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# The Effect of Temperature on the Destruction of Salmonellas in Activated Sludge

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## ABSTRAK

*Kajian ini meneliti kesan suhu terhadap penghapusan* Salmonella *di dalam enapcemar aktif. Hasil kajian menunjukkan bahawa kadar penghapusan* Salmonella *meningkat apabila suhu 'mixed liquor' dinaikkan di dalam lingkungan 5° hingga 25°C, tetapi pada suhu 30°C, kadar penghapusannya adalah rendah. Ini ialah kerana kegiatan protozoa ciliata adalah rendah pada suhu setinggi ini, dan keadaan ini membolehkan* Salmonella *terus hidup. Pengurangan bilangan* Salmonella *pada suhu 30°C mungkin akibat kekurangan zat makanan dan mekanisma-mekanisma lain seperti tindakan virus bakteria. Implikasi hasil kajian ini terhadap operasi loji pembersihan air buangan dibincangkan.*

## ABSTRACT

*The effect of temperature on the survival of* salmonellas *in activated sludge was examined. The results showed that the* Salmonella *destruction rates increased in line with increasing temperature of the mixed liquor from 5° to 25°C, whereas at 30°C the rate declined markedly. This is most probably due to inactivation of ciliate protozoa at the higher temperature resulting in increased survival of the pathogens. The slow reduction in the number of* salmonellas *at 30°C may be the result of nutrient starvation, in addition to other possible mechanisms such as the lytic action of bacterial viruses.*

## INTRODUCTION

Laboratory data and field studies have indicated that activated sludge systems are generally more effective than trickling filters in destroying bacterial pathogens from wastewaters (Yaziz and Lloyd, 1979). Although there are many reports in the literature confirming the beneficial effects of increased temperature (in the range of 10° – 30°C) on the chemical quality of the final effluent from a wastewater treatment system, there is a lack of comparable data concerning the specific influence of temperature on the destruction of bacterial pathogens during wastewater treatment. The limited information available indicates that bacterial destruction efficiencies are generally higher in warm tropical climates than in temperate regions (Mara *et al.*, 1979). However, it is difficult to make a meaningful comparison of the data since the treatment processes used in the tropical regions (mainly lagoon systems) differ markedly from those of the temperate climates (conventional systems including the activated sludge and trickling filter process). In a completely mixed activated sludge system, for example, temperature affects the growth kinetics of bacteria and protozoa, the settling characteristics of the sludge, and the oxygen transfer characteristics of the system.

The aim of this study was to examine the specific influence of temperature on the survival of salmonellas in the activated sludge process and to determine the optimum operating temperature which would result in maximum pathogen destruction within the shortest hydraulic retention time possible. It has been shown earlier that the primary mechanism responsible for the destruction of salmonellas in activated sludge was ingestion by ciliate protozo (Yaziz and Lloyd, 1982).

## MATERIALS AND METHODS

### *Laboratory Model of the Activated Sludge Tank*

The aeration vessel consists of a cylindrical polypropylene container of 6 litres capacity and fitted with a removable lid. Filtered air was bubbled through the activated sludge at a rate of 120 l/h (Orion PoPo II air pump) via two flexible tubes, and the contents of the vessel was stirred to increase aeration efficiency. A condenser with a cotton wool plug was fitted to the lid of the vessel to prevent aerosols from escaping and also to prevent loss of liquid through evaporation. The whole vessel was immersed in a water jacket and the temperature of the sample was kept constant by maintaining the water temperature at a fixed value using a Grant Circulator. Sampling and inoculation was performed through a port cut into the lid and stoppered with a rubber bung.

### *Bacterial Culture*

*Salmonella enteritidis* was isolated from the activated sludge earlier and was then identified biochemically and serologically. It was initially purified on Xylose Lysine Deoxycholate selective media and then streak-purified on nutrient agar with incubation at 37°C.

### *Effect of Temperature*

Samples of activated sludge measuring four litres each were obtained from a nearby sewage treatment plant and were aerated in the laboratory model of the activated sludge tank at predetermined constant temperatures. The range of temperature selected were 5°, 10°, 15°, 20°, 25° and 30°C. At zero time, a 5 ml suspension of *S. enteritidis* in Ringer's solution (Optical Density at 650 nm = 1.70) was inoculated into the activated sludge sample and allowed to equilibrate for 15 mins. Following this, 25 ml samples were withdrawn from the vessel at 2 h intervals for a period of 24 h, and were examined for salmonellas as described by Yaziz and Lloyd, (1982).

## RESULTS

Increasingly greater numbers of salmonellas were destroyed from the activated sludge during the first 10 h of aeration in line with the increase of temperature from 5° to 25° (Table 1). For example, at 10°C, there was a log 1.9 reduction in the number of salmonellas in the mixed liquor compared to a log 2.9 reduction at 25°C. However, at 30°C, the number of salmonellas destroyed was very much lower than that at 25°C and was comparable to the destruction that occurred at 5°C.

In this study, T-90 is defined as the time taken to achieve a 90% reduction in the number of salmonellas in the mixed liquor at any given

TABLE 1
The destruction of salmonellas after 10 hours aeration at different temperatures

| Bacterial conc./ml | Temperature (°C) 5 | 10 | 15 | 20 | 25 | 30 |
|---|---|---|---|---|---|---|
| Initial ($\times 10^6$) | 4.50 | 4.72 | 4.30 | 5.32 | 4.30 | 5.40 |
| After 10 hours | $1.64 \times 10^5$ | $7.00 \times 10^4$ | $3.57 \times 10^4$ | $4.00 \times 10^4$ | $1.00 \times 10^4$ | $2.50 \times 10^5$ |
| Destruction (%) | 21.6 | 27.4 | 31.4 | 30.6 | 39.7 | 19.8 |

TABLE 2
T-90 for S. enteritidis in activated sludge at different temperatures

| Temperature (°C) | Initial concentration ($\times 10^6$ cells/ml) | T-90 (h) |
|---|---|---|
| 5 | 4.50 | 4.40 |
| 10 | 4.72 | 3.05 |
| 15 | 4.30 | 2.80 |
| 20 | 5.32 | 2.20 |
| 25 | 4.30 | 2.00 |
| 30 | 5.40 | 6.55 |

temperature. The T-90 values against temperature are presented in Table 2 and graphically depicted in *Figure 1*. Considering the similar initial bacterial concentrations in the mixed liquor, the values of T-90 may be taken to represent the effect of temperature and not the concentration.

