Effect of sodium chloride concentration on the heat resistance and recovery of Salmonella typhimurium

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ABSTRACT

The survival of *Salmonella typhimurium* (ATCC 13311) heated and recovered in media with 0%, 1%, 2%,3%,4% or 5% added sodium chloride was investigated. A protective effect in the heating medium and an inhibitory effect in the recovery medium were observed. The results showed an interaction between the effect, on D58 values, of sodium chloride concentration in both media. Lower concentration in the heating media led to a greater effect of the sodium chloride concentration in the recovery media. When the sodium chloride concentration was the same in both media, the protective effect exerted in the heating media prevailed over its inhibitory effect in the recovery media.

Keywords : *Salmonella typhimurium*, Sodium chloride, Heat treatment, Recovery, Modelling

INTRODUCTION

Salmonella spp are pathogenic bacteria responsible for one of the most frequent foodborne diseases. Since 1970, the number of isolates of different *Salmonella* serotypes has increased steadily (Tauxe, 1991). *Salmonella enteritidis* and *Salmonella typhimurium* are the serotypes most frequently involved in food poisoning outbreaks. Although , *S. enteritidis* became more important in the late 80's and early 90s than *S. typhimurium*, currently it seems that the latter *S. typhimurium* is increasing species (Zeidler, 1997). This micro-organism is mostly found in foods of animal origin and its inactivation in foods can be achieved by an adequate heat treatment.

The addition of sodium chloride to foods to inhibit or delay microbial growth is a very old and widespread practice. However, some micro-organisms are able to grow even in media with high concentrations of sodium chloride. *S. typhimurium*, at its optimum growth temperature, is able to develop in media with 7 % sodium chloride (Matches and Liston, 1979). A mathematical model of *S. typhimurium* growth at different temperatures in media of different pH with different sodium chloride concentrations was developed by Gibson *et al.* (1988). This model was worked out with data obtained with unheated cells. The behaviour of heated cells in these media is not known.

Heat treatments kill a fraction of a bacterial population, but they also generate damaged cells. The ability of heated cells to survive depends on the recovery conditions . If they are favourable, they can repair heat damage and grow. Heat is supposed to damage some cellular structures (cytoplasmic membrane, ribosomes, RNA, enzymes...). As a consequence, cell membrane permeability is altered and leads

to leakage of proteins, aminoacids, potassium ions etc... Heat injured cells also become more sensitive to sodium chloride. The addition of sodium chloride to the recovery medium has been used by authors to measure the proportion of heat damaged cells. Tomlins and Ordal (1976) and Mackey and Derrick (1982) have enumerated heat damaged *S. typhimurium* cells by using recovery media with added 2 and 3 % of sodium chloride, respectively. Therefore, the addition of sodium chloride to the recovery medium leads to the estimation of lower apparent D values than those calculated using an optimum recovery medium.

It has been shown that heat resistance of *Salmonella*, as that of other bacterial species, can increase if cells are preincubated (Ng, 1982) or heated in low water activity media, such as those obtained by adding sodium chloride (Cotterill and Glauert, 1969, Palumbo *et al.*, 1995) or other solutes such as sucrose, glycerol and fructose (Goepfert *et al.*, 1970, Sumner *et al.*, 1991). In most of these studies, the solute concentrations were very high (> 6% of NaCl). Such concentrations are only found in some special foods like egg products, sausage, ham. The conclusions of these investigations cannot be extrapolated to the majority of food products. The influence of low sodium chloride concentrations on the heat resistance of *S. typhimurium* is not well known. When salt is added to the recovery medium it has the opposite effect (Tomlins and Ordal, 1976, Mackey and Derrick, 1982). Published data do not allow us to predict which of the effects is going to prevail at the sodium chloride concentrations of most foods.

The purpose of this investigation was to determine the influence of sodium chloride concentration in the heating and recovery medium on the capacity of *S. typhimurium* to survive heat treatment .

MATERIALS AND METHODS

Bacterial culture and media

The *S. typhimurium* strain (STCC 443, ATCC 13311) used in this study was supplied by the Spanish Type Culture Collection. It was maintained on slants of Tryptic Soy Agar (Biolife; Milan; Italy) with 0.6% of Yeast Extract added (Biolife) (TSAYE).

