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# Extrinsic Control Parameters for Ozone Inactivation of Escherichia Coli Using a Bubble Column

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1 Title: “Extrinsic control parameters for ozone inactivation of  
2 *Escherichia coli* using a bubble column”

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21

22 **Abstract**

23 **Aims:** This study aimed to investigate the effect of extrinsic control parameters for ozone  
24 inactivation of *E. coli* in a bubble column.

25 **Methods and Results:** Ozone inactivation of *Escherichia coli* ATCC 25922 in Tryptic Soya  
26 Broth was examined. The parameters studied included temperature (ambient, 20, 25 and  
27 30°C), exposure time (up to 30 min), gas flow rate (0.03, 0.06, 0.12, 0.25, 0.5, and 0.75 L min<sup>-1</sup>)  
28 and concentration level (5 different levels). The efficacy of ozone treatment was a function  
29 of the parameters investigated and optimum control parameters of flow rate (0.12L min<sup>-1</sup>),  
30 temperature (ambient) and ozone concentration (75 µg mL<sup>-1</sup>) resulted in a t<sub>d5</sub> (time required to  
31 achieve 5 log reduction) of 20 minutes.

32 **Conclusions:** Optimum control parameters of gas flow rate, ozone concentration and  
33 temperature are reported for *E. coli* inactivation within a bubble column.

34 **Significance and Impact of Study:** In 2001, the FDA approved use of ozone as a direct  
35 additive to food and in 2004, issued guidelines for the use of ozone in liquid systems.  
36 However, these guidelines highlighted gaps in the literature for ozonation of liquid foods.  
37 This study provides useful information regarding optimum extrinsic control parameters for *E.*  
38 *coli* inactivation in liquid media using a bubble column to ensure microbiological safety.

39 **Keywords:** *E. coli*, Ozone, Bubble column, Weibull model, Non thermal technology

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## 44 **1. Introduction**

45 Ozonation is a relatively new method for food preservation which can be applied in order to  
46 meet consumer's demand for fresher and safer ready-to-eat products. Ozone destroys micro-  
47 organisms by progressive oxidation of vital cellular components. Activated oxygen species  
48 resulting from ozone decomposition includes singlet oxygen, hydroxyl radical, superoxide  
49 anion (perhydroxyl radical at low pH) and hydrogen peroxide which elicit potent cidal  
50 activity against a broad-spectrum of microorganisms (Korycka-Dahl & Richardson 1978).  
51 The bacterial cell surface has been suggested as the primary target of ozonation. The biocidal  
52 effect of ozone is caused by its high oxidation potential, reacting up to 3000 times faster than  
53 chlorine with organic material (EPRI 1997), and its ability to readily diffuse through  
54 biological cell membranes. Khadre and Yousef (2001), found that ozone was more effective  
55 than hydrogen peroxide against food borne *Bacillus* species spores. Treatments with ozone  
56 (1.6 and 2.2 ppm) for 1 min decreased *Shigella sonnei* population in water by 3.7 and 5.6 log,  
57 respectively. In addition, *S. sonnei* counts were reduced by 1.8 log units in lettuce treated with  
58 5 ppm for 5 min (Selma *et al.* 2007). Oztekin *et al.* (2005) reported on the effects of ozone  
59 treatment on the micro flora of dried figs, where the application of gaseous ozone at 5 or 10  
60 ppm for 3 to 5 h resulted in significant reductions in total bacteria, coliform and yeast/mould  
61 counts. Habibi Najafi and Haddad Khodaparast (2009) concluded that a minimum of one hour  
62 ozone treatment at 5 ppm could be successfully used for reducing both coliform and  
63 *Staphylococcus aureus* populations on date fruits, but that longer exposure times are required  
64 for elimination of the total mesophilic bacteria as well as yeast/mould. Fan *et al.* (2007)  
65 reported that gaseous ozone effectively inactivated *Listeria innocua* on solid media at  
66 concentrations of 50 and 100 nl l<sup>-1</sup> during short exposure times at both 5 and 20°C. Ozone has  
67 been shown to reduce populations of *E. coli* O157:H7 in phosphate buffer (Byun *et al.* 1998)  
68 while its preservation efficacy has been also evaluated in a variety of food products, including

69 milk, gelatin, albumin, casein, and meat products (Kim *et al.* 1999). Williams *et al.* (2004)  
70 reported that ozone treatment ( $0.9\text{ g h}^{-1}$ ) of apple cider and orange juice at  $4^{\circ}\text{C}$  or ozone in  
71 combination with mild heating ( $50^{\circ}\text{C}$ ) may provide an alternative to thermal pasteurisation for  
72 reduction of *E. coli* O157:H7 and *Salmonella* in apple cider and orange juice. The major  
73 advantage of ozone is auto decomposition, excess ozone auto-decomposes rapidly to produce  
74 oxygen thus leaving no residues in food (Khadre *et al.* 2001). Ozone is readily soluble in  
75 water and its solubility increases as the temperature of water decreases (Steenstrup and Floros  
76 2004). *Escherichia coli* O157:H7 is an enteric pathogen that is associated with a number of  
77 outbreaks of food-borne illnesses (CDC 1996). These outbreaks led the U. S. Food and Drug  
78 Administration (FDA) to issue hazard analysis critical control point (HACCP) regulations for  
79 safe and sanitary processing of liquid foods such as fruit juice (USFDA 2001). A primary  
80 performance standard required by this HACCP regulation is a minimum 5-log reduction in the  
81 juice being processed (USFDA 2001).

