Influence of Enterococcus Faecalis and Bacillus sp with Added Calcium Lactate on Engineering Concrete Properties and Enhancement of Self Healing in Concrete Under Unsterilized Condition

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For my beloved parents, siblings and friends
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Thank you
ABSTRACT

Concrete is one of the most used construction material in the world. The constant development and research for better concrete has led to bioconcrete, which is concrete infuse with micro-organisms that benefits the concrete by production of calcium carbonate. However, the production of calcium carbonate is limited to the calcium available in the cement. Therefore, the purpose of this study is to add calcium nutrient source in the form of calcium lactate in bioconcrete and study the engineering concrete properties and self-healing of micro-cracks. The bacteria used in this study is 3% Enterococcus faecalis and 5% Bacillus sp. Whereas the calcium lactate that is added into this study is in concentrations of 0.22 g/L, 1.09 g/L and 2.18 g/L. Concrete with dimensions of 100 mm × 100 mm × 500 mm for prisms, cylinders of 150 Ø × 300 mm and cubes of 150 mm × 150 mm ×150 mm are used to test for the engineering concrete properties at 7th, 14th and 28th day and self-healing of micro-cracks in concrete is in the range of 0-100 days. This research has contributed significantly to the finding of overall improvement of concrete properties from the addition of calcium lactate in bioconcrete. This is confirmed with the improvement of engineering concrete properties and self-healing of micro-cracks with the addition of 2.18 g/L of calcium lactate for both bacteria. UPV and micro-structure analysis were conducted to verify self-healing. Based on overall results, concrete with Bacillus sp with 2.18 g/L is most ideal.
Konkrit adalah salah satu daripada bahan binaan yang paling banyak digunakan. Pembangunan dan penyelidikan yang berterusan untuk konkrit yang ideal telah membawa kepada penjumpaan biokonkrit, iaitu konkrit yang mempunyai tambahan mikro-organisma yang memberi manfaat kepada konkrit dengan pengeluaran kalsium karbonat. Walau bagaimanapun, pengeluaran kalsium karbonat adalah terhad kepada kalsium yang terdapat dalam simen. Oleh itu, tujuan kajian ini adalah untuk menambah sumber nutrient kalsium dalam bentuk kalsium laktat dalam biokonkrit untuk mengkaji sifat-sifat konkrit kejuruteraan dan pembaikan semulajadi retakan mikro di dalam biokonkrit. Bakteria yang digunakan dalam kajian ini ialah 3% Enterococcus faecalis dan 5% Bacillus sp. Selain itu, Kalsium laktat yang ditambah dalam biokonkrit adalah dalam kepekatan 0.22 g/L, 1.09 g/L dan 2.18 g/L. Konkrit yang keras dengan dimensi 100 mm × 100 mm × 500 mm untuk prisma, silinder 150 Ø × 300 mm dan kiub 150 mm × 150 mm × 150 mm telah digunakan untuk menguji sifat-sifat konkrit kejuruteraan pada 7th, 14th dan 28th hari kematangan dan penyembuhan diri retakan mikro di dalam konkrit dianalisis dalam tempoh 0-100 hari. Ujikaji ini telah menyumbang kepada ilmu bahawa penambahan kalsium laktat dalam biokonkrit boleh menambah baik sifat-sifat kejuruteraan konkrit dan pembaikan semulajadi keretakan mikro di dalam konkrit dengan penambahan 2.18 g/L kalsium laktat untuk kedua-dua jenis bakteria. Penetuan pembaikan semulajadi keretakan mikro telah dilakukan dengan UPV dan Analisa mikro-struktur. Berdasarkan keputusan yang didapati, konkrit dengan Bacillus sp dan 2.18 g/L kalsium laktat adalah paling ideal.
## CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>TITLE</td>
<td>i</td>
</tr>
<tr>
<td>DECLARATION</td>
<td>ii</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>iii</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENT</td>
<td>iv</td>
</tr>
<tr>
<td>ABSTRACT</td>
<td>v</td>
</tr>
<tr>
<td>CONTENTS</td>
<td>vii</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>xv</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>xvii</td>
</tr>
<tr>
<td>LIST OF SYMBOLS AND ABBREVIATIONS</td>
<td>xxii</td>
</tr>
<tr>
<td>LIST OF APPENDICES</td>
<td>xxiv</td>
</tr>
</tbody>
</table>

### CHAPTER 1 INTRODUCTION

1.1 Introduction                              1
1.2 Problem statement                         4
1.3 Objectives                                6
1.4 Scope of study                            6
1.5 Importance of study                       7
1.6 Organization of thesis                    8

