

**UNIVERSITY TEKNOLOGI MARA**

**DETERMINATION OF OPTIMAL GROWTH  
PHASE AND INOCULUM SIZE OF *Enterobacter  
aerogenes* (ATCC 13048) FOR LONG TERM  
STORAGE (STOCK CULTURE)**

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## AUTHOR'S DECLARATION

I hereby declare that based on my original work and has not been submitted previously or currently for other degree in Universiti Teknologi MARA (UiTM) or any other institution.

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## ABSTRACT

### **Determination of Optimal Growth Phase and Inoculum Size of *Enterobacter aerogenes* (ATCC 13048) For a Long-Term Storage of Stock Culture**

Stock culture is the microorganism which is maintained under a specific condition to ensure the viability of microorganisms for a longer storage. *Enterobacter aerogenes* (ATCC 13048) was chosen for preparation of stock culture and it is commonly associated with nosocomial infection. Preparation of bacteria culture can reduce expenses of purchasing from overseas and can supply continuous bacterial stock for study purposes. Pure culture of *E. aerogenes* (ATCC 13048) was obtained on 5% sheep blood agar and was subcultured on MacConkey agar. Biochemical tests showed positive reaction for citrate, Voges-Proskauer, motility and acid/acid with gas for triple sugar iron (TSI) test. Optimal growth phase can be identified through a growth curve graph plotted using concentration measurement at OD600 nm and colony count (CFU/ml) of *E. aerogenes* (ATCC 13048) against every hour of incubation at 35°C in Trypticase Soy Broth (TSB) media. The growth curve shows that *E. aerogenes* (ATCC 13048) has a short lag phase which lasted 2 hours in 35°C incubation in TSB media. The absorbance (OD600) and colony forming unit (CFU/ml) for *E. aerogenes* (ATCC 13048) have same starting point of exponential phase growth which started at 2<sup>nd</sup> hour and ended at 4<sup>th</sup> hour in 35°C incubation before the start of stationary phase. The study has shown that mid-exponential phase of *E. aerogenes* (ATCC 13048) in TSB was successfully determined at 3<sup>rd</sup> hour in 35°C incubation with concentration at OD600 nm 1.92 and corresponding colony count  $9.9 \times 10^8$  CFU/ml as the optimal growth phase for harvesting bacterial cells for storage. Temperature used for storage using glycerol stocks were 4°C, -20°C and -80°C while for microbeads vials were -20°C and -80°C. In conclusion, all stock cultures from glycerol stocks and microbeads vials of *Enterobacter aerogenes* (ATCC 13048) were recovered in pure cultured and viability maintained after one month storage at 4°C, -20°C and -80°C.

**Keywords:** *Enterobacter aerogenes*, preservation, glycerol stock, microbead, colony count