82 The FDA's approval of ozone as a direct additive to food in 2001 triggered interest in ozone  
83 applications. A number of commercial fruit juice processors in the US and Europe began  
84 employing ozone to treat the products resulting in industry guidelines being issued by the  
85 FDA (FDA 2004). However, these guidelines highlighted gaps with respect to lack of  
86 knowledge regarding the critical control parameters for ozone inactivation in liquid systems.  
87 The objective of this study was to investigate the effect of extrinsic control parameters for  
88 ozone inactivation of *E. coli*.

89

## 90 **2. Materials and Methods**

### 91 **2.1 Bacterial culture conditions**

92 Stock cultures of *E. coli* ATCC 25922 were maintained using protect beads (Technical  
93 Services Consultants Ltd, UK) at  $-70^{\circ}\text{C}$ . One protect bead was used to inoculate one tryptic

94 soy agar (TSA, Scharlau Chemie, Spain) plate, and incubated at 37°C overnight. An isolated  
95 colony of *E. coli* ATCC 25922 was inoculated into 5 ml tubes containing tryptic soy broth  
96 (TSB) and incubated overnight at 37°C. The bacterial density was determined by measuring  
97 absorbance at 550nm using McFarland standard (BioMérieux, Marcy -l'Etoile, France)  
98 working inoculum corresponding to  $1.0 \times 10^8$  CFU ml<sup>-1</sup> was prepared. For ozone treatment,  
99 cells were adjusted to a density of  $1.0 \times 10^6$  CFU ml<sup>-1</sup> in TSB.

## 100 **2.2 Ozone generation and measurements**

101 Ozone was produced by a corona discharge generator (Model OL80, OzoneLab™, Ozone  
102 services, Canada). Pure oxygen was supplied via an oxygen cylinder (Air Products Ltd.,  
103 Dublin, Ireland) and the flow rate was controlled using an oxygen flow regulator. To  
104 determine the effect of gas flow rate and ozone concentration, experiments were carried out in  
105 a 100 ml glass bubble column. In order to determine the effect of temperature, experiments  
106 were carried out in a 250 ml glass bubble column with heating jacket (Fig.1). Ozone  
107 concentration was varied from 28-120 µg ml<sup>-1</sup> (Table 1). Ozone concentration was recorded  
108 using an ozone analyzer. Temperature was controlled in the 250 ml bubble column by  
109 circulating water at the selected temperature through the heating jacket. Steady state ozone  
110 production was achieved prior to media treatment by passing ozone through sterile distilled  
111 water for 10 min at the required flow rate and temperature. To prevent excess foaming, 5-10  
112 µL sterile anti-foaming agent (Sigma Aldrich Ireland Ltd., Dublin, Ireland) was added to the  
113 medium before each ozone treatment.

## 114 **2.3 Experimental design**

115 A working concentration of  $1 \times 10^6$  CFU ml<sup>-1</sup> *E. coli* ATCC 25922 inoculum was treated at  
116 gas flow rates of 0.03, 0.06, 0.12, 0.25, 0.5 and 0.75 L min<sup>-1</sup>. Inoculated samples of 90ml for  
117 the 100ml column or 200ml for the 250ml column were transferred to the bubble column

118 reactors, and treated for up to 30 min. Experiments were also carried out to determine the  
119 effect of ozone concentration and temperature on the efficacy of ozone for inactivation of  $1 \times$   
120  $10^6$  CFU ml<sup>-1</sup> *E. coli* ATCC 25922 . Different ozone concentrations in the range of 28-120  
121  $\mu\text{g ml}^{-1}$  and 17-75  $\mu\text{g ml}^{-1}$  were tested. For the temperature studies, different temperatures (12-  
122 15°C, 20°C, 25°C and 30°C) were tested at optimum flow rate of 0.12L min<sup>-1</sup> achieved on the  
123 basis of flow rate studies. All experiments were carried out in duplicate and then replicated.  
124 To quantify the effects of ozone treatment parameters, 1 ml samples were removed at 5  
125 minute intervals ; samples were serially diluted in maximum recovery diluent (MRD,  
126 Scharlau Chemie, Spain), and 0.1ml aliquots of appropriate dilutions were plated on TSA in  
127 duplicate, incubated at 37°C for 24h and counted. Control experiments were performed where  
128 the working inoculum was exposed to oxygen only.

## 129 **2.4 Mathematical modelling**

### 130 **2.4.1 Primary Modelling**

131

132 The GInaFiT tool was employed to perform the regression analysis of the microbial  
133 inactivation data (Geeraerd *et al.* 2005). The Weibull model (Mafart *et al.* 2002) was used to  
134 model the experimental death curves, which displayed a downward concave trend. This model  
135 has been used previously to describe the inactivation kinetics for ozone (Bialka *et al.* 2008).  
136 The model used was:

$$137 \quad \text{Log}_{10} \left( \frac{N}{N_0} \right) = - \left( \frac{t}{\alpha} \right)^{\beta} \quad (1)$$

138 where  $N$  is the number of microorganisms,  $N_0$  (CFU mL<sup>-1</sup>) is the initial number of  
139 microorganisms,  $\alpha$  [min] (time for the first decimal reduction) and  $\beta$  [-]. are parameters  
140 relating to the scale and shape of the inactivation curve, respectively. The numerical values of

141  $\alpha$  and  $\beta$  were used to calculate a desired log reduction. The time required to obtain a 5 log  
142 reduction ( $t_{d5}$ ) was calculated using eqn 2, where  $D_5$  is 5log reduction

$$143 \quad t_{d5} = \alpha \times (D_5)^{\frac{1}{\beta}} \quad (2)$$

144 The Weibull distribution corresponds to a concave upward survival curve if  $\beta < 1$  and concave  
145 downward if  $\beta > 1$  (van Boekel 2002).