### CHAPTER 2 LITERATURE REVIEW

10
2.1 Introduction 10
2.2 Bioconcrete 11
2.3 Bacteria 12
  2.3.1 Types of bacteria that are used in concrete 14
  2.3.2 Enterococcus faecalis 18
  2.3.3 Bacillus sp 18
  2.3.4 Function of bacteria in concrete 19
2.4 The importance of calcium carbonate in concrete 21
2.5 Addition of calcium nutrient source 22
2.6 Enzymatic pathway of bacteria 24
2.7 Determination of precipitation of calcium carbonate 26
  2.7.1 Micro-structure test 26
  2.7.2 Elements in concrete 28
  2.7.3 Chemical composition 29
  2.7.4 Mineralogy of concrete 30
2.8 Cracks in concrete structures 31
  2.8.1 Category of cracks 34
  2.8.2 Conventional methods for crack repair 35
  2.8.3 Bacteria as self-healing agent for concrete 36
  2.8.4 Analyzing crack repairs 38
2.9 Effect of bioconcrete on engineering concrete properties 40
  2.9.1 Compressive strength 41
  2.9.2 Relationship between compressive strength and splitting tensile strength 44
  2.9.3 Flexural strength with addition of bacteria and Calcium lactate 47
  2.9.4 Relationship between compressive-flexural strength 49
  2.9.5 Durability and permeability 50
    2.9.5.1 Water penetration 51
2.10 Concluding remark 53

CHAPTER 3 METHODOLOGY
3.1 Introduction 55
3.2 Methodology of study 55
3.3 Material preparation 58
3.3.1 Fine Aggregate 58
3.3.2 Coarse aggregate 59
3.3.3 Cement 60
3.3.4 Bacteria 62
  3.3.4.1 Bacteria identification 62
  3.3.4.2 Growth curve of bacteria 65
  3.3.4.3 Serial dilution and colony count 65
3.4 Calcium lactate 66
3.5 Concrete casting 68
  3.5.1 Concrete mix design 68
  3.5.2 Batching of concrete 69
  3.5.3 Fresh concrete tests 70
3.6 Experimental program 70
  3.6.1 Compressive strength test 70
  3.6.2 Tensile strength test 72
  3.6.3 Flexural strength of prism 74
  3.6.4 Water penetration 75
3.7 Self-healing by bacteria with calcium lactate 77
  3.7.1 Standardized cracks 77
  3.7.2 Realistic cracks 79
  3.7.3 Analyzing self-healing 80
    3.7.3.1 Ultrasonic pulse velocity (UPV) 80
    3.7.3.2 Stereomicroscope 81
3.8 Analyses of concrete 82
  3.8.1 Micro-structure analyses 82
  3.8.2 Elements of concrete 83
  3.8.3 Chemical composition 83

CHAPTER 4 RESULTS AND DISCUSSION
4.1 Introduction 85
4.2 Bacteria identification 85
  4.2.1 Gram staining (Morphology observation) 86
  4.2.2 DNA quantification 87
    4.2.2.1 Polymerase chain reaction (PCR) 88
    4.2.2.2 Phylogenetic tree 89
4.2.3 Bacteria growth chart 92
4.2.4 Bacteria counting 93

4.3 Concrete properties 94
4.3.1 Workability of fresh concrete 94

4.4 Engineering Concrete properties 94
4.4.1 Compressive strength for concrete specimens 95
4.4.1.1 Compressive strength of control 95
   E. faecalis and B. sp
4.4.1.2 Compressive strength results for concrete 96
   with E. faecalis and E. faecalis (with 0.22 g/L,
   1.09 g/L and 2.18 g/L of calcium lactate)
4.4.1.3 Compressive strength results for concrete 97
   with B. sp and with (0.22 g/L, 1.09 g/L and 2.18
   g/L of calcium lactate)
4.4.1.4 Comparison between E. faecalis and B. sp 99

4.4.2 Tensile strength test 100
4.4.2.1 Tensile strength of normal and 100
   bioconcrete
4.4.2.2 Tensile strength of normal and bioconcrete 101
   With calcium lactate
4.4.2.3 Tensile strength of concrete containing 102
   B. sp and calcium lactate
4.4.2.4 Comparison between E. Faecalis and B. Sp 104

4.4.3 Flexural strength test 104
4.4.3.1 Flexural strength of control and 105
   bioconcrete
4.4.3.2 Flexural strength of control concrete and 106
   bioconcrete containing E. Faecalis and
   calcium lactate
4.4.3.3 Flexural strength of control and concrete 107
   with B. Sp and concentrations of calcium
   lactate
4.4.3.4 Comparison between E. faecalis and B. sp 108
   with concentrations of calcium lactate
4.4.4 Relationship between compressive-tensile strength

