Development of a mathematical model of Cryptosporidium inactivation by ozonation

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Abstract A new mathematical model was developed to express the processes of Cryptosporidium inactivation by ozonation. In this model, five different stages were considered as state variables of Cryptosporidium oocysts for accurate expression of the inactivation. ATP, in vitro excystation and DAPI/PI permeability assays were used to describe the oocyst amounts of different stages. Some reaction constants were estimated by the structure components of oocysts or by the stoichiometry of reactions, while the others were by the oocysts population changes in ozonation. The calculated values of this model were well consistent with experimental inactivation data. Three-log inactivation of sporozoites required about 0.04 mgO₃ per unit oocyst (mgC) from the simulation results. Before ozone reacts with sporozoites, more ozone was consumed to oxidize other parts of oocysts and DOC produced. The main path of inactivation of oocysts by ozonation was estimated to be $P_1$ (intact oocysts) $\rightarrow P_2$ (oocysts with damaged outer oocyst wall) $\rightarrow P_3$ (oocysts without excystation function) $\rightarrow P_4$ (oocysts with inactivated sporozoites and no excystation function) from experimental and simulated results.

Keywords ATP; Cryptosporidium parvum; inactivation; mathematical model; ozonation

Introduction Cryptosporidium parvum, a coccidian intestinal parasite, infects humans and may cause gastroenteric disease, and the oocysts are extremely resistant to chlorine and other commonly used disinfectants. It is reported that ozone is the most effective disinfectant as an alternative to chlorine for inactivation of Cryptosporidium (Peeters et al., 1989; Korich et al., 1990; Finch et al., 1993). However, no agreement has been reached on the inactivation kinetics and mechanism of oocysts by ozonation. A C. parvum oocyst consists of oocyst walls, sporozoites and residuals (Reduker et al., 1985). Since ozonation is supposed to have different effects on these parts, it is difficult to estimate the oxidation performance on these three components of oocysts. Little attention has been given to this aspect of oocyst inactivation by ozonation. The objective of this research was to propose a model on the oocyst inactivation by ozonation and to verify the model by using experimental data.

Development of the model

Inactivation process of oocysts and its mathematical expressions

Figure 1 shows the fundamental concept on inactivation process of oocysts by ozonation. In this study, the inactivation of oocysts by ozone is illustrated with two phases. One is the damage of excystation function and another is the inactivation of sporozoites. The inactivation process of intact oocysts was modeled as follows: Five different stages of oocysts ($P_1$, $P_2$, $P_3$, $P_4$ and $P_5$ shown in Figure 1) are assumed to appear simultaneously during the ozonation of oocysts. Intact oocyst ($P_1$) is transformed into $P_2$ form of oocyst by the complete oxidation of the outer wall. The inner wall of the $P_2$ form of oocyst is punched by ozone, and then ozone can penetrate into the inside of the oocyst and inactivate sporozoites. At this stage ($P_3$) the suture of oocyst does not react with ozone, that is, it still has an excystation function. $P_4$ form of oocyst represents the stage where ozone reacts with the inner wall of $P_2$ form and simultaneously oxidizes the suture controlling excystation. At this
stage sporozoites do not contact with ozone, that is, sporozoites are still active. $P_5$ stage of oocyst is produced by oxidation of suture of $P_3$ form of oocyst or the inactivation of sporozoites of $P_4$ form of oocyst by ozone. Cryptosporidium oocysts are completely inactivated at this stage.

The biomass of oocysts was represented as the amount of carbon. In the preliminary study, a linear relationship was observed between TOC concentration and the number of $C.\ parvum$ oocysts in the range of $1.0 \times 10^3 – 2.5 \times 10^6$ oocysts/ml, carbon quota in an individual oocyst was estimated as $4.5 \times 10^{-9}$ mg. This result shows that the biomass of oocysts can be represented by TOC concentration in the model. It was assumed that dissolved organic carbon (DOC) and carbon dioxide were formed with the oxidation of oocysts. The reaction between ozone and oocysts was assumed by a first order reaction. Reaction between DOC and ozone was also considered to be a first order reaction.