146

### 147 **2.4.2 Secondary modelling**

148 To characterise the dependence of the Weibull parameters ( $\alpha$  and  $\beta$ ) on the ozone processing  
149 variables, the following steps were taken:

- 150 1. Individual nonlinear regressions of all experiments with eqn (1) were performed.
- 151 2. Inspection of the variation of  $\alpha$  and  $\beta$  with processing temperature (T) and ozone flow  
152 rate per reactor volume ( $fO_3$ ) ( $\text{mg min}^{-1} \text{ml}^{-1}$ ).
- 153 3. Experiments at each processing condition were averaged.
- 154 4. Polynomial relationships of  $\ln(\alpha)$  and  $\beta$  with the ozone flow rate and the temperature  
155 were proposed.
- 156 5. Additional terms to the model were investigated and compared with the base model  
157 using a log-likelihood ratio test.

158 All nonlinear regressions were performed using the R statistical software libraries (R Core  
159 Development Team, 2007)

160

## 161 **3. Results**

### 162 **3.1 Effect of gas flow rate**

163 The inactivation of *E. coli* ATCC 25922 was found to be dependant on gas flow rate.

164 Survival curves of *E. coli* ATCC 25922 in TSB treated with ozone ( $0.045\text{-}0.170 \text{ mg min}^{-1}\text{mL}^{-1}$ )



165 <sup>1</sup>) at the selected flow rates are shown in Figure 2. Each curve shows a noticeable lag time  
166 prior to reduction, due to ozone demanding substances present in the TSB medium.  
167 Regardless of the lag time, after 25 minutes, complete inactivation was achieved using the  
168 following flow rates: 0.06, 0.12, 0.25 and 0.5 L min<sup>-1</sup>. However, complete inactivation was  
169 not achieved with flow rates of 0.03 and 0.75 L min<sup>-1</sup> after 30 minutes treatment. The  
170 optimum flow rate was 0.12 L min<sup>-1</sup>, with a t<sub>d5</sub> value of 20 minutes (Table 2). Exposure of *E.*  
171 *coli* to pure oxygen for 30 min resulted in no inactivation (data not shown).

### 172 **3.2 Effect of ozone concentration**

173 The optimum flow rate of 0.12 L min<sup>-1</sup> was chosen to investigate the effect of ozone  
174 concentration on inactivation. Based on results reported by Tiwari *et al.* (2008), a second flow  
175 rate of 0.06 L min<sup>-1</sup> was investigated as this flow rate was found to incur the least amount of  
176 colour degradation for freshly squeezed orange juice. Five different levels of ozone  
177 concentration were investigated; ranging from 28-120 µg ml<sup>-1</sup> ozone in the case of 0.06 L  
178 min<sup>-1</sup>, and from 17-75 µg ml<sup>-1</sup> ozone in the case of 0.12 L min<sup>-1</sup> (Fig. 3 and Table 1). For both  
179 flow rates, the highest concentration (level 5) was the most effective to inactivate *E. coli*  
180 ATCC 25922 (Fig. 3a, b). The flow rate of 0.06 L min<sup>-1</sup> yielded a t<sub>d5</sub> of 24.2 minutes and the  
181 0.12 L min<sup>-1</sup> flow rate yielded a t<sub>d5</sub> value of 20 minutes. From the graph it is evident that there  
182 is a stepwise decrease in the efficiency of *E. coli* inactivation at the lower concentration levels  
183 (1 and 2), where inactivation was insufficient to calculate t<sub>d5</sub> values.

### 184 **3.3 Effect of temperature**

185 Temperature effects on the efficacy of ozone inactivation on *E. coli* ATCC 25922 were  
186 examined at the optimum inactivation flow rate of 0.12 L min<sup>-1</sup>. Four different temperatures  
187 were investigated: ambient temperature (12-15°C), 20°C, 25°C and 30°C. Survival curves are

188 shown in Figure 4. Ambient temperature gave the best inactivation levels with a  $t_{d5}$  value of  
189 20 minutes (Table 2).

190

### 191 **3.4 Modelling of the inactivation kinetics and assessment of the process**

192

193 Each individual experiment was fit to a Weibull model. The Weibull model adequately  
194 described the microbial inactivation (Fig 5). The relationship between model parameters ( $\alpha$   
195 and  $\beta$ ) and processing parameters of ozone flux ( $fO_3$ ) and temperature (T) are shown in Figure  
196 6. The characteristic time relationship with the ozone flux through the reactor showed an  
197 increase in the inactivation rate as ozone flux increased until a flux of ca. 0.1mg  $O_3$  per  
198 processing minute per mL of reactor volume. The dependence of the characteristic time with  
199 temperature showed that lower processing temperatures achieved higher inactivation rates.  
200 The shape parameter  $\beta$  showed no clear dependence with  $fO_3$ , however, a sharp increase of  $\beta$   
201 was found with increasing temperature. At higher processing temperatures the concavity of  
202 the Weibull curve will increase and the lag time prior to inactivation will be shorter. This  
203 effect is opposite to the effect that temperature has on  $\log(\alpha)$ , however, in the present study  
204 the temperature effect on  $\log(\alpha)$  prevails and consequently at higher temperatures (up to  
205 25°C) less inactivation was found.

