RADIOMETRIC DETECTION OF BACTERIA

Pages with reference to book, From 34 To 38

Syed J. Khurshid (Nuclear Chemistry Division, Pakistan Institute of Nuclear Science & Technology, P.O. Nilore, Rawalpindi.)
M. Mubashir A. Khan, B. Shahnaz (Pakistan Medical Research Council, National Institute of Health, Islamabad.)

Abstract

The radiometric method has been used to detect the presence of bacteria in serial dilutions of nine bacterial species with and without agitation. It was also found that the detection time is proportional to the dilution showing high sensitivity down to one colony forming unit and the agitation resulted in different manners depending upon bacterial species (JPMA 37 : 34 , 1987).

INTRODUCTION

The automation in thç clinical microbiology has eased the diagnosis and treatment of the patients by providing the information with rapidity, accuracy, and uniformity. The radio-metric methodology is now utilized in variety of microbiological techniques such as to detect bacteria in blood[1-5] rapid anti-biotic susceptibility test[6-8] detection of bacteria in food[9] and cosmetics, detection of white cell metabolism[10] and sterility testing of radiopharmaceuticals[11-12].This technique was developed and used by NASA to search life on planets (Mars) [13].
The results with the radiometric method of detection of bacteria has been reported by number of workers. Our experience with the automated Bactec 460 (Figure 1),

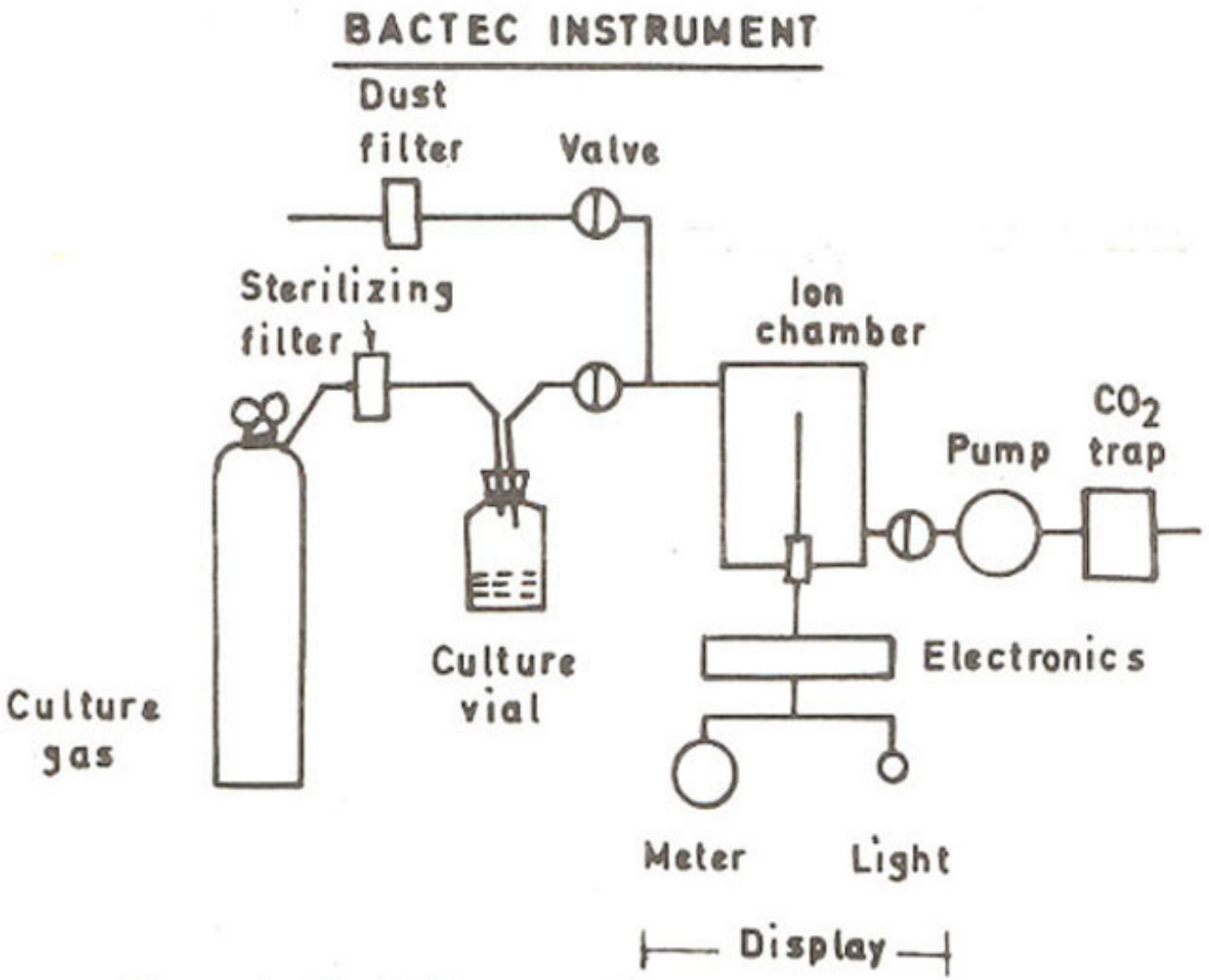

Figure 1. Block Diagram of the principle of operation of Bactec unit.

an instrument using radio-metric procedure for detection of radiolabelled metabolites is reported here.

