with 0.05 mL of ClearPAC/L. The CF treatment was conducted at room temperature. After mixing, the flocculated water was poured in 175-mL conical tubes and allowed to settle. Supernatant and sludge were sampled at 1, 2, 4 and 8 h and plated on MacConkey agar medium supplemented with Rif and Tet. The detection limit was 10 CFU/mL.

**Plasmid transfer**

Conjugation experiments using *E. coli* MG1655RifR bearing pVCR94 as the donor strain and *E. coli* MG1655NalR as the recipient strain were assayed at two bacterial concentrations, 10⁴ and 10⁸ CFU/mL. Both strains were added to Creek 2 water, which was treated by CF as described above. The experiment was conducted at room temperature and 35 °C. The flocculated water was settled for 2, 8, 24, 72 and 120 h in conical tubes. In a control experiment, water was inoculated with bacteria but not treated with PACL. Exconjugants were determined by plating water and sludge on MacConkey agar medium supplemented with Nal and Tet. The frequency of spontaneous mutations for Nal resistance in the donor strain (false exconjugants) was also determined by plating water and sludge on MacConkey supplemented with Nal, Rif and Tet, and this bacterial count was subtracted from the number of NalR TetR colonies to give the number of true exconjugants. The detection limit was 10 CFU/mL. When the number of putative exconjugants was small, the presence of the plasmid in NalR TetR colonies was verified by colony PCR (polymerase chain reaction) amplifying the variable region of the pVCR94-borne class 1 integron using the primer pair In-F 5'-GGCATCCAAGCGAAGC-3' and In-B 5'-AAGCAGCAGTGTGACCIGAT-3', respectively (Ceccarelli et al. 2006). The frequency of exconjugant formation was expressed as the number of exconjugant CFU per recipient CFU in the mating mixture at the time of plating.

**RESULTS**

**Experimental water**

The physicochemical and microbiological characteristics of the surface water samples collected for the CF experiments are presented in Table 1. All samples had relatively high bacterial counts and required disinfection treatment to comply with the CQM Program. Turbidity was generally above the recommended range of 1–10 NTU for available on-site disinfection technologies, such as UV irradiation, ultrafiltration and chlorination (WHO 1996; USEPA 2005, 2006). Turbidity was consistently higher in the samples collected in January than in July. In the 5 days preceding the January sampling, 32 mm of rain was recorded at a nearby weather station, and the snow cover had decreased from 61 to 11 cm (Environment Canada 2010). Surface runoff probably increased turbidity of the water sources.

The water samples also had UVA₂₅₄ nm values above maxima recommended for efficient disinfection by UV irradiation (0.125–0.300 cm⁻¹; USEPA 2006), indicating high DOM content in all water sources. The difference between the January and July samples was not consistent across all water sources. For River and Creek 2, UVA₂₅₄ nm was higher in the samples collected in January, while it was considerably higher in the July samples from the Pond, and similar in both samples from Creek 1. Results thus indicated that all water sources required a pretreatment prior to disinfection treatment, but also that water characteristics that could potentially determine optimum coagulant doses, such as turbidity or UVA, are not necessarily correlated.

**Optimum coagulant dose at 15 °C**

The optimum PACL dose was defined as the dose required to reduce the water turbidity to 1.0 ± 0.1 NTU after 2 h of settling. This set point was selected because disinfection systems are generally optimized at turbidity levels ≤1 NTU for incoming water. The CF treatment also decreased the
UVA254 nm of the water to levels suitable for UV disinfection. At optimum dose, the UVA254 nm of all but one water sample ranged from 0.11 to 0.19 cm$^{-1}$, while the water from the Pond in July was decreased from 2.23 to 0.37 cm$^{-1}$. CF treatment removes DOM by charge neutralization, which reduces the solubility of the organic matter and favors adsorption onto the metal hydroxide flocs (Duan & Gregory 2003). Treating the water by CF also reduced iron concentrations below detection limit, but had no effect on hardness, conductivity, aluminum concentration, alkalinity, pH and nitrate concentration.

Figure 1 presents the optimum dose at 15 °C for all samples collected in July and January. For two of the water sources (River and Creek 1), similar doses were required for both samples. Creek 2, on the other hand, required a considerably higher dose in January than July, while the Pond showed the opposite trend with a much higher dose in July than January. Results thus indicated that there is no consistent trend in optimum dose within each sampling site, and in subsequent analyses all eight samples were considered as independent samples.

