

**UNIVERSITI TEKNOLOGI MARA**

**THE POTENTIAL OF EFFECTIVE  
MICROORGANISM (EM) INCLUSION IN  
ENHANCING THE PROPERTIES OF  
CEMENT PASTE AND CONCRETE**

**NOORLI BINTI ISMAIL**

Thesis submitted in fulfillment  
of the requirements for the degree of  
**Doctor of Philosophy**

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## CONFIRMATION BY PANEL OF EXAMINERS

I certify that a Panel of Examiners has met on 12 April 2019 to conduct the final examination of Noorli binti Ismail in **Doctor of Philosophy** thesis entitled “The Potential of Effective Microorganism (EM) Inclusion in Enhancing the Properties of Cement Paste and Concrete” in accordance with Universiti Teknologi MARA Act 1976 (Akta 173). The Panel of Examiner recommends that the student be awarded the relevant degree. The Panel of Examiners was as follows:

Lee Wei Koon , PhD  
Senior Lecturer  
Faculty of Civil Engineering  
Universiti Teknologi MARA  
(Chairman)

Marzuki Ab.Rahman, PhD  
Senior Lecturer  
Faculty of Civil Engineering  
Universiti Teknologi MARA  
(Internal Examiner)

Megat Azmi Megat Johari, PhD  
Professor  
School of Civil Engineering  
Engineering Campus  
Universiti of Sains Malaysia  
(External Examiner)

Saroj Mandol, PhD  
Professor  
Department of Civil Engineering  
Universiti of Jadavpur, India  
(External Examiner)

**PROF SR TS DR HAJI ABDUL  
HADI HAJI NAWAWI**

Dean  
Institute of Graduates Studies  
Universiti Teknologi MARA  
Date: 29 May 2019

## AUTHOR'S DECLARATION

I declare that the work in this thesis was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the results of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

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Name of Student : Noorli binti Ismail

Student I.D. No. : 2011505645

Programme : Doctor of Philosophy – EC990

Faculty : Civil Engineering

: The Potential of Effective Microorganism (EM)  
Inclusion in Enhancing The Properties of Cement  
Paste and Concrete

:

Signature of Student : *noorli* .....

Date : May 2019

## ABSTRACT

Recently, Effective Microorganism (EM) has shown the potential to be used as new additives in cement based materials. Previous research works reported the incorporation of EM increased the compressive and splitting tensile strength of cement based, significantly. However, extensive evaluation of other aspects need to be investigated to study their potential since the incorporation of EM in cement based materials is still new area. Experimental works in this present study showed the incorporation of EM in cement paste increased up to 40% compressive strength and leading to produce low porosity which decreased at least 19% corresponding to cement paste without EM. However, the hydration process was completed longer than specimens without EM due to the earlier reaction of dehydration and decarbonation detected using thermogravimetric (TGA/DTG) test and presence of potassium thulium chloride in EM solution. This can be a reasoning to the delayed initial and final setting time in the microbed cement paste. Furthermore, the high compressive strength and low porosity also leading to improve the internal densification of cement matrix which reported the formation of calcium silicate hydrate (CSH) and ettringite by detecting from formation of bundle shape and needles like pine leaves, acicular, thin, narrow and pointed shape, respectively at later age of 28 days and 60 days using scanning electron microscopy (SEM). The denser and lesser void in internal microstructure for the microbed cement paste also the factor contributes towards high compressive strength. Due to high resulted compressive strength, survivability of EM bacteria in cement paste was successfully detected using Biolog Microbial Identification System (BMIS) by the presence of EM bacteria species *Microbacterium Flavescens*, *Leuconostoc Fallax* and *Achromobacter xylosoxidans* which was able to survive up to 28 days in cement paste. Subsequently, the establishment of relationship between compressive strength and total porosity was established for low, normal and high concrete at the age of 3, 28, 60 and 180 days. Incorporation of EM also produce lower porosity of concrete which was conducted by mercury intrusion porosimetry (MIP). Also the relationship of compressive strength from destructive and non-destructive tests was successfully established. Multivariable linear regression was chosen to predict the compressive strength by applying dual regression equation which considered two (2) independent variables together; pulse velocity and rebound number obtained from the experimental results. The establishment of multivariable regression equation for both specimens was proposed as;

$$\text{Control, } f_c = 0.0245\text{UPV} - 0.1572\text{RN} - 79.422, R^2 = 0.844$$

$$\text{Microbed, } f_c = 0.00247\text{UPV} - 0.247\text{RN} - 83.803, R^2 = 0.915$$

where  $f_c$  = compressive strength, UPV = ultrasonic pulse velocity, RN = rebound number

Overall, the incorporation of EM in cement paste and concrete showed the potential to use as new additives in enhancing compressive strength associated with low porosity.

