

The effect of interfering substances on the disinfection process: a mathematical model

R.J.W. Lambert and M.D. Johnston

Unilever Research Colworth, Sharnbrook, Bedfordshire, UK

781/2/01: received 9 February 2001, revised 18 April 2001 and accepted 18 April 2001

R.J.W. LAMBERT AND M.D. JOHNSTON. 2001.

Aims: To gain a greater understanding of the effect of interfering substances on the efficacy of disinfection.

Methods and Results: Current kinetic disinfection models were augmented by a term designed to quantify the deleterious effect of soils such as milk on the disinfection process of suspended organisms. The model was based on the assumption that inactivation by added soil occurred at a much faster rate than microbial inactivation. The new model, the fa_r -soil model, was also able to quantify the effect of changing the initial inoculum size (1×10^7 – 5×10^7 ml⁻¹ of *Staphylococcus aureus*) on the outcome of the suspension tests. Addition of catalase to the disinfection of *Escherichia coli* by hydrogen peroxide, resulted in changes to the shape of the log survivor/time plots. These changes were modelled on the basis of changing biocide concentration commensurate with microbial inactivation.

Conclusions: The reduction in efficacy of a disinfectant in the presence of an interfering substance can be quantified through the use of adaptations to current disinfection models.

Significance and Impact of the Study: Understanding the effect of soil on disinfection efficacy allows us to understand the limitations of disinfectants and disinfection procedures. It also gives us a mechanism with which to investigate the soil tolerance of new biocides and formulations.

INTRODUCTION

Disinfection is the reduction in numbers of organisms, principally of those capable of causing a deleterious effect to some operation or product. Disinfection is but one part of a process to create a hygienic production line or work area after or before use. In a normal two-step process, cleaning and rinsing occur before disinfection. Removal of post-production debris and rinsing to remove the cleaning fluids allows optimum disinfection to occur. In an investigation of the effect of surface cleaning Schmidt (1989) concluded: 'different amounts of dirt and bacteria are left behind on surfaces depending on the thoroughness of cleaning. The inactivation of disinfectants by dirt residues can be cancelled out by increasing concentration and extending the time that the disinfectants are allowed to act.'

Disinfectants can be seriously affected by the presence of organic matter; for example, iodophor and chlorine disinfectants are 'notoriously sensitive to organic soil' (Pryor and Brown 1975). Hard water also reduces the effectiveness of disinfectants and this effect is included in the AOAC official tests of detergents and sanitizers and also in the European Union's legislative disinfectant efficacy tests (Davis 1990; Holah 1995).

Bloomfield and Miles (1979) reported that in the presence of milk soil there was a rapid loss of chlorine available from hypochlorite solutions for the disinfection of *Escherichia coli*. At 2% milk soil, the efficacy of disinfection had been reduced by 8 log₁₀ numbers. They suggested that the loss was caused by organic material, either in the form of bacterial cells or milk, reacting with the disinfectant. Jono *et al.* (1986) examined the effect of bovine serum albumin (BSA) on the bactericidal activity of quaternary ammonium compounds (QACs). It was demonstrated that in 2% BSA, up to 80% of the dose of QAC given was bound up with the BSA. In the presence of human serum or yeast extract between 10 and 100 times more QAC was required to achieve a specified level of disinfection.

Correspondence to: Dr Ronald J.W. Lambert, Unilever Research Colworth, Sharnbrook, Bedfordshire MK44 1PT, UK
(e-mail: ronnie.lambert@unilever.com).

Residual cleaning fluids can also have a deleterious affect on disinfection power: anionic surfactants neutralize QAC disinfectants on a molar basis, whereas phenolic-type disinfectants are completely incompatible with non-ionic surfactants (Pryor and Brown 1975).

One simple way to show the effect of soil on a disinfectant is to compare the disinfectant's minimum inhibitory concentration (MIC) against its biocidal concentration. Normally an MIC is lower than a biocidal concentration, since an MIC is the level required to inhibit growth, whereas a biocidal concentration is one that kills off organisms. Baldry (1983) reported that the MIC of peracetic acid was 0.33 mM whereas the biocidal concentration was about three times lower at 0.13 mM. The biocide test differs from the MIC test in that the latter test is performed in growth medium, which may be capable of reacting with the test disinfectant. In work carried out in this laboratory on a hypochlorite-based disinfectant, the MIC was found to be greater than 0.2% in Tryptone Soya Broth, whereas five log reductions occurred in 5 min with only 0.04% when the test was performed with distilled water. We believe this difference was due to the reaction of the hypochlorite with the 'soil' of the growth medium used in the MIC experiment.

