THE CHARACTERISTICS OF STRUCTURAL CONCRETE USING BACTERIA IMPROVEMENT

RAMIN ANDALIB

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ALHAMDULILLAH

All Praise For God, Creator Of This Universe
Thanks For the Precious Faith You Blessed On Me
And Thanks For All The Strength And Knowledge You Granted On Me

Thanks

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ABSTRACT

Concrete is a porous material and its porosity is accompanied by ingress of aggressive agents to decrease its strength and durability. Natural ingredient with filling capacity can be added to concrete to improve its characteristics. There are some natural waste materials which are known as good materials for partial replacement of ordinary Portland cement in the production of concrete. Construction problems and mix design complexity using these waste materials are the reasonable evidences for a need for another type of concrete. In recent times, the application of microorganism in cementations materials has received a lot of interest. One significant area of interest is bacterial concrete. The concept is to introduce bacteria in concrete, which will aid in mineral precipitation of pores and tiny cavity areas. Bacillus is a type of bacteria that can produce $CaCO_3$ as a binding filler material to enhance concrete characteristics. This study provided an insight of a new biotechnological method based on calcite precipitation to improve the strength of structural concrete. It is clear that mineral precipitation has the potential to enhance construction material resistance towards degradation processes. Different cell concentrations of bacteria from 10^3 to 10^7cfu/ml were introduced in concrete and significant increase in the compressive strength was obtained in the case of 10^5cfu/ml at different ages. Subsequently, to the structural concrete, five different cell concentrations of bacteria (10×10^5 to 50×10^5cfu/ml) were introduced and significant increase in the compressive and flexural strength were obtained when 30×10^5cfu/ml of bacteria was added to concrete at different ages (7, 14, 28 and 60 days). This appropriate cell concentration (30×10^5cfu/ml) was applied again to the various grades of structural concrete (30, 35, 40, 45, and 50MPa) by mixing water (per ml) in the current experimental approach. It was found that the compressive strength of the higher grade of structural bacterial concrete has improved as compared to lower grade due to more precipitation of calcite. In order to study the durability of structural concrete against aggressive agents, specimens with appropriate cell concentration were immersed in different types of acids solution (sulphuric and hydrochloric acids) to compare their effects on 60th, 90th and 120th day. The experiment demonstrated that bacterial concrete had decreased in weight and strength losses when compared to the ordinary Portland cement concrete without bacteria as control, especially in the highest grade of structural bacterial concrete (50MPa).It was also found that maximum compressive strength and weight loss occurred during sulphuric acid immersion. Microbial calcite precipitation was also quantified by X-ray diffraction (XRD) analysis, visualized by scanning electron microscopy (SEM) and analysed by energy dispersive spectrometer (EDS). An increase in density and uniformity of bacterial concrete was observed compared to the ordinary Portland cement concrete because of calcite deposition. Eventually, it was discovered that addition of Bacillus species bacteria had a positive effect on the strength and durability of structural concrete.