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## LIST OF SYMBOLS

### Symbols

MPa	Mega pascal
mL/g	Milliliter per gram
$\mu\text{m}$	Micrometer
$^{\circ}\text{C}$	Degree celcius



PT TA UTHM  
PERPUSTAKAAN TUNKU TUN AMINAH



## LIST OF ABBREVIATIONS

### Abbreviations

EM	Effective Microorganism
UPV	Ultrasonic pulse velocity
RN	Rebound number
BS	British Standard
ASTM	American Society for Testings and Materials
MIP	Mercury Intrusion Porosimetry
DTG	Derivative thermogravimetric
TGA	Thermogravimetric Analysis
XRD	X-Ray Diffraction
XRF	X-Ray Fluorescence
C-S-H	Calcium silicate hydrates
Ca(OH) <sub>2</sub>	Calcium hydroxide
CaCO <sub>3</sub>	Calcium carbonate
EMS	Effective Mixed Solution
SEM	Scanning Electron Microscope
FESEM	Field Emission Scanning Electron Microscope
EDX	Energy dispersive X-Ray spectroscopy

# CHAPTER ONE

## INTRODUCTION

### 1.1 Research Background

Generally, Effective Microorganism (EM) is classified into two types namely EM Non Liquid and EM Liquid. EM Non Liquid is not in liquid form consists of only one pure bacteria species. The bacteria species that have been used in EM Non Liquid for cement based materials is *Bacillus* species (Ramachandran et al., 2001; Dick et al., 2006; De Muynck et al., 2008; Jonkers and Schlangen, 2009; Tittelboom et al., 2010; Arunachalam et al., 2010; Afifudin et al., 2011; Siddique and Kaur, 2011; Navneet, et al., 2012a and Navneet et al., 2012b, Srinivasa et al., 2013; Mondal et al., 2016 and Krishnapriya and Shahinrahman, 2017). The other types of bacteria are *Shewanella* species and *Escherichia Coli* (Ghosh et al., 2003, 2005, 2009 and Siddique and Kaur, 2011). However, EM Non Liquid is not the focus of the present study. Conversely, EM Liquid comes in liquid form consists of more than one bacteria species and widely used in agriculture (Higa, 1991; Szymanski and Petterson, 2003; Lee et al., 2008; Mayer et al., 2008; Karthick et al., 2011; Wolejko et al., 2016 and Kusznierevicz et al., 2017).

Originally, EM Liquid was developed in 1970's at the University of the Ryukyus, Okinawa, Japan by Dr. Teruo Higa, a horticultural Professor (Higa, 1991). The main ingredients of EM are categorised into three or more types of microorganisms namely lactic acid bacteria, photosynthetic bacteria, yeast and actinomyces (Higa, 1999 and Szymanski and Petterson, 2003). These microorganisms are blended with the molasses which is the sugar cane based product resulting from refining process in the factory and it is thick and blackstrap solution. The addition of molasses in EM Liquid as a nutrient medium to EM bacteria in order to promote the bacteria growth. This solution is maintained at a low pH ranges between 3.0 and 4.0 under ambient condition (Lee et al., 2008; Mayer et al., 2010) for the agriculture purposes.