**Table 1** | Physicochemical and microbiological characteristics of the water samples collected in the winter and summer months of 2008, for the coagulation-flocculation experiments

<table>
<thead>
<tr>
<th>Physicochemical parameters</th>
<th>Pond (January)</th>
<th>Pond (July)</th>
<th>River (January)</th>
<th>River (July)</th>
<th>Creek 1 (January)</th>
<th>Creek 1 (July)</th>
<th>Creek 2 (January)</th>
<th>Creek 2 (July)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DM (mg/L)</td>
<td>346</td>
<td>311</td>
<td>401</td>
<td>342</td>
<td>329</td>
<td>220</td>
<td>403</td>
<td>792</td>
</tr>
<tr>
<td>SS (mg/L)</td>
<td>68.6</td>
<td>12.9</td>
<td>111.0</td>
<td>&lt;4.1</td>
<td>44.3</td>
<td>BDL</td>
<td>52.9</td>
<td>11.4</td>
</tr>
<tr>
<td>Turbidity (NTU)</td>
<td>73.2</td>
<td>26.5</td>
<td>143.0</td>
<td>5.4</td>
<td>61.4</td>
<td>15.7</td>
<td>73.5</td>
<td>11.2</td>
</tr>
<tr>
<td>UVA254 nm (cm$^{-1}$)</td>
<td>0.83</td>
<td>2.23</td>
<td>0.54</td>
<td>0.32</td>
<td>0.57</td>
<td>0.59</td>
<td>1.58</td>
<td>0.48</td>
</tr>
<tr>
<td>Alkalinity (mg CaCO$_3$/L)</td>
<td>190</td>
<td>112</td>
<td>117</td>
<td>221</td>
<td>126</td>
<td>124</td>
<td>93.2</td>
<td>301</td>
</tr>
<tr>
<td>Hardness (mg CaCO$_3$/L)</td>
<td>184</td>
<td>120</td>
<td>148</td>
<td>240</td>
<td>132</td>
<td>120</td>
<td>108</td>
<td>344</td>
</tr>
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<td>pH</td>
<td>7.80</td>
<td>7.80</td>
<td>7.70</td>
<td>8.30</td>
<td>7.90</td>
<td>8.10</td>
<td>7.60</td>
<td>8.10</td>
</tr>
<tr>
<td>Conductivity (μS)</td>
<td>469</td>
<td>287</td>
<td>415</td>
<td>600</td>
<td>438</td>
<td>386</td>
<td>379</td>
<td>1351</td>
</tr>
<tr>
<td>NO$_3$-N (mg/L)</td>
<td>0.58</td>
<td>1.49</td>
<td>5.27</td>
<td>6.94</td>
<td>2.25</td>
<td>1.56</td>
<td>2.68</td>
<td>1.95</td>
</tr>
<tr>
<td>Iron (mg/L)</td>
<td>0.92</td>
<td>1.94</td>
<td>0.15</td>
<td>0.02</td>
<td>0.48</td>
<td>0.40</td>
<td>1.01</td>
<td>0.03</td>
</tr>
<tr>
<td>Aluminum (mg/L)</td>
<td>1.11</td>
<td>1.32</td>
<td>0.21</td>
<td>0.03</td>
<td>0.67</td>
<td>0.25</td>
<td>1.32</td>
<td>0.01</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Microorganisms (CFU/100 mL)$^a$</th>
<th>Winter</th>
<th>Summer</th>
<th>Winter</th>
<th>Summer</th>
<th>Winter</th>
<th>Summer</th>
<th>Winter</th>
<th>Summer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total coliforms</td>
<td>88</td>
<td>113</td>
<td>76</td>
<td>52</td>
<td>32</td>
<td>225</td>
<td>NA$^b$</td>
<td>120</td>
</tr>
<tr>
<td>Fecal coliforms</td>
<td>45</td>
<td>164</td>
<td>36</td>
<td>52</td>
<td>19</td>
<td>119</td>
<td>NA$^b$</td>
<td>55</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>9</td>
<td>142</td>
<td>24</td>
<td>12</td>
<td>10</td>
<td>111</td>
<td>NA$^b$</td>
<td>&lt;4</td>
</tr>
<tr>
<td>Enterococci</td>
<td>31</td>
<td>420</td>
<td>48</td>
<td>33</td>
<td>9</td>
<td>329</td>
<td>NA$^b$</td>
<td>15</td>
</tr>
<tr>
<td><em>Yersinia</em> spp.</td>
<td>44</td>
<td>28</td>
<td>32</td>
<td>&lt;4</td>
<td>15</td>
<td>7</td>
<td>NA$^b$</td>
<td>&lt;4</td>
</tr>
</tbody>
</table>

DM = dry matter; SS = suspended solids; UVA = ultraviolet absorbance.