ABSTRAK

Konkrit adalah bahan berliang dan kewujudan liang ini membolehkan kemasukan ejen agresif yang akan mengurangkan kekuatan dan ketahanannya. Bahan tambah semulajadi yang mempunyai keupayaan mengisi boleh ditambah bagi meningkatkan ciri-ciri konkrit. Terdapat beberapa bahan buangan semulajadi yang baik untuk dijadikan bahan gantian separa bagi mengganti sebahagian simen Portland biasa untuk menghasilkan konkrit. Masalah pembinaan dan kerumitan untuk mereka bentuk konkrit yang menggunakan bahan buangan ini menyebabkan kajian untuk menghasilkan satu jenis konkrit yang lain diperlukan. Akhir-akhir ini, penggunaan mikroorganisma dalam bahan bersimen telah menerima banyak faedah. Satu aplikasi yang signifikan adalah konkrit bakteria. Konsep ini adalah untuk memasukan bakteria ke dalam konkrit, yang akan membantu dalam penghapusan liang dan rongga kecil. Bacillus adalah sejenis bakteria yang boleh menghasilkan CaCO3 sebagai bahan pengisi mengikat untuk meningkatkan ciri-ciri konkrit. Kajian ini memberi gambaran tentang sesuatu kaedah bioteknologi baru berdasarkan mendakan kalsit untuk meningkatkan kekuatan struktur konkrit. Adalah jelas bahawa mendakan galian mempunyai potensi untuk meningkatkan ketahanan bahan binaan terhadap proses degradasi. Kepekatan sel yang berbeza bagi bakteria dengan pencairan dari 10^3 hingga 10^7 cfu/ml diperkenalkan dalam konkrit dan peningkatan kekuatan mampatan yang signifikan diperolehi dalam kes 10^5 cfu/ml pada peringkat pertumbuhan yang berbeza. Selepas itu, untuk konkrit struktur, lima kepekatan sel yang berbeza daripada bakteria (10×10^5-50×10^5 cfu/ml) telah diperkenalkan dan peningkatan yang ketara dalam kekuatan mampatan dan lenturan diperolehi dalam kes 30×10^5 cfu/ml pada peringkat pertumbuhan yang berbeza (7, 14, 28 dan 60 hari). Kepekatan sel yang sesuai (30×10^5 cfu/ml) telah digunakan sekali lagi untuk pelbagai gred konkrit struktur (30, 35, 40, 45, dan 50 MPa) dengan cara mencampurkan air (per ml) untuk eksperimen semasa. Hasil nya menunjukkan bahawa peningkatan kekuatan mampatan gred yang lebih tinggi daripada konkrit bakteria struktur adalah lebih berbanding dengan gred yang lebih rendah disebabkan oleh mendakan yang lebih daripada kalsit. Dalam usaha untuk mengkaji ketahanlasakan konkrit struktur terhadap agen agresif, spesimen dengan kepekatan sel sesuai di rendam dalam pelbagai jenis asid (asid sulfurik dan asid hidroklorik) untuk membandingkan kesan pada peringkat pertumbuhan 60, 90 dan 120 hari. Kajian menunjukkan bahawa konkrit bacteria menghasilkan pengurangan berat dan kekuatan yang lebih sedikit berbanding dengan konkrit simen Portland biasa sebagai konkrit kawalan, terutama di peringkat gred tertinggi iaitu pada 50 MPa. Didapati juga bahawa kekuatan mampatan dan kehilangan berat maksimum berlaku selepas direndam dalam asid sulfurik berbanding dengan asid hidroklorik. Mikrob mendakan kalsit juga dinilai oleh pembelauan sinar-X (XRD) analisis, digambarkan dengan mengimbas mikroskopi elektron (SEM) dan dianalisis oleh tenaga spektrometer serakan (EDS). Adalah didapati bahawa peningkatan ketumpatan dan keseragaman kepada konkrit bakteria berbanding dengan konkrit simen Portland biasa disebabkan pemendapan kalsit. Akhirnya, penggunaan bakteria dalan konkrit menggunakan spesis Bacillus telah menghasilkan kesan positif kepada kekuatan dan ketahanan struktur konkrit.

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LIST OF ABBREVIATIONS

ACI	-	American Concrete Institute
ASTM	-	American Society of Testing of Materials
$Al_2 O_3$	-	Aluminum Oxide
BP	-	Bacteria Suspended in Phosphate Buffer
BS	-	British Standard
BU	-	Bacteria Suspended in Urea – $CaCl_2$
BW	-	Bacteria Suspended in Water
C	-	Cement
CFUs	-	Colony Forming Units
CH	-	Calcium Hydrate
C-S-H	-	Calcium Silicate Hydrate
Ca^{2+}	-	Calcium Ion
$Ca Cl_2$	-	Calcium chloride
$Ca CO_3$	-	Calcium Carbonate
Ca O	-	Calcium Oxide
$Ca (OH)_2$	-	Calcium Hydroxide
$C_2H_3NaO_2S$	-	Thioglycollate
$CO (NH_2)_2$	-	Carbamide or Urea
CO_2	-	Carbon Dioxide
CO_3^{2-}	-	Carbonate Ion
DO	-	Dissolved Oxygen
DOE	-	Department Of Environment
EDS	-	Energy Dispersive Spectrometer
EN	-	European standards
Eqn.	-	Equation
Fe^{2+}	-	Iron Ion
Fe S	-	Iron Sulfide