However, study on EM Liquid and its effect towards concrete properties is still new. Only a few studies have been reported (Nobuyuki et al., 2004; Jamaludin et al., 2009; Andrew et al., 2012 and Isa et al., 2016). The preliminary results revealed that

the workability of fresh concrete improved and the initial strength increased when EM Liquid was added to concrete mix. The types of EM Liquid which have been used and found to have potential to improve concrete properties are EM-No.1 (Nobuyuki et al., 2004; Jamaludin et al., 2009, Andrew et al., 2012 and Isa et al., 2016), EM-No.3 (Nobuyuki et al., 2004), EM-X (Nobuyuki et al., 2004) and EM Ceramic (Nobuyuki et al., 2004). Nevertheless, their studies are limited to conclude the effectiveness of the EM Liquid in enhancing the properties in cement paste, mortar and concrete. Furthermore, the influence of incorporating EM Liquid to internal microstructure and profile of pore distribution of cement needs to be extensively evaluated. Conclusive findings about the influence of bacterial activity in EM Liquid towards phase identification of hydration products and also quantitative measurement of mass loss associated with the temperature are rarely published to understand the cement hydration of microbed cement based.

In this present study, the type of EM Liquid used is EM-No.1 with brand name Bio-Booster which is available from Farmer's Organisation Johor. The selection of Bio-Booster is based upon its affordable price and availability. The content of Bio-Booster is yeast, molasses, lactic acid bacteria and phototropic (photosynthetic) bacteria. The intention to determine the influence of incorporating EM.No-1:Bio-Booster to concrete properties and the mechanism underlies is becoming the primary interest in this study. Commonly, EM Liquid was mixed with molasses and distilled water to activate the bacteria content in EM. Based on the findings of Jamaludin et al., (2009), only one set percentage of activation EM Liquid namely 5% of EM.No-1, 5% of molasses and 90% of distilled water was adopted and mixed in concrete. This limited selected ratio is based upon the optimum percentage of each material used in agriculture purpose denoted by Lee et al., (2008). Contents of molasses and EM.No-1 were optimised in cementitious material looking into the influence towards high compressive strength of the resulting cement paste. Within the knowledge of the author, the optimum ratio of EM Liquid to be used in concrete and molasses has not been reported elsewhere. Therefore, it is the intention of the present study to explore the utilisation of EM Liquid in enhancing concrete properties after determining the optimum content of molasses and EM.No-1 of the EM solution.

## 1.2 Problem Statement

Generally, EM Liquid are able to improve the quality and quantity of plants and fruits (Priyardi et al., 2005; Khaliq et al., 2006; Mayer et al., 2008; Lee et al., 2008 and Muthaura et al., 2010; Alagakannan and Ashokkumar, 2015; Wolejko et al., 2016 and Kusznierevicz et al., 2017). Meanwhile, a few researchers (Nobuyuki et al., 2004; Jamaludin et al., 2009; Andrew et al., 2012 and Isa et al., 2016) were reported the potential of EM Liquid in enhancing the concrete properties using limited ratios of EM in concrete. Yet, no comprehensive study has been reported in determining the effect of adding various ratios of the constituents in EM towards concrete properties. Further, no study has confirmed the optimum of molasses content to be added into cement based material to give the most enhanced effect on its mechanical properties. Possibility, no additional molasses is required in the mixture of EM due to the contents of EM.No-1:Bio Booster already contained molasses. Therefore, the preliminary study was carried out to confirm the requirement of molasses in terms of compressive strength of the resulting cement paste.

Regarding the EM Liquid contents, Szymanski and Petterson (2003) reported that the bacteria species in Lactic acid bacteria can be *Lactobacillus plantarum*, *Lactobacillus casei*, *Streptococcus lactis*, Yeast (*Saccharomyces cerevisiae*, *Candida utilis*) and Photosynthetic bacteria (*Rhodospseudomonas palustris*, *Rhodobacter spaeroides*). In the present study, isolation and characterisation of bacteria were carried out in order to identify the bacteria species contain in the Bio Booster: EM.No-1 which have not been identified in the previous works. Besides, no study was reported to evaluate the viability of EM in microbed cement based material. For that reason, the investigation was performed to examine the existence of bacteria species in EM Liquid and its viability when it is included in cement paste.