A broth subculture was prepared by inoculating with one single colony from a plate, a test tube containing 5 ml of sterile Tryptic Soy Broth (Biolife) with 0.6% of Yeast Extract added (Biolife) (TSBYE). After inoculation, this tube was incubated at 37°C for 18 hours. Erlenmeyer flasks (250 ml) with 50 ml of sterile TSBYE were inoculated with this subculture to a final concentration of 10^6 cells/ml approx. The flasks were then incubated for 30 h. at 37°C under agitation (130 rev/min) (Selecta; mod. Rotabit; Spain). After that time cells had reached stationary phase and maximum thermo-tolerance. Cell suspensions thus prepared were stored under refrigeration, without agitation, for up to 30 d. Previous experiments showed that, during storage, heat resistance did not vary (data not shown).

Heat treatments

Heat treatments were carried out in duplicate in a thermoresistometer TR-SC as described elsewhere (Condón *et al.* 1993). TSBYE medium with 0%, 1%, 2%, 3%, 4%, and 5% added NaCl (Probus, Barcelona, Spain) were used as heating media.

Once the heating medium (350 ml) temperature had attained stability (T±0.05°C), it was inoculated with 0.2 ml of an adequately diluted cell suspension. During heat treatments 2 ml samples were periodically collected into sterile test tubes. From each one of these tubes an aliquot of 0.1 ml was immediately pour-plated in TSAYE containing 0%, 1%, 2%, 3%, 4%, and 5% NaCl added (TSAYE-S).

Incubation of heated samples and survival counting

After heat treatments, the plates were incubated at 37°C for 48 hours. Previous experiments showed that longer incubation times did not increase survivor counts.

Colonies were counted with an Image Analyser Automatic Counter (Protos, Analytical Measuring Systems, Cambridge, UK) as described elsewhere (Condón *et al.* 1996).

Heat-damaged cells were estimated by the difference in the number of CFU formed by heated suspensions in TSAYE or TSAYE-S medium.

Heat resistance parameters and data fitting

D_T values were calculated from the slope of the straight portion of survival curves. Survival curves were drawn by plotting log of number of survivors vs their corresponding heating times.

Correlation coefficients (r_o) were calculated with the appropriate statistical package (Statview 512; D. Feldman and J. Gagnon, BrainPower Inc., Calabasas, CA). The statistical significance of differences ($p > 0.05$) between D_T values was tested as described by Steel and Torrie (1960).

Multiple linear regressions used to fit polynomial models were carried out with the STAT-ITCF software (Institut Technique des Céréales et du Fourage, France).

RESULTS AND DISCUSSION

The effect of NaCl concentration in the heating and recovery media on the heat resistance (D values) of our *S. typhimurium* strain is shown in Figure 1. *Salmonella* heat resistance values published in literature can vary widely. The heat resistance of our strain heated in TSBYE and recovered in TSAYE (D_{58}°C =0.4 min) was similar to that reported by D´Aoust *et al.* (1987) for several *Salmonella* strains.

The addition of sodium chloride to the heating medium led to higher D values, while sodium chloride addition to the recovery medium made them lower. These results are in agreement with those of other authors who also found that sodium chloride in the heating medium increased heat resistance of *Salmonella* (Cotterill and Glauert, 1969, Ng, 1982, Palumbo *et al.*, 1995, Blackburn *et al.*, 1997), while the recovery medium prevented the recovery of heat injured cells (Tomlins and Ordal, 1976, Mackey and Derrick, 1982)).

The magnitude of the effect of NaCl in the heating media on the heat resistance of our strain was similar to that reported by Baird-Parker *et al.* (1970) for other *Salmonella* serotypes. The addition of 5% sodium chloride to the treatment medium increased the $D_{58°C}$ value of our strain, recovered in TSAYE medium, from 0.4 to 0,66 min. The higher microbial heat resistance in low water activity media has long been known (Corry ,1974). It is supposed to be due to a dehydration of the cell´s

cytoplasm (plamolysis), that would make enzymes and structural proteins inside more heat-stable (Corry ;1974).

The addition of 5% sodium chloride to the recovery medium decreased D_{58} value of cells heat treated in TSBYE from 0.4 to 0.08 min. This effect of sodium chloride in the recovery medium on the heat resistance of our strain could not be explained solely by the effect of the lower water activity of this medium on microbial growth . Perhaps, as suggested by some authors, heat treatment altered the selective permeability of cell membrane, allowing sodium chloride to go into the cytoplasm (Tomlins and Ordal, 1976) where it would interfere with vital metabolic activities. The action mechanisms of sodium chloride inside the cell remain still unknown. It has been suggested that sodium ions would inhibit some enzymatic activities (Kyzlink, V., 1990), and/or displace magnesium ions from ribosomes (Lee and Goepfert, 1975). Ribosomes would require magnesium ions to repair heat damage.