206

207 Considering the evidence for curvature on the  $\log(\alpha)$  and  $\beta$  values with ozone and  
208 temperature from Figure 6, a model with quadratic effects of ozone and temperature on the  
209 characteristic time and shape parameter was built. Additional parameters of dependence of the  
210  $\log(\alpha)$  with the ozone concentration and the flow rate were found to be significant. Table 4  
211 shows the estimated parameters from the Weibull model fit (All parameters were significant  
212 at  $p < 0.05$ ).

#### 213 4. Discussion

214 The extrinsic parameters investigated had significant influence on the efficacy of ozone. 5 log  
215 reductions were achieved in TSB, depending on the flow rate applied to the cell suspension.  
216 Others have reported on the effects of intrinsic factors on ozone inactivation including  
217 medium type and organic matter content. Ozone has a high oxidation potential, it reacts with  
218 microorganisms as well as with other particles and compounds if placed in an environment  
219 such as food systems that are rich in organic matter (Kim *et al.* 1999). The survival curves of  
220 *E. coli* in TSB treated with ozone showed a noticeable lag time before cell death was  
221 observed. Lag times were also observed in other studies, Chen *et al.* 1992 reported ca. 10 min  
222 lag time in 0.8% saline solution when *E. coli* was treated with 5mgO<sub>3</sub> L<sup>-1</sup> at a flow rate of  
223 0.1L min<sup>-1</sup>. Williams *et al.* (2005), studied the inactivation of *E. coli* in orange juice, and  
224 found the efficacy of ozonation was reduced in the presence of ascorbic acid and organic  
225 matter. A defined lag time was also observed in the current work when treatment was carried  
226 out in TSB. The ozone flow rate was found to be an important factor for inactivation. This  
227 may be due to the effect of gas flow rates on bubble size. A possible explanation could be that  
228 the bubble size generated at the higher flow rates was too large. At high flow rates a small  
229 number of large bubbles are produced, which rise to the liquid surface quickly, thereby  
230 escaping the medium quickly. The resulting poor gas dissolution reduces the contact time,  
231 leading to a lower inactivation rate. At low flow rates, small bubbles are produced, however  
232 as the amount of ozone applied is low, the corresponding inactivation is slow.

233 Lag times tended to increase with increasing temperature. Herbold *et al.* (1989) showed that  
234 ozone inactivation of hepatitis A virus and *E. coli* was faster at 10°C than at 20°C. Ozone  
235 solubility in water is 13 times that of Oxygen at 0-30°C (Rice 1986). The solubility ratio for  
236 ozone increases as the temperature of water decreases (Bablon *et al.* 1991). Ozone  
237 decomposition is faster at higher water temperatures (Rice *et al.* 1981). As the temperature

238 increases ozone becomes less soluble and less stable with an accompanying increase in the  
239 decomposition rate. Achen and Yousef (2001) treated *E. coli* contaminated apples with ozone  
240 at 4, 22 and 45°C, and observed that counts of the bacterium on the surface decreased by 3.3,  
241 3.7 and 3.4 log<sub>10</sub>-units, respectively. Steenstrup and Floros (2004), studied the effect of  
242 temperature (5–20°C) at 860 ppm (v/v) ozone and different gaseous ozone concentrations  
243 above 1,000 ppm on inactivation of *E. coli* O157:H7 in apple cider and reported D values  
244 ranging from 0.6 to 1.5 min at 20°C and 5°C, respectively. The temperatures employed in this  
245 study ranged from ambient to 30°C. *E. coli* is a mesophilic micro-organism and would not be  
246 inactivated at these temperatures in the absence of ozone. In the current study, a reduction >  
247 5 log cycles was achieved at ambient temperature (12-15°C) within 25 min. The antimicrobial  
248 activity of ozone decreased with increasing temperatures. This result showed that the efficacy  
249 of ozone inactivation depended on the medium type and organic matter content. Based on  
250 these investigations, to optimise the use of ozone for liquid systems, temperatures should be  
251 maintained at low to ambient, the maximum obtainable ozone concentration should be  
252 employed and the flow rate should be selected to maximise solubility thus minimising gas  
253 escape from the free surface. Tiwari *et al.* (2008a, b; 2009) recently studied the effects of  
254 ozone on quality and nutritional parameters for a range of fruit juices, highlighting significant  
255 losses in nutritional quality which were dependent on ozone control parameters of ozone  
256 concentration and gas flow rate. However, achieving rapid microbial inactivation using  
257 optimised control parameters may mitigate losses in nutritional quality. Ozone inactivation  
258 efficacy will be dependent on the food system employed and various fruit juices should be  
259 investigated further. The ozone inactivation kinetics of *E. coli* was well described by the  
260 Weibull model with high values for R<sup>2</sup>, indicating goodness of fit. The inactivation of *E. coli*  
261 displayed a downward concavity, with a  $\beta$  parameter > 1, indicating the susceptibility of  
262 remaining cells to the ozone treatment (van Boekel 2002).

263 The fitting of the microbial inactivation parameters with the process variables yielded a model  
264 that presented a dependence not only on the amount of ozone introduced per minute, but also  
265 a dependence on the total gas flux and the ozone concentration in the bubble. The final model  
266 points to a compromise region of intermediate ozone flow rates (around  $0.1 \text{ mg O}_3 \text{ min}^{-1} \text{ mL}^{-1}$ )  
267 <sup>1</sup>) at ambient temperatures.

## 268 **5. Conclusions**

269 This work shows the optimised critical control parameters governing ozone inactivation of *E.*  
270 *coli* in a liquid system. Control of these parameters is essential for optimised inactivation to  
271 meet food safety requirements. Inactivation of *E. coli* was rapid at ambient temperature, this  
272 has the advantage of less energy consumption than traditional thermal pasteurisation  
273 processes, while minimising the effects on quality parameters.