Fe_2O_3	-	Iron Oxide
g	-	gram
HCl	-	Hydrochloric Acid
H^+	-	Hydrogen Ion
HCO_3^-	-	Hydrogen Carbonate Ion
H_2CO_3	-	Carbonate Acid
H_2O	-	Hydrogen Oxide or Water
H_2O_2	-	Hydrogen Peroxide
H_2S	-	Hydrogen Sulfide
H_2SO_4	-	Sulfuric Acid
KCl	-	Potassium chloride
K_2O	-	Potassium Oxide
L	-	Litter
LOI	-	Loss On Ignition
mg	-	milligram
MgO	-	Magnesium Oxide
MICP	-	Microbiologically Induced Calcite Precipitation
mL	-	milliliter
mm	-	millimeter
MPa	-	Mega Pascal
N	-	Nioton
Na_2O	-	Sodium Oxide
NASA	-	National Aeronautics and Space Administration
$Na_2S_2O_3$	-	Sodium Thiosulfate
NH_2COOH	-	Carbomate
NH_3	-	Ammonia
NH_3^-N	-	Ammoniacal Nitrogen
NH_4^+	-	Ammonium Ion
NH_4^+N	-	Ammonium Nitrogen
O_2	-	Oxygen
OH	-	Hydroxide Ion
OPC	-	Ordinary Portland Cement
P_2O_3	-	Phosphorus Oxide

ppt	-	parts per thousand
psi	-	pound over inch square
PU	-	Polyurethane
RHPC	-	Rapid Hardened Portland Cement
SEM	-	Scanning Electron Microscopy
Si O_2	-	Silicon Oxide
SRPC	-	Sulfate resistant Portland Cement
TDS	-	Total Dissolved Solids
TEM	-	Transmission Electron Microscope
UTM	-	Universiti Teknologi Malaysia
USA	-	United States Of America
W	-	Water
W_c	-	Water for coarse aggregate
W_f	-	Water for fine aggregate
XRD	-	X Ray Diffraction
µm	-	micrometer

LIST OF SYMBOLS

f_c	-	Characteristic Strength
f_m	-	Mean Strength
K	-	Constant
PH	-	Level of Acidity by Indicator
S	-	Standard deviation
$\%$	-	Percentage

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CHAPTER 1

INTRODUCTION

1.1 Background

The studies revealed that without microorganisms, biological knowledge would be a highly restricted area of study. Microorganisms are microscopic organisms, which not just provide the base for the fundamental research concerned with biological knowledge; they also assist to make the biological green building materials.

While a great number of successful concrete buildings are annually established throughout the world, there are so many concrete structures that become hazardous in consequence of deterioration. Hence the continuous progressions in the civil engineering areas have improved a growing requirement to comply more with environmental conditions in addition to structural requirements.

Concrete is a porous material and its porosity is faced by aggressive agents, to decrease its strength and durability seriously. Hence an added ingredient to fill the concrete pores and its tiny cavity areas can improve its characteristics and consequently the maintenance cost will be considerably reduced.

Bacterial concrete is a novel research domain can be used for cementitious materials that cure themselves automatically by bio-mineralization mechanism. The concept is to introduce bacteria in concrete, which aids to precipitate calcite in pores and tiny cavity areas. The presence of pores and micro-cracks in the hydrated

cement paste can highly influence the concrete characteristics and as a result they can provide a path through which moisture, chlorides, carbon dioxide and other aggressive agents can penetrate. Mostly the micro-cracks without suitable and immediate attention can expand, thus causing the deterioration and weakening of the concrete strength.