The basic equation of disinfection kinetics is the Chick–Watson model (CW) (Chick 1908; Watson 1908). That model states that the change in the logarithm of the viable numbers of organisms is directly related to the disinfectant concentration raised to a power, known as the dilution coefficient (Hugo and Denyer 1987) (eqn 1.1). However, the CW model assumes a constant biocide concentration throughout the disinfection. If the biocide concentration decreased due to reaction with 'soil' then the efficacy of the disinfection would be expected to decrease by the power of the dilution coefficient. For biocides such as peroxide this exponent is approximately 1, whereas phenolic biocides have dilution coefficients of approximately 6. In the former case, reduction of biocide by half reduces the effectiveness by half, whereas in the latter case reduction by half reduces the effectiveness to under 2% of its previous value.

$$\log R = K[B]^n t \quad (1.1)$$

where $\log R$ = the decimal log reduction in microbial numbers, K = the disinfection rate constant, $[B]$ = biocide concentration, n = dilution coefficient and t = time.

The Intrinsic Quenching model (IQ, eqn 1.2) attempts to take into account the ability of 'microbial soil' to limit the effectiveness of the biocide (Lambert and Johnston 2000). The Quenching parameter, Q , is a measure of the intrinsic, microbial, level of quenching within a disinfectant test, and is made up made up of two constants: K_q the rate of biocide inactivation and n the dilution coefficient of the disinfectant. Figure 1 displays the effect of a reducing biocide concentration on the rate of disinfection for a biocide with $n = 3$.

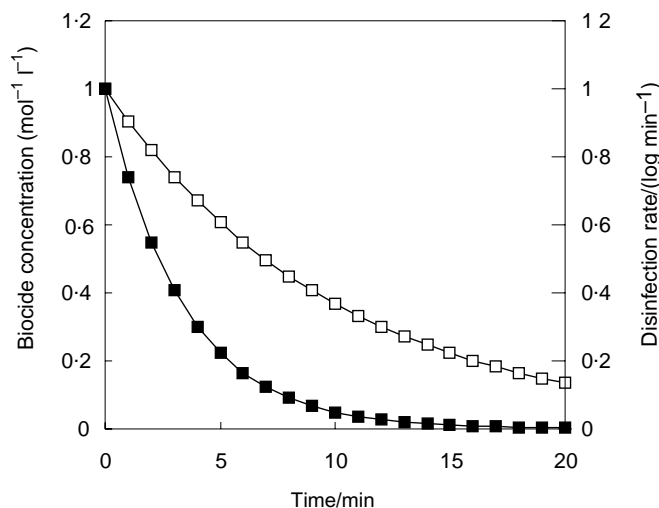


Fig. 1 The effect of decreasing biocide concentration on disinfection rate for a hypothetical disinfectant examined using the IQ model: disinfection parameters of $K = 1 \log R \min^{-1}$, $K_q = 0.1 \min^{-1}$, $B = 1 \text{ mol l}^{-1}$ and $n = 3$: ■, disinfection rate; □, biocide concentration

$$\log R = K[B]^n \left(\frac{1 - \exp(-Qt)}{Q} \right) \quad (1.2)$$

where $Q = K_q n$.

In general, kinetic experiments in this laboratory are carried out in 'clean' conditions, i.e. distilled water with no added soil such as bovine albumin or milk. The disinfection obtained can therefore be considered as the optimum disinfection rate for a given level of biocide. To obtain such results in a factory environment would require efficient cleaning and no complicating factors such as hard water, which can reduce the effectiveness of certain disinfectants (Johns 1947, 1948; Pryor and Brown 1975). However, such conditions are rarely met within a factory environment: inefficient cleaning, residual cleaning fluids and hard water are the realities.

To be able to understand the limitations that the presence of soil places on the effectiveness of disinfectants a mathematical model of the situation was required. Herein is reported our attempts to quantify the effect of soil on the disinfection process.

METHODS, MICROBES AND MODELS

Preparation of bacterial suspensions

Staphylococcus aureus (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 15442) or *Escherichia coli* (ATCC 11229) was grown overnight in a flask containing 80 ml Tryptone Soya Broth, TSB (Oxoid CM 129) shaking at 30°C. The culture was centrifuged at 4000 r.p.m. (510 g, Sigma model 3K-1) for 10 min. The resulting cell pellets were pooled and resuspended

in 0.1% peptone water. An inoculum level of $3 \times 10^8 \text{ ml}^{-1}$ was used, to ensure a good degree of reproducibility optical density was used via reference to a standardized O.D._{600nm}/cell-numbers curve.

Suspension tests

The disinfection method has been published previously (Lambert *et al.* 1998; Johnston *et al.* 2000; Lambert 2001), the method of analysis uses the ratio of the area under a test O.D.-time curve to the area of the control O.D.-time curve (Lambert 2001). For the milk soil studies, four surfactant biocides – three cationic QACs and the amphoteric surfactant EmpigenBB were chosen and a concentration, for each, which gave approximately 5 log reductions in *Staph. aureus* ($3 \times 10^8 \text{ ml}^{-1}$) in 36 min obtained. To this concentration of biocide was added the required level of milk (semi-skimmed, purchased daily from a local supermarket), immediately prior to the disinfection test.