MATERIAL AND METHOD

In this automated procedure a 3-5m1 sample is incubated at 35.37°C in a sealed rubber septum vial with a liquid^{14} C-labelled sterilized substrate with an activity of: 2 uCi per vial. If bacteria are present they metabolize carbohydrate or protein, the components of the substrate as energy source, releasing^{14} co2 by catabolizing glucose or by decarboxylation of aminoacid produced during incubation. The sterile needles of Bactec 460 pierce through rubber septum into the vial above nutritive media and the^{14} C02 produced during the incubation period is then aspirated from the test vial through sterilizing filter into the ionization chamber, the electrometer present in Bactec unit then measures the current produced in the ionization chamber. This measurement is converted to growth index (GI) reading which is an arbitrary liner scale related to the amount of radioactivity in the ionization chamber. The amount of 14 CO2 liberated is proportional to the amount of the bacterial growth in the nutrient media. A reading of 100 GI corresponds to 0.025 microcurie of^{14} C. A threshold GI may be set which is usually 30 for aerobic vials and 20 for anaerobic vials, a reading above threshold level indicates the presence of

bacteria.
The ionization chamber of Bactec is exhausted through a CO2 trap to prevent release of radioactive material into room air. The model Bactec 460 which we have, allows 60 vials to be placed on it at one time and do sequential testing, printing the data simultaneously, flagging the sample as positive above threshold GI reading, taking only one minute for each sample. The bacterial concentrations of 10,3 102. 10 and 1 of nine species were prepared in peptone water and used for this experiment. These bacterial species namely were Escherichia co/i 0111, Salmonella typhi, Shigella flexnerii, Salmonella paratyphi B, Proteus mirabilis, Pseudomonas peutrifaciens, Pseudomonas aeruginosa, Providencia steuartti, Salmonella enteritidis, Citrobacter freudii and 2 ml of each bacterial concentration injected in 6B aerobic vial containing tryptic soy broth and ^{14} C labelled substrate and incubated at 37°C to find the effect of concentration on detection time. Another set of each species was prepared in duplicate, one set was incubated without agitation and one with agitation at a speed of 200 strokes min^{1} at 37°C to find the difference between the behaviour of agitated and non-agitated samples. The samples were checked after every 2 hours upto 12 hours and then after 24 hours, 48 hours, 72 hours and 96 hours.

RESULTS AND DISCUSSION

The results of bacterial dilution are plotted against the time of detection in Figure 2.

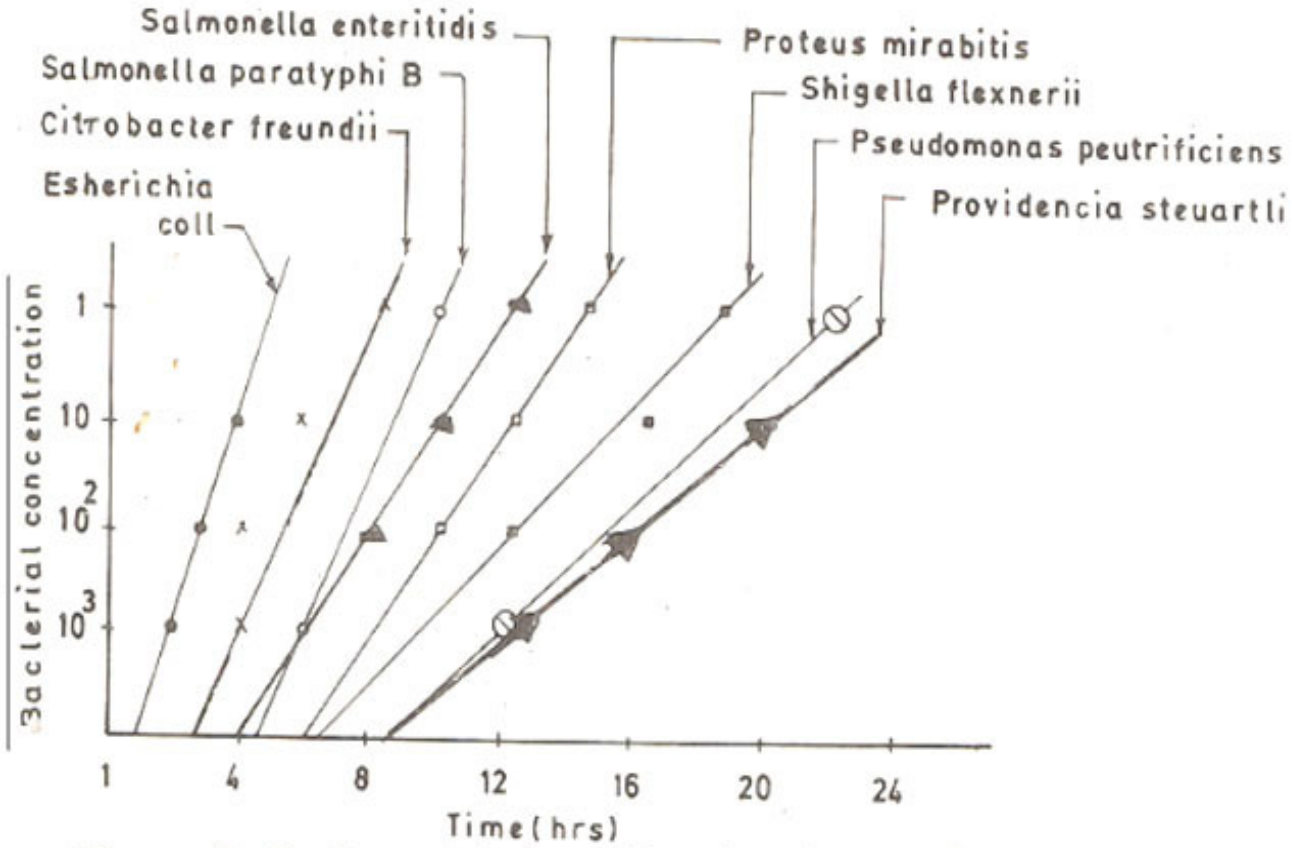

Figure 2. Radiometric detection time for serial dilutions of eight different bacterial species.

The results show that the bacterial detection time is proportional to the initial concentration of bacterial suspension which varies from species to species. Our results have shown bacterial recovery in a little longer time as compared to others depending on the use of 200 strokes min^{1} instead to 250 strokes

min^{1}. It also shows high sensitivity of the radiometric method, showing that the method can be used for quantification of the bacterial contents in different samples.
The results of the agitated and non-agitated samples have also been plotted as growth index versus time (Figure 3-11).