In order to success against the traditional filling additives deficiency, new materials with bacterial filling capacity can be applied efficiently. The application of urease producing microorganisms to survive and grow within concrete can solve these problems effectively. Urease assists microorganisms to precipitate mineral, by urea hydrolysing existent in the medium. It actually allows leaving CO_2 from urea to mix with calcium ions to create calcium carbonate in the form of calcite.

In recent times, biological strengthening application of cementitious materials has gotten a lot of attention. One significant region of attention regarding strengthening in cement based materials is bio-mineralization.

The bacterial concrete can be constructed using bacteria that are able to precipitate calcite. A new-technique based on bio-mineralization is the process by which living organism produce minerals to harden or stiffen the existing tissues. It was found that the bio-mineralization process will not interfere with the setting time of concrete (Seshagiri Rao et.al, 2013).

This circumstance is so called as MICP (microbiologically induced calcite precipitation). The fundamental law is to produce ammonia using the microbial urease enzyme. The equations [Eqn. (1) to (7)] show a sequence of biochemical reactions that occur to form calcium carbonate with the help of ureolytic bacteria (Wu et al., 2012).

Some significant researches on carbonate precipitation have been carried out by using ureolytic bacteria. Ureolytic bacteria have ability to affect the calcite precipitation by the creation of a urease enzyme.

Urease is the significant enzyme engaged in the calcite precipitation procedure induced by bacteria to hydrolyse urea. Urea can be used as a nitrogen source (Burne and Chen, 2000) in the sequence of biochemical reactions to increase the pH of the environment with calcite precipitation as a result of biological activity (Burne and Marquis, 2000).

Carbamide or urea as an essential compound with the $CO(NH_2)_2$ chemical formula is hydrolysed to carbamate (salt of carbamic acid) and ammonia in presence of urease enzyme as shown in Eqn. (1).

$$CO(NH_2)_2 + H_2O \xrightarrow{\text{Urease enzyme}} NH_2COOH + NH_3 \quad \text{Eqn. (1)}$$

Carbamate is hydrolysed to form ammonia and carbonic acid in a spontaneous manner as shown in Eqn. (2).

$$NH_2COOH + H_2O \rightarrow NH_3 + H_2CO_3 \quad \text{Eqn. (2)}$$

Carbonic acid is hydrolysed to form bicarbonate and hydrogen ions as shown in Eqn. 3.

$$H_2CO_3 + H_2O \leftrightarrow HCO_3^- + H^+ \quad \text{Eqn. (3)}$$

Ammonia hydrolyses to form ammonium and hydroxide ion as seen in Eqn. 4.

$$2NH_3 + 2H_2O \leftrightarrow 2NH_4^+ + 2OH^- \quad \text{Eqn. (4)}$$

The reaction in Eqn. (4) continuously produces hydroxide ion, which gives rise to increase in pH that shifts the overall equilibrium of bicarbonate ion (HCO_3^-) towards the formation of carbonate ions as shown in Eqn. (5).

$$HCO_3^- + H^+ + 2NH_4^+ + 2OH^- \leftrightarrow CO_3^{2-} + 2NH_4^+ + 2H_2O \quad \text{Eqn. (5)}$$

Bacteria cell wall has negative charge and because of this reason, it is able to attract positively charged calcium ions (Ca^{2+}) to deposit on their cell wall surface (Eqn. 6). The Ca^{2+} ions then react with CO_3^{2-} ions leading to the precipitation of calcium carbonate ($CaCO_3$) at the cell surface as shown in Eqn. (7). This precipitation serves as nucleation site.

$$Ca^{2+} + Cell \rightarrow Cell - Ca^{2+} \quad \text{Eqn. (6)}$$

$$Cell - Ca^{2+} + CO_3^{2-} \rightarrow CaCO_3 - Cell \downarrow \quad \text{Eqn. (7)}$$

Bacillus is a type of bacteria that can produce $CaCO_3$ as a binding filler material to reduce concrete capillary pores to improve its strength and durability. There are some spp of *Bacillus* that produce urease to precipitate calcite associated with bio-cementation (De Muynck et al., 2008; Achal et al., 2009).

