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The effect of biostats on the ciliate Tetrahymena pyriformis and on mixed populations of T. pyriformis and Klebsiella pneumoniae.

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The effect of biostats on the ciliate Tetrahymena pyriformis and on mixed populations of T. pyriformis and Klebsiella pneumoniae

17 Projektledare/~~Författare~~

A.H. Neilson

18 Sammandrag (ange gärna målsättning, metod, teknik, resultat m m)

The effect of biostats on strains of Klebsiella and on the ciliate Tetrahymena pyriformis was investigated. It was shown that the ciliate was more sensitive than the bacteria. The direct effect of these compounds cannot therefore be ignored. In experiments with two-component cultures it was shown that bacteria previously exposed to the biostats were less desirable nutrient sources for the ciliate than unexposed bacteria. It cannot be assumed that deleterious effects on the ciliate population are not caused by the presence of biostats in process waters.

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Introduction

It is well established that the control of natural populations is frequently governed by the number of predators present. The importance of phages, species of Bdellovibrio and ciliated protozoans as predators upon bacteria has been examined. It seems that the first two are of minor importance but that the role of protozoans in controlling bacterial populations in biological treatment plants and elsewhere has been clearly established.

One important problem in paper mills is the accumulation of slime deposits apparently caused by polysaccharide synthesis by members of the Enterobacteriaceae which may be present in large numbers. Control of these populations and therefore of the slime is generally accomplished by addition of slimicides which generally function as bacteriostats (biostats). Ideally, residual amounts in the water should be broken down chemically and that taken into the cells degraded before the water enters the biological treatment plant.

The present investigation set out to determine the differential toxicity of several slimicides towards bacteria and the ciliate Tetrahymena pyriformis, and to examine whether bacteria exposed to such compounds were toxic to populations of T. pyriformis when fed to them as the principal food source. Such studies have a more general interest in the context of accumulation of toxic substances in the higher trophic levels of a food chain.

General plan of the experiments

Some general comments may be made here about the way in which the experiments were carried out and about the strains used.

Micro-organisms used

Several strains of Klebsiella pneumoniae were used: these had been isolated from paper mill process waters and one (HA-2) had previously been shown to be relatively tolerant of biostats. All strains were typical K. pneumoniae as revealed by biochemical

tests: their source and API (Analytab Products Inc.) code number are given in Table 1. K. pneumoniae was chosen partly because it is probably the commonest member of the Enterobacteriaceae present in process waters and partly because it has been established that this is a convenient food source for the chosen protozoan. This was the ciliate T. pyriformis and was obtained as an axenic culture from the Cambridge Collection of Algae and Protozoa.

It was shown in the course of the experiments that after growth in a rich medium, the ciliate showed only low feeding rates, presumably due to the accumulation of substantial amounts of reserve material. Cultures were therefore routinely starved in a synthetic process water for 48 hour before use: after such treatment utilization of the bacterial food source was rapid. The synthetic process water (SPW) which was used contained (mg/l tap water): glucose, 38: ammonium sulphate, 2.8: KH_2PO_4, 1.32.

Biostats tested

Four different biostats were examined and throughout they have been referred to by their contracted trade names: R-80, V-10, T-9, J-26. Their trade name, the manufacturer, the class and the chemical structures have been assembled in Tables 2 and 3.

Assay of effect

In all of the experiments, the effect of the biostats was measured by the loss in viability of relatively dense populations (10^8 bacteria/ml: 10^{4-5} protozoa/ml). This design provides at the same time an experimental situation in which viability can be measured accurately and relatively easily, and also a realistic model of the conditions which could occur in the biological treatment systems. It would be expected that the ratio of biostat to biomass was low.

A. The effect of biostats on bacteria.

Cultures of K. pneumoniae were grown aerobically in nutrient broth for 18 h at 35° C. Cells were harvested from 100 ml of culture by centrifugation at room temperature (450 x g, 10 minutes), the cells washed twice with sterile phosphate buffer (pH 7), re-centrifuged and suspended in buffer (10 ml). The amounts of biostats used are given in Figs,1-4 and were added to SPW(100ml), the washed bacterial suspension added (2 ml) and the cultures shaken at 25°C. Samples (1 ml) were removed at 0, 5, 24 and 48 h, diluted in phosphate buffer, mixed with melted Tryptone/ Yeast extract/glucose agar and the number of colonies counted after incubation at 35°C for 48 hours.

The results of these experiments have been assembled in Figures 1 to 4.

B. The effect of biostats on T. pyriformis

The growth medium contained (g/l distilled water): proteose peptone, 10.0: yeast extract, 2.5: liver digest, 2.0. Cultures were grown in 500 ml of medium in 1.8 l Fernbach flasks which were shaken gently for 72 hours at 25°C. Cells were harvested by centrifugation (450 x g, 10 minutes), washed twice with sterile phosphate buffer and after centrifugation suspended in buffer (10 ml). The biotests were used at concentrations of 0, 5, 10, 25, 50 and 100 ppm and were first mixed well with SPW (100 ml). Washed cells (2 ml) of T. pyriformis were added and the cultures shaken gently at 25°C. Samples (1 ml) were removed at 0, 5, 24, 48 and 72 hours, mixed with 2 drops of Lugol's iodine solution, and the number of cells counted in a Fuchs-Rosenthal counter 0.2 mm deep with 0.0625 mm^2 divisions. The number of cells occurring in all sectors was counted, the total divided by 256 and multiplied by 8×10^4 to give the total number of cells in 1 ml sample. Lysed cells and smaller rounded cells which were not viable could readily be distinguished and were not included in the total count.