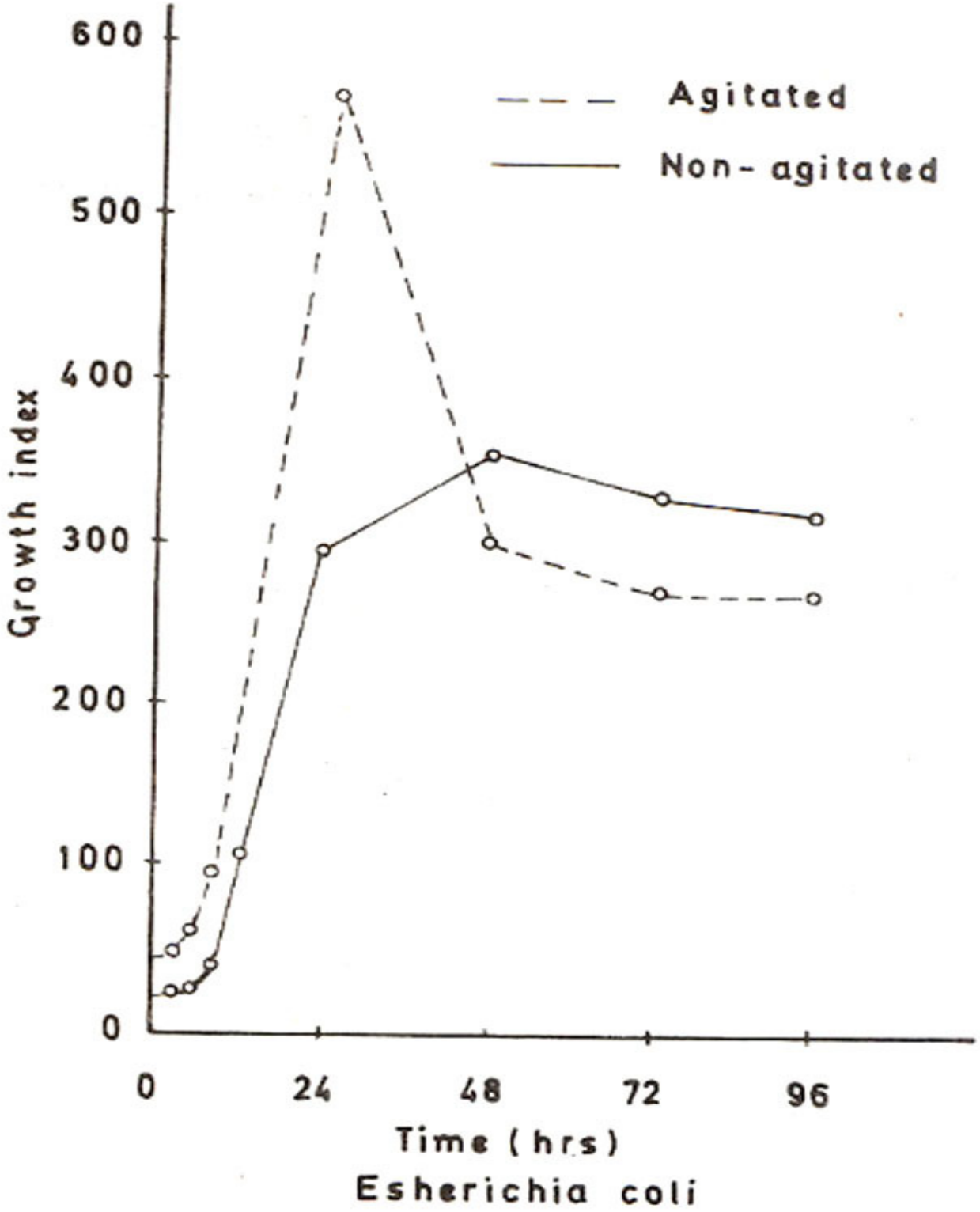

Figure 3. Effect of agitation upon bacterial detection.