Induction of tailing by enzymes

Hydrogen peroxide (Sigma-Aldrich, Dorset, UK) was diluted to appropriate concentrations (w/v) in distilled water prior to use. The quenching agent used in these experiments was catalase-universal quench. This was prepared each day by mixing 9 ml of universal quenching agent with 100 μl of a 1% filter sterilized catalase solution (Sigma-Aldrich). Disinfectant tests were carried out as described above, except that concentrations of catalase were added to the bacterial suspension prior to addition of the disinfectant.

Models used

Although the IQ model (1.2) is an adequate descriptor of disinfection kinetics recent developments in kinetics, Lambert (2001) has offered a more suitable model: the f_{a_t} model of disinfection states that:

$$f_a = \exp \left\{ - \left(\frac{x}{P_1} \right)^{P_2} t^{P_3} \right\} \quad (2.1)$$

where f_a is the fractional area (the ratio of test area to control area, obtained from the O.D.-time curves), x is the biocide concentration, t is the time of biocide contact, P_1 is the concentration of biocide at the inflexion point of the f_{a_t} – log x curve, P_2 is the concentration exponent and P_3 is the time exponent. It has also been shown that log reduction in the numbers of organisms and f_a are equivalent measures of microbial inactivation when analysed by the Bioscreen method (Lambert 2001) and are related by the equation:

$$\log R = M(1 - f_a) \quad (2.2)$$

where M is the maximum number of log reductions achievable (equivalent to log I_0).

For a constant concentration of biocide equation 2.1 reduces to

$$f_a = \{-KT^{P_3}\} \quad (2.3)$$

Inoculum size model

To examine any inoculum size dependency on the disinfection test we assumed that the constant, K , is directly related in some way to the initial inoculum size, I_0 .

$$K = K' I_0^i \quad (2.4)$$

where K' is a constant and i is the inoculum exponent, giving:

$$f_a = \exp \{-K' I_0^i t^{P_3}\} \quad (2.5)$$

Disinfection–soil models

The reduction in disinfection efficacy is hypothesized to be due to the soil reacting in some way with the disinfectant and ‘quenching’ its effect. Essentially, the effective disinfectant concentration is reduced. There are two ways of approaching the problem of modelling the effect of soil: (1) assume that the reaction with the soil is faster than microbial inactivation, giving a residual disinfectant concentration model, or (2) assume that the inactivation of biocide by soil is commensurate with microbial inactivation.

For a constant concentration of biocide and inoculum size the following empirical law, the f_{a_t} -soil model (2.6), was used for the effect of added soil on the disinfection test. When the added soil level is zero, $f(\text{soil}) = 0$, equation 2.1 is regained.

$$f_a = \exp \{-K(1 + f(\text{soil}))^m t^{P_3}\} \quad (2.6)$$

where $(1 + f(\text{soil}))^m$ is an empirical function of the soil.

The simplest function that has been found for the soil is

$$f(\text{soil}) = S^r \quad (2.7a)$$

where S = percentage of soil present and r is termed the soil exponent. Other empirical soil functions such as:

$$f(\text{soil}) = K_1 S^2 + K_2 S \quad (2.7b)$$

have also been used successfully.

The IQ model of disinfection (Lambert and Johnston 2000) attempts to model the non-linearity of log-survivor curves by hypothesizing that microbial loading itself acts as a soil towards the disinfectant, reducing its effective concentration during the disinfection test. The appropriate modifications to the f_{a_t} model can be made to encompass this idea:

$$fa = \exp \left\{ -K(1 + f(\text{soil}))^m \left(\frac{1 - \exp(-Qt)}{Q} \right) \right\} \quad (2.8)$$

where Q is the level of intrinsic (microbial) quenching.

In all of these models, the inactivation of the biocide with the soil is considered to be faster than the microbial inactivation of the biocide. In essence the term $(1 + f(\text{soil}))^m$ is a factor reducing the magnitude of K , through inactivation of biocide.

Statistics and modelling package

The models were fitted to the observations using the JMP statistics package (SAS Institute, Cary, NC, USA). Using the package in the non-linear fitting platform, the parameters were obtained. The root mean square error (RMSE) has been quoted as the goodness of fit statistic. In Tables 1, 2, 3 and 4, the R^2 statistic refers to the R^2 of the plot of observable against calculated values.