The results of these experiments are given in Figure 5.

C. The effect of feeding bacteria treated with biostats to T. pyriformis

For these experiments, K. pneumoniae: strain 300 was chosen and the two biostats showing the greatest differential toxicity to the two organisms viz. V-10 and T-9: they were each used at only one concentration (50 and 200 ppm respectively). Strain 300 was grown as in section A. the cells removed by centrifugation, washed twice with sterile phosphate buffer and re-suspended in 10 ml buffer.

T. pyriformis was grown as in section B, the cells harvested by centrifugation, washed twice with sterile phosphate buffer, resuspended in buffer and added to 500 ml SPW in a 1.8 l Fernbach flask. The cultures were then starved for 48 hours during which time they were gently shaken at 25°C. The cells were then harvested by centrifugation and resuspended in 20 ml buffer. The biostats were added to 100 ml SPW and well mixed. The following combinations were then prepared:

1. Bacterial suspension (treated with biostat, 2 ml) + ciliate (5 ml)
2. Bacterial suspension (untreated, 2 ml) + ciliate (5 ml)
3. Bacterial suspension (untreated, 2 ml)
4. Ciliate (5 ml)

These were incubated at 25°C with gentle shaking. Samples (2 ml) were removed at 0, 5, 24 and 48 hours and divided into two portions. 1 ml was serially diluted, mixed with molten TGE medium as in section A and the total number of bacteria estimated as before. 1 ml was treated with Lugol's solution as in section B and the number of ciliates estimated microscopically.

The results have been presented in Figures 6 and 7.

Discussion

It is obvious from the figures that the tolerance of the bacteria towards different biostats varies widely. J-26 and T-9 have almost no effect and it appears that, with increasing time, there is substantial recovery from the effect of R-80. The difference in the tolerance of the various strains does not seem to be significant except that strain HA-2 is most tolerant of R-80. By contrast, T. pyriformis is relatively sensitive towards all of the biostats except J-26: the effect of V-10 appears to be irreversible, while with increasing time, there is substantial recovery from the effects of the other two. Detailed interpretation of the data is complicated by the probable occurrence of chemical decomposition of the compounds in the medium during the experiments and by lack of knowledge of the fate of the compounds in bacteria exposed to them.

In the experiments with mixed cultures, the increase in the number of protozoans and corresponding decrease in number of bacteria were less when the bacteria had previously been exposed to biostat. Further experiments, however, are clearly necessary to establish the mechanism of the growth inhibition.

Conclusions

1. T. pyriformis is clearly more sensitive to the effect of the biostats than any of the bacteria tested. The direct effect of these compounds cannot therefore be discounted.

2. Bacteria exposed to the two biostats examined are clearly less desirable nutrient sources for T. pyriformis than unexposed bacteria.

3. It cannot therefore be assumed that deleterious effects on the protozoan population are not caused by the presence of biostats in process water.

References

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Fry, J.C., and Staples, D.G. (1976). Distribution of Bdellovibrio bacteriovorus in sewage works, river water and sediments. Appl. Environment. Microbiol. 31, 469-474.

Enzinger, R.M., and Cooper, R.C, (1976). Role of bacteria and protozoa in the removal of Escherichia coli from estuarine waters. Appl. Environment. Microbiol. 31, 758-763.

Pretorius, W.A. (1962). Some observations on the role of coliphage on the number of Escherichia coli in oxidation ponds. J. Hyg. 60, 279-281.

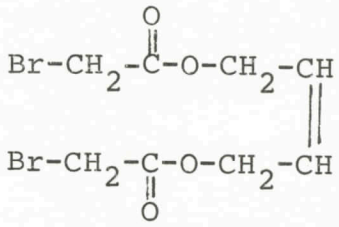
Table 1. Klebsiella strains isolated from various samples of paper mill process waters.

IVL:nr	Taxon	API:nr	Locality
HA-2	Klebsiella pneumoniae	5215 773	Hallsta
273	Klebsiella pneumoniae	5215 773	Iggesund
298	Klebsiella pneumoniae	5215 573	Hallsta
300	Klebsiella pneumoniae	5215 773	Nymölla

Table 2.

	Class	Manufactural	Trade Name
V-10	2	Gullviks	Slimicide V-10
T-9	2	CDM	Chemviron Thiocyanat T-9
R-80	3	Sanbolagen	Paraclox R-80
J-26	3	CDM	Chemviron Amin J-26

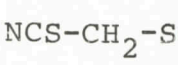
Table 3.