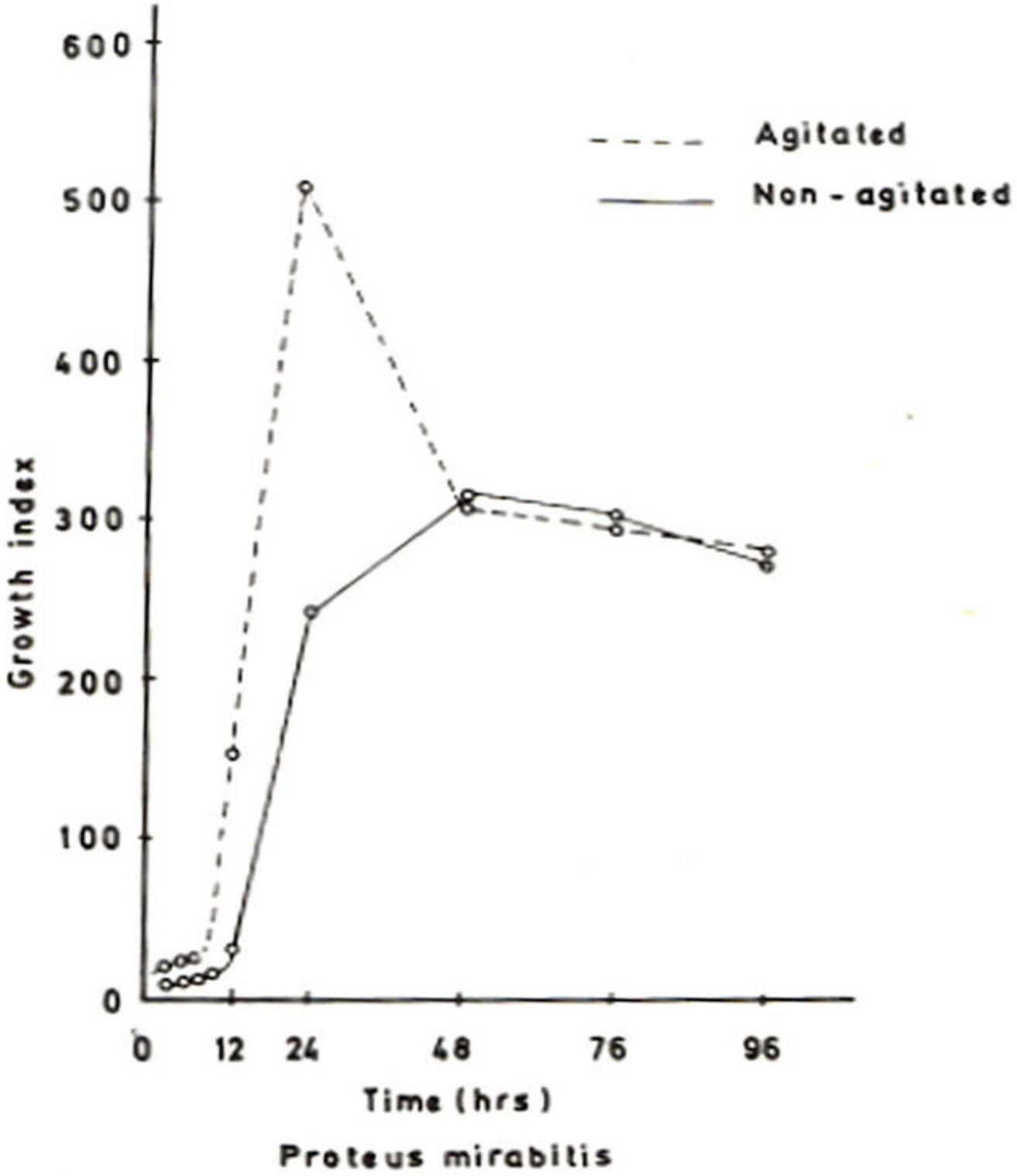

Figure 4. Effect of agitation upon bacterial detection.

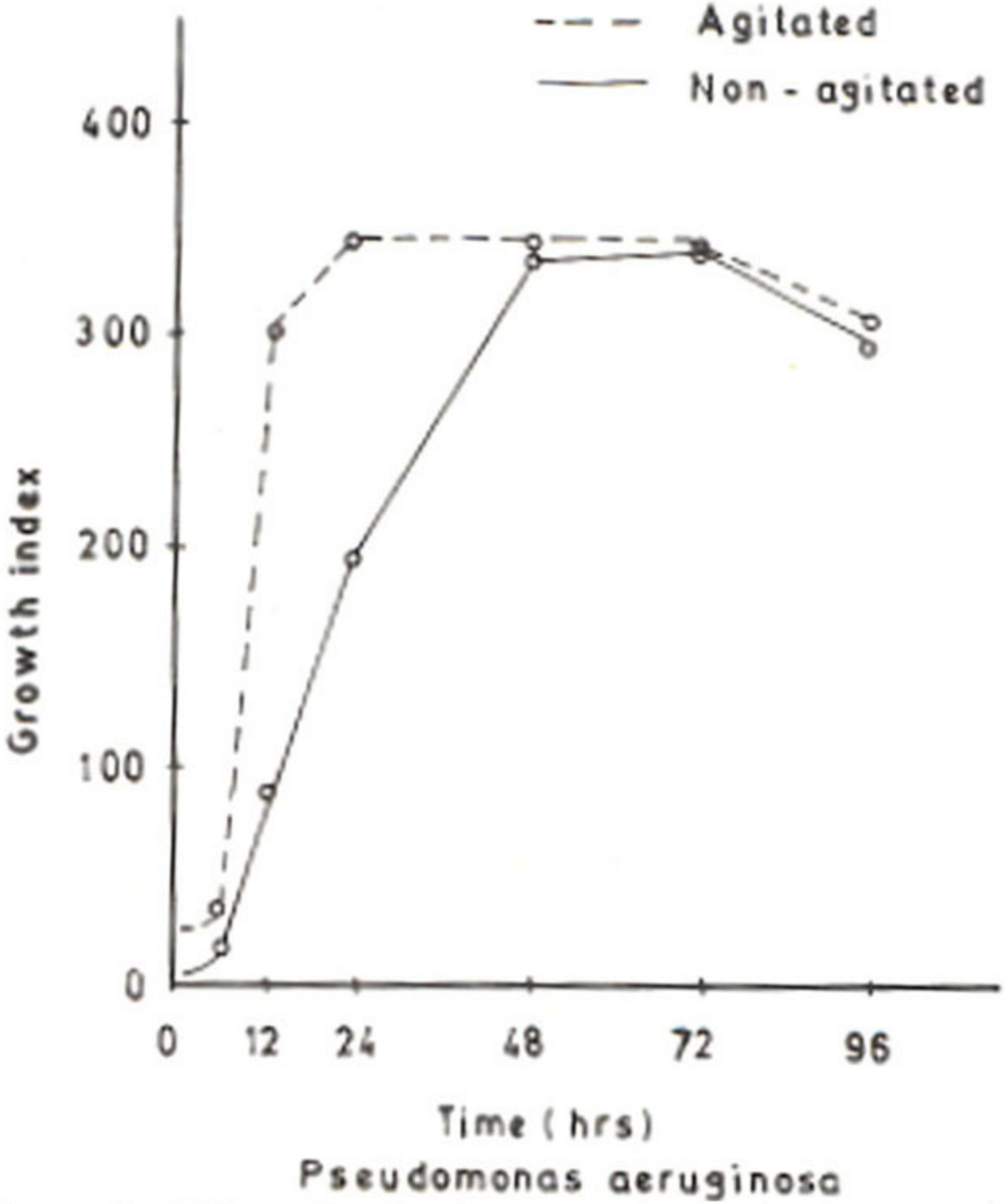

Figure 6. Effect of agitation upon bacterial detection.