RESULTS

Inoculum size effects

Benzalkonium chloride (0.0002%) gave approximately 5 log reductions in 36 min against a $1 \times 10^7 \text{ ml}^{-1}$ culture of *Staph. aureus*. Increasing the inoculum size from $1 \times 10^7 \text{ ml}^{-1}$ to $5 \times 10^7 \text{ ml}^{-1}$ reduced the efficacy of the disinfection. An inoculum size of $3 \times 10^8 \text{ ml}^{-1}$ exposed to

this concentration of benzalkonium chloride gave no reductions in microbial viable numbers. Results of the analysis of the data obtained using eqn 2.5 are given in Table 1. An examination of the inoculum size effect on the disinfection of *Staph. aureus* with hypochlorite (0.00125%) gave similar results. From the values of the inoculum exponent, i , for benzalkonium and hypochlorite the level of inactivation is dependent, approximately, on the inverse of the square root of the inoculum size, for SDS at pH 4, the relationship is approximately inversely related.

Disinfection of *Staph. aureus* in the presence of milk soil

Addition of milk soil to the surfactant biocides caused a severe decrease in disinfection efficacy. At the highest levels of soil used (1%) complete attenuation of disinfection occurred. The data were amenable to analysis by the fa_t -soil model (2.6) and the IQ-variation of this model (2.8); the results are given in Table 2. The values obtained for K are similar, reflecting the use of concentrations of the biocides to achieve a similar level of disinfection in 36 min. Through an analysis of the r and m exponents, the activity of EmpigenBB is reduced to 56% in the presence of 0.1% milk soil, and to 1.6% of activity in the presence of 1% milk soil. Similarly, C12QAC is reduced to 51% and 8% activity in the presence of 0.1 and 1% milk soil, respectively. Figure 2 shows a stereo-view of the effect of added milk soil on the disinfection of *Staph. aureus* by C14QAC, the calculated grid (fa_t -soil model), and the superimposed observations are in good agreement. These findings are also in general agreement with those found by Bloomfield and Miles (1979) where efficacy was reduced by 8 log numbers with 2% milk soil.

The P_3 (and the Q) values obtained from the model show a small standard deviation in their value (results not shown). This suggests that inactivation with milk soil had occurred before addition to the microbes, i.e. the assumption that reaction or inactivation with the milk soil would be faster than inactivation of the microbes appears valid. If a large

Table 1 Modelled parameters for the disinfection of *Staph. aureus*

fa_t -Inoculum model (eqn 2.5)	Conc.	K'	i	P_3	RMSE (R^2)
Hypochlorite	0.00125%	29.86	-0.44	1.556	0.053 (0.950)
Benzalkonium	0.0002%	2007	-0.515	0.258	0.027 (0.954)
SDS/pH 4	0.03%	1.4×10^8	-1.26	0.908	0.065 (0.947)

Table 2 Modelled parameters for the disinfection of *Staph. aureus* in the presence of milk soil

Disinfectant	model	K	r	m	P_3/Q^*	RMSE (R^2)
EmpBB	fa_t -soil	0.121	0.990	-5.99	0.775	0.032 (0.983)
EmpBB	fa_t -IQ	0.091	0.996	-5.93	0.039*	0.025 (0.990)
C12QAC	fa_t -soil	0.133	0.696	-3.69	0.579	0.048 (0.918)
C12QAC	fa_t -IQ	0.0789	0.698	-3.66	0.077*	0.040 (0.942)
C14QAC	fa_t -soil	0.115	0.681	-4.97	0.916	0.038 (0.980)
C14QAC	fa_t -IQ	0.107	0.691	-4.89	0.0198*	0.035 (0.984)
C18QAC	fa_t -soil	0.188	0.909	-3.24	0.796	0.067 (0.948)
C18QAC	fa_t -IQ	0.137	0.901	-3.24	0.026*	0.069 (0.945)

fa_t -soil model (eqn 2.6) fa_t -IQ (eqn 2.8). Q^* = value of Q in fa_t -IQ model.

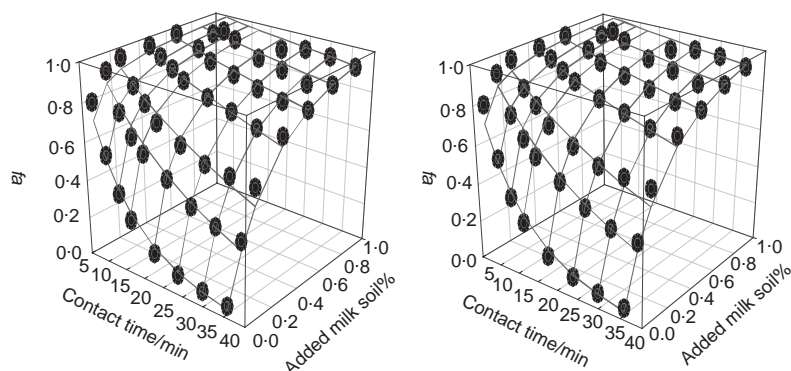


Fig. 2 Stereoview of the disinfection of *Staph. aureus* by C14QAC in the presence of added milk soil. The grid is the calculated fractional area (f_a , eqn 2.6), the symbols are the observations

deviation had occurred this would be indicative of inactivation during the disinfection test itself, and would be dependent on the levels of milk soil present, which was not the case.