274

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359 **Table 1:** Ozone concentrations at two selected gas flow rates

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<b>Levels of Ozone concentration</b>	<b>Flow rate 0.06L min<sup>-1</sup> concentration (µg/ml)</b>	<b>Flow rate 0.12L min<sup>-1</sup> concentration (µg/ml)</b>
Level 1	28-49	17-28
Level 2	60-70	33-40
Level 3	82-87	46-56
Level 4	103-114	67-70
Level 5	115-120	72-75

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382 **Table 2:** Parameters of the Weibull model and extrinsic control parameters for ozone  
 383 inactivation of *E. coli*

Extrinsic parameters	$fO_3$ [mg O <sub>3</sub> min <sup>-1</sup> ml <sup>-1</sup> ]	$\alpha$ [min]	$\beta$ []	$R^2$	$t_{d5}(\text{min})$ inactivation time
<b>Gas flow rate (L min<sup>-1</sup>)</b>					
0.03	0.045	22.98±0.51*	3.07±0.23	0.99	38.82
0.06	0.078	16.88±0.20	4.48±0.13	0.99	24.2
0.12	0.098	5.53±2.62	1.25±0.4	0.95	20
0.25	0.145	11.65±1.67	2.27±0.4	0.98	23.7
0.5	0.144	16.60±0.81	3.83±0.44	0.99	29.5
0.75	0.170	15.31±0.67	2.28±0.19	0.99	31
<b>Temperature(°C)</b>					
Ambient	0.098	5.53±2.62	1.25±0.4	0.95	20
20	0.087	19.18±0.93	4.08±0.13	0.99	28.5
25	0.087	27.09±0.37	4.22±0.42	0.99	39.7
30	0.087	23.6±0.45	7.46±0.48	0.99	29.3
<b>Concentration (levels)</b>					
0.06L min <sup>-1</sup> flow rate					
1	0.025	-	-	-	-
2	0.043	-	-	-	-
3	0.056	25.68±0.71	2.38±0.38	0.98	50.54
4	0.072	22.40±0.39	2.54±0.22	0.99	42.2
5	0.078	16.88±0.20	4.48±0.13	0.99	24.18
<b>Concentration (levels)</b>					
0.12L min <sup>-1</sup> flow rate					
1	0.03	-	-	-	-
2	0.048	37.01±4.28	1.52±0.39	0.96	107.04
3	0.068	18.3±0.61	3.70±0.37	0.99	28.27
4	0.087	15.50±0.34	3.51±0.16	0.99	24.5
5	0.098	5.53±0.62	1.25±0.37	0.95	20

384 (\* Standard error, (-) not determined, ( $R^2$ ) Coefficient of determination

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389 **Table 3: Final candidate model for the inactivation of *E. coli* with ozone bubbling. All**  
 390 **parameters significant at p<0.05 level.**

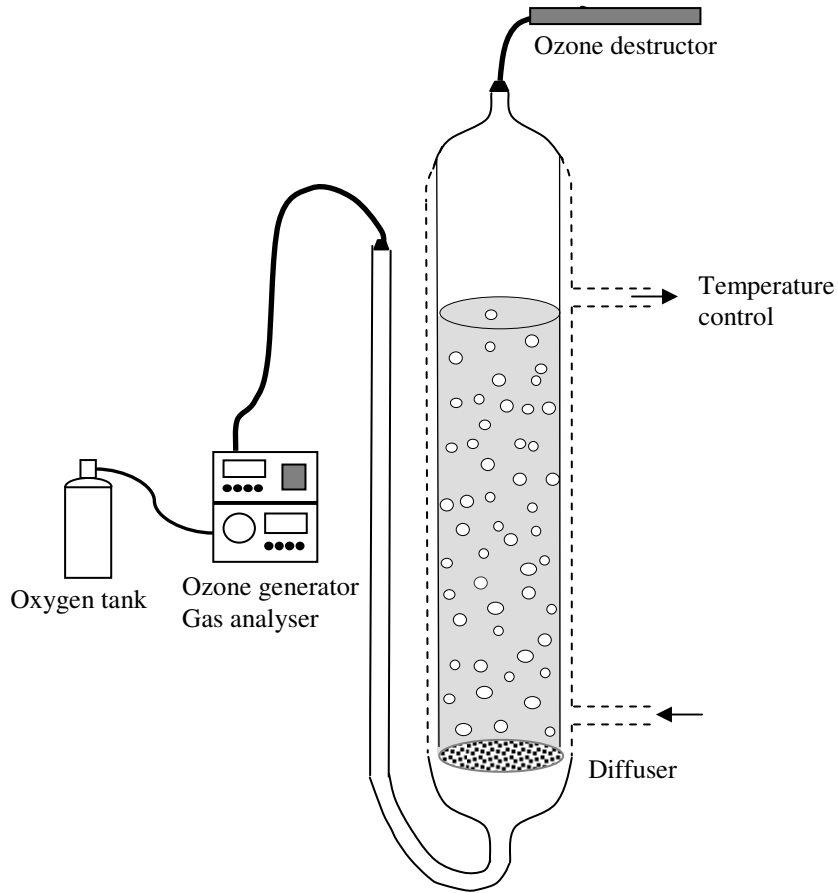
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Parameter	Estimates
Log $\alpha$	$1.7_{(0.3)} - 47_{(5)} \times fO_3 + 192_{(23)} \times fO_3^2 + 0.281_{(0.02)} \times T -$ $0.0053_{(0.0005)} \times T^2 + 1.1_{(0.2)} \times \text{flow.rate} + 0.006_{(0.001)} \times [\text{Ozone}]$
$\beta$	$-5.2_{(1.1)} + 0.52_{(0.08)} \times T$
	<b>Model Fit</b>
RMSE*	0.732
SSQ	172.81
$R^2$	0.91
$R^2_{\text{adj}}$	0.90
n	115