4.4.4.1 Relationship between compressive-tensile strength for bioconcrete

4.4.4.2 Relationship between compressive-tensile strength for *E. faecalis* and calcium lactate

4.4.4.3 Compressive-tensile strength for *B. sp* and concentration of calcium lactate

4.4.5 Relationship between compressive-flexural strength.

4.4.5.1 Relationship between compressive-tensile strength for bioconcrete

4.4.5.2 Relationship between compressive-flexural strength for *E. faecalis* and calcium lactate

4.4.5.3 Relationship between compressive-flexural strength for *B. sp* and calcium lactate

4.4.6 Water penetration

4.4.6.1 Water penetration of concrete and bioconcrete

4.4.6.2 Water penetration of *E. Faecalis* and *E. faecalis* with concentrations of calcium lactate

4.4.6.3 Water penetration of *B. sp* and *B. sp* with calcium lactate

4.4.6.4 Comparison between *E. Faecalis* and *B. sp*

4.5 Microstructure and morphology of bioconcrete

4.5.1 Chemical composition identification using X-ray Spectroscopy (XRF)

4.5.2 Microstructure analysis

4.5.2.1 Microstructure analysis for normal and bioconcrete

4.5.2.2 Microstructure analysis results for concrete with *E. faecalis* and calcium lactate.
4.5.2.3 Microstructure of concrete specimens with B.sp and calcium lactate.
4.5.2.4 Discussion for micro-structure
4.5.3 Element
  4.5.3.1 Elements in normal and bioconcrete
  4.5.3.2 Elements in concrete with E. faecalis concentrations of calcium lactate
  4.5.3.3 Elements in concrete with B.sp and calcium lactate
  4.5.3.4 Comparison between E. faecalis and B.sp
4.6 Self-healing properties
  4.6.1 Ultrasonic pulse velocity (UPV)
    4.6.1.1 Standard crack (Concrete cube with metal strip) of normal and bioconcrete
    4.6.1.2 Standard crack (Concrete cube with metal strip) of concrete with E. faecalis and calcium lactate
    4.6.1.3 Standard crack (Concrete cube with metal strip) of B.sp and calcium lactate
    4.6.1.4 Comparison between E. faecalis and B. sp with concentrations of calcium lactate
  4.6.2 Standard crack (Prism)
    4.6.2.1 Standard crack (Prism) of normal and bioconcrete
    4.6.2.2 Standard crack (Prism) of concrete for Normal and concrete with E. faecalis and Calcium lactate
    4.6.2.3 Standard crack (Prism) of normal concrete specimens and bioconcrete with calcium lactate
    4.6.2.4 Comparison between bioconcrete containing E. faecalis and B. sp
4.6.3 Stereomicroscope

4.6.3.1 Standard crack (Prism) of control concrete specimens and bioconcrete specimens

4.6.3.2 Standard crack (Prism) of E. faecalis and E. Faecalis with concentrations of calcium lactate

4.6.3.3 Standard crack (Prism) of B. sp and B. sp with concentrations of calcium lactate

4.6.3.4 Comparison of self-healing between E. faecalis and B. sp

4.6.4 Realistic crack (Circular disk)

4.6.4.1 Stereomicroscope of realistic crack (Circular disk) of normal and bioconcrete

4.6.4.2 Stereomicroscope of realistic crack (Circular disk) of E. faecalis with calcium lactate

4.6.4.3 Stereomicroscope of realistic crack (Circular disk) of B. sp with calcium lactate

4.6.4.4 Comparison of self-healing of E. faecalis and B. sp with different concentrations of calcium lactate

4.7 Concluding remark

CHAPTER 5 CONCLUSION AND RECOMMENDATION

5.1 Introduction

5.2 Conclusion

5.2.1 Isolation and identification of Enterococcus faecalis and Bacillus sp

5.2.2 Concrete properties

5.2.2.1 Compressive strength

5.2.2.2 Tensile strength

5.2.2.3 Flexural strength

5.2.2.4 Water penetration

5.2.3 Micro-structure analysis

5.2.3.1 Chemical composition identification using
X-ray spectroscopy (XRF)

5.2.3.2 Micro-structure analysis 168
5.2.3.3 Elements 168

5.2.4 Evaluation of self-healing 169
5.2.4.1 UPV (Prism) and standardized crack 169
5.2.4.2 Stereomicroscope 169

5.2.5 Overall conclusion 170

5.3 Research contribution 170
5.4 Recommendation 170

REFERENCES 172
APPENDICES 184
LIST OF TABLES

2.1 Various bacteria used in concrete 15
2.2 Chemical composition of cement and blast furnace slag using XRF 29
2.3 Category and description of cracks (Technical note-No 30, 2010) 34
2.4 Compressive strength of values 7 and 28 days tests with bacteria influence mortar 41
2.5 Equation for compressive-tensile strength relation 45
2.6 Previous research on bacteria 47
2.7 The result of testing beam for high strength concrete and normal concrete with added fiber 49
2.8 Relationship between compressive-flexural strength 50
2.9 Treatment available for water Permeability 52
3.1 Chemical composition of cement 61
3.2 Amount of calcium lactate used 66
3.3 Mix proportion used in this study 68
3.4 Fabrication batch 69
3.5 Number of specimens used for compressive strength test 71
3.6 Number of specimens used for tensile strength test 72
3.7 Number of specimens used for flexural strength test 74
3.8 Number of specimens used for water penetration test 76
4.1 The concentration and quality of DNA using Nanodrop 88
4.2 PCR parameter 89
4.3 Slump test for concrete workability 94
4.4 Compressive strength of normal and bioconcrete 95
4.5 Compressive strength of concrete with *E. Faecalis* and calcium lactate 97
4.6 Compressive strength of concrete with *B. sp* and calcium lactate 98
4.7 Tensile strength results for normal and bioconcrete
4.8 Tensile strength of concrete with *E. faecalis* with calcium lactate
4.9 Concrete with *B. sp* and various concentration of calcium lactate
4.10 Flexural strength of concrete for normal and bioconcrete
4.11 Flexural strength of concrete with *E. Faecalis* and calcium lactate
4.12 Results of bioconcrete with calcium lactate
4.13 The relationship between compressive-tensile between bioconcrete
4.14 Compressive-tensile relationship of *E. Faecalis* and calcium lactate
4.15 Compressive-tensile of concrete with *B.sp* and calcium lactate
4.16 Relationship between compressive-flexural strength for bioconcrete
4.17 Relationship between compressive-flexural strength for *E. faecalis*
4.18 Relationship between compressive-flexural strength for *B. Sp*
4.19 Water penetration of bioconcrete
4.20 Water penetration of concrete with *E. Faecalis* and calcium lactate
4.21 Results of water penetration of concrete with *B.sp* and calcium lactate
4.22 Chemical composition of bioconcrete
4.23 Ultrasonic pulse velocity of control and bioconcrete
4.24 UPV data for control and *E. faecalis* with different concentrations of calcium lactate
4.25 Data obtained for *B. sp* and concentrations of calcium lactate
4.26 Ultrasonic pulse velocity of concrete prism
4.27 Ultrasonic pulse velocity of concrete prism with *E. faecalis*
4.28 Percentage of healing of concrete prism containing *B.sp* and calcium lactate
4.29 Percentage of healing for control and bioconcrete
4.30 Percentage of healing for bioconcrete and calcium lactate
4.31 Percentage of healing for bioconcrete specimens containing *B. sp* and calcium lactate
4.32 Percentage of healing Percentage of healing for Bioconcrete specimens
4.33 Percentage of healing for Bioconcrete specimens containing different concentrations of calcium lactate
4.34 Healing of concrete containing *B. sp* and concentrations of calcium lactate
## LIST OF FIGURES