$^a$Mean value from three samples collected in December 2007, January and March 2008 (winter), and in May, June and August 2008 (summer) for the Pond, the River and Creek 1, and one sample collected in July 2008 for Creek 2.

$^b$NA, not analyzed.

**Figure 2** | PACI dose (as milliliters of ClearPAC per liter of water) required to reduce turbidity to 1.0 NTU after 2 h of settling at 15 °C.

**Temperature effect on optimum coagulant dose**

Figure 2 presents the effect of temperature on optimum PACI dose. Decreasing process temperature from 15 to
8 °C increased the required dose for six of the eight water samples, while increasing temperature to 28 °C slightly decreased the required dose of four samples. However, the variations in PACI requirements were generally low, except for the two samples with high UVA254 nm (Pond in July and Creek 2 in January). Moreover, variations in PACI requirements appeared to be mostly due to differences in settling rate with temperature. Figure 3 presents decreases in water turbidity with settling time during CF treatment of one water sample (Creek 2 in January) at three temperatures. A dose of 0.15 mL ClearPAC/L, corresponding to the optimum dose at 15 °C, was used at all temperatures. Figure 3 shows that floc settling rate decreased as water temperature was reduced, probably because of increased viscosity. Water viscosity increases from 0.81 cp at 28 °C to 1.34 cp at 8 °C. The target value of 1 NTU was reached after 90 and 120 min of settling at 28 and 15 °C, respectively, and data suggest that it would have been reached at 8 °C, if additional settling time had been allowed. This tendency was observed with all samples. PACI is known to be less affected by variations in temperature than non-prehydrolysed coagulants, such as alum (Van Benschoten & Edzwald 1990).

Optimum dose and physicochemical characteristics

Figure 4 presents optimum coagulant dose at 15 °C with respect to turbidity and UVA254 nm, two parameters that can be easily monitored online, and have been shown to correlate with required dose (Bazer-Bache et al. 1990; Gagnon et al. 1997; Wu & Lo 2008). Optimum dose was significantly correlated to UVA254 nm ($R^2 = 0.96; p < 0.05$), but not turbidity. The linear correlation between optimum dose and UVA254 nm was also significant ($p < 0.05$) at the three other tested temperatures. Other studies have shown that DOM controls the coagulation process, when water contains both mineral particles and organic matter, because of the high surface area and charge of organics compared with minerals (Rebhun & Lurie 1993; Duan & Gregory 2005). Optimum dose was also correlated to iron concentration ($R^2 = 0.88; p < 0.05$), but radiation at 254 nm is strongly absorbed by iron ions in water (Savoye et al. 2001). In this study, iron was highly correlated to UVA254 nm ($R^2 = 0.90; p < 0.05$).

However, results also showed that the relation between UVA254 nm and dose was weak at low UVA254 nm values. At UVA254 nm below 0.85 cm$^{-1}$, although optimum PACI dose ranged from 0.0125 to 0.0750 mL ClearPAC/L, a dose of 0.05 mL/L provided a turbidity $\leq 1.9$ NTU after 2 h of settling with all water samples and at all temperatures. At water UVA254 nm above 1 cm$^{-1}$, on the other hand, a dose of 0.05 mL/L had no effect on water quality, and dose had to be increased three to five times to reach the target of 1 NTU after 2 h of settling.

The results of the study thus indicated that for water sources with low variations in UVA254 nm or with UVA254 nm remaining below 0.85 cm$^{-1}$, a single dose would
be adequate throughout the year if increased settling time is provided in winter. However, for water with high variations in organic matter content, a relatively simple online UVA$_{254}$ nm meter could be used to automatically adjust dose, while settling time could be increased when water temperature is low. The UVA$_{254}$ nm-based dose would apply to a relatively large range of turbidities (5–143 NTU).

**Bactericidal effect of PACl**

The water collected from Creek 2 and used to test the survival of bacteria during CF treatment had a turbidity of 136 NTU and a UVA$_{254}$ nm of 0.72 cm$^{-1}$. It contained 120 CFU/100 mL of total coliforms and *E. coli* was <10 CFU/100 mL. The low indigenous bacterial concentration already in place in the water minimized the interaction of the existing bacterial population on subsequent experiments. A dose of 0.05 mL ClearPAC/L resulted in a residual turbidity of 1 NTU after 2 h of settling at 20 and 30 °C.