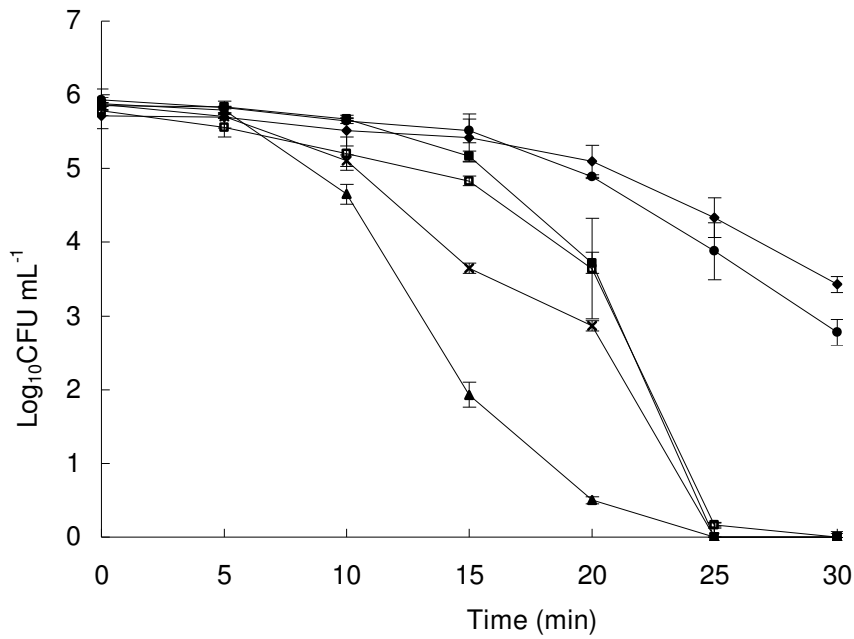
392 \*RMSE: Root Mean Square error, SSQ: Sum of squares of data,  $R^2$ : Coefficient of  
 393 determination,  $R^2_{\text{adj}}$ : Adjusted R-square, n: Number of experiments

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**Figure 1: Schematics of the ozone processing equipment.**



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433 **Figure 2:** Ozone inactivation of *E. coli* at different flow rates. Flow rates: (◆) 0.03, (■) 0.06,

434 (▲) 0.12, (×) 0.25, (□) 0.5, (●) 0.75. Error bars represent Standard Deviation. .

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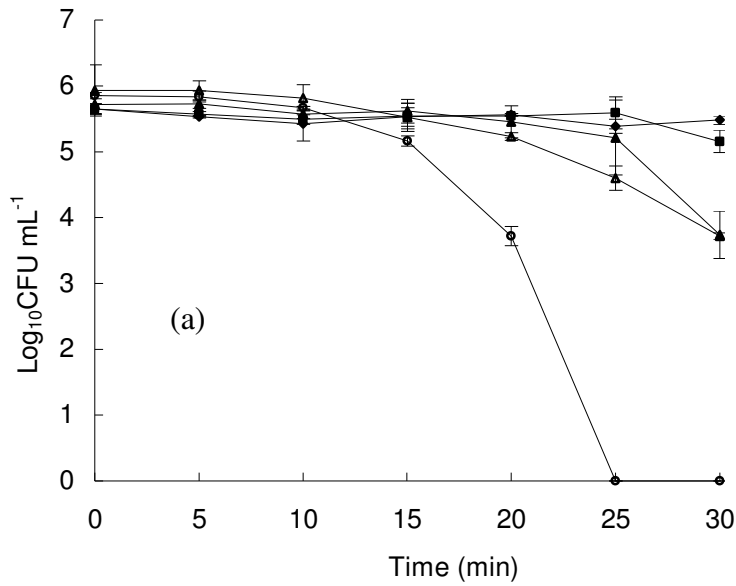
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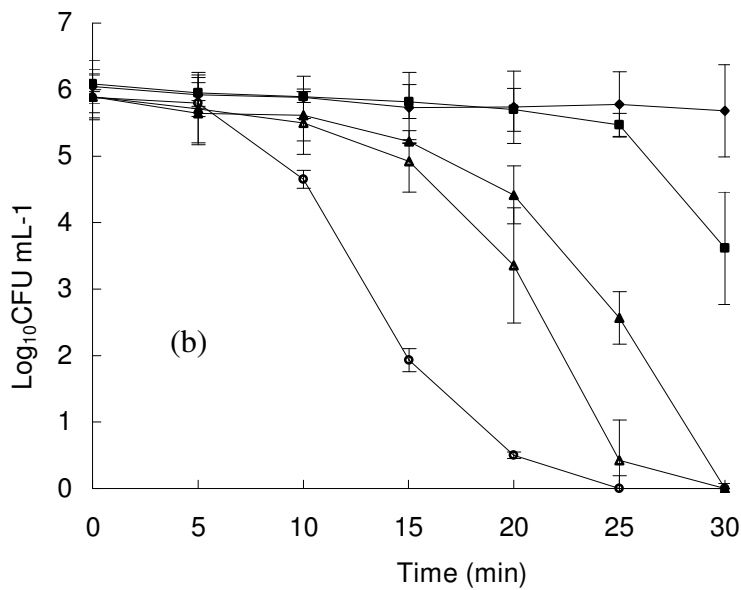
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446 **Figure 3:** Effect of ozone concentration on the inactivation of *E. coli*(a) At 0.06L min<sup>-1</sup> flow