<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Factors influencing concrete durability</td>
<td>2</td>
</tr>
<tr>
<td>2.1</td>
<td>Image of bacteria with calcium carbonate formation of the cell wall</td>
<td>13</td>
</tr>
<tr>
<td>2.2</td>
<td>Microstructure analysis of biosealant removed from inside cracks of Concrete: (a), (c) and (e) are concrete with added bacteria, whereas (b), (d) and (f) are concrete with bacteria and calcium lactate.</td>
<td>27</td>
</tr>
<tr>
<td>2.3</td>
<td>Chemical constituents of precipitation of calcium carbonate. The high Amount of Ca present is evident and the precipitation is inferred to as calcite (CaCO$_3$) crystals</td>
<td>28</td>
</tr>
<tr>
<td>2.4</td>
<td>Concrete containing fungi with image</td>
<td>28</td>
</tr>
<tr>
<td>2.5</td>
<td>Diffractograms of consolidated sand with bacteria</td>
<td>30</td>
</tr>
<tr>
<td>2.6</td>
<td>Concrete structure subjected to corrosion</td>
<td>33</td>
</tr>
<tr>
<td>2.7</td>
<td>Cracking which initiates the steel corrosion process</td>
<td>33</td>
</tr>
<tr>
<td>2.8</td>
<td>Before and after experiment for self-healing of concrete using bacteria</td>
<td>38</td>
</tr>
<tr>
<td>2.9</td>
<td>Method to measure UPV (EN12504-4:2004) (a) Direct method (b) and (c) Indirect method</td>
<td>39</td>
</tr>
<tr>
<td>2.10</td>
<td>Image from Stereomicroscope after 100 days</td>
<td>40</td>
</tr>
<tr>
<td>2.11</td>
<td>Different bacteria concentration with different amount of silica fume of (a) 28 days (b) 91 days</td>
<td>42</td>
</tr>
<tr>
<td>2.12</td>
<td>The compressive strength of cement mortar of control and addition of Bacterial cells</td>
<td>43</td>
</tr>
<tr>
<td>2.13</td>
<td>Linear relationship between compressive and tensile strength</td>
<td>46</td>
</tr>
<tr>
<td>2.14</td>
<td>Flexural strength of mortar beams with addition of bioremediase</td>
<td>48</td>
</tr>
<tr>
<td>2.15</td>
<td>The influence of surface treatment on the rate of water absorption versus Time for mortar cubes with water-cement ration of 0.6</td>
<td>51</td>
</tr>
<tr>
<td>3.1</td>
<td>Research flowchart</td>
<td>56</td>
</tr>
<tr>
<td>Section</td>
<td>Description</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>-------------</td>
<td></td>
</tr>
<tr>
<td>3.2</td>
<td>Bacteria liquid culture and calcium lactate flowchart</td>
<td></td>
</tr>
<tr>
<td>3.3</td>
<td>Sand used during casting</td>
<td></td>
</tr>
<tr>
<td>3.4</td>
<td>Sieve analysis result for fine aggregate</td>
<td></td>
</tr>
<tr>
<td>3.5</td>
<td>Coarse aggregate within the sieve limit</td>
<td></td>
</tr>
<tr>
<td>3.6</td>
<td>Sieve analysis result for coarse aggregate</td>
<td></td>
</tr>
<tr>
<td>3.7</td>
<td>Mix Portland cement</td>
<td></td>
</tr>
<tr>
<td>3.8</td>
<td><em>Enterococcus faecalis</em> and <em>Bacillus sp</em> used in this study</td>
<td></td>
</tr>
<tr>
<td>3.9</td>
<td>Bacteria identification procedure</td>
<td></td>
</tr>
<tr>
<td>3.10</td>
<td>Materials for preparation bacteria with nutrient source (<em>Enterococcus faecalis</em> / <em>Bacillus sp</em>)</td>
<td></td>
</tr>
<tr>
<td>3.11</td>
<td>Serial dilution of bacteria</td>
<td></td>
</tr>
<tr>
<td>3.12</td>
<td>Calcium lactate pre-measured for casting</td>
<td></td>
</tr>
<tr>
<td>3.13</td>
<td>Calcium lactate dissolved before fabrication</td>
<td></td>
</tr>
<tr>
<td>3.14</td>
<td>Universal testing machine for compressive strength test</td>
<td></td>
</tr>
<tr>
<td>3.15</td>
<td>Cubes use for compressive strength test</td>
<td></td>
</tr>
<tr>
<td>3.16</td>
<td>Cylinder sample placed in UTM machine for splitting tensile test</td>
<td></td>
</tr>
<tr>
<td>3.17</td>
<td>Fabrication of concrete cylinder sample</td>
<td></td>
</tr>
<tr>
<td>3.18</td>
<td>Flexural test for concrete conducted using UTM</td>
<td></td>
</tr>
<tr>
<td>3.19</td>
<td>Mold of prism used to fabricate prism used in flexural strength test</td>
<td></td>
</tr>
<tr>
<td>3.20</td>
<td>Testing machine for water penetration test</td>
<td></td>
</tr>
<tr>
<td>3.21</td>
<td>Metal strip pushed into fresh concrete to create cracks</td>
<td></td>
</tr>
<tr>
<td>3.22</td>
<td>View of prism with steel reinforcement (2R8)</td>
<td></td>
</tr>
<tr>
<td>3.23</td>
<td>Sample of prism with standard crack</td>
<td></td>
</tr>
<tr>
<td>3.24</td>
<td>Circular disk sample for self-healing observation and analyses</td>
<td></td>
</tr>
<tr>
<td>3.25</td>
<td>Ultrasonic pulse velocity set-up</td>
<td></td>
</tr>
<tr>
<td>3.26</td>
<td>Ultrasonic pulse velocity specimen</td>
<td></td>
</tr>
<tr>
<td>3.27</td>
<td>Specimens with crack used for monitoring</td>
<td></td>
</tr>
<tr>
<td>3.28</td>
<td>EDX machine for analysis</td>
<td></td>
</tr>
<tr>
<td>3.29</td>
<td>Hand-hydraulic press used to create pellets</td>
<td></td>
</tr>
<tr>
<td>3.30</td>
<td>Pellets formed for XRF analyses</td>
<td></td>
</tr>
<tr>
<td>4.1</td>
<td>Image of <em>Enterococcus faecalis</em> under microscope</td>
<td></td>
</tr>
<tr>
<td>4.2</td>
<td>Image of <em>Bacillus sp</em> under microscope</td>
<td></td>
</tr>
<tr>
<td>4.3</td>
<td>Agarose gel of PCR product</td>
<td></td>
</tr>
<tr>
<td>4.4</td>
<td>Phylogenetic position of <em>Bacillus sp</em> and closely related taxa (Sulphate</td>
<td></td>
</tr>
</tbody>
</table>
Reduction bacteria)