Recovery media with low sodium chloride concentrations (2-3%), incapable of influencing growth of unheated cells, but inhibiting growth of heat damaged cells, have been used by authors to assess heat damage (Tomlins and Ordal, 1976; Mackey and Derick; 1982). By this procedure, the number of heat damaged cells is enumerated by the difference between the number of CFU developed by heated cells in recovery media with or without added sodium chloride.

However the influence of sodium chloride concentration in the recovery medium on D values obtained with cells heated in a medium with different concentrations of sodium chloride has never been investigated.

Our results (Fig. 1) showed a clear interaction between the concentration of sodium chloride in both media. The magnitude of the difference between the D_{58}°C values, obtained by heating in media with different concentrations of sodium chloride, was bigger for the highest concentrations of sodium chloride in the recovery medium. For recovery medium without sodium chloride added, D_{58} values obtained increased from 0.4 to 0.66 minute when heating medium sodium chloride concentration was increased from 0% to 5%. However when a recovery medium with 5% of sodium chloride was used, D_{58} values obtained increased ten fold, from 0.08 to 0.80 minute. D_{58}°C values were always lower for the highest concentration of sodium chloride in the recovery medium. However, this influence of sodium chloride concentration in the recovery medium on the magnitude of D_{58}°C values obtained, was smaller for the highest sodium chloride concentration in the heating medium. In a medium with a 5% sodium chloride added it was almost slight. This interaction between the concentration of NaCl in both media is well illustrated by Figures 2 and 3. Survival curves obtained (Fig. 2) seemed to indicate that the damage inflicted in cells heat treated in a medium with a 1% sodium chloride added was quite different from that inflicted on cells in a heating medium with a 5% sodium chloride. In the cell suspension heat treated in a medium with 1% sodium chloride added, the percentage of damaged cells increased with heating time, as shown by the difference among counts obtained in a recovery medium with 1% or 5% added sodium chloride. However, in the cell suspension heat treated in a medium with a 5% sodium chloride added the percentage of damaged cells remained practically the same regardless of heating time (fig. 3). This behaviour is in agreement with the data of Mafart and Leguérinel (1997) who showed that recovery ratio was independent of heating time when the decimal reduction time was not reduced with regard to observed optimum recovery conditions.

D values of Fig 1 could be fitted according to the following polynomial equation:

$$\log D' = 0.4397 + 0.2824\ x - 0.1549\ x^2 + 0.005543\ y^2 + 0.02534\ xy \quad (1)$$

with $r^2 = 0{,}925$

where x was the sodium chloride concentration added to the heating medium and y, the salt concentration added to the recovery medium.

In practice, the food is both the heating and the recovery medium (x=y).Therefore the case in which x=y is particularly interesting. Under these conditions, our polynomial model becomes:

$$\log D' = a_0 + a_1 x + a_2 x^2$$

This equation could be reparameterized to

$$\log D' = \log D_0 - \frac{x}{x_{opt}}\left(\log \frac{D_0}{D_{\max}}\right)\left(2x_{opt} - x\right)$$

Where D_0 denotes the D value without salt and x_{opt} the NaCl concentration at which the D value is maximum (Dmax). The relationship between these values and coefficients a_0, a_1 and a_2 is illustrated in appendix.

The inactivation rate of bacteria is supposed to be ruled by first order kinetics. However, deviations from the linearity of survival curves have been frequently reported (Cole *et al.*, 1993, Condón *et al.*, 1992). Some survival curves obtained in this investigation did not follow a strict logarithmic course of destruction and sometimes a clear biphasic profile was obtained. Comparing the curve corresponding to 1% sodium chloride in the heating medium and a 5% sodium chloride in the recovery medium with that corresponding to 5% in the heating and the recovery medium, the number of survivors after 5 seconds of treatment were, approximately the same (Figure 2). After that, cells heated in a 5% sodium chloride medium showed much greater heat resistance than cells heated in a 1% sodium chloride medium. Perhaps the drop in counts after the first 5 seconds of heating in media containing 5% of NaCl could be due to an osmotic shock inactivating most (90% aprox .) of cells in the suspension, the remaining showing a much higher heat resistance. It could also happen that heat resistance of cells in 1% and 5% NaCl were the same, but cells in the 5% suspension were able to adapt to the environment by dehydrating their cytoplasm, during the first five seconds. As a consequence, they would increase their heat resistance, and/or their capacity to repair heat damage. A similar adaptation phenomenon had been observed by Ng (1982), and Cotterill and Glauert (1969).