Slimicide V-10

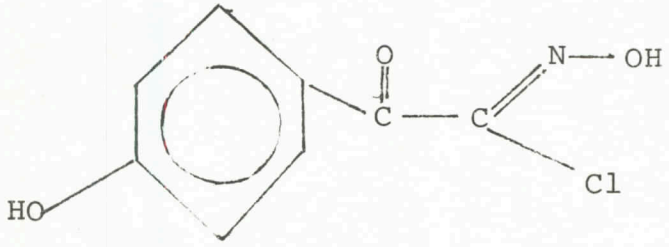
Bis-1,4-bromacetoxy-2-butene

Chemviron Thiocyanat T-9



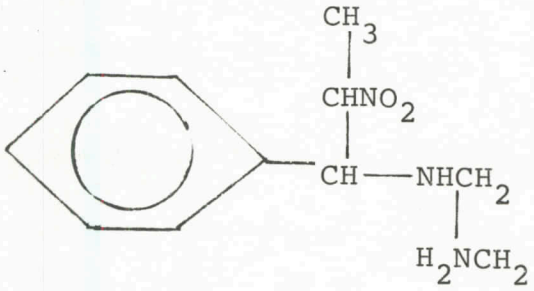
Methylenebisthiocyanate

Paraclox R-80



Parahydroxy-2-oxophenylacetohydroxamic acid chloride

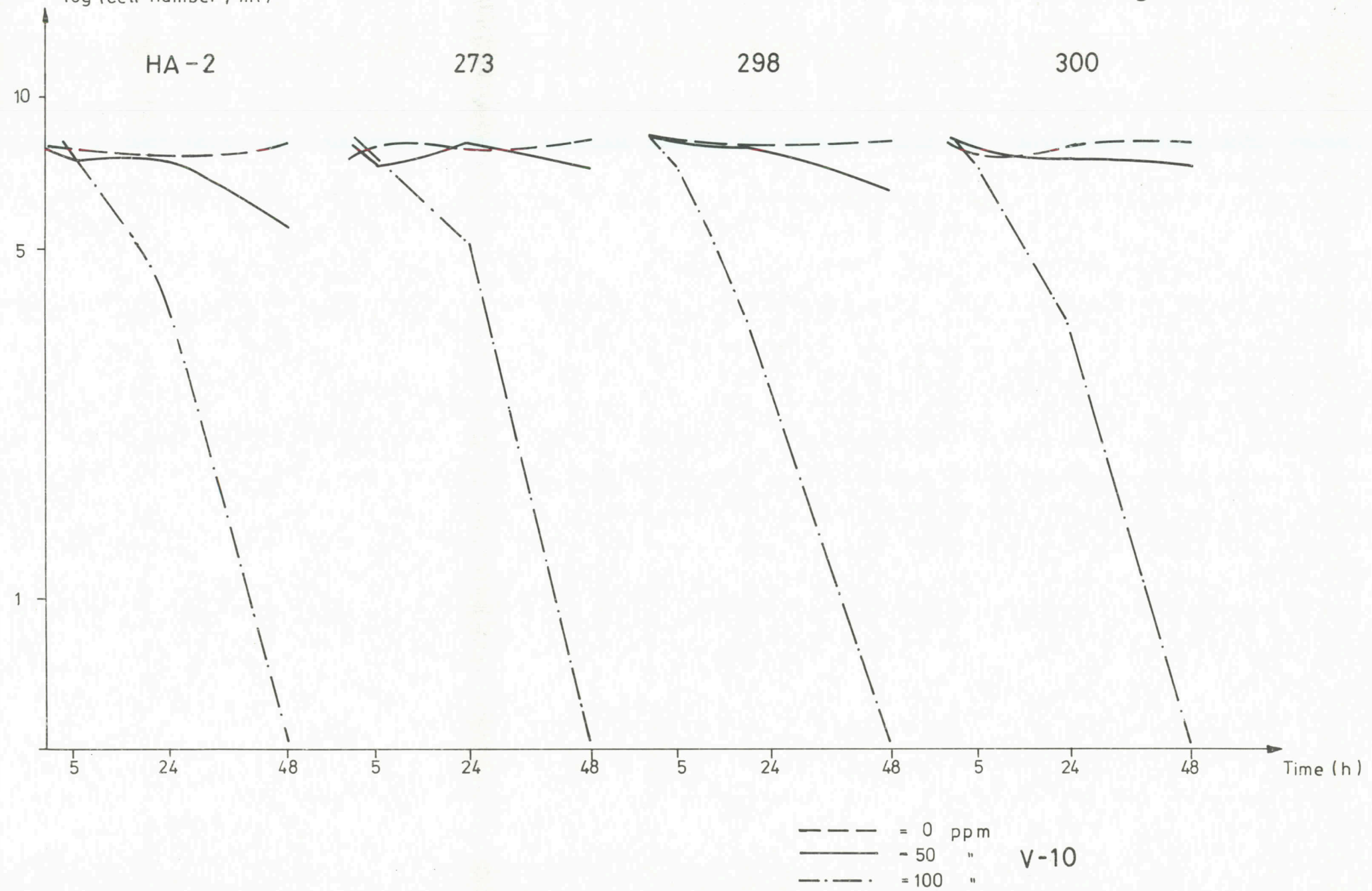
Chemviron Amin J-26



N-[α-(1-nitroethyl)benzyl]-ethylenediamine

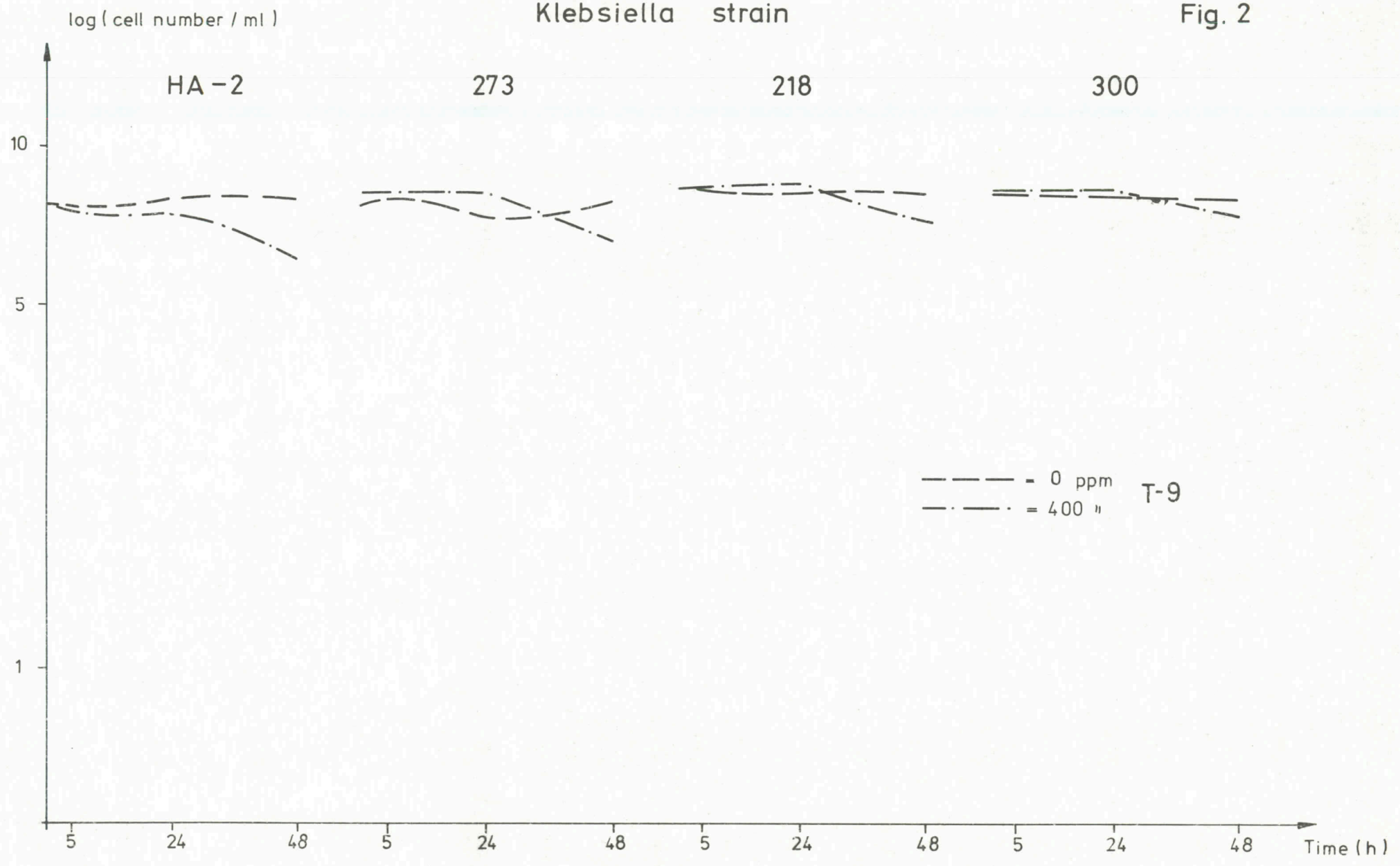
Klebsiella strain

Fig.1