4.5 Phylogenetic position of *Enterococcus faecalis* and closely related taxa (Ureolytic bacteria)

4.6 The absorbance value of bacteria

4.7 Relationship of Absorbance (nm) VS number of cells for *E. faecalis*

4.8 Relationship of Absorbance (nm) VS number of cells for *B. sp*

4.9 Compressive strength of normal and bioconcrete with age of curing

4.10 Compressive strength of concrete with *E. Faecalis* and calcium lactate with time

4.11 Compressive strength of concrete with *B. sp* and *B. sp* with calcium lactate

4.12 Tensile strength test (left) before and (right) after testing

4.13 Tensile strength for control and bioconcrete samples

4.14 Tensile strength for control and concrete with *E. Faecalis* and calcium lactate

4.15 Tensile strength for control and concrete containing *B. sp* and calcium lactate

4.16 Prism before (left) and after (right) flexural strength test

4.17 Comparison of flexural strength of control and concrete containing *E. Faecalis* and *B. sp*

4.18 Flexural strength of concrete for control and bioconcrete with calcium lactate

4.19 Flexural strength of concrete with *B. sp* and calcium lactate

4.20 Comparison compressive-tensile between each empirical strength relation in bioconcrete

4.21 Comparison of compressive-tensile strength relation in bioconcrete with calcium lactate

4.22 Comparison between compressive-tensile strength relation in bioconcrete containing *B. sp* and calcium lactate

4.23 Comparison between compressive-flexural relation in bioconcrete

4.24 Compressive-flexural strength relation of bioconcrete with calcium lactate

4.25 Comparison between compressive-flexural strength relation of bioconcrete with calcium lactate

4.26 (a) Shows the water penetration test set-up and (b) shows the water penetration depth after testing
4.27 Relationship of water penetration between bioconcrete