Mechanism on how the EM Liquid densifies the internal cement microstructure has not been well explained in the literature. Besides, the inclusion of EM Non Liquid in cement paste (Jin et al., 2017), mortar (Ghosh et al., 2005) and concrete (Navneet et al., 2012a; 2012b; 2013 and George et al., 2010) showed the growth of fibrous filler material in the pores due to the existence of calcium carbonate crystals. Simultaneously, Kim et al., (2013) and Krishnapriya et al., (2015) proved that the internal microstructure of the concrete containing EM Non Liquid was denser and finer while the control concrete specimens was stacked like gravel and loosely

connected to each other. Therefore, the feeling of curiosity drives the attempt to investigate the influence of EM Liquid to the internal microstructure of cement by using Scanning Electron Microscopy (SEM) and Field Emission Scanning Electron Microscopy (FESEM) techniques and substantiated with Electron Disperse X-Ray (EDX) together with thermal analysis using Thermogravimetric Analysis (TGA) and Derivative Thermogravimetric (DTG).

Generally, the smaller range size of pore diameter distribution and low porosity in cement based specimens is leading to high compressive strength (Odler and Rößler, 1985; Kumar and Bhattacharjee, 2003a; Kumar and Bhattacharjee, 2003b; Chen et al., 2014; Bu et al., 2016 and Wang et al., 2017) and it is indicated good durability of cement based materials. Seshagiri et al., (2014) concluded the pore diameter of microbed concrete was distributed in smaller range than the concrete specimens without EM Non Liquid. It can summarised, the incorporation of EM Non Liquid able to decrease the porosity of concrete. While, Ramachandran and Ramakrishnan (2001); Bang et al., (2001); Bachmeier et al., (2002); Skinner and Jahren (2004); Ghosh et al., (2005); DeJong et al., (2006); De Muynck et al., (2007); Jonkers et al., (2010); Kim et al., (2013) and Thiyagarajan et al., (2016) reported that high intensity of calcium carbonate ( $\text{CaCO}_3$ ) were reported due to the process of nucleation on bacterial cell walls when EM Non Liquid added in the specimens. This process produced the compound of calcite (Ca) and vaterite (V) which were classified as hydration products. However, the influence of EM Liquid inclusion to the hydration products and pore size distribution of cement have not been published from the previous works. Therefore in this present study, X-Ray Diffraction (XRD) and X-Ray Fluorescence (XRF) analysis were carried out to verify the composition and morphology of cement hydration products due to the presence of calcium carbonate ( $\text{CaCO}_3$ ) and calcium silicate hydrates (CSH). Whereas, Mercury Intrusion Porosimetry (MIP) test was adopted to investigate the influence of EM Liquid addition on the pore size distribution of microbed cement paste.

Within the limited scope of the existing literature, only destructive strength of microbed concrete had been investigated with the concrete grade of thirty (30) without the correlation. It has been established between destructive and non-destructive strength of concrete with various EM contents till the age of 28 days (Andrew et al., 2012). In addition, none of the published works was attempted to relate the splitting tensile strength and compressive strength of microbed concrete as well as the

compressive strength and porosity. Therefore, in this present study non-destructive tests namely Rebound Hammer (RH) and Ultrasonic Pulse Velocity (UPV) were carried out on concrete specimens in order to correlate the compressive strength with these non-destructive tests. The concrete grades of 20 MPa, 30 MPa and 40 MPa were prepared to establish the correlation between compressive strength, splitting tensile strength, rebound number and UPV values with respect to different maturity day. Furthermore, pore distribution profiles of concrete was also established. The extensive study based on the previously mentioned testing is required to understand the effectiveness of EM Liquid in enhancing concrete properties and bring towards more added value to the existing knowledge with regards to the use of microorganisms in concrete.

### 1.3 Objectives

The objectives of this research are as follows;

- a) To determine the optimum percentage of constituent in molasses and EM to give the most enhanced effect to cement paste.
- b) To confirm the influence of EM on the microstructural, chemical, mineralogical, thermal and porosity change within cement matrix using full term of Field Emission Scanning Electron Microscope (FESEM) substantiated with Electron Dispersive X-Ray (EDX), X-Ray Fluorescence (XRF), X-Ray Diffraction (XRD), Thermogravimetric Analysis (TGA) and Mercury Intrusion Porosimetry (MIP).
- c) To identify the species of EM bacteria in order to investigate the survivability of EM bacteria in hardened cement paste.
- d) To establish the relationship of concrete strength obtained based on destructive and non-destructive testing methods for microbed concrete Grade of 20, 30 and 40.
- e) To establish the relationship between the compressive strength and porosity for microbed concrete Grades of 20, 30, and 40 with and without EM.