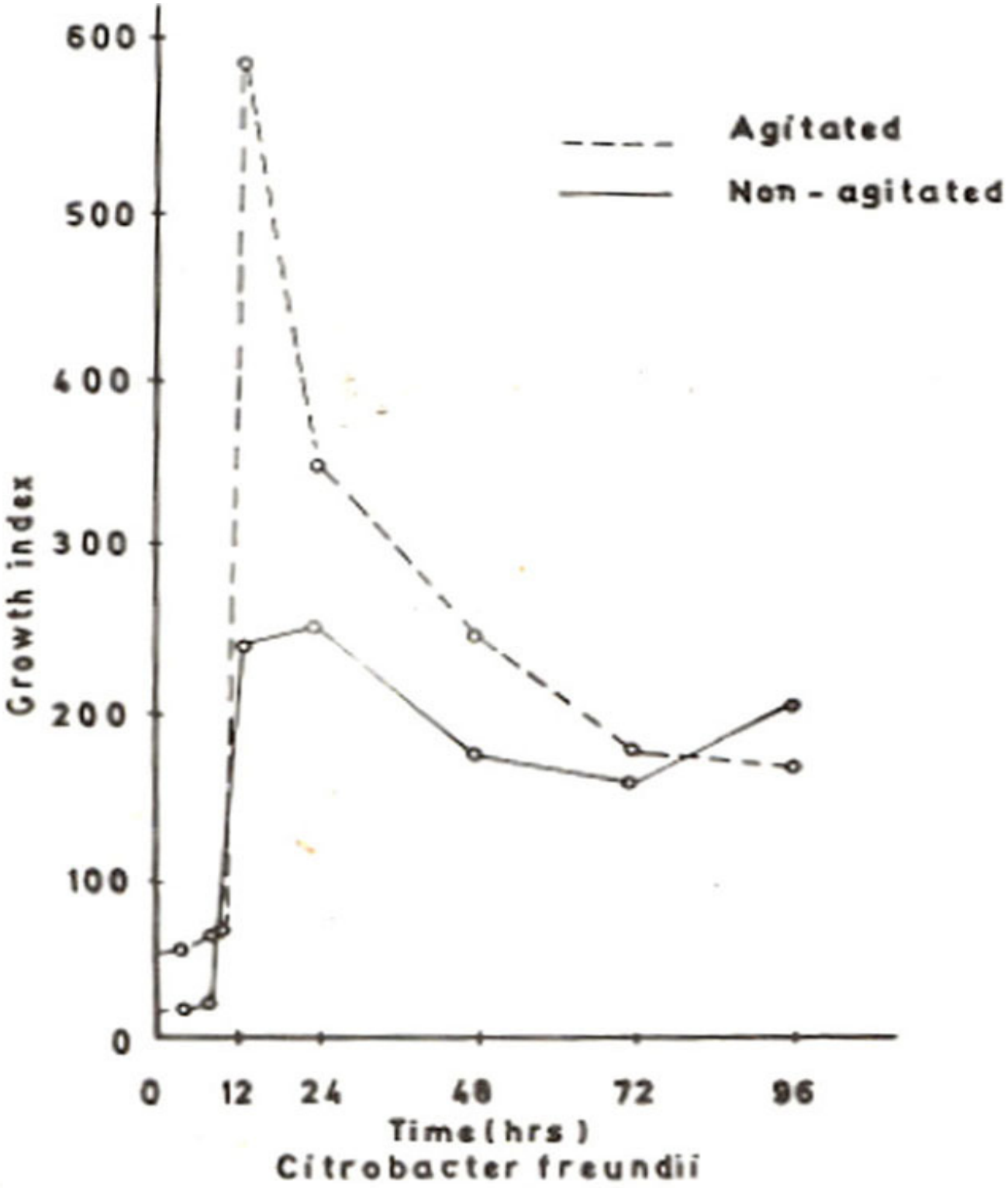

Figure 5. Effect of agitation upon bacterial detection.