4.28 Water penetration of concrete with *E. Faecalis* and calcium lactate

4.29 Relationship between concrete with *B.sp* and calcium lactate

4.30 Comparison between two different bacteria

4.31 Microstructure of normal concrete

4.32 Concrete sample with *E. faecalis*

4.33 Microstructure of concrete sample with *B.sp*

4.34 Microstructure of *E. faecalis* and 0.22 g/L of calcium lactate

4.35 Concrete sample with *E. faecalis* and 1.09g/L calcium lactate

4.36 Microstructure of *E. Faecalis* and 2.18g/L calcium lactate

4.37 Microstructure of *B.sp* and 0.22 g/L of calcium lactate

4.38 Sample with *B. sp* and 1.09g/L calcium lactate

4.39 Concrete with *B. sp* and 2.18g/L calcium lactate

4.40 The image and graph of elements for control sample by EDX

4.41 The image and graph of elements with 3% *Enterococcus faecalis* by EDX

4.42 The image and graph of elements for sample with 5% *Bacillus sp* by EDX

4.43 Comparison of calcium mass in control and sample with bacteria

4.44 EDX image and element composition for 3% *Enterococcus faecalis* with 0.22g/L of calcium lactate

4.45 Element composition for 3% *E. faecalis* with 1.09 g/L of calcium lactate

4.46 Element composition of 3% *E. Faecalis* with 2.18g/L calcium lactate

4.47 Comparison of calcium mass in control and *E. faecalis* sample with Calcium lactate

4.48 EDX image and element composition for 5% *Bacillus sp* with 0.22g/L of calcium lactate

4.49 EDX image and element composition for 5% *Bacillus sp* with 1.09 g/L of calcium lactate

4.50 5% *B.sp* with 2.18g/L Calcium lactate

4.51 Amount of calcium in concrete sample of normal and bioconcrete

4.52 Comparison of calcium mass of bioconcrete with different Concentrations of calcium lactate

4.53 Comparison of healing percentage of control and bioconcrete samples

4.54 Percentage of healing of control, bioconcrete and concrete with calcium lactate
4.55 Comparison of healing capabilities of control, concrete with B. sp and calcium lactate
4.56 Percentage of healing comparison between, normal E. faecalis and B. sp
4.57 Percentage of healing for control and bioconcrete
4.58 Comparison between different concentrations of calcium lactate with bioconcrete containing E. faecalis
4.59 Comparison of concrete containing B. sp with concentrations of calcium lactate
4.60 Percentage of healing for bioconcrete
4.61 Percentage of healing for bioconcrete specimens with E. faecalis and calcium lactate
4.62 Comparison of healing between concrete specimens with B. sp and calcium lactate
4.63 Comparison percentage of healing between E. Faecalis and B. sp
4.64 Comparison for healing percentages among bioconcrete specimens
4.65 Healing capabilities comparison for specimens containing E. Faecalis and calcium lactate
4.66 Comparison between bioconcrete specimens containing different concentrations of calcium lactate
4.67 Comparison of healing between E. Faecalis and B. sp
## LIST OF SYMBOLS AND ABBREVIATIONS

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E. \ faecalis$</td>
<td>-</td>
<td>Enterococcus faecalis</td>
</tr>
<tr>
<td>$B. sp$</td>
<td>-</td>
<td>Bacillus sp</td>
</tr>
<tr>
<td>$kg$</td>
<td>-</td>
<td>Kilogram</td>
</tr>
<tr>
<td>$m^3$</td>
<td>-</td>
<td>$m \times m \times m$ or volume</td>
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<tr>
<td>$kg/m^3$</td>
<td>-</td>
<td>Density</td>
</tr>
<tr>
<td>SCC</td>
<td>-</td>
<td>Self compacting concrete</td>
</tr>
<tr>
<td>$CaCO_3$</td>
<td>-</td>
<td>Calcium Carbonate</td>
</tr>
<tr>
<td>%</td>
<td>-</td>
<td>Percentage</td>
</tr>
<tr>
<td>$k$</td>
<td>-</td>
<td>Growth Rate</td>
</tr>
<tr>
<td>$k_1$</td>
<td>-</td>
<td>Carbonation rate constants</td>
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<tr>
<td>$g$</td>
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<td>Gram</td>
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<tr>
<td>$CO_2$</td>
<td>-</td>
<td>Carbon Dioxide</td>
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<tr>
<td>pH</td>
<td>-</td>
<td>Power of Hydrogen</td>
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<tr>
<td>cells/ml</td>
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<td>Cells per 1 ml</td>
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<tr>
<td>$FA$</td>
<td>-</td>
<td>Fly Ash</td>
</tr>
<tr>
<td>$SF$</td>
<td>-</td>
<td>Silica Fume</td>
</tr>
<tr>
<td>$\frac{dq}{dt}$</td>
<td>-</td>
<td>The rate of fluid, $m^3/s$</td>
</tr>
<tr>
<td>$L$</td>
<td>-</td>
<td>Thickness of sample in m</td>
</tr>
<tr>
<td>$A$</td>
<td>-</td>
<td>Cross sectional area of the sample in $m^3$</td>
</tr>
<tr>
<td>$\Delta h$</td>
<td>-</td>
<td>Drop in hydraulic head through the sample, m</td>
</tr>
<tr>
<td>$K$</td>
<td>-</td>
<td>Coefficient of permeability in $m/s$</td>
</tr>
<tr>
<td>$m/s$</td>
<td>-</td>
<td>Meter per second</td>
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<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>-------------</td>
<td>-------------------------------------</td>
<td></td>
</tr>
<tr>
<td>kPa</td>
<td>Kilo pascal</td>
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</tr>
<tr>
<td>BS</td>
<td>British Standard</td>
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<tr>
<td>BS EN</td>
<td>British Standard European Norm</td>
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</tr>
<tr>
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<td>Gram</td>
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</tr>
<tr>
<td>mm</td>
<td>Milimeter</td>
<td></td>
</tr>
<tr>
<td>DOE</td>
<td>Department of Environment</td>
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</tr>
<tr>
<td>L</td>
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<tr>
<td>NaOH</td>
<td>Sodium Hydroxide</td>
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<tr>
<td>ml</td>
<td>Millilitre</td>
<td></td>
</tr>
<tr>
<td>NTU</td>
<td>Nephelometric Turbidity Unit</td>
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</tr>
<tr>
<td>CFU</td>
<td>Colony Forming Unit</td>
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<tr>
<td>CFU/ml</td>
<td>Colony Forming Unit per Millilitre</td>
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<tr>
<td>UB</td>
<td>Ureolytic Bacteria</td>
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<tr>
<td>cm</td>
<td>Centimeter</td>
<td></td>
</tr>
<tr>
<td>RPM</td>
<td>Revolution Per Minute</td>
<td></td>
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<tr>
<td>NCBI</td>
<td>National Centre for Biotechnology Information</td>
<td></td>
</tr>
</tbody>
</table>
# LIST OF APPENDICES