The differential equations on each concentration of $P_i$, DOC, CO$_2$ and ozone are derived from the fundamental concept on inactivation process of $C.\ parvum$ oocysts by ozonation shown in Figure 1. The equations are expressed as follows:

$$\frac{dP_1}{dt} = -(k_{12} + k_{1c} + k_{1d})OP_1 \quad (1)$$
$$\frac{dP_2}{dt} = k_{12}OP_1 - (k_{23} + k_{2c} + k_{2d})OP_2 \quad (2)$$
$$\frac{dP_3}{dt} = k_{23}OP_2 - (k_{35} + k_{3c} + k_{3d})OP_3 \quad (3)$$
$$\frac{dP_4}{dt} = k_{24}OP_2 - (k_{45} + k_{4c} + k_{4d})OP_4 \quad (4)$$
$$\frac{dP_5}{dt} = k_{35}OP_3 + k_{45}OP_4 - (k_{5c} + k_{5d})OP_5 \quad (5)$$
$$\frac{dD}{dt} = k_{1d}OP_1 + k_{2d}OP_2 + k_{3d}OP_3 + k_{4d}OP_4 + k_{5d}OP_5 - k_{DC}OD \quad (6)$$
$$\frac{dC}{dt} = k_{1c}OP_1 + k_{2c}OP_2 + k_{3c}OP_3 + k_{4c}OP_4 + k_{5c}OP_5 + k_{DC}OD \quad (7)$$
$$\frac{dO}{dt} = -(\alpha_1 k_{1c} + \alpha_2 k_{1d})OP_1 - (\alpha_1 k_{2c} + \alpha_2 k_{2d})OP_2 - (\alpha_1 k_{3c} + \alpha_2 k_{3d})OP_3 - (\alpha_1 k_{4c} + \alpha_2 k_{4d})OP_4 - (\alpha_1 k_{5c} + \alpha_2 k_{5d})OP_5 - (\alpha_1 + \alpha_2)k_{DC}OP_5 - k_{O3}O \quad (8)$$

where $P_1, P_2, P_3, P_4$ and $P_5$ are the concentrations of oocysts for different stages as expressed by organic carbon (mgC/l), $O$ is the concentration of dissolved ozone (mgO$_3$/l),

Figure 1 Fundamental concept on inactivation of Cryptosporidium oocysts. $P_1$ is intact oocysts, $P_2$ is active oocysts with damaged outer walls, $P_3$ is oocysts with excystation function and inactivated sporozoites, $P_4$ is oocysts with active sporozoites and no excystation function, and $P_5$ is oocysts without active sporozoites nor excystation function.
D is the concentration of dissolved organic carbon (mgC/l) and C is the concentration of carbon dioxide (mgC/l). On the other hand, \( k_{12}, k_{23}, k_{24}, k_{35} \) and \( k_{45} \) are reaction rate constants for oocyst stage transfer (l/mgO\(_3\)/min), and \( k_{1D}, k_{2D}, k_{3D}, k_{4D} \) and \( k_{5D} \) are reaction rate constants for the formation of dissolved organic carbon (l/mgO\(_3\)/min). While \( k_{1C}, k_{2C}, k_{3C}, k_{4C} \) and \( k_{5C} \) are reaction rate constants for formation of carbon dioxide from each state of oocysts (l/mgO\(_3\)/min). \( k_{DC} \) is the reaction rate constant for formation of carbon dioxide from dissolved organic carbon (l/mgO\(_3\)/min) and \( k_{O3} \) is rate constant of ozone self-decomposition (1/min). \( \alpha_1 \) is the amount of ozone required for the oxidation of oocysts into carbon dioxide (mgO\(_3\)/mgC). \( \alpha_2 \) is the amount of ozone required for the oxidation of oocysts into dissolved organic carbon (mgO\(_3\)/mgC). Finally, \( t \) is the reaction time.