A full disinfection study of C12QAC in clean conditions was conducted and the results analysed by the f_a -model (2.1). Figure 3 shows a plot of the f_a vs disinfection contact time for 0.0025% C12QAC calculated using 2.1 and that calculated from 2.6 using the parameters in Table 2. Good agreement between the two models was found for the zero soil level. Figure 3 also shows the effect of increasing soil

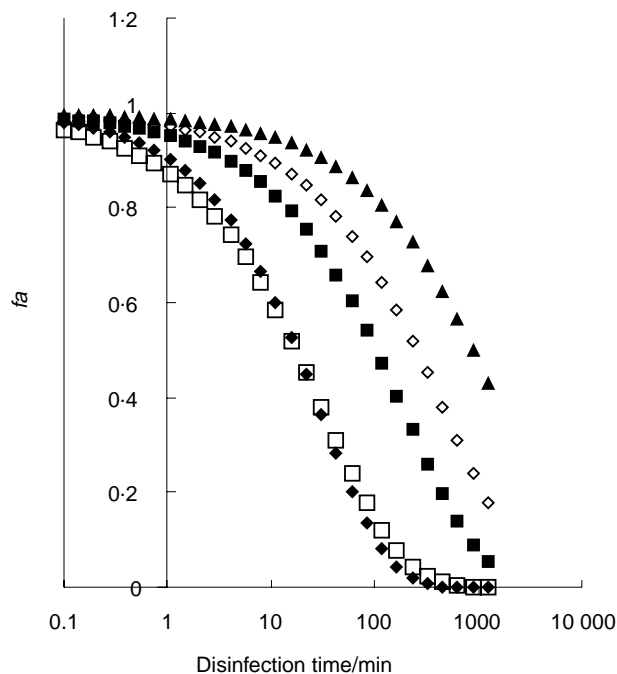


Fig. 3 The calculated (eqn 2.6, Table 2) effect of added milk soil on the disinfection of C12QAC (0.0025%) against *Staph. aureus*: added milk soil; □, 0%; ■, 0.2%; ◇, 0.4%; ▲, 0.8%; Calculated f_a values for C12QAC (0.0025%) (eqn 2.1) with parameters $P_1 = 0.008$, $P_2 = 1.99$, $P_3 = 0.68$, ◆

levels on the time to achieve any specific level of disinfection.

SDS and added dead cell contents vs *Staph. aureus*

In these experiments (for method see Johnston *et al.* 2000), a level of dead cell contents were added to a test sample of *Staph. aureus* (1×10^8 ml $^{-1}$). Thus, we argued, the added dead cell contents would act as a soil in the disinfection process and therefore the f_a -soil model (2.6) should be applicable. Previously, a power relationship with the soil, i.e.

$$f(\text{soil}) = S^r$$

had been used, in this case we assumed that $f(\text{soil}) = K_i I_+$, i.e.

$$f_a = \exp\{-K(1 + K_i I_+)^m t^{P_3}\} \quad (3.1)$$

where I_+ is the level of dead cell contents added in microbes ml $^{-1}$, and K_i is a constant.

Table 3 gives the parameters obtained and Fig. 4 shows a plot of the observed f_a against the calculated f_a .

Catalase addition experiments

In the previous studies, the disinfectant and the soil were mixed prior to addition to the live microbes. This allowed the modelling to be conducted on the basis of a 'residual' disinfectant concentration. The small deviation in the P_3 value was taken as evidence that this assumption was

Table 3 Modelled parameters (eqn 3.1) for the disinfection of *Staph. aureus* by sodium dodecyl sulphate in the presence of added dead cell contents

K	m	P_3	K_i	RMSE (R^2)
0.696	-7.35	0.559	2.8×10^{-9}	0.0244 (0.988)

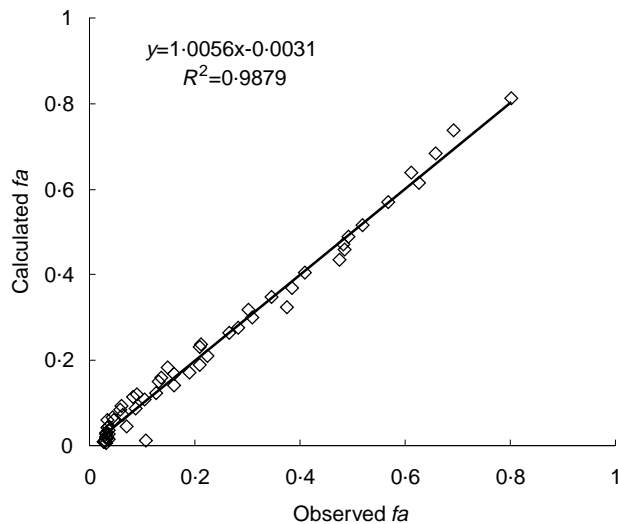


Fig. 4 Relationship between observed and calculated fractional area (fa) for the addition of dead cell contents to the disinfection of *Staph. aureus* by SDS. Calculated = $1.006(\text{observed}) - 0.003$, $r^2 = 0.988$

appropriate. If, we hypothesized, the reduction in biocide occurred during the disinfection test then changes in the P_3 value dependent on the concentration of soil should occur. To test this hypothesis we investigated the effect of catalase on the disinfection of *E. coli* by hydrogen peroxide (1%).