<table>
<thead>
<tr>
<th>APPENDIX</th>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>DOE MIX DESIGN</td>
<td>184</td>
</tr>
<tr>
<td>B</td>
<td>DNA SEQUENCE</td>
<td>186</td>
</tr>
<tr>
<td>C</td>
<td>RAW DATA FOR UPV</td>
<td>188</td>
</tr>
<tr>
<td>D</td>
<td>RAW DATA FOR STEREOMICROSCOPE</td>
<td>194</td>
</tr>
</tbody>
</table>
CHAPTER 1

INTRODUCTION

1.1 Introduction

Concrete that is made from coarse granular aggregate, sand, cement and water is the most widely used construction material in the world. Concrete is the preferred construction material for a wide range of buildings, bridges and other civil engineering structures. The material has been successful and popular due to the ease of casting into various shaped and sizes, it is easy to procure in the market and it is cheaper compared to other building materials. The main strength of concrete lies in its compressive strength which is higher than conventional building materials such as bricks and stone masonry (Unnikrishna and Devdas, 2003). The development of concrete technology had triggered various grade and type of concrete namely, foam concrete, light-weight concrete, high strength concrete and others. An option of concrete in the market is concrete infuse with microorganisms known as bioconcrete.

The use of concrete is gaining even more momentum as many more plans and development are taking place due to the Country’s economic growth and development. As the demand for concrete increases, the need to create a better, longer lasting and more durable concrete is much in need. This is due to concrete shortcomings that are known as concrete degradation which occurs in the form of cracking, scaling, occurrence of efflorescence and many more. According to Jackson
and Dhir (1996), concrete is required to sustain the load and be more durable. The durability is defined as resistance to deterioration which occurs by means of internal or external factors. Figure 1.1 shows several factors that influence the durability of concrete.

Based on Figure 1.1, deterioration in concrete is caused by several factors such as physical deterioration, chemical deterioration and reinforcement corrosion. These deteriorations leads to cracking in structures, leaching of chemicals, carbonation and chlorination. If left untreated, these deteriorations causes the lifespan of a building to reduce and affect the structural integrity of a building.

In the current market, a lot of methods have been adopted in the construction industry to minimize the concrete degradation. The concrete degradation is minimized by adding various chemical admixtures, concrete hardeners, damp-proofing admixtures and etc (Tittelboom et al., 2012b). These methods used are not environmentally friendly and pose several drawbacks on the environment such as air pollution, soil and water contamination (Guadalupe et al., 2014).

There are many studies conducted which focuses on the reduction of concrete deterioration by adding environmentally friendly materials such as waste from factory production in order to come out with alternative solution to concrete problem (Kartini et al., 2010; Panda and Bal, 2013; Bashar et al., 2013; Norlia et al., 2013;
The common waste and industrial by-products that are normally being used are rice husk, recycle waste material, fly ash and silica fume.

Kartini et al., (2010) had stated that rice husk ash is able to improve the durability of concrete in G30 concrete by lowering the permeability, thus lowering the absorption characteristics and increasing the resistance of concrete to chloride ion penetration. Apart from that, Panda and Bal (2013) had stated that recycle aggregate used as partial replacement yielded more desirable compressive strength as compared to full replacement of course aggregate in self compacting concrete. Conventional way to increase strength without adding waste in concrete, normally relies on chemical solution or adding more cement. Development and research are conducted in order to provide a more environmentally friendly alternative to concrete durability. There are studies that focus on prolonging the life of concrete structure by inducing self healing capability. This prevents crack from becoming unmanageable which leads to expensive repairs and reduction of structural integrity of the building.