Equations 1 to 5 show the time-derivatives of oocyst concentration. The biomass of oocysts is represented by the amount of carbon. Intact oocyst (\( P_1 \)) is transformed into \( P_2 \) stage with reaction rate \( k_{12} \) by the complete degradation of the outer wall. The \( P_2 \) stage is transformed into \( P_3 \) and \( P_4 \) stage with reaction rate \( k_{23} \) and \( k_{24} \). The \( P_5 \) stage is produced by the oxidation of \( P_3 \) and \( P_4 \) stage oocysts with reaction rate \( k_{35} \) and \( k_{45} \) by ozone. For all the stages, ozonation of oocysts produces DOC with reaction rates of \( k_{1D}, k_{2D}, k_{3D}, k_{4D} \) and \( k_{5D} \) and CO\(_2\) with reaction rates of \( k_{1C}, k_{2C}, k_{3C}, k_{4C} \) and \( k_{5C} \). Equations 6 and 7 account for the solubilization and the mineralization processes in oocyst decomposition by ozonation. In the equation 6, DOC is produced from all of the oocyst degradation processes by ozone and is transformed into CO\(_2\) with reaction rate \( k_{DC} \). In the Eq. 7, CO\(_2\) is produced by complete oxidation of oocysts and DOC. Equation 8 accounts for the changes in ozone concentration. The reaction between ozone and oocysts was expressed by a first order reaction rate. The DOC produced consumes ozone and its reaction rate is also expressed by a first order reaction on DOC and ozone concentration. The differential Eqs. 1 to 8 were numerically and simultaneously solved by the Runge-Kutta method.

**Verification experiments**

*Cryptosporidium* oocysts used in this study were HNJ-1 strain obtained from Dr. M. Iseki (Osaka City University Medical School, Osaka, Japan), who originally isolated them from an immunologically normal Japanese patient with diarrhoea. Fresh feces from infected SCID (severe combined immunodeficiency) mice were purified by Sucrose density flotation and IMS methods to obtain *C. parvum* oocysts. Purified oocysts were stored in pure (Milli-Q\(^\circ\), Millipore Co.) water containing antibiotics at 4ºC until used (Jenkins et al., 1997). Oocyst concentration in stock suspension was determined with a direct hemocytometer count and it was diluted tenfold to prepare low oocyst concentrations.

For the identification of this mathematical model, some experiments were conducted in two 500-ml batch reactors at 20ºC. Batch experiments were conducted under different concentrations of ozone. Ozone was bubbled for 1 hr in a 500-ml gas absorption flask filled with 500 ml Milli-Q\(^\circ\) water (Millipore Co.) to obtain the designated concentration of ozone. Before starting the experiment, 1 ml of the purified *C. parvum* suspension was injected into one reactor to make a final concentration of approximately \( 1.0 \times 10^3 \) to \( 1.5 \times 10^5 \) oocysts/ml, while a test sample with no addition of oocyst was made for the control reactor. Thirty millilitres of sample were collected at appropriate intervals with sterilized pipettes for the determination of oocyst viability, TOC and DOC, and then were mixed with excess sodium thiosulfate in sterile test tubes for deozonation. Additional samples were withdrawn intermittently from the reactor for the determination of ozone concentration. Ozone concentration was measured by the Indigo method at a wavelength of 600 nm. TOC concentration in samples was analyzed by TOC analyzer (Sievers 810, USA).

Oocyst viability was assessed by *in vitro* excystation, DAPI/PI permeability and ATP assays. *In vitro* excystation and DAPI/PI permeability assay were performed according to...
the method of Campbell et al. (1992), and ATP assay was performed according to the method of Somiya et al. (2000). In the ATP assay, samples were pretreated to extract ATP either by excystation (ATPe) or by sonication (ATPs). ATPe assay can detect selectively the amount of excystable oocysts, while ATPs assay represents the total activity of oocysts in the sample (Somiya et al., 2000; Kim et al., 1999).

Experimental results

Table 1 summarizes the experimental conditions, and inactivation of C. parvum oocysts by ozone at 20ºC.