447 rate ozone concentrations: (◆) 0.025 mg O<sub>3</sub> min<sup>-1</sup> ml<sup>-1</sup>, (■) 0.043 mg O<sub>3</sub> min<sup>-1</sup> ml<sup>-1</sup>, (▲) 0.056

448 mg O<sub>3</sub> min<sup>-1</sup> ml<sup>-1</sup>, (△) 0.072mg O<sub>3</sub> min<sup>-1</sup> ml<sup>-1</sup>, (○) 0.078 mg O<sub>3</sub> min<sup>-1</sup> ml<sup>-1</sup>

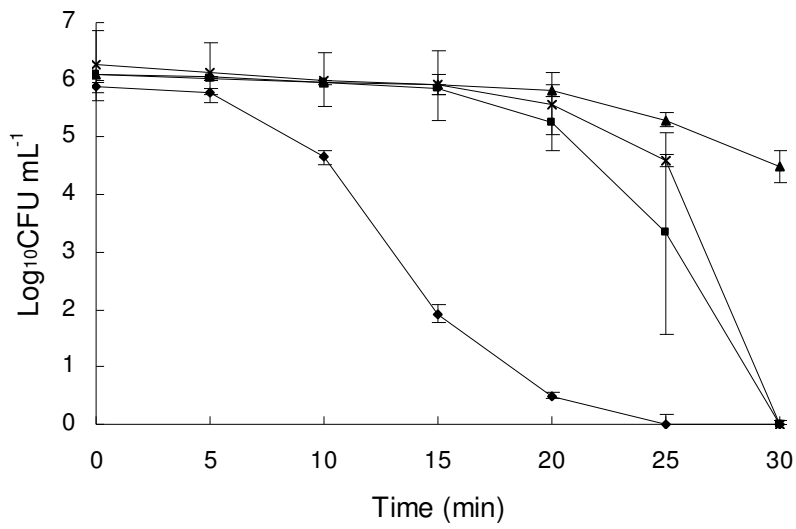
449 (b) At 0.12L min<sup>-1</sup> flow rate ozone concentrations: (◆) 0.03 mg O<sub>3</sub> min<sup>-1</sup> ml<sup>-1</sup>, (■) 0.048 mg

450 O<sub>3</sub> min<sup>-1</sup> ml<sup>-1</sup>, (▲) 0.068 mg O<sub>3</sub> min<sup>-1</sup> ml<sup>-1</sup>, (△) 0.087 mg O<sub>3</sub> min<sup>-1</sup> ml<sup>-1</sup>, (○) 0.098 mg O<sub>3</sub> min<sup>-1</sup>

451 ml<sup>-1</sup>. Error bars represent Standard Deviation.

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455 **Figure 4:** Effect of temperature on the efficacy of ozone inactivation of *E. coli*

456 (◆) ambient, (■) 20°C, (▲) 25°C, (×) 30°C. Error bars represent Standard Deviation.

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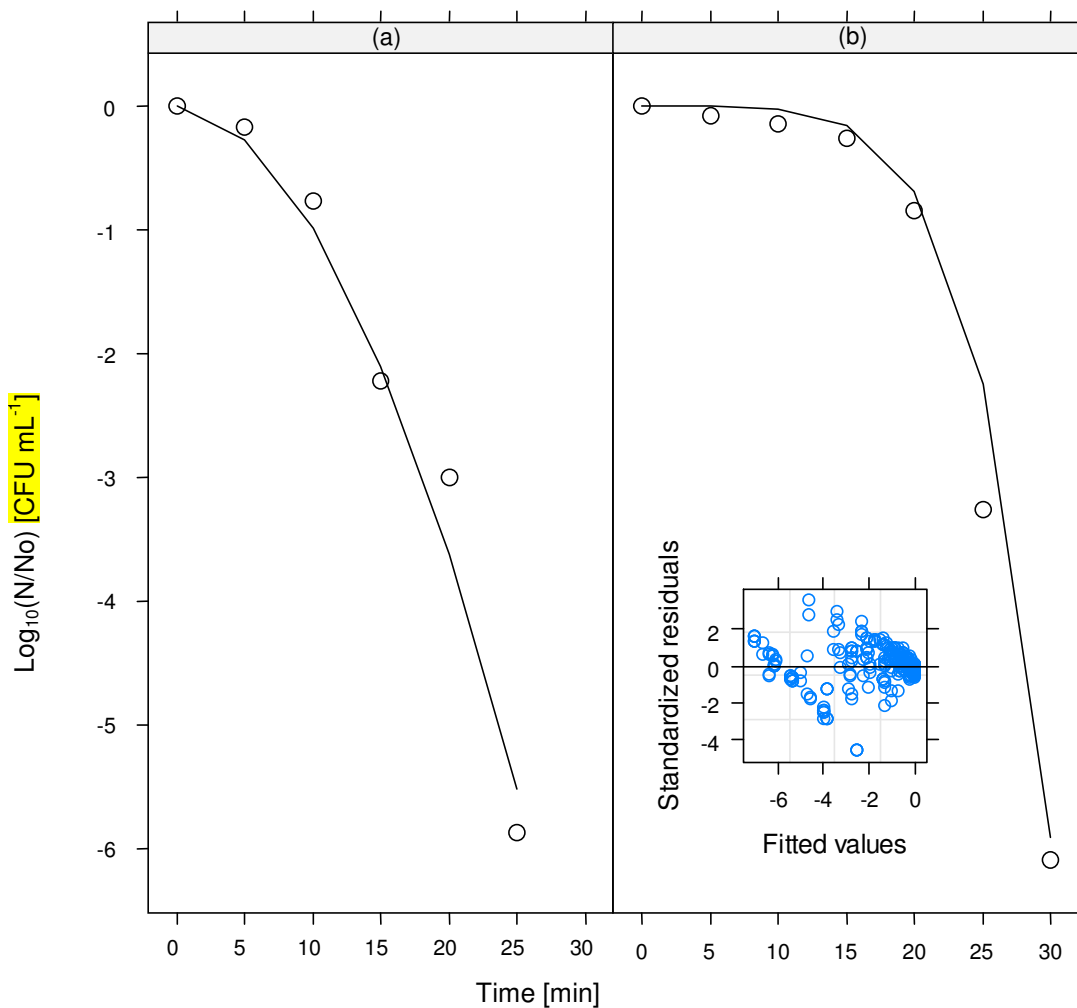
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469 **Figure 5:** Typical Weibull model fit (continuous line) for the ozone inactivation experimental  
 470 data. The insert of (b) shows the standardized residual vs the fitted values, indicating a  
 471 satisfactory fit.