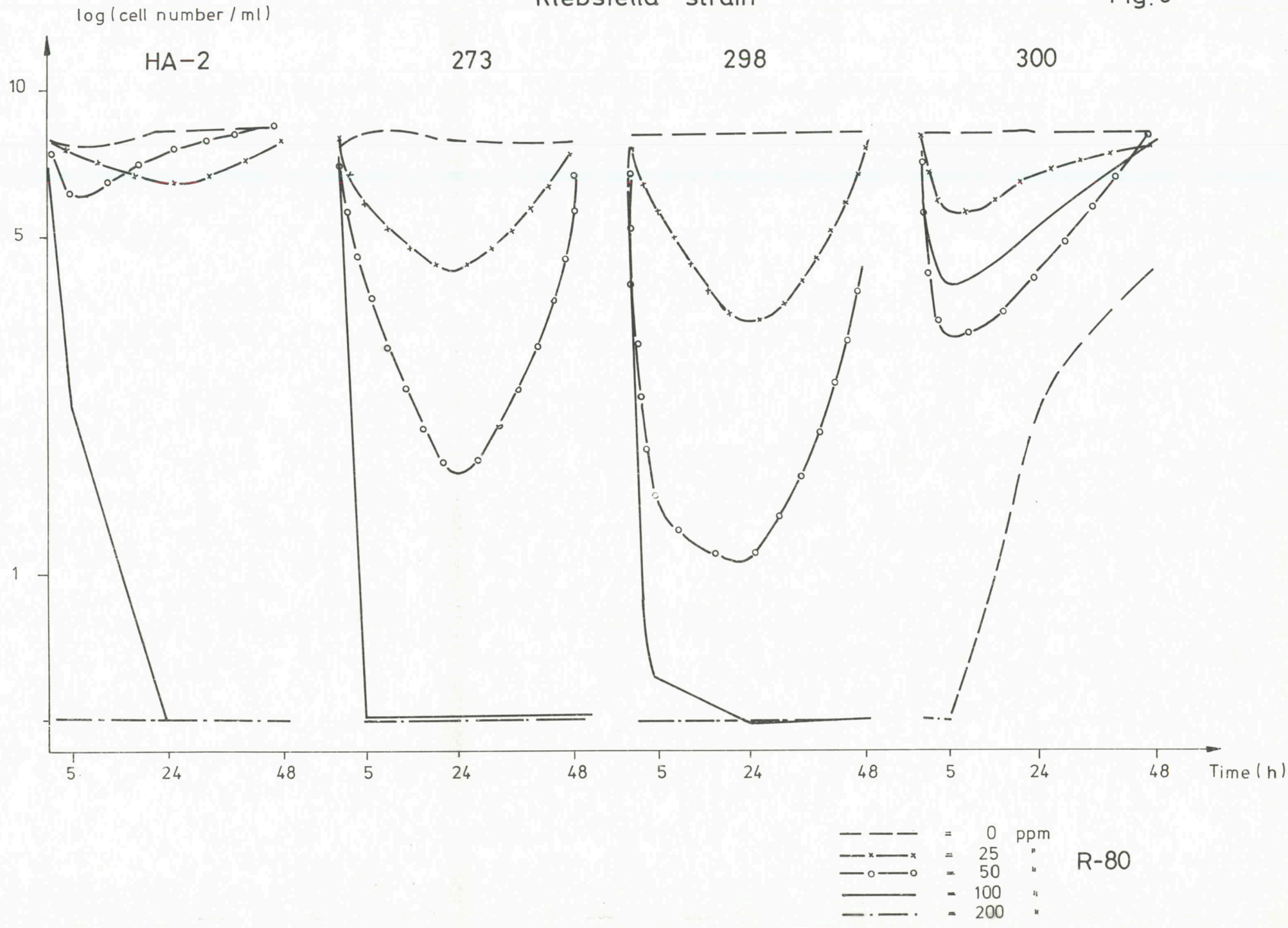
Klebsiella strain

Fig. 2



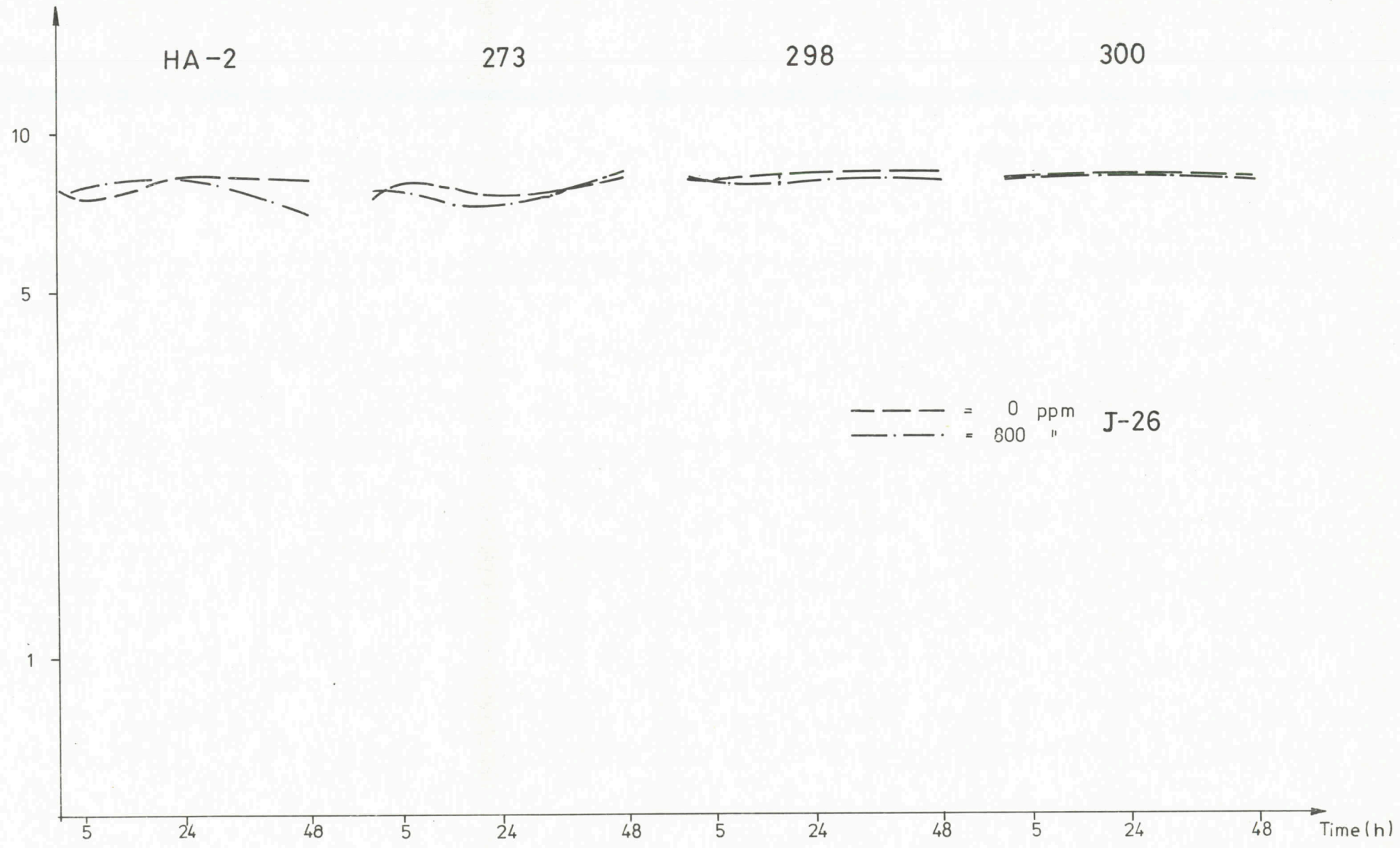
Klebsiella strain

Fig. 3



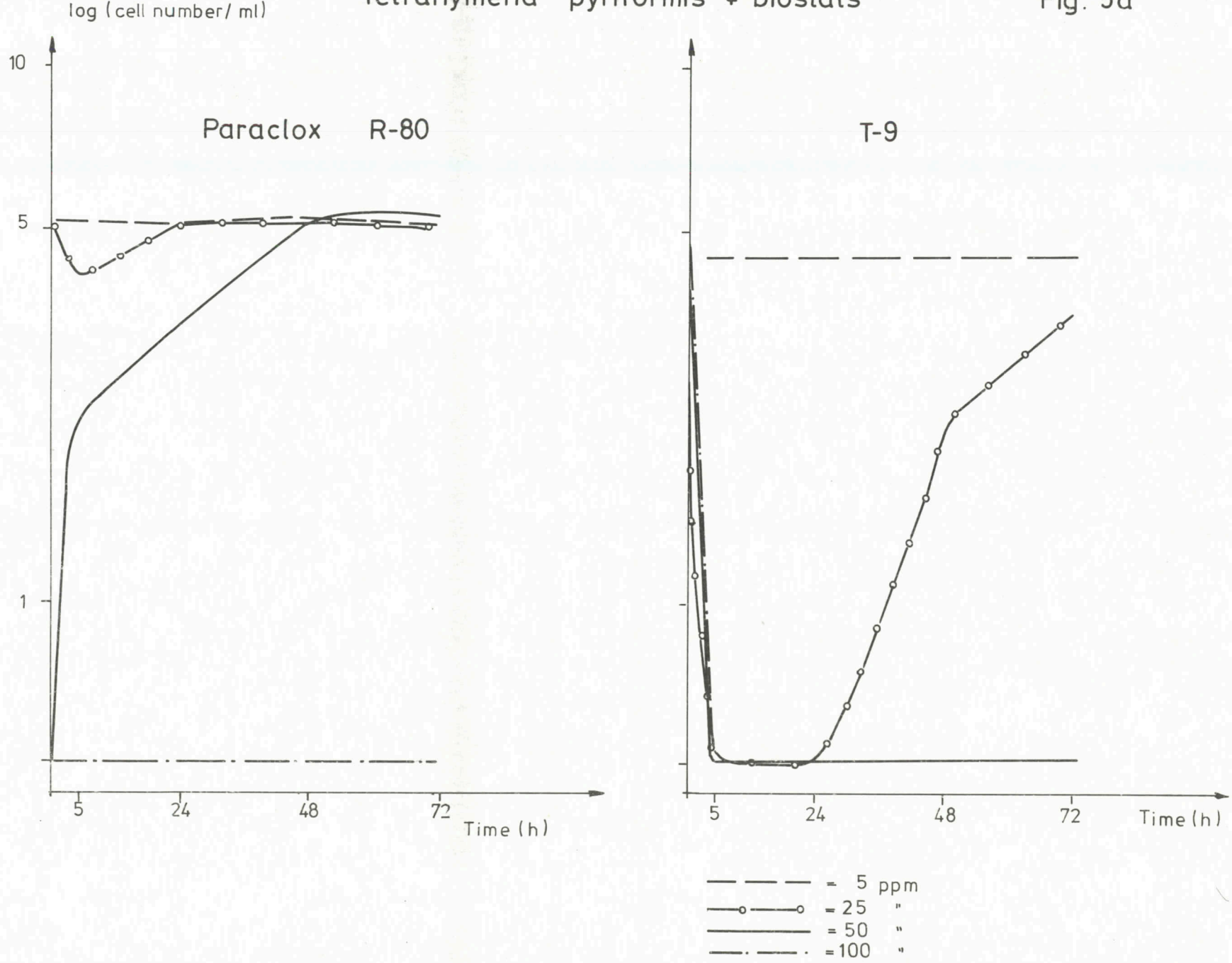
Klebsiella strain

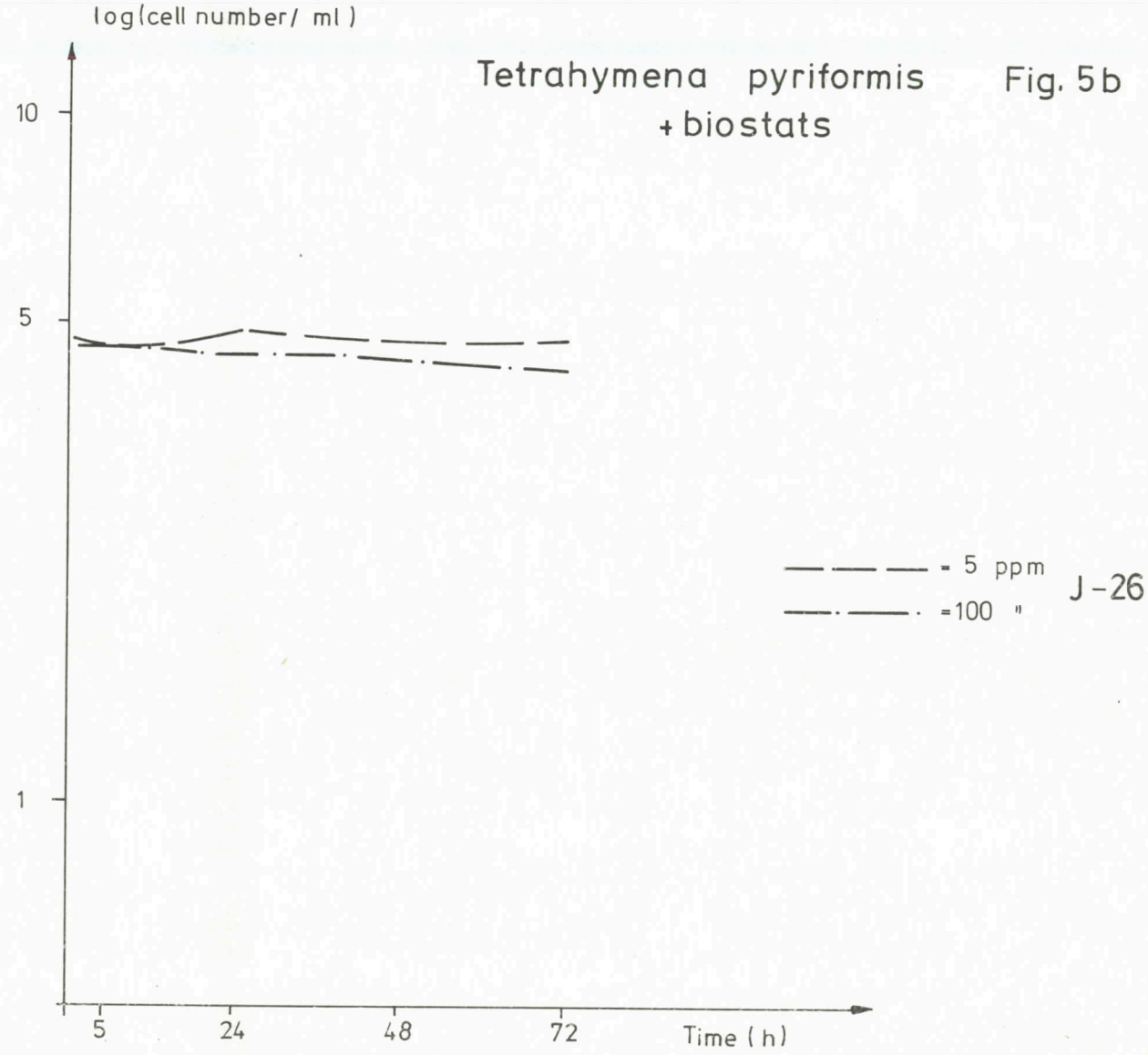