The results in *Fig. 1* clearly show the advantage of aerating activated sludge at higher than ambient temperatures in the temperate regions.

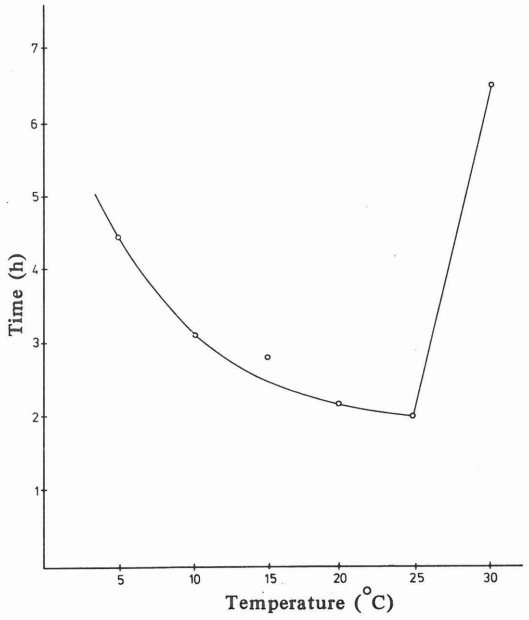


*Fig. 1. T-90 for* **S. enteritidis** *in activated sludge at different temperatures.*

*Salmonella* destructions of $> 90\%$ may be achieved at relatively short hydraulic retention times in the temperature range of 15° – 20°C, but clearly the optimum working temperature for bacterial destruction is at 25°C.

## DISCUSSION

Much of the research relating to the effect of temperature on the activated sludge process has been restricted to the effects on the chemical quality of the final effluent. There have been few reports in the literature concerning the effects of temperature on the survival of bacterial pathogens in activated sludge. Ruchhoft (1934) reported an 86% reduction in the number of *S. typhi* in diluted activated sludge after 5.5 h storage at 22°C. In another study, Van Der Drift *et al.* (1977) indicated a 2.4 log reduction in the number of *E. coli* organisms after 10 h aeration in the mixed liquor at 17°C. This is in close agreement with the results of this study for the destruction of salmonellas in activated sludge (2.1 log reduction after 10 h aeration at 15°C). Raising the temperature above 25°C resulted in poorer salmonellae destruction efficiencies.

It is known that the maximum temperature tolerance for many peritrich protozoa in activated sludge is between 23° – 25°C (Table 3). Thus, when the temperature of the mixed liquor is increased to 30°C, most of the *Peritrichia* would be damaged by the heat and cease or deccelerate their feeding activities. This accounts for the better survival of the pathogens

TABLE 3
Temperature tolerance of several species of *Peritrich* protozoa

| Organism | Extreme temperature tolerences (°C) |
|---|---|
| *Vorticella microstome* | 0 – 30 |
| *V. campanula* | 0 – 23 |
| *V. convallaria* | 2 – 23 |
| *V. nebulifera (similis)* | 4 – 20 |
| *V. striata* | 2 – 23 |
| *Carchesium polypinum* | 0 – 25 |
| *Epistylis plicatilis* | 4 – 25 |
| *Opercularia nutans* | 2 – 25 |

Data complied from "Ciliated Protozoa" Ed. Hartmut Bick. WHO 1972.

at 30°C than at 25°C. Hence increasing the temperature of the mixed liquor up to the optimum temperature for ciliated protozoa would result in faster pathogen destruction but at higher temperatures (>30°C) the decrease in salmonellae concentration is most likely due to nutrient starvation since it was shown in another experiment (Yaziz, 1979) that nutrient availability and temperature conditions control the survival of salmonellas in the absence of predation by ciliated protozoa. Also, the possibility of the action of other mechanisms such as the lytic action of bacterial viruses cannot be excluded. In contrast, at the lower temperatures (5° – 25°C), protozoa may feed actively on the salmonellas present in the mixed liquor thus contributing to their destruction.

The temperature of activated sludge in the temperate regions may vary from 7°C in winter to a mean of 16°C in summer. Thus the process is normally operating well below the optimum temperature range for bacterial pathogen removal throughout the year. Nonetheless, it must be borne in mind that the efficiency of the laboratory model is inevitably greater than the operational plant because of the batch nature of the model, and therefore some care must be exercised when extending the information obtained here to full scale plants. In addition the effects of temperature are less noticeable in the field because of the small daily variations (<2°C) and also because this factor cannot be considered independently of other elements, such as flow rates, detention times, organic loads, and oxygen penetration that influence the efficiency of the system. Operational factors such as flow rates and organic loads, and the concentration of toxic metals can seriously affect the growth and survival of protozoa in activated sludge which in turn directly affects the efficient destruction of bacteria and the quality of the final effluent. Nonetheless, if the temperature could be maintained at high levels continuously, for example through the use of heated water from cooling towers of power plants, the efficiency of the activated sludge process will most certainly be augmented, both in terms of producing chemically higher quality effluents and achieving huge reductions in the number of bacterial pathogens.

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