We had already shown that catalase positive bacteria at high inoculum levels can quickly breakdown added hydrogen peroxide such that no reduction in viable organisms was observed (Johnston *et al.* 2000). The breakdown of peroxide to water and oxygen by catalase occurs during the experiment, therefore it was believed that addition of quantities of catalase to the disinfection test would affect the shape of the fa_t curves.

The effect of the addition of catalase on the log reduction-time curves is shown in Fig. 5a. With increasing amounts of catalase, the rate of disinfection is reduced substantially. The data was found to fit the following equation:

$$fa = \exp \left\{ -K(1 + K_2 \text{Cat})t^{(P_3 + P_4 \text{Cat})} \right\} \quad (3.2)$$

The parameters obtained are given in Table 4. Without the catalase dependency on the time exponent, the root mean square error (RMSE) was 0.087. Figure 5b compares the disinfection of hydrogen peroxide (1%), calculated using the fa_t model, with the levels of disinfection obtained from eqn 3.2. A small discrepancy exists between the fa -time profile for the zero catalase experiments. Increasing the amount of added catalase decreases the rate of disinfection. On a logarithmic time scale, the shape of the fa -time plot changes with increasing catalase – compare with Fig. 3, which has a constant curve shifted to the right with increasing milk soil.

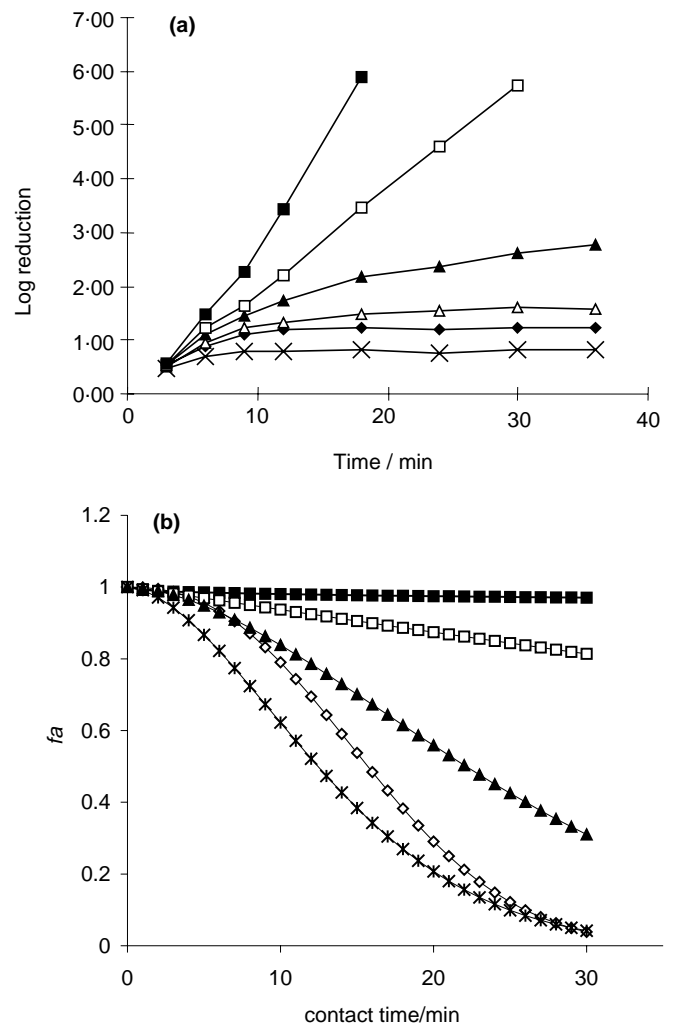


Fig. 5 (a) Effect of added catalase on the disinfection of *E. coli* by hydrogen peroxide. Added catalase: ■, 0%; □, 0.0006%; ▲, 0.001%; △, 0.0011%; ◆, 0.0012%; ×, 0.0015%. (b) The calculated (eqn 3.2, Table 4) effect of added catalase on the disinfection of *E. coli* with hydrogen peroxide (1%): added catalase; ◇ 0%; ■ 0.0015%; □ 0.001%; ▲ 0.0005%. Calculated fa values for hydrogen peroxide (1%, eqn 2.1) with parameters $P_1 = 5.8$, $P_2 = 2.69$, $P_3 = 1.73$ ×