Whatever the reason, the magnitude of the decrease in counts after the first 5 seconds of treatment depended on the amount of sodium chloride added to heating medium (Fig 4). This drop in counts could be described by the following polynomial equation:

$$\log \frac{N_1}{N_0} = -0.4287 + 0.1244\ x - 0.0385\ x^2 - 0.0534\ y^2 + 0.0478\ xy \qquad (3)$$

with r^2=.0.913

where x represented the NaCl concentration added to the heating medium and y , the NaCl concentration added to the recovery medium.

The number of survivor after any treatment time under all environmental conditions investigated could be calculated by the equation:

$$\log N_t = \log N_1 - \frac{(t-5)}{D'} \quad (4)$$

where D´values could be provided by equation 1 or 2 and N_1 values by equation 3.

Fig. 5 illustrates ,for the whole count of the experimental design (n = 506), the relationship between the experimentally and the theoretically calculated survival counts. The correlation between both values was quite satisfactory (r^2=0,946).

As our results show, the effect on the survival of *Salmonella typhimurium* after a heat treatment, of the presence of NaCl in the medium (at least up to a concentration of 5%) was very limited. The inhibiting effect of NaCl on heated cells was mostly compensated for the increase in heat resistance of cells during treatment. In some instances the addition of NaCl even increased the probability of survival. This was the case when the concentration of sodium chloride was the same in both media, as it is normally the case in foods. When the concentration of sodium chloride was the same in both media (x=y) survival increased slightly.

Data presented in this work will help to predict the safety of salted cooked or pasteurised foods with regard to *Salmonella typhimurium.*

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APPENDIX

Reparameterization of polynomial model

The data were fitted according to a simple polynomial model :

$$\log D = a_0 + a_1 x + a_2 y + a_3 x^2 + a_4 y^2 + a_5 xy$$

x : [NaCl] in the heat treatment medium

y : [NaCl] in the recovery medium

In the case of pasteurized foods, the recovery medium is also the heating menstrum then, x = y

The model becomes :

$$\log D = a_0 + a_1 x + a_2 x^2$$

with according to the data,

a_0 = -0.4397

a_1 = 0.1275

a_2 = -0.01317

The coefficient do not have any physical or biological significance, so that the following reparameterization was proposed.

1) Without NaCl, x = 0 and logD = $\log D_0$

(D-value without salt $a_0=\log D_0$)

2) $\frac{d(\log D)}{dx} = a_1 + a_2 x$

If x_{opt} is the NaCl concentration at which the D-value is maximum ($D = D_{max}$)

$$a_1 + 2a_2 x_{opt} = 0 \quad \Rightarrow \quad a_1 = -2a_2 x_{opt}$$

$$\log D = \log D_0 - 2a_2 x_{opt} x + a_2 x^2 \quad \Rightarrow \quad \log D = \log D_0 - a_2 x(2x_{opt} - x)$$

3) $\log D_{max} = \log D_0 - a_2 x_{opt}^2 \quad \Rightarrow \quad a_2 = \frac{1}{x_{opt}^2} \log \frac{D_0}{D_{max}}$

The reparameterized model is then :

$$\log D = \log D_0 - \frac{x}{x_{opt}^2}\left(\log \frac{D_0}{D_{max}}\right)(2x_{opt} - x)$$

with $D_0 = 10^{a_0} = 0.363 \text{ min}$

$$x_{opt} = -\frac{a_1}{2a_2} = 4.84\%$$

$$D_{max} = 10^{a_0 - a_2 x_{opt}^2} = 0.739 \text{ min}$$

Figure legends

Fig. 1. Influence of NaCl concentration on the heating and recovery media on D_{58} values of *S. typhimurium*.