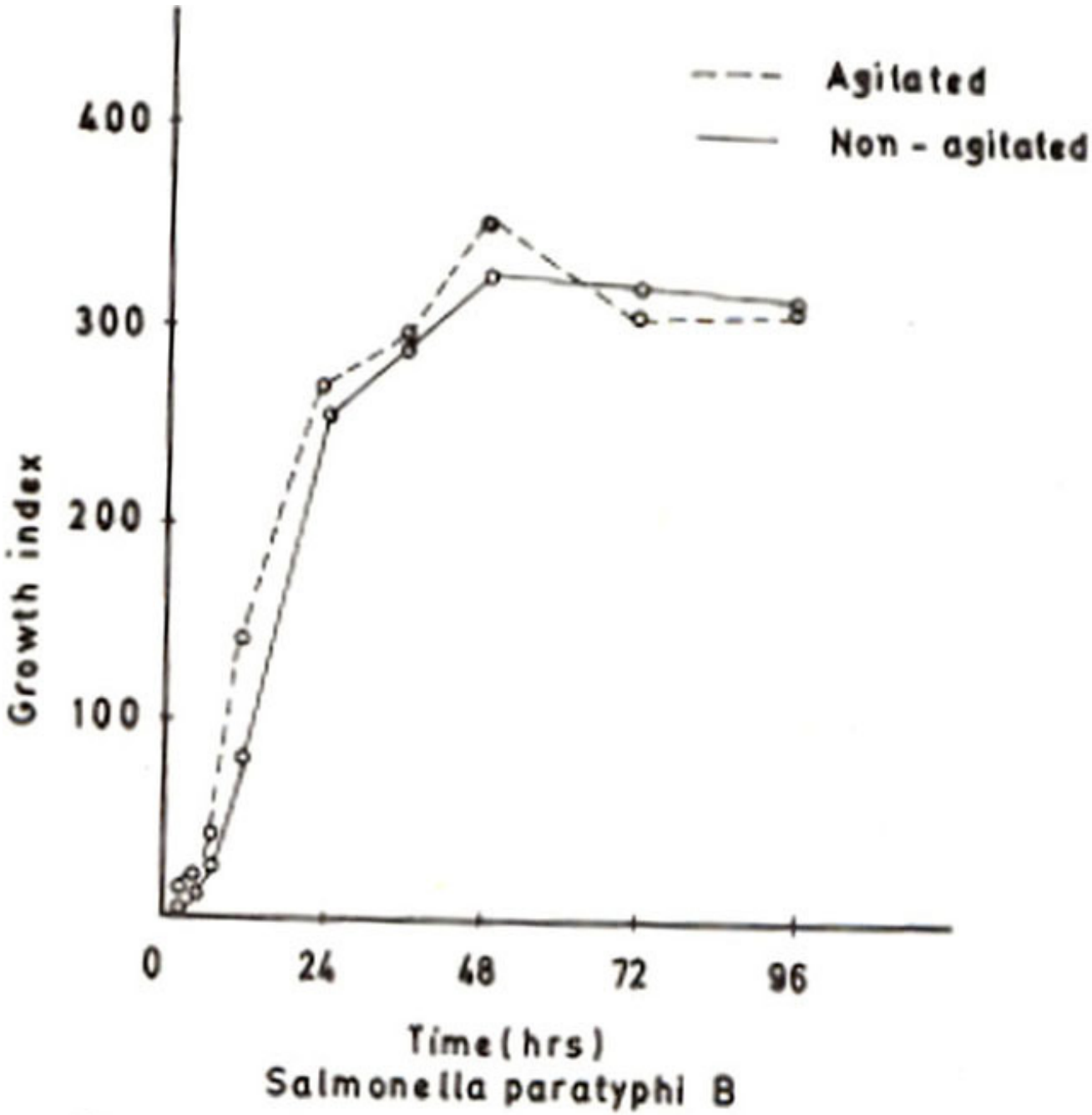

Figure 7. Effect of agitation upon bacterial detection.

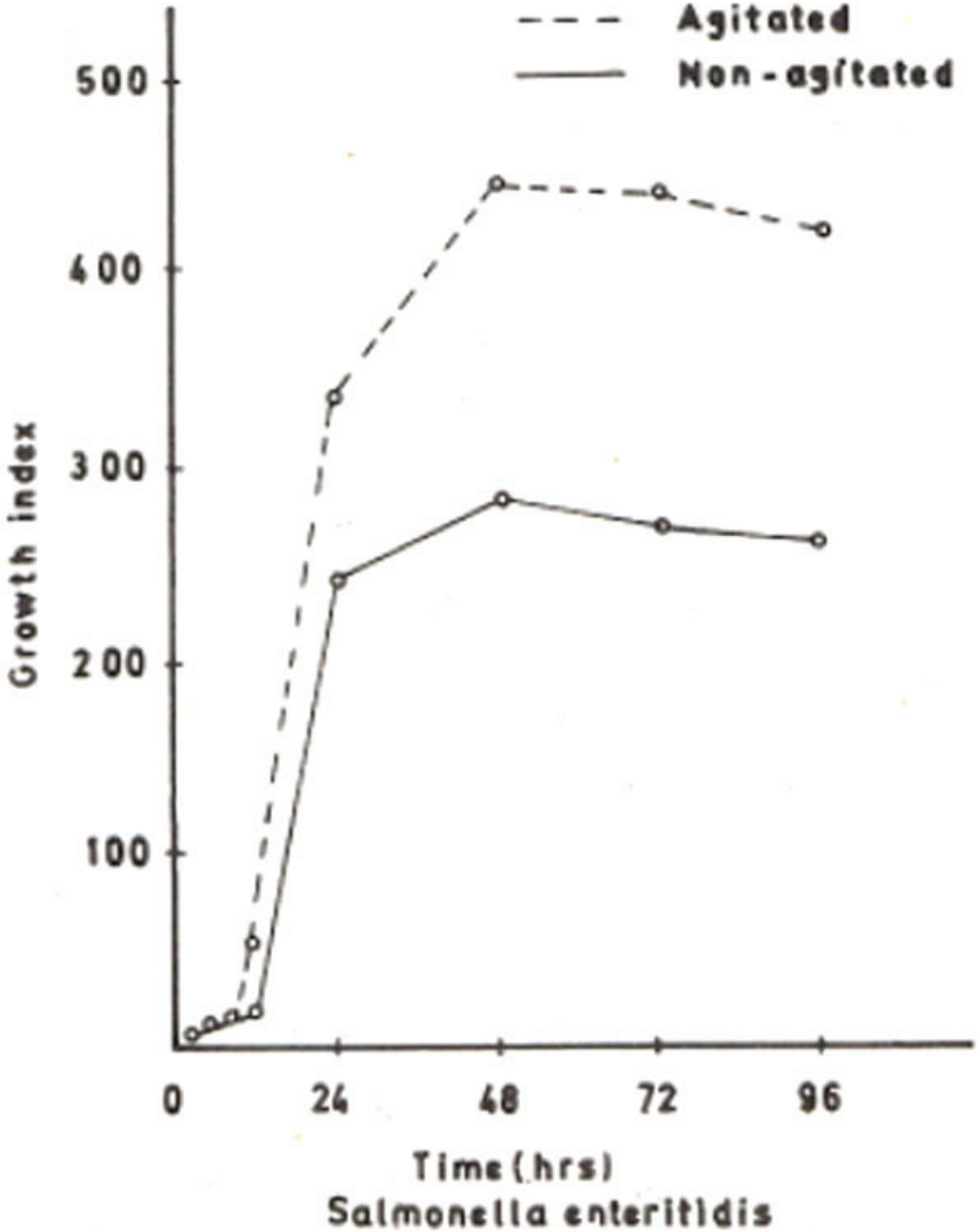

Figure 8. Effect of agitation upon bacterial detection.