<table>
<thead>
<tr>
<th>Dose of oocysts (oocysts/l)</th>
<th>Initial ozone (mg/l)</th>
<th>Residual ozone (mg/l)</th>
<th>Control ozone (mg/l)</th>
<th>Contact time (min)</th>
<th>In vitro excystation (%)</th>
<th>DAPI/PI (%)</th>
<th>ATPe (%) #</th>
<th>ATPs (%) #</th>
<th>TOC (mg/l)</th>
<th>DOC (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5 10^5</td>
<td>0.70</td>
<td>0.70</td>
<td>0.64</td>
<td>0</td>
<td>84.8</td>
<td>95.7</td>
<td>100</td>
<td>100</td>
<td>0.68</td>
<td>0.00</td>
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<tr>
<td>1.5 10^5</td>
<td>0.70</td>
<td>0.65</td>
<td>0.58</td>
<td>5</td>
<td>11.9</td>
<td>82.2</td>
<td>6.8</td>
<td>27.4</td>
<td>0.66</td>
<td>0.11</td>
</tr>
<tr>
<td>1.5 10^5</td>
<td>0.70</td>
<td>0.47</td>
<td>0.48</td>
<td>10</td>
<td>3.4</td>
<td>82.7</td>
<td>1.6</td>
<td>17.3</td>
<td>0.67</td>
<td>0.19</td>
</tr>
<tr>
<td>1.5 10^5</td>
<td>1.13</td>
<td>1.13</td>
<td>1.13</td>
<td>0</td>
<td>66.9</td>
<td>74.5</td>
<td>100</td>
<td>–</td>
<td>0.76</td>
<td>0.00</td>
</tr>
<tr>
<td>1.5 10^5</td>
<td>1.13</td>
<td>0.95</td>
<td>1.03</td>
<td>5</td>
<td>2.8</td>
<td>17.1</td>
<td>3.0</td>
<td>–</td>
<td>0.73</td>
<td>0.22</td>
</tr>
<tr>
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<td>1.13</td>
<td>0.95</td>
<td>1.02</td>
<td>10</td>
<td>3.5</td>
<td>3.9</td>
<td>&lt;1</td>
<td>–</td>
<td>0.70</td>
<td>0.38</td>
</tr>
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<td>1.5 10^5</td>
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<td>1.01</td>
<td>1.01</td>
<td>0</td>
<td>82.1</td>
<td>93.4</td>
<td>100</td>
<td>100</td>
<td>0.48</td>
<td>0.06</td>
</tr>
<tr>
<td>1.5 10^5</td>
<td>1.01</td>
<td>0.92</td>
<td>0.94</td>
<td>5</td>
<td>4.7</td>
<td>20.4</td>
<td>3.6</td>
<td>17.8</td>
<td>0.47</td>
<td>0.29</td>
</tr>
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<td>0.88</td>
<td>0.92</td>
<td>10</td>
<td>1.9</td>
<td>3.0</td>
<td>0.9</td>
<td>4.0</td>
<td>0.48</td>
<td>0.29</td>
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<td>1.5 10^5</td>
<td>1.14</td>
<td>1.14</td>
<td>1.15</td>
<td>0</td>
<td>90.5</td>
<td>89.1</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1.5 10^5</td>
<td>1.14</td>
<td>1.04</td>
<td>1.11</td>
<td>5</td>
<td>4.8</td>
<td>15.3</td>
<td>–</td>
<td>5.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1.5 10^5</td>
<td>1.14</td>
<td>0.94</td>
<td>1.10</td>
<td>10</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>–</td>
<td>6.8</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1.5 10^5</td>
<td>3.10</td>
<td>3.10</td>
<td>3.10</td>
<td>0</td>
<td>90.5</td>
<td>89.1</td>
<td>–</td>
<td>100</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1.5 10^5</td>
<td>3.10</td>
<td>3.09</td>
<td>3.05</td>
<td>1</td>
<td>1.8</td>
<td>18.8</td>
<td>–</td>
<td>8.0</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>1.5 10^5</td>
<td>3.10</td>
<td>2.53</td>
<td>3.00</td>
<td>5</td>
<td>&lt;1</td>
<td>1</td>
<td>–</td>
<td>4.7</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