Since the coagulant product can be concentrated in the sludge over time, the survival of exogenously added bacteria was determined at three ClearPAC doses (0.005, 0.05, 0.5 mL/L), while a negative control did not receive PACl. To maximize exposure of bacteria to PACl, settling of flocs was prevented by vortexing the bacterial suspensions throughout these experiments conducted at room temperature. Bacterial viability was not affected in the negative control and at the lowest dose of 0.005 mL/L; 83–104% of the initial inocula were recovered after 2 or 8 h for all bacterial concentrations tested ($10^3$–$10^7$ CFU/mL) and for both strains (MG1655Rif$^R$ and MG1655Nal$^R$, tested separately). A dose of 0.05 mL/L had some bactericidal effect and caused a 1.2–1.3 log$_{10}$ decrease of the lowest starting inoculum (3.7–3.8 log$_{10}$ CFU/mL) of both strains after 8 h of contact. The highest dose of PACl (0.5 mL/L) completely prevented detection of any of the two strains after 2 and 8 h of contact for the two lowest inocula (3.7–3.8 and 4.7–4.8 log$_{10}$ CFU/mL), but 37–85% of the highest inoculum (6.7–6.8 log$_{10}$ CFU/mL) was still detected after 2 h of PACl exposure.

Results indicate that bacteria are likely to survive a 2-h PACl exposure at doses representative of those necessary to optimally treat natural water sources. These data are consistent with Finch & Smith (1986) who showed that bacteria concentrated in sludge are able to survive after 27 h of contact with alum.

**Survival of bacteria in CF sludge from jar tests**

Since PACl can be concentrated in the sludge, its bactericidal activity may be enhanced. On the other hand, bacteria may also be concentrated in the sludge along with organic matter and thus be more resistant to the bactericidal effect of PACl. Additionally, even if the CF process may take as little as 2 h, the sludge may be stored for several hours or days.

The next set of experiments evaluated bacterial survival in sludge and treated water in a jar test at room temperature. With a starting inoculum of $3.9 \log_{10}$ CFU/mL and a PACl dose of 0.05 mL/L, the concentration of viable bacteria was $5.5 \log_{10}$ CFU/mL in the sludge after 1 h of settling and this amount only slightly dropped to $5.3 \log_{10}$ CFU/mL after 8 h. Some residual bacteria were also found in the treated water but concentrations were near the detection limit beyond 2 h of settling time. These data show that bacteria can be concentrated and survive for an extended period of time at room temperature in the sludge produced by a representative dose of PACl.

**Plasmid transfer**

Since a certain amount of bacteria survived in the presence of PACl, it was justified to presume that genetic exchanges might occur, especially in the sludge where bacteria are concentrated and in close proximity. As an initial proof of concept, high inocula ($\sim 8 \log_{10}$ CFU/mL) were used both for donor and recipient *E. coli* strains that were mixed together in a jar test performed at 35 °C. Figure 5(a) presents detection of both donor and recipient strains as well as the number of exconjugants detected in the sludge over time. Data indicated that the donor and recipient strains survived at high numbers ($> 6.38 \log_{10}$ CFU/mL) in the sludge up to 120 h while the detection of exconjugants that have acquired plasmid pVCR94 rapidly increased in the first 24 h and remained stable up to 120 h ($4.14–4.72 \log_{10}$ CFU/mL). The increased frequency of plasmid transfer measured as a function of time (Figure 5(b)) was statistically
significant \( (P < 0.05, \text{one factor analysis of variance (ANOVA) test}) \). The frequency of plasmid transfer from donor to recipient in water with no PACl added for the same period of time was also examined. In such optimal experimental conditions (high inocula and 35 \(^\circ\)C), there was only a slight increase (10.8-fold on average) in the detection of exconjugants in the sludge compared with that seen in water between 8 and 120 h (Figure 5(b)). Although no plasmid transfer was detected in water at 2 h, there was no significant difference in plasmid transfer in water (i.e. without PACl) or sludge over time \( (p > 0.05, \text{two factors ANOVA test}) \). When the same experiment was repeated at room temperature, the frequency of plasmid transfer decreased to very low levels and a frequency of transfer was not accurately measurable. However, Nal and Tet-resistant exconjugants, in which plasmid pVCR94 was detected by PCR, were obtained and confirmed the occurrence of transfer in these experimental conditions (Table 2). Similarly, using low starting inocula \( (\sim 10^8 \text{CFU/mL}) \) that are closer to those found in natural sources of water \( (\text{Ibekwe et al. 2002}) \), the frequency of plasmid transfer by genetic conjugation was near or below the detection limit at room temperature and 35 \(^\circ\)C. The occurrence of plasmid transfer was however confirmed at 35 \(^\circ\)C after 120 h (Table 2).