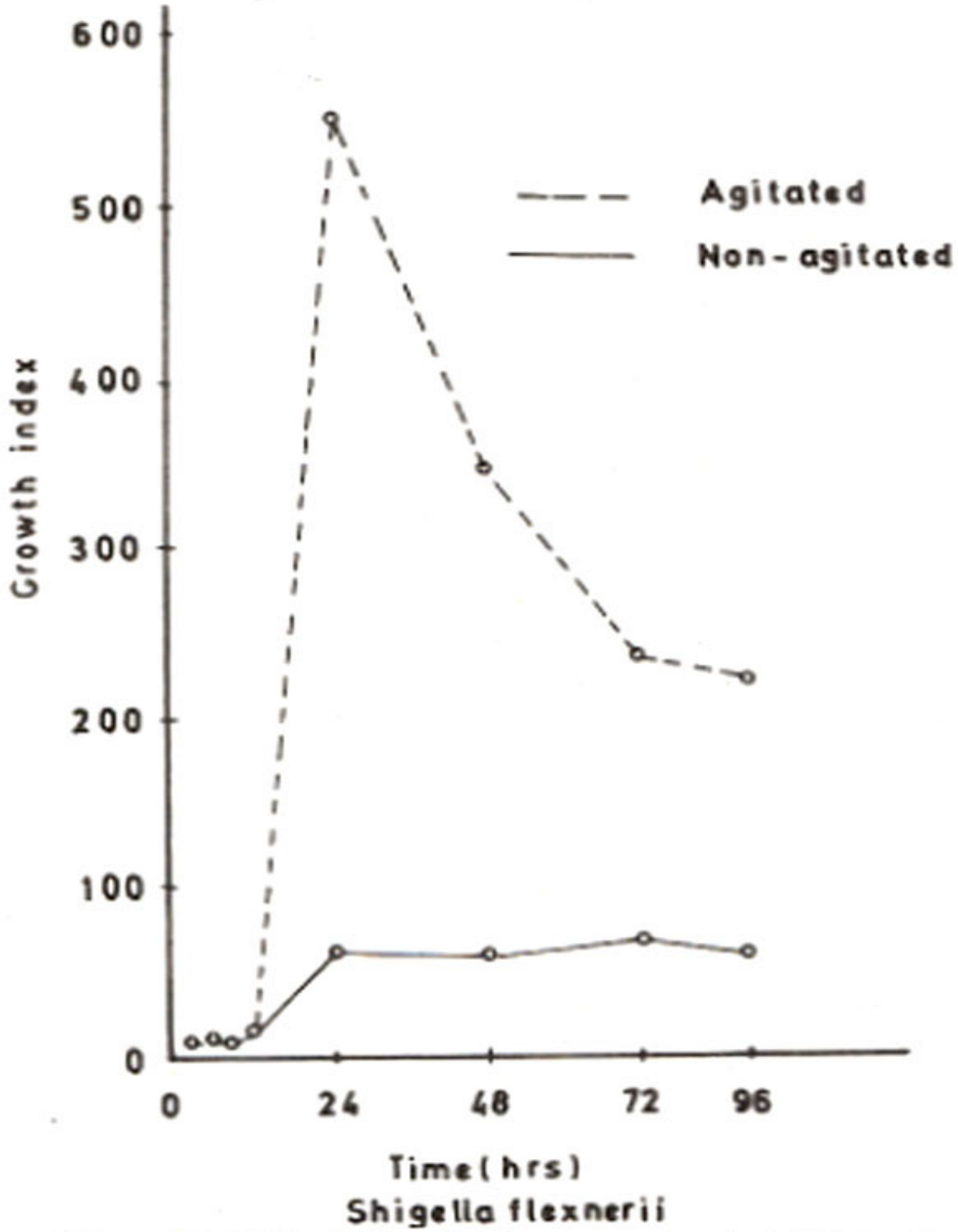

Figure 9. Effect of agitation upon bacterial detection.