#### 1.4 Scope of Work and Limitations

This research however is subjected to several limitations. The incorporation of molasses and EM.No-1 in cement paste were limited to 25% due to the adverse contribution of the former. While, 10% EM.No-1 was incorporated in concrete specimens which based on the optimum content of EM.No-1 resulting high compressive strength in cement paste. Three (3) major stages were performed to accomplish the objectives of this present study and it was further explained in Chapter 3. The first stage represents the activities conducted in identifying the bacteria species contained in the EM Liquid with brand name of Bio-Booster. The single species of bacteria for each group of lactic acid and photosynthetic bacteria including yeast and actinomyces were revealed by isolating and sub-culturing the mixed colonies to obtain the pure culture of single species. The culture media of nutrient agar, nutrient broth and slant agar were prepared prior to the process of isolation and sub-culture begin. The proportions of 10% EM.No-1 and 90% distilled water was adopted to ferment and process the isolation and sub-culturing the EM solution begin. Upon the completion of fermentation periods, the serial dilution technique was conducted to isolate the mixed colony in EM Liquid. The process of isolation and subculture was conducted by transferring the mixed colony to other new media using four (4) general applications from solid to solid media, solid to liquid media, liquid to solid media and liquid to liquid media. The pure species was identified using Biolog Microbial Identification System (BMIS) by culturing in microplate. After determining the pure species, the macroscopic and cellular morphology characteristics of colony was also observed.

The second stage concludes the activities performed in incorporating the EM Liquid in cement paste. Molasses and EM.No-1 contents were optimised in cement paste through several designated series representing different contents of molasses and EM.No-1. The selection of optimum contents was made based on the highest compressive strength of the resulting cement paste. Further investigation was carried out in determining the characteristics of microbed cement paste containing EM Liquid in terms of oxide composition and cement hydration products by implementing X-Ray Fluorescence (XRF) and X-Ray Diffraction (XRD) analysis, respectively. The phase identification of calcium silicate hydrates (CSH), calcium hydroxide  $\text{Ca(OH)}_2$  and calcium carbonate ( $\text{CaCO}_3$ ) was also performed by conducting thermogravimetric analysis (TGA). The influence of EM Liquid to the pore size distribution and porosity

was performed using Mercury Intrusion Porosimetry (MIP). Simultaneously, the densification of the internal structure of microbed cement paste matrix was captured to compare the internal structure of cement paste with and without EM using Field Emission Scanning Electron Microscope (FESEM) with Energy Dispersive X-Ray (EDX). Eventually, the viability of the EM bacteria was conducted to examine the survival period of bacteria in cement paste by implementing viable count and captured of bacteria by serial dilution and microscope, respectively.

Lastly, the third stage involved concludes the activities conducted in incorporating the EM Liquid in three (3) concrete grades namely Grade 20, 30 and 40 which was designated as M20, M30 and M40, respectively. Ten percent (10%) amount of EM from the total amount of water used was fermented for seven (7) days prior to the addition in the concrete specimens. Testing of aggregate includes tests for sieve analysis and specific gravity. The mix design of all concrete grades was finalised prior to the testing of aggregate. The testing of compressive strength, tensile splitting strength including the non-destructive testing via rebound hammer (RH) and ultrasonic pulse velocity (UPV) were carried out on the cylindrical specimens with size of 150 mm in diameter and 300 mm in length. Subsequently, the testing of Mercury Intrusion Porosimetry (MIP) was conducted to analyse the pore size distribution of low, normal and high concrete grade with and without EM. Eventually, the correlation between strength based on destructive and non-destructive testings was established by plotting these three (3) parameters on the three dimensional graph.

### **1.5 Significance of Study**

Effective Microorganism (EM) liquid is classified as the sustainable material based on the environmental friendly application. It is non-toxic, harmless microorganism and beneficial for animal health, plants, food processing, environmental protection, biological fertiliser and pesticides as well as human consumption. In addition, this microbial technology has shown promising findings in many areas of application such as in agriculture and waste water treatment. However, EM has not been widely used in concrete application. With the promising findings in this present study proved the capability of local product of EM in concrete technology and able to contribute to human, benefit to environmental, economy and the country.