# Samples were pretreated to extract ATP from oocysts either by excystation (ATPe) or by sonication (ATPs)

Experimental results

Table 1 summarizes the experimental conditions, and inactivation of C. parvum oocysts by ozone at 20ºC. Ozone concentration in the control reactor was utilized to estimate the ozone self-decomposition rate \( k_{o3} \). In the experimental conditions of pH 5.9–6.7 and temperature 20ºC, ozone self-decomposition rate was calculated to be 0.007–0.01 1/min. Viability of oocysts was measured by in vitro excystation, DAPI/PI permeability and ATP assays as surrogate. Viability of oocysts decreased rapidly when higher ozone concentration was applied. About 90% and more of oocysts lost an excystation function within 10 minutes in the ozone concentration of 0.7 mg/l. However, when the ozone concentration of 3.1 mg/l was applied, the excystation function of more than 90% of oocysts was damaged within 1 minute. In the case of an initial ozone concentration of 0.7 mg/l, most sporozoites were still alive for 10 minutes contact time, while ATPe of oocysts decreased with elapsed time and diminished almost within 10 minutes. It is considered that an ATP method is sensitive in expressing the level of oocyst inactivity. No significant changes of TOC concentration were observed with elapsed time, but DOC concentration increased in the elapsed time as shown in Table 1. This means the main reaction of organic carbon with ozone was not mineralization.

Model verification and numerical analysis

Reaction rate constants

The reaction rate constants were obtained from experimental data and/or literature, and these values are summarized in Table 2. Reduker et al. (1985) reported that the oocyst wall of C. parvum was 49.7 nm thick, and composed of two layers. The outer layer was reported...
to be irregular in thickness, averaging 10 nm, and a thin, electron-lucent space (2.5 nm) existed between the outer and inner layers. The above information was used to evaluate the volume and carbon content of the outer and inner layers, suture and oocyst. Reaction rate constants, $k_{12} - k_{DC}$ were calculated based on $K_1 - K_5$, which were determined from the results of in vitro excystation, DAPI/PI permeability and ATP assays. $K_2$ was determined from the ATP decline of oocysts by ozonation, while $K_3$ and $K_4$ were determined from the results of in vitro excystation and DAPI/PI permeability assays, respectively. Rate constant of ozone self-decomposition was determined from experimental data. The amount of ozone required for complete oxidation of an oocyst was determined from the observed ozone consumption and carbon dioxide production. The ozone consumption was measured experimentally, and carbon dioxide production was calculated from the decrease of TOC in solution.

### Verification and numerical analysis

Ozonation test of *C. parvum* was conducted in batch reactors for the verification data of the developed model. ATP assay can detect only the amount of oocysts with excystation function and active sporozoites. The value of ATP, therefore, represents the summation of $P_1 + P_2$ oocysts. In vitro excystation assay was considered to detect oocysts with intact suture only.