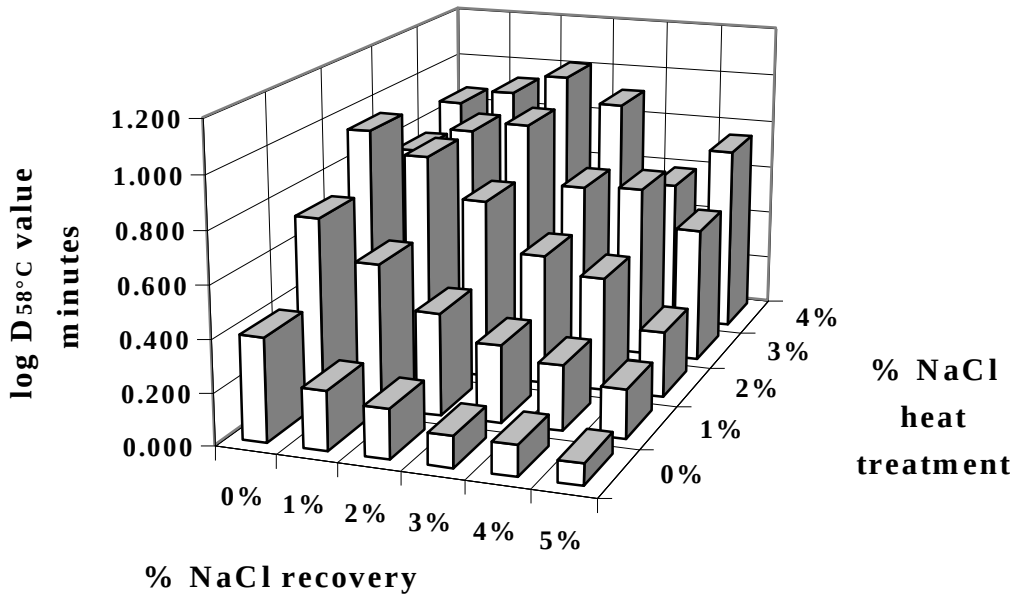
Fig. 2. Survival curves at 58°C of *S. typhimurium* heated in TSBYE added with 1% NaCl (closed symbol) or 5% NaCl (opened symbol) the recovered medium is TSAYE added with 1% (circle) and 5% NaCl added (square).

Fig. 3. Ratio of number of UFC for recovery conditions 5% NaCl added / 1% NaCl added, at different heating time at 58°C in TSBYE with 1% NaCl ○ and 5% NaCl □ added

Fig. 4. Influence of NaCl concentration on the heating and recovery media on *S. typhimurium* counts obtained after 5 seconds of treatment.

Fig. 5. Correlation between experimentally and theoretically predicted counts (n=506).

Figure 1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1.000
0.800
0.600
0.400
0.200
0.000
0%
1%
2%
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4%
5%
% NaCl recovery
4%
3%
2%
1%
0%
% NaCl
heat
treatment

