

**UNIVERSITY TEKNOLOGI MARA**

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

**NURUL NAJIHAH AB HAMID**

Thesis submitted in partial fulfilment of the requirement for

**Bachelor of Medical Laboratory Technology (Hons.)**

**Faculty of Health Sciences**

July 2019

## 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.

Name of Student : Nurul Najihah binti Ab Hamid

Student ID Number : 2016409398

Programme : Bachelor of Medical Laboratory Technology (Hons)

Faculty : Health Sciences

Thesis Title : Determination of Optimal Growth Phase and Inoculum Size of *Enterobacter aerogenes* (ATCC 13048) for a Long-term Storage (Stock Culture)

Student's Signature : .....

Date : July 2019

## **ACKNOWLEDGEMENT**

Firstly, I would like to wish my gratitude to my supervisor, Dr Roslinah Mohamad Hussain for her guidance, encouragement and advice in completing my Final Year Project. Her guidance and commitment to teach her student throughout all the process in completing this study. I would like to thank all the lecturers in Department of Medical Laboratory Technology, Faculty of Health Sciences who always share their thought, knowledge and advice throughout my study in UiTM Puncak Alam.

I would like to acknowledge the lab staff in Centre of Microbiology, Department of Medical Laboratory Technology for their cooperation to help and guidance during research process.

Finally, special thanks to all lecturer involved, supportive teammate, all family members and friends for their encouragement until completing this study.

## TABLE OF CONTENTS

TITLE.....	i
AUTHOR’S DECLARATION .....	ii
APPROVAL.....	iii
ACKNOWLEDGEMENT .....	iv
TABLE OF CONTENTS .....	v
LIST OF TABLES .....	vii
LIST OF FIGURES .....	viii
LIST OF ABBREVIATIONS .....	ix
ABSTRACT .....	x
INTRODUCTION .....	1
1.1 Background of Study .....	1
1.2 Problem Statement .....	3
1.3 Research Objective .....	4
1.3.1 General Objective .....	4
1.3.2 Specific Objective .....	4
1.4 Hypothesis .....	4
1.4.1 Alternative Hypothesis .....	4
1.4.2 Null Hypothesis .....	4
1.5 Significance of Study .....	5
LITERATURE REVIEW.....	6
2.1 American Type Culture Collection (ATCC) .....	6
2.2 <i>Enterobacter aerogenes</i> .....	6
2.3 Identification and Classification of Bacteria .....	8
2.4 Stock Culture for Storage .....	9

## 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