Fig. 4



Tetrahymena pyriformis + biostats

Fig. 5a





Tetrahymena pyriformis + biostats

Fig. 5b

J-26

Fig. 6

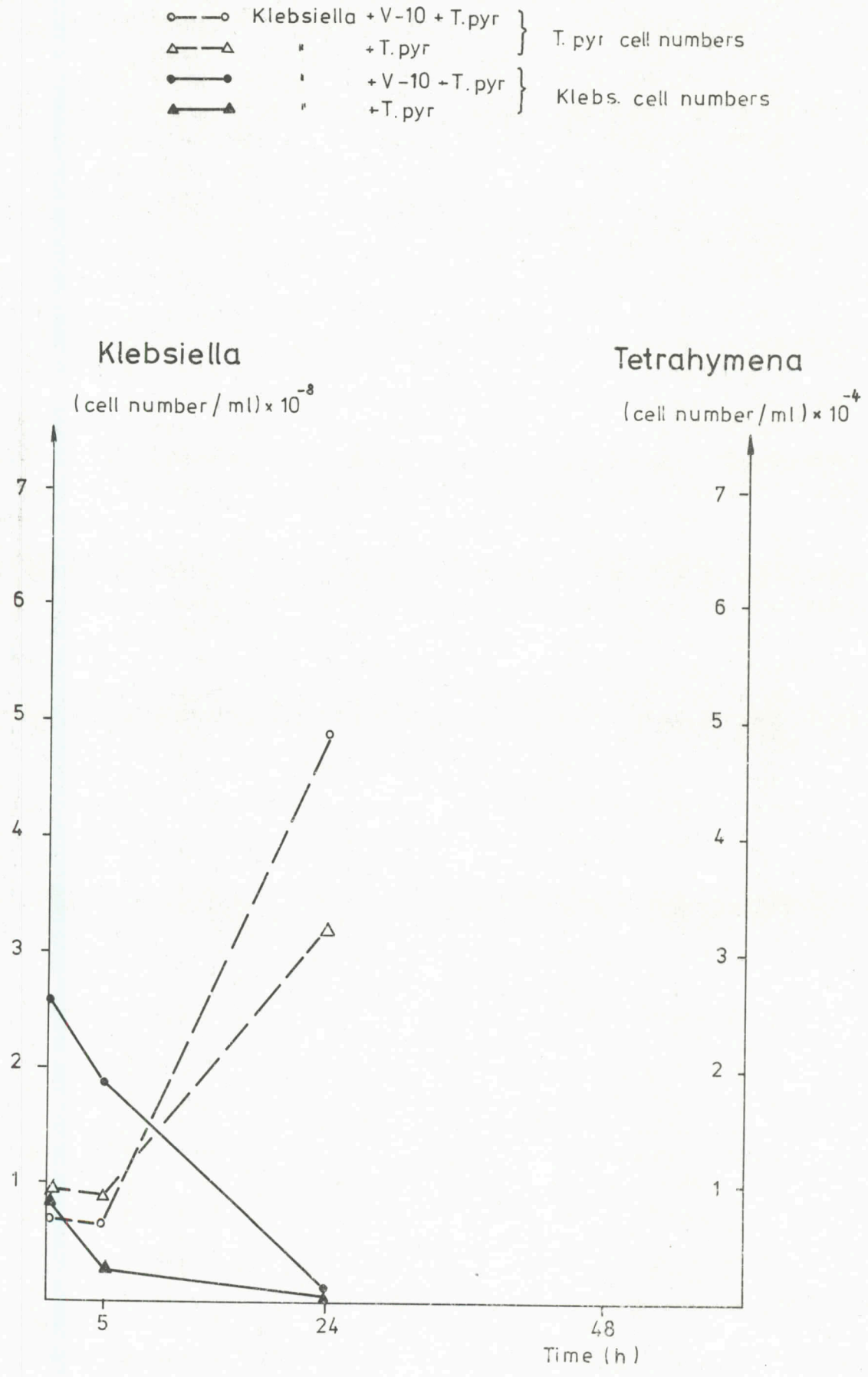
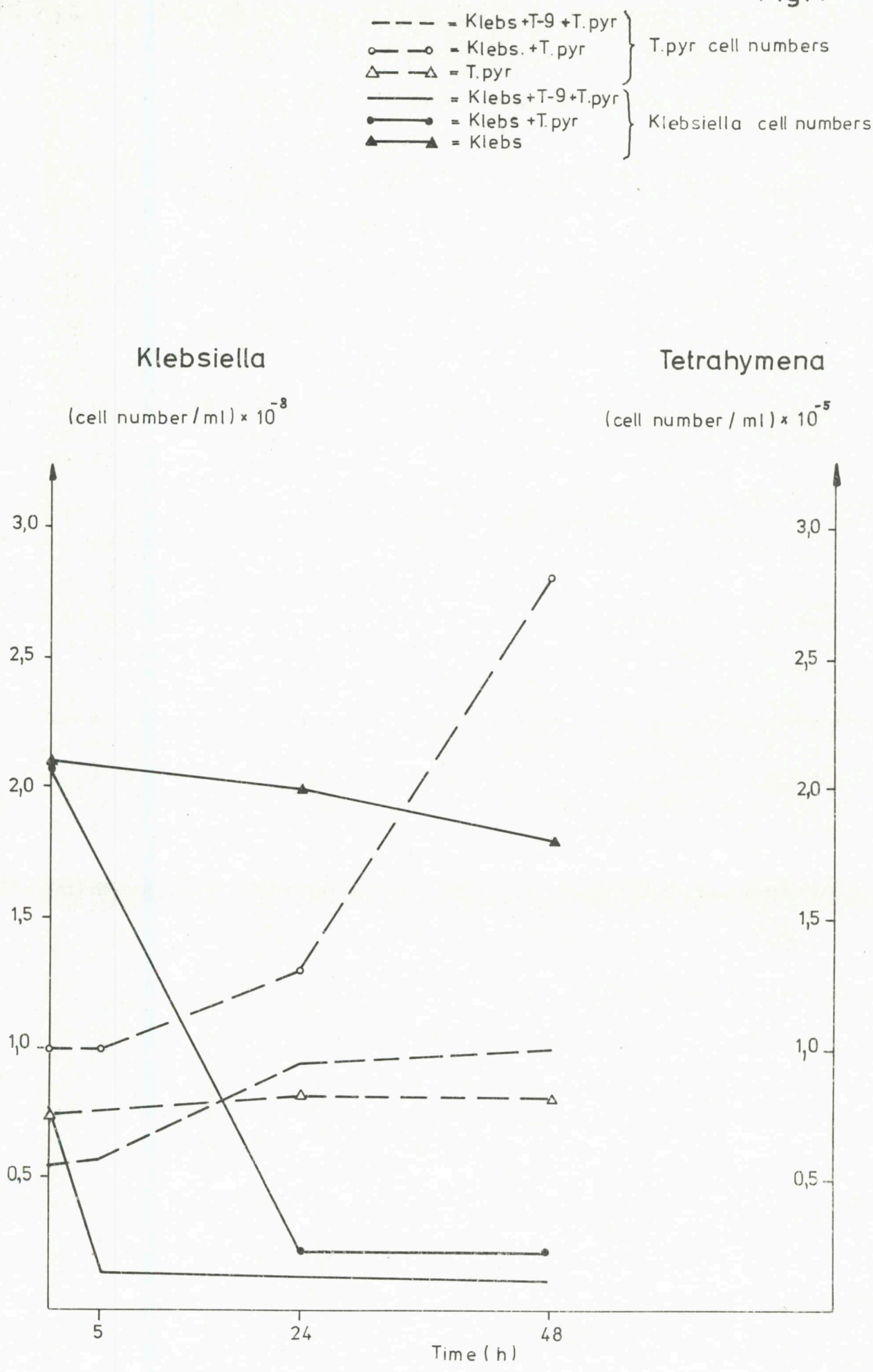

Klebsiella + V-10 + T.pyr
" + T.pyr
" + V-10 + T.pyr
" + T.pyr
T. pyr cell numbers
Klebs. cell numbers
Klebsiella
(cell number / ml) × 10^-8
Tetrahymena
(cell number / ml) × 10^-4
7
6
5
4
3
2
1
5
24
48
Time (h)

Fig. 7


= Klebs +T-9 +T.pyr
= Klebs. +T.pyr
= T.pyr
T.pyr cell numbers
= Klebs +T-9 +T.pyr
= Klebs +T.pyr
= Klebs
Klebsiella cell numbers
Klebsiella
(cell number / ml) × 10⁻⁸
Tetrahymena
(cell number / ml) × 10⁻⁵
3,0
2,5
2,0
1,5
1,0
0,5
5
24
48
Time (h)