### Table 2 Rate constants and related equations

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Definition</th>
<th>Unit</th>
<th>Values</th>
<th>Related equations and reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>$K_1$</td>
<td>Reaction rate constant of $P_1$ oocysts with ozone</td>
<td>$1/mgO_3/min$</td>
<td>3.0 ($1.836$)</td>
<td>$k_{12} \beta_1 K_1$, $k_{1D} = (1-\beta_1) K_1$; $K_1 = k_{12} + k_{1D} + k_{1C}$</td>
</tr>
<tr>
<td>$K_2$</td>
<td>Reaction rate constant of $P_2$ oocysts with ozone</td>
<td>$1/mgO_3/min$</td>
<td>1.12</td>
<td>$k_{23} \beta_2 K_2$, $k_{24} = (1-\beta_2) K_2$; $K_2 = k_{23} + k_{24} + k_{2D} + k_{2C}$</td>
</tr>
<tr>
<td>$K_3$</td>
<td>Reaction rate constant of $P_3$ oocysts with ozone</td>
<td>$1/mgO_3/min$</td>
<td>0.67</td>
<td>$k_{35} \beta_3 K_3$, $K_3 = k_{35} + k_{3D} + k_{3C}$</td>
</tr>
<tr>
<td>$K_4$</td>
<td>Reaction rate constant of $P_4$ oocysts with ozone</td>
<td>$1/mgO_3/min$</td>
<td>0.48</td>
<td>$k_{45} \beta_4 K_4$, $K_4 = k_{45} + k_{4D} + k_{4C}$</td>
</tr>
<tr>
<td>$K_5$</td>
<td>Reaction rate constant of $P_5$ oocysts with ozone</td>
<td>$1/mgO_3/min$</td>
<td>0.01</td>
<td>$K_5 = k_{5D} + k_{5C}$</td>
</tr>
<tr>
<td>$k_{O3}$</td>
<td>Decomposition rate constant of ozone</td>
<td>$1/min$</td>
<td>0.01</td>
<td>Observed</td>
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<tr>
<td>$\beta$</td>
<td>Ratio of oocyst into DOC</td>
<td>–</td>
<td>0.99</td>
<td>Using the changes of concentration of TOC</td>
</tr>
<tr>
<td>$\delta_1$</td>
<td>Ratio between $P_1$ and $P_2$ form of oocysts as unit carbon</td>
<td>$mgC/mgC$</td>
<td>0.994</td>
<td>Reduker et al. (1985)</td>
</tr>
<tr>
<td>$\delta_2$</td>
<td>Ratio between $P_2$ and $P_3$ form of oocysts as unit carbon</td>
<td>$mgC/mgC$</td>
<td>0.990</td>
<td>Reduker et al. (1985)</td>
</tr>
<tr>
<td>$\delta_3$</td>
<td>Ratio between $P_3$ and $P_4$ form of oocysts as unit carbon</td>
<td>$mgC/mgC$</td>
<td>0.990</td>
<td>Reduker et al. (1985)</td>
</tr>
<tr>
<td>$\delta_4$</td>
<td>Ratio between $P_4$ and $P_5$ form of oocysts as unit carbon</td>
<td>$mgC/mgC$</td>
<td>0.454</td>
<td>Reduker et al. (1985)</td>
</tr>
<tr>
<td>$\delta_5$</td>
<td>Ratio between $P_2$ and $P_3$ form of oocysts as unit carbon</td>
<td>$mgC/mgC$</td>
<td>0.455</td>
<td>Reduker et al. (1985)</td>
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<tr>
<td>$\gamma$</td>
<td>Residual Ratio of carbon in oocysts oxidized with ozone</td>
<td>–</td>
<td>0.45</td>
<td>Using the residual of particulate organic carbon</td>
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<tr>
<td>$\alpha_1$</td>
<td>Amount of ozone required for oxidation of an oocyst to carbon dioxide</td>
<td>$mgO_3/ mgC$</td>
<td>2.70</td>
<td>Using the concentration of ozone consumption and carbon dioxide calculated</td>
</tr>
<tr>
<td>$\alpha_2$</td>
<td>Amount of ozone required for oxidation of an oocyst to DOC</td>
<td>$mgO_3/ mgC$</td>
<td>0.20</td>
<td>Using the concentration of ozone consumption and DOC</td>
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<tr>
<td>$\sigma$</td>
<td>Transfer ratio from $P_2$ to $P_3$ form of oocysts</td>
<td>–</td>
<td>0.11</td>
<td>Probability and trial and error method</td>
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</table>
(i.e. \( P_1 + P_2 + P_3 \)). DAPI/PI permeability assay was introduced to estimate the amount of oocysts with active sporozoites (i.e. \( P_1 + P_2 + P_4 \)). In the model, initial condition was set at \( P_i = P_1, P_2 = P_3 = P_4 = P_5 = 0 \).