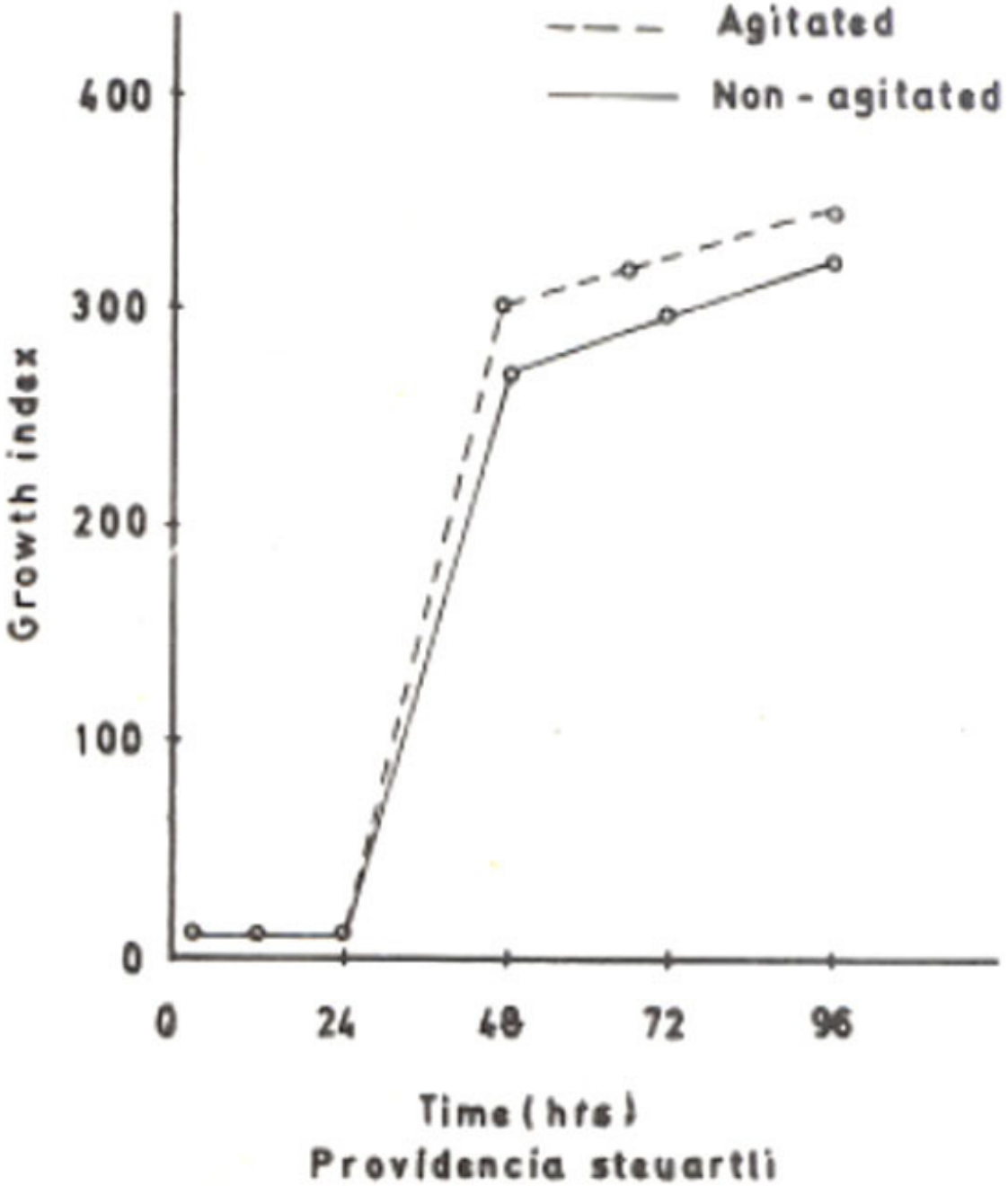

Figure 10. Effect of agitation upon bacterial detection.

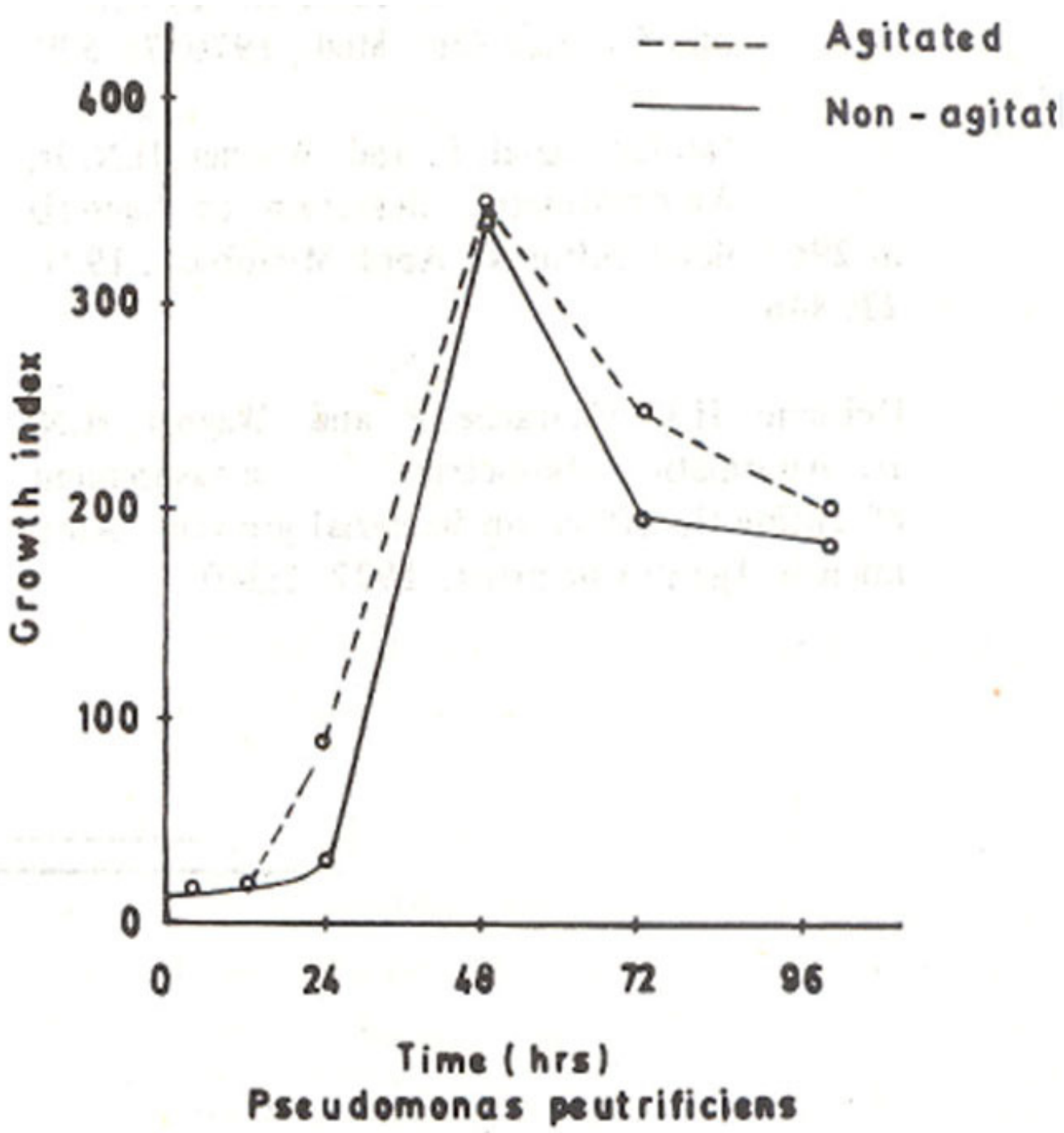

Figure 11. Effect of agitation upon bacterial detection.

The results show that agitation affects the detection time and growth of bacterial species in the following manner but again our results have shown a late maxima in the peaks due to less agitation speed. This also shows that as we increase the speed of the agitation the recovery increases to certain extent beyond which damage can occur.

a. The species Escherichi.a coli, Proteus mirabilis and Citrobacterfreundll have shown faster detection besides a higher growth index.

b. The Pseudomonas aeruginosa, Salmonella paratyphi B have also shown a faster detection but the same growth index.

c. The Pseudomonas peutrifici ens, Providencia steuartti have shown no or little effect on detection

time as well as on growth index.
These findings have shown that usually the agitation affects the detection time and growth index.
The advantage of radiometric method is standardized methodology, short time required for detection of growth compared to conventional system, repetitive monitoring, detection of organisms to single colony, detection of nonvisualized bacteria, identification of Neisseria in 3 hours[14] suspectibility test of mycobacteria in 3-6 days[15] and detection of mycobacterium tuberculosis in 10-12 days[16] estimation of gentamycin and vitamin B12 in patients serum[17] and sterility testing of short live radiopharmaceuheals.[17]

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