The existing study will demonstrate that bio-mineralization in cementitious materials can be an appropriate technique to improve the structural concrete characteristics. This thesis actually displays the consequences of a detailed study to realize the effect of bacteria, specifically *Bacillus* sp, since it is integrated on the interior of cement paste.

1.2 Problem Statement

Currently the application of concrete is quickly increasing throughout the world due to its basic ingredients availability. It is obvious that cement, the main ingredient of concrete, has a high environmental impact on global warming since around 10% of the total CO_2 emission to atmosphere is due to the cement industry. Therefore the progression of a sustainable concrete is immediately required for

environmental justification and it has become necessary that the study efforts in using of natural materials gain greater attention.

However geo-polymer concrete are known as good alternative for the ordinary Portland cement concrete, costly alkaline solutions, construction problem in terms of workability, and mix design complexity are some reasonable evidences to welcome another type of concrete that called bacterial concrete.

To compare geo-polymer and bacterial concrete economically just based on chemical consumption which is essential for geo-polymerisation and bio-mineralization processes, Palm oil fuel ash-fly ash concrete and bacterial concrete as a sample are estimated. A significant reason to select Palm oil fuel ash-fly ash concrete was several studies that conducted to investigate the feasibility of applying Palm oil fuel ash in construction materials. The total amount of chemical was around 167 kg/m^3 in palm oil fuel ash-fly ash concrete (Ariffin et al., 2012), since the chemical quantity of bacterial concrete was around 10.25 kg/m^3 (Andrew et al., 2012). Regarding construction problem in terms of workability, despite the amount of water was 29 kg/m^3 in palm oil fuel ash-fly ash concrete, its quantity in bacterial concrete was around 205 kg/m^3. Hence the bacterial concrete is more economical and more workable than geo-polymer concrete based on chemical consumption and constructability. For mix design complexity, it was found that the bio-mineralization process will not interfere with the setting time of concrete (Seshagiri Rao et.al, 2013) and because of that, concrete mix design standard (i.e. British code) can be used for bacterial concrete mix design. The palm oil fuel ash-fly ash concrete mix design is also accompanied by doubt and difficulty due to lacking of standard. Hence bacterial concrete as a new technique based on bio-mineralization is a good alternative for ordinary Portland cement concrete towards sustainable construction.

In recent times, it is discovered that bacterial calcite precipitation from biological activities of bacteria can enhance the overall behaviour of concrete. The urease enzyme existent in bacteria, breaks down urea by water to produce carbonate and in attendance of a calcium origin, calcite is voluntarily precipitated under these situations. Recent research has shown that specific species of bacteria can actually be

useful to fill the concrete pores automatically by calcite precipitation as a result of microbial activities.

This new technique significantly reduce the bacterial concrete maintenance costs due to its service life span growth and subsequently will reduce the CO_2 emission to atmosphere to help global warming partially by reduction of cement demand. Hence, in order to obtain more ability to realize the effects of bacteria in concrete, investigation on its characteristics is necessary.

1.3 Research Aim and Objectives

The main aim of this research is to investigate the strength and durability of structural concrete by using isolated soil bacteria. To achieve this aim the following objectives are conducted:

1- To identify the appropriate bacteria with calcite precipitation capacity to introduce in structural concrete to enhance its properties.

2- To establish the appropriate concentration of bacteria to achieve the appropriate strength of structural concrete in terms of compressive and flexural strengths.

3- To investigate the durability of bacterial structural concrete based on the appropriate concentration of bacteria in short term exposure to acidic environment.

4- To investigate the micro-structure of strengthened bacterial structural concrete to confirm the appropriate concentration of bacteria based on calcite intensity, its elements values, and micro-scan observations.

1.4 Scope of the Study

The scope of work involved the following:

Soil *Bacillus* bacteria from tropical environment are identified to apply in structural concrete.