The decomposition of ozone in presence and absence of oocysts is shown in Figure 2. Dissolved ozone was rapidly decreased within one minute in presence of oocysts. The reaction between ozone and oocyst reached almost completion within two minutes. The plotted line shows the simulation results on the experiment condition. Decreasing patterns of dissolved ozone in the reactor were well simulated by the model.

Figure 3 shows the changes in the ATPe, DAPI+PI- (or DAPI-PI-) staining and excystation ratios of oocysts with the elapsed time. At an initial ozone concentration of 1.1 mg/l, 90% of oocysts lost the excystation function within two minutes, and most of the sporozoites were inactivated in 10 minutes. The amount of oocyst ATPe decreased in a pattern similar to the decline tendency of excystation. Figure 3 indicates that the DAPI+PI- (or DAPI-PI-) staining ratio is always higher than the excystation ratio at the same contact time. This implies that some sporozoites still remain active even though the excystation function is damaged. Since the oxidation by ozone is not site specific, the possibility of oxidation of suture part by ozone, which is situated on the inner wall, would be higher than that of sporozoites. Thus, \( P_3 \) form of oocysts would be readily oxidized and transformed into the next stage (\( P_5 \)).

Figure 4 illustrates the experimental results and the simulation results on the change of carbon amount fractions with time. The figure clearly shows that the reaction of POC, the production of DOC and carbon dioxide were almost finished in the initial two minutes of contact time. This is attributed to rapid oxidation of oocyst walls with ozone in the initial two minutes of contact time. The simulated results show good coincidence with the experimental data on carbon balance.

Figure 5 shows the simulation results of the utilized ozone concentration for 90, 99, 99.9% inactivation of oocysts respectively. Three-log inactivation or sporozoites required
about 0.042 mgO3/l. Before ozone reacts with sporozoites, however, more ozone was
consumed to oxidize other parts of oocysts and DOC from oocysts. This implies that the
observed ozone concentration could not directly express the inactivation degree of oocysts.
Therefore, the reevaluation of the meaning of CT value would be needed for the
inactivation of C. parvum oocysts.

Figure 6 shows the simulation results of the changes in the different forms of oocysts
with time. The intact oocyst (P1) reacted with ozone and transformed into P2 form of
oocysts at once. Most of P2 stage of oocyst was rapidly transformed into P4 form of oocyst.
The transformation of P2 form to P3 was relatively small. Therefore, the main path of in-
activation of oocysts was concluded as P1 → P2 → P4 → P5 from the experimental and simu-
lated results.

Conclusions
A new mathematical model was developed for the establishment of Cryptosporidium
inactivation processes by ozonation and verified with the experimental data. The main
conclusions obtained in this study are summarized as follows.
1. In the developed model, five different stages of C. parvum were considered for accurate
expression of the inactivation process. ATPe, in vitro excystation and DAPI/PI perme-
ability assays were used to estimate the amounts of different stages of oocysts. The reac-
tion rate constants were estimated from either batch experimental data or literature. The
calculated values by this model were well consistent with experimental inactivation
data.
2. Dissolved ozone was rapidly decreased within one minute in presence of oocysts. The
reaction between ozone and oocyst reached almost completion within two minutes.
Decreasing patterns of dissolved ozone in the reactor were well simulated by the model.
3. No significant changes of TOC concentration were observed with elapsed time, but DOC concentration increased in the initial two minutes by the experimental and simulated results. This is attributed to rapid oxidation of oocyst walls with ozone.

4. Three-log inactivation of sporozoites required about 0.04 mgO₃ per unit oocyst (mgC) from the simulation results. Before ozone reacts with sporozoites, more ozone is needed to oxidize other parts of oocysts and DOC produced.

5. The main path of inactivation of oocysts is expected to be $P_1$ (intact oocysts) $\rightarrow P_2$ (oocysts with damaged oocyst wall) $\rightarrow P_4$ (oocysts without excystation function) $\rightarrow P_5$ (oocysts with inactivated sporozoites and no excystation function) from experimental and simulated results.

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References