figure 2

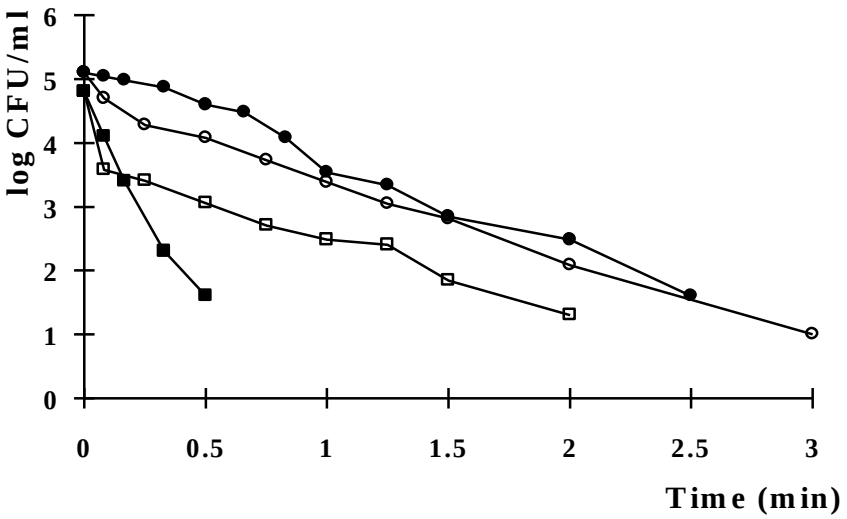


figure 3

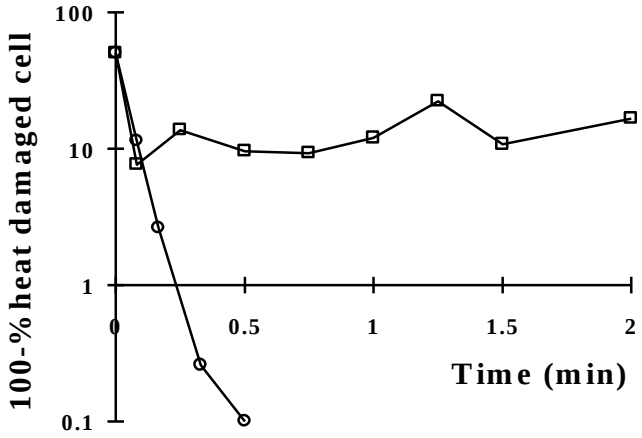


figure 4

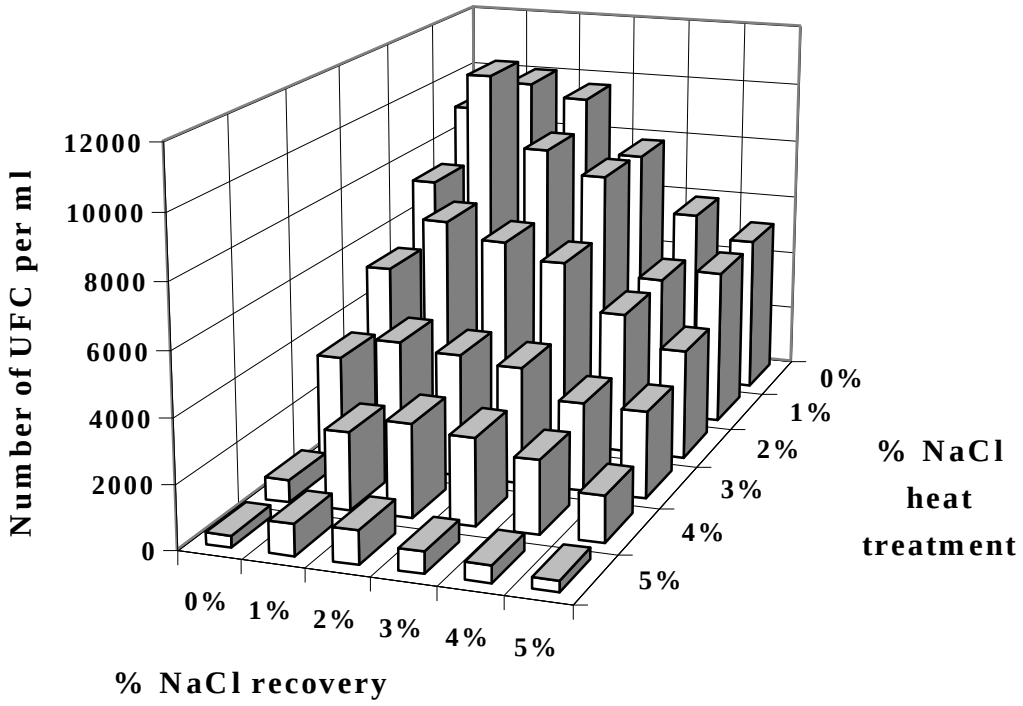
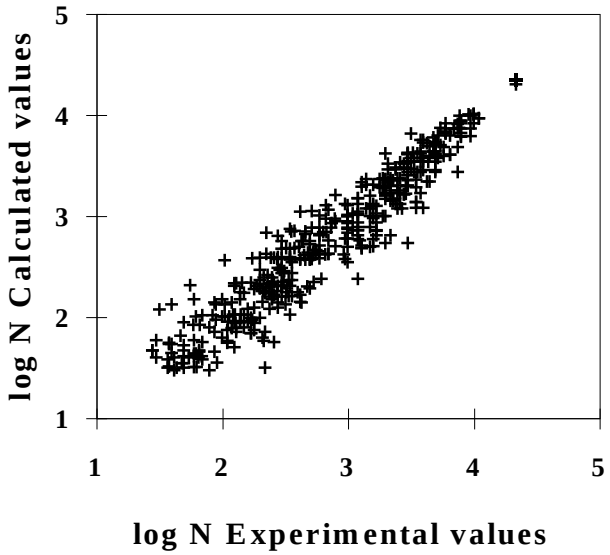

Number of UFC per ml
12000
10000
8000
6000
4000
2000
0
0%
1%
2%
3%
4%
5%
% NaCl recovery
0%
1%
2%
3%
4%
5%
% NaCl heat treatment

figure 5


log N Calculated values
5
4
3
2
1
1
2
3
4
5
log N Experimental values