The bacterial structural concrete specimens are prepared using different cell concentration of bacteria relevant to10^3, 10^5, and 10^7 serial dilution factors that are introduced in concrete based on 30 MPa to obtain appropriate serial dilution's factor in terms of compressive strength.

The bacterial structural concrete specimens are prepared using five different cell concentrations of bacteria (extracted from appropriate serial dilution's stage) that are introduced in concrete to compare with controlled structural concrete specimens by compressive strength test (35 and 40 MPa) and flexural strength test (35 MPa) to obtain appropriate cell concentration.

The bacterial structural concrete specimens prepared using appropriate cell concentration (extracted from five different cell concentrations of bacteria) that are introduced in concrete based on 30, 35, 40, 45, and 50 MPa to compare with controlled structural concrete specimens to investigate the effect of appropriate cell concentration of bacteria in terms of compressive strength.

The bacterial structural concrete specimens are prepared using appropriate cell concentration (extracted from five different cell concentrations of bacteria) that are introduced in concrete based on 40, 45, and 50 MPa to compare with controlled structural concrete specimens for durability study in terms of acidic condition (sulphuric and hydrochloric acids).

The calcite deposition in bacterial structural concrete specimens are quantified and visualized to compare with controlled structural concrete specimens in

terms of micro-structural investigation [SEM (scanning electron microscopy**),** XRD (X-ray diffraction**)**, and EDS (Energy Dispersive Spectrometer) tests].

1.5 Significance of the Study

In reaction to the replacing of concrete progress, the prosperity of this study will be useful for the construction industry by using bacterial concrete towards green construction materials. This research describes a novel technique using living microorganism which investigates the process of ureolytic bacteria isolation to introduce in concrete to improve its characteristics by calcite precipitation. Moreover, this research provides a novel alternative material to Malaysia to prevent the problem of early concrete deterioration. The significance of the research may contain the following effects:

1- However, in majority of the previous studies, microorganisms were purchased from Europe, USA and other collections in the world; bacteria in this research can be isolated from tropical environment directly.

2- In most of the previous studies, the concentrations of bacteria were not clear and the numbers of colonies were not mentioned as an ingredient. In this research, five different cell concentrations of microorganism from appropriate stage of serial dilution can be introduced in different grades of concrete to obtain appropriate concentration of bacteria.

3- In majority of the previous studies, the focus of researchers was on mortar and only few studies focused on concrete. In this research, the focus is on structural concrete and it covers high strength bacterial concrete.

4- The micro-structural study on acid attacked specimens of bacterial structural concrete in acidic condition has not investigated yet. Hence, the micro-structural study can be developed by consequences of this research.

1.6 Research Methodology

The major purpose of the current experimental research is to achieve special empirical data, which aids to realize the bacterial concrete and its strength and durability. In the existent research, investigations are performed on the behaviour of hardened characteristics of structural concrete with and without appropriate concentration of *Bacillus* bacteria. The hardened characteristics such as compressive strength, flexural strength, and durability of structural bacterial concrete in terms of acidic condition are obtained by performing some of the acceptable laboratory experiments.

Regarding the growth of microorganism, the pure culture of *Bacillus* bacteria is isolated from the soil sample that is taken from Universiti Teknologi Malaysia. Soil bacteria isolation is a meaningful first stage in a process of many biological experiments. The soil samples are suspended into nutrient broth in a sterile conical flask separately and the flasks are placed in water bath and incubator shaker at certain temperatures respectively. A loopful of nutrient broth is also streaked onto nutrient agar plate for each sample to obtain a pure medium culture.

During this study, spread plate technique is carried out after serial dilution as to reduce the number of bacteria per unit sample volume, then the streak plate technique is performed using agar medium in order to isolate the individual bacterial cells and further the standard viable plate count is used to determine the colony-forming units (CFUs).