DISCUSSION

It is known that in the presence of soils, such as blood and dairy soils, disinfectants are less active (Jones *et al.* 1986). It is believed that the disinfectants are either adsorbed by the soil and are therefore inactivated, or react with the soil and become inactivated. Thus in any system containing microbes and soil, there will be two parts to the biocide inactivation – the microbial (or intrinsic) quenching and the soil (or extrinsic) quenching. Legislative, disinfectant suspension tests (e.g. DIS/TSS2 1979; Anon 1994; Holah 1995) apply a narrow range of microbial numbers (e.g. 1×10^8 – 5×10^8

Table 4 Modelled parameters (eqn 3.2) for the disinfection of *Staph. aureus* by hydrogen peroxide in the presence of added catalase

K	K_2	P_3	P_4	RMSE (R^2)
9.58×10^{-4}	4997	2.39	-1339	0.022 (0.991)

organisms ml^{-1}), and also allow for the presence of hard water and interfering substances such as bovine albumin. These additions to the tests attempt to control known problems and ensure relevance (Bloomfield 1995). In the work described herein we have attempted to quantify some of these effects, and to show that (1) the effects *can* be quantified and that (2) the observations can be rationalized from the common platform of disinfection kinetics.

Within the kinetics of disinfection, the constant K encompasses all that the experimentalist strives to control rigidly within the experiment. Small differences in the size of the initial inoculum (especially on a log scale) are assumed to have little effect on the value of K . Added soil is known to have a large effect and introducing strict guidelines into standard tests caters for this. We have simply added an addendum to the disinfection constant, that $K = K'(1 + f(\text{soil}))^m$. An examination of eqn 2.5 also shows that this factor can be used for the inoculum size effect, since $(1 + 1 \times 10^7)$ is, certainly within any experimental error, the same as 1×10^7 .

By quantifying the effect of a small range of initial inoculum sizes on disinfection, we can immediately ascertain whether the prescribed range can seriously affect the outcome of any expensive legislative tests.

The models used for the milk soiling assumed that the disinfectant inactivation was quicker than the microbial inactivation. The time exponent was considered to be an indicator for this. The rationale behind the catalase addition experiments was that the disinfectant reduction would be commensurate with microbial inactivation, and that the time exponent would show a strong dependence on catalase concentration. This was indeed the case. However, this result also poses a problem with respect to the inoculum size effect and the hypothesis behind the IQ model.

If, as the IQ model supposes, that the quenching parameter, Q , is related to the intrinsic microbial quenching, then an inoculum dependency on the Q (and the time exponent) parameter should have been observed. An examination of the standard deviation of the P_3 parameter (2.5) and a re-analysis of the data with the IQ model showed no significant dependence on the inoculum size. Either the change in P_3 over the range 1×10^7 – $5 \times 10^7 \text{ ml}^{-1}$ was too small within experimental error, else the hypothesis that this parameter is based on microbial self-quenching of the disinfectant is in error. Current work is pursuing this anomaly.

The work herein, we believe, has two principle benefits: (1) it can be used to examine disinfectants in the presence of soil, perhaps helping to develop new, soil tolerant ones and (2) it helps to underpin the need for good hygienic practice: disinfection without substantive cleaning may limit effectiveness or even simply be a wasteful exercise. Good standards of hygiene, actively practised, are required to prevent simple infections or cross-contamination. In a study of hospital hygiene in the Federal Republic of Germany, Bergler (1991), quotes nosocomial infection (720 000 cases) as the most common disease. Of those interviewed, 93% agreed that hygiene rules were needed but only 63% were aware of those rules in their own workplace. At the start of 2001, the UK government announced an increase of £30 million to hospitals to improve hygiene practice. The lack of hygiene awareness encompasses professionals as well as the general public. In the home, cleaning with disinfection can significantly reduce the number of contamination possibilities (Bloomfield and Scott 1997; Cogan *et al.* 1999; Barker and Bloomfield 2000). Cleaning alone cannot ensure a significant reduction in the possibilities of contamination. From the literature already cited, disinfection without cleaning is known to lead to a reduction in efficacy. Whether the hygiene standards are borne in a hospital, factory or home, the same criteria apply: the reduction of risk can be accomplished by good practices – actively encouraged and seen to be operating, with a good cleaning and disinfection regime.