472 (a) 0.145 mg Ozone min<sup>-1</sup> ml<sup>-1</sup> at ambient temperature

473 (b) 0.087 mg Ozone min<sup>-1</sup> ml<sup>-1</sup> at 20°C.

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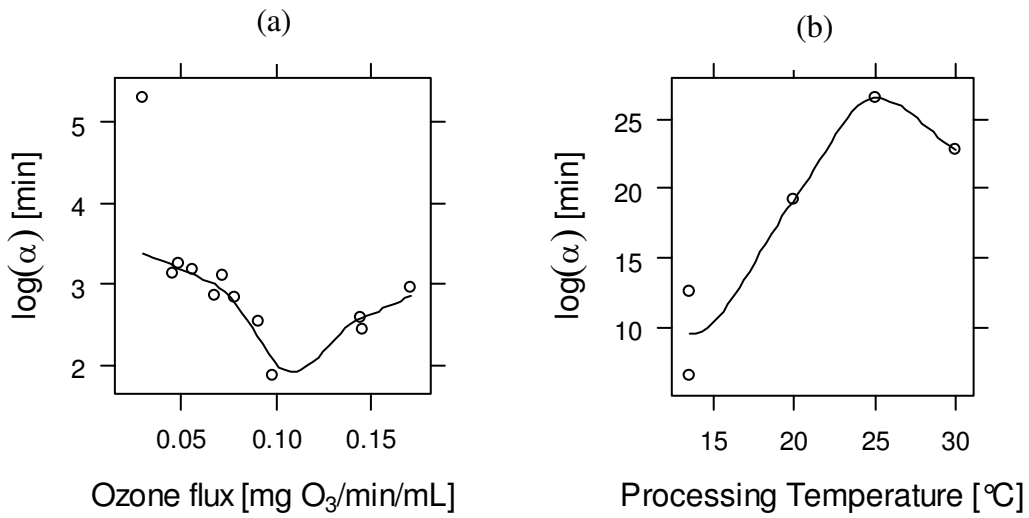
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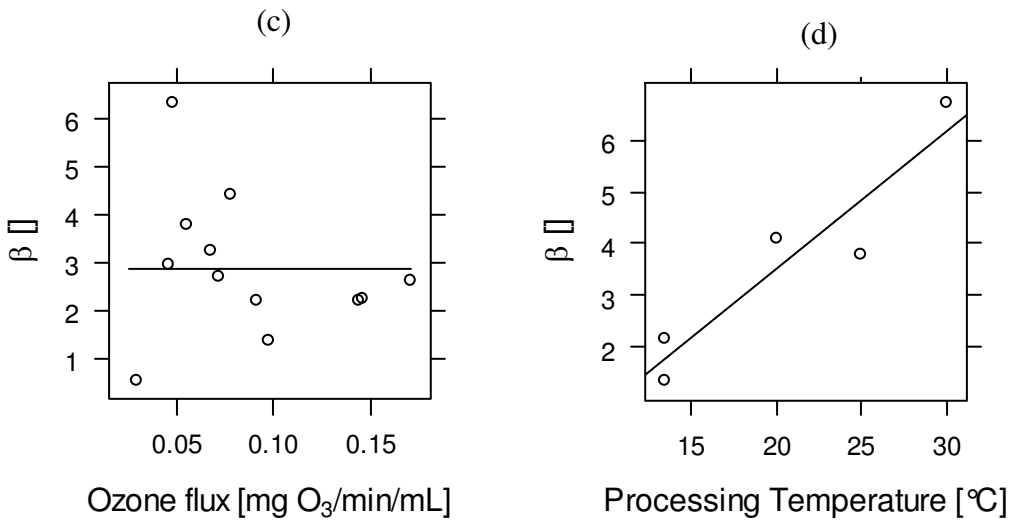
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483 **Figure 6:** Relationship of the log of the characteristic time  $\alpha$  (a) with the ozone processing  
484 conditions at ambient temperature and (b) with the processing temperature at  $0.09 \text{ mg O}_3 \text{ min}$   
485  $^{-1} \text{ mL}^{-1}$  and the shape parameter relationship  $\beta$  with (c) the ozone processing conditions at  
486 ambient temperature and (d) the temperature at  $0.09 \text{ mg O}_3 \text{ min}^{-1} \text{ mL}^{-1}$  of reactor volume. The  
487 continuous line represents a smoothing of the estimates.