Results thus indicated that sludge provided no specific advantage for genetic conjugation. This seems especially true considering that the maximum water temperature registered in six surface water sources from Eastern Ontario over a 2-year sampling period ranged from 16.0 to 24.7 \(^\circ\)C (unpublished data), and that bacterial concentrations found in surface water from farmlands only occasionally reach \( 10^3-10^4 \text{CFU/mL} \) \( (\text{Ibekwe et al. 2002}) \). When similar conditions were used \( (22-25 \^\circ\)C and bacterial inocula of \( \sim 10^8 \text{CFU/mL} \) ), no plasmid transfer was observed (Table 2). Nevertheless, gene transfer might occur in appropriate conditions such as high bacterial concentrations and/or high temperatures. It is conceivable that such appropriate conditions may arise when water is highly contaminated with animal feces or if batches of standing water or sludge are exposed to sun.

**CONCLUSION**

Results from this study indicated that for water sources with UV absorbance remaining below 0.85 cm\(^{-1}\), a single PACl dose will be adequate throughout the year to achieve efficient CF, but additional settling time could be required in the winter when water temperature is below 10 \(^\circ\)C. For water with high variations in organic matter content, however, an online UV\(\lambda_{254} \text{nm} \) sensor could be used to automatically control coagulant dose. The CF process should adequately treat water for any subsequent disinfection treatments (UV, ozonation, nanofiltration).
Results showed that bacteria remained viable in sludge produced by the CF process and that gene transfer may occur under optimal conditions. However, this study could not assess whether these optimal conditions could arise in the field and if special handling of sludge residues, other than storage with the manure prior to land application, is warranted.

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**Table 2** Transfer of plasmid pVCR94 (Tet<sup>b</sup>) from *E. coli* MG1655RifR to MG1655Nal<sup>b</sup> at the indicated high and low inocula, water temperatures and time spent in the sludge.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Time (h)</th>
<th>Donor</th>
<th>Recipient</th>
<th>Number of Nal&lt;sup&gt;a&lt;/sup&gt;, Tet&lt;sup&gt;b&lt;/sup&gt; colonies&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Number of exconjugant confirmed&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Transfer</th>
</tr>
</thead>
<tbody>
<tr>
<td>22–25</td>
<td>2</td>
<td>8.7</td>
<td>8.4</td>
<td>6</td>
<td>4</td>
<td>Detected</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>8.5</td>
<td>8.3</td>
<td>7</td>
<td>3</td>
<td>Detected</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>7.8</td>
<td>8.1</td>
<td>4</td>
<td>3</td>
<td>Detected</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>7.4</td>
<td>7.6</td>
<td>BDL&lt;sup&gt;c&lt;/sup&gt;</td>
<td>–</td>
<td>ND&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>7.5</td>
<td>7.4</td>
<td>BDL</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>22–25</td>
<td>2</td>
<td>4.6</td>
<td>4.7</td>
<td>1</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4.6</td>
<td>4.9</td>
<td>BDL</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>5.0</td>
<td>4.8</td>
<td>1</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>4.8</td>
<td>4.7</td>
<td>1</td>
<td>0</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>4.8</td>
<td>4.6</td>
<td>BDL</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td>35</td>
<td>2</td>
<td>4.9</td>
<td>4.8</td>
<td>BDL</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>4.7</td>
<td>4.9</td>
<td>BDL</td>
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<td>ND</td>
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<td></td>
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<td>4.9</td>
<td>5.0</td>
<td>BDL</td>
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<tr>
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<td>4.4</td>
<td>4.7</td>
<td>BDL</td>
<td>–</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>120</td>
<td>4.5</td>
<td>4.6</td>
<td>4</td>
<td>4</td>
<td>Detected</td>
</tr>
</tbody>
</table>

<sup>a</sup>Total number of colonies recovered from triplicate agar plates containing Nal and Tet (possible mixture of exconjugants and donor strain with spontaneous Nal resistance).

<sup>b</sup>Total number of Nal and Tet-resistant isolates in which plasmid pVCR94 was detected by PCR (true exconjugants).

<sup>c</sup>BDL, below detection limit (1 CFU/300 μL, non-diluted).

<sup>d</sup>ND, not detected.


climateData/canada_e.html?& (accessed 20 October 2011).


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