REFERENCES

- Anonymous (1994) Chemical antiseptics and disinfectants: quantitative suspension test for the evaluation of bactericidal activity of chemical disinfectants and antiseptics for use in food, domestic and industrial areas – test method and requirements. Provisional European Norm – PREN 1276. London: British Standards Institute.
- Baldry, M.G.C. (1983) The bactericidal, fungicidal and sporicidal properties of hydrogen peroxide and peracetic acid. *Journal of Applied Bacteriology* **54**, 417–423.
- Barker, J. and Bloomfield, S.F. (2000) Survival of *Salmonella* in bathrooms and toilets in domestic homes following salmonellosis. *Journal of Applied Microbiology* **89**, 137–144.
- Bergler, R. (1991) Barriers in hospital hygiene – psychological aspects. *Zentralblatt Fur Hygiene und Umweltmedizin* **191**, 117–158.
- Bloomfield, S.F. (1995) Reproducibility and predictivity of disinfection and biocide tests. In *Microbiological Quality Assurance Screening and Bioassay: a guide towards relevance and reproducibility of inocula* eds Brown, M.R.W. and Gilbert, P. pp. 189–215. Boca Raton, FL: CRC Press.
- Bloomfield, S.F. and Miles, G.A. (1979) The relationship between residual chlorine and disinfection capacity of sodium hypochlorite and sodium dichloroisocyanurate solutions in the presence of *Escherichia coli* and of milk. *Microbios Letters* **10**, 33–43.
- Bloomfield, S.F. and Scott, E.A. (1997) Cross contamination and infection in the domestic environment and the role of chemical disinfectants. *Journal of Applied Bacteriology* **83**, 1–9.

- Chick, H. (1908) An investigation of the laws of disinfection. *Journal of Hygiene (Cambridge)* **8**, 92–158.
- Cogan, T.A., Bloomfield, S.F. and Humphrey, T.J. (1999) The effectiveness of hygiene for prevention of cross-contamination from chicken carcasses in the domestic kitchen. *Letters in Applied Microbiology* **29**, 354–358.
- Davis, B. (1990) Surfactant–biocide interactions. In *Recent Developments in the Technology of Surfactants, Critical Reports on Applied Chemistry*, Vol. 30, ed. Porter, M.B. pp. 65–131. New York: Elsevier Science.
- DIS/TSS2 (1979) *Efficacy Data Requirements*. EPA publication, 25 January. Washington DC 20460: US EPA.
- Holah, J.T. (1995) Progress report on CEN/TC 216/working group 3: disinfection test methods for food hygiene, institutional, industrial and domestic applications. *International Biodeterioration and Biodegradation* **36**, 355–365.
- Hugo, W.B. and Denyer, S.P. (1987) Concentration exponent of Disinfectants and Preservatives (Biocides). In *Preservatives in the Food, Pharmaceutical and Environmental Industries* eds Board, R.G., Allwood, M.C. and Banks, J.G. pp. 281–291. Oxford: Blackwell Scientific Publications.
- Johns, C.K. (1947) A method of assessing the sanitising efficacy of quaternary ammonium and hypochlorite products. *American Journal of Public Health* **37**, 1322–1327.
- Johns, C.K. (1948) Germicides for the food industry. *Canadian Food Industry* **19**, 24–27.
- Johnston, M.D., Simons, E.-A. and Lambert, R.J.W. (2000) An explanation for the variability of the bacterial suspension test. *Journal of Applied Microbiology* **8**, 237–242.
- Jones, M.B., Miller, J.J. and Brown, W.E. (1986) Effectiveness of a quaternary ammonium compound in the presence of hard water and organic soil. *Developments in Industrial Microbiology* **25**, 771–777.
- Jono, K., Takayama, T. and Kuno, M. and Higashide, E. (1986) Effect of alkyl chain length of benzalkonium chloride on the bactericidal activity and binding to organic materials. *Chemical and Pharmaceutical Bulletin* **34**, 4215–4224.
- Lambert, R.J.W. (submitted) (2001) Advances in disinfection testing and modelling. *Journal of Applied Microbiology* **91** (in press).
- Lambert, R.J.W., Johnston, M.D. and Simons, E.-A. (1998) Disinfectant testing: use of the Bioscreen Microbiological Growth Analyser for laboratory biocide screening. *Letters in Applied Microbiology* **26**, 288–292.
- Lambert, R.J.W., Johnston, M.D. and Simons, E.-A. (1999) A kinetic study of the effect of hydrogen peroxide and peracetic acid against *Staph.aureus* and *Ps. aeruginosa* using the Bioscreen disinfection method. *Journal of Applied Microbiology* **87**, 782–786.
- Lambert, R.J.W. and Johnston, M.D. (2000) Disinfection kinetics: a new hypothesis and model for the tailing of log-survivor/time curves. *Journal of Applied Microbiology* **88**, 907–913.
- Pryor, A.K. and Brown, R.S. (1975) Quaternary ammonium disinfectants: an updated perspective. *Journal of Environmental Health* **27**, 326–330.
- Schmidt, U. (1989) Cleaning and disinfection methods: effect of rinsing on surface bacterial count. *Fleischwirtschaft* **69**, 71–74.
- Watson, H.E. (1908) A note on the variation of the rate of disinfection with change in the concentration of the disinfectant. *Journal of Hygiene (Cambridge)* **8**, 536–542.