The cultivation of bacteria in pure culture is necessary to observe bacterial colony morphology. It is indicated to the observable colony characteristics which are essentially different in appearance from other bacterial species. However bacterial colonies are different from the individual features of their appearance, a colony essentially seems a spot growing on the medium. This spot is consisted of millions of bacteria that appeared through cell division into two parts from one initial bacterium. The shape of colony, the margins or edges, and the surface characteristics as well as colony’s colour are some features of a colony to identify it.

To help in the more accepted identification of bacteria, a series of biochemical tests (catalase test, starch test, urease test, oxidase test, lactose fermentation test, indole test, and H_2S test) are applied to distinguish even closely associated microorganisms. These experiments are also included an easy to read table that enables to rapidly identify an unknown isolated bacteria on the basis of colour changes. Bergey manual of systematic bacteriology is also used as the main resource for determining the identity of bacteria.

In this study, DOE technique is applied to design concrete ingredients in accordance with department of environment of United Kingdom. Mix design concrete is specified as the procedure to choose appropriate ingredients of concrete to calculate their relative ratios with the aim of concrete creating on the basis of desired strength and durability.

However the compressive strength of concrete is measured by testing cubes (100*100*100 mm) under a laboratory test machine according to BS 1881: Part 116: 1983, the flexural strength of the concrete prism (100*100*500 mm) is determined based on. BS 1881: Part 118: 1983.For durability study in terms of acidic condition, the specimens are immersed in 5% of sulphuric acid (H_2SO_4-95%) and hydrochloric acid (HCl-37%) solutions to calculate the strength and weight loses of structural bacterial concrete.

To find out the relationship between the compressive strength growth and the bacterial calcite deposition, SEM and XRD analysis are visualized and quantified respectively. EDS is also considered to determine the sample chemical properties description. In this study, microstructure chemical analysis of acid attacked bacterial concrete is also done to justify the durability improvement in terms of acidic condition (5% solution of H_2SO_4 and HCl) in comparison to the ordinary Portland cement concrete.

1.7 Thesis Layout

The thesis is divided into seven chapters that are composed of Chapter 1 (Introduction), Chapter 2 (Bacteria Isolation and Identification), Chapter 3 (Bacterial Concrete), Chapter 4 (Methodology), Chapter 5 (Microorganism Identification to Achieve Appropriate Bacterial Concrete Mix), Chapter 6 (Micro-Structural Investigations on Bacterial Structural Concrete), and Chapter 7 (Conclusions and Recommendations).

The descriptions for each chapter are as follows:

1- Chapter 1 demonstrates the general background of the study and summaries of the problem statements, research aim and objectives, scope of the study, and significance of research. The research methodology and thesis layout are also reported in this chapter.

2- Chapter 2 displays a brief review of bacteria isolation, concentration, and identification by biological, morphological, and biochemical procedures.

3- Chapter 3 expresses a concise review of concrete, bacterial concrete, bacterial effect on concrete strength and durability, and eventually micro-structural investigation on bacterial concrete.

4- Chapter 4 describes the manufacture of test specimens and presents the detail of the test program. The significant purpose of the current practical investigations is to obtain particular experimental data, which aids to interpret the bacterial concrete and its properties. This chapter actually presents the details of programs consisting of material preparation, bacteria isolation and identification, concrete mix design based on DOE method, testing procedures concentrating on compressive and flexural strengths, durability of concrete in terms of acidic conditions and eventually some micro-structural investigation such as XRD (X-ray

diffraction), SEM (scanning electron microscopy) and EDS (Energy Dispersive Spectrometer).

5- Chapter 5 discusses on the analysis of bacteria isolation and identification, bacterial structural mix design, the mechanical properties of the bacterial structural concrete such as compressive and flexural strengths, and the durability of the bacterial structural concrete in terms of acidic conditions.

6- Chapter 6 investigates the micro-structure of strengthened bacterial structural concrete to confirm the appropriate concentration of bacteria based on calcite intensity and its elements values, and micro-scan observations.

7- Chapter 7 makes a decision about the research consequences with recommendations for further study.

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