Worldwide, researchers have studied on the use of bacteria in concrete with various improvement in concrete properties and self-healing (Muynck et al., 2008; Tittelboom et al., 2010; Jonkers., 2011; Abo-El-Enein., 2013; Varenyam et al., 2013; Parmar et al., 2013). Certain group of bacteria have the capability in prolonging the life of concrete by using specific enzyme to precipitate calcium carbonate (Muynck et al., 2008).

Some researchers added calcium based nutrient in bioconcrete to facilitate with the process of producing calcium carbonate. Introducing additional calcium nutrient source in the formed of calcium lactate, calcium acetate and calcium chloride were used and it was discovered that significant improvement was found on mechanical properties and durability of concrete. Self-healing of concrete were also found to have increase significantly with addition of calcium based nutrient (Abo-El-Enein et al., 2013; Xu and Yao., 2014; Xu et al., 2015). Suitability of bacteria in concrete depends on factors such as the ability of the bacteria to live in little to no oxygen environment and environment which is high in alkaline. Some researchers have device methods to seal the bacteria and calcium nutrient source in capsules of light weight aggregate (LWA) to protect the bacteria from the roughness of concrete fabrication and protect the bacteria from high alkaline environment of bacteria. The bacteria and calcium source within the capsules or LWA are released once a crack is
formed through external pressure or loading. (Jonkers and Erik., 2008; Tittelboom et al., 2012b; Wang et al., 2012; Rajesh et al., 2015).

1.2 Problem statement

Concrete is an important element in construction and it is the most used material worldwide. Concrete is used to construct buildings from foundations to building structure, road curbs and drainage. Construction of different parts of buildings requires different standard or strength of concrete. In order to achieve the concrete standard require of different buildings or sub-structure, different strength of concrete are used. Methods to increase strength of concrete are to reduce the water ratio of concrete mixture, increase cement content or adding chemical admixture. Reduction of water reduces workability thus making it hard to cast while addition of cement content is costly. Chemicals used in chemical admixture to improve concrete properties are not as costly compared to adding extra cement but the use of chemicals itself is harmful towards the environment. Additional natural resources are depleted in order to manufacture the chemicals used to improve engineering concrete properties (Mehta, 2002).

Concrete has many shortcomings. These shortcomings come in the form of high water penetration, cracking, steel reinforcement deterioration and etc. Constructed building and infrastructures after the second half of the last century has seen a declined in lifespan due to rapid deterioration. The enhancement of building durability has been the aim of many researchers as longer lifespan reduces the amount of raw material used, carbon dioxide emission and energy consumption related to construction (Schlangen and Sangadj, 2013). New materials are soughtout to construct new building and the production of cement releases carbon dioxide to the atmosphere. Regular maintenance could reduce the chance of faster building deterioration. Cracks are often the trigger to problems, micro-cracks formed which are not treated could potentially become serious through moisture or water entering the cracks. The moisture or water that enter the cracks causes the steel reinforcement to rust and therefore reduce the structure’s integrity (Tittelboom et al., 2010).

The use of chemicals are often the solution to industrial problems. Chemicals used in order to fix and repair concrete have considerable negative effects on the
environment (Tittelboom et al., 2012b). Production of these chemicals requires a vast amount of resources, thus depletion of the world resources due to usage and high demands. Apart from that, the use of chemicals on concrete such as epoxy and synthetic fillers are temporary and often requires re-application when cracks re-emerges. Alternative methods that are more environmentally friendly need to be explored for improving degradation of concrete (Muynck et al., 2007).

Ramachandran et al., (2001) had argue that cracks in concrete is a very common phenomenon. However, cracks in structure have to be treated in order to prevent the crack from expanding and causing major problems. There are many available treatments in the market to cope with cracking in structure, among them are epoxy, resins, epoxy mortar and other synthetic mixtures. Unfortunately, the synthetic filler which is commonly used acts as a temporary solution and reapplication is needed depending on the formation of other cracks.

Jonkers., (2011a) had proven that high binder content of concrete mixture resulted to delay crack formation. Concrete has a certain autonomous healing of micro crack which is related to the composition of concrete mixtures. Mixtures that contain high binder content shows high crack healing properties which are due to the secondary or delayed hydration. Unfortunately, this ability to heal crack is only limited to cracks which are smaller than 0.2mm and also due to the global sustainable reasons the use of high binder content cement is not encourage. It is costly to use more cement in concrete and using more encourages higher production of cement. This induces carbon dioxide emissions which eventually leads to global warming.

Researchers such as Abo-El-Enein et al., (2012) and Xu and Yao (2014) have researched the possibility of adding calcium source into bioconcrete. In the study conducted by Xu and Yao (2014), it was found that the type of calcium source added have a profound effect on the degree of crystallinity of precipitated calcite by bacteria. Organic and inorganic calcium sources were used, inorganic calcium source such as calcium chloride were found to have lowest amount of precipitated CaCO₃ while using organic calcium source such as calcium lactate were found to achieve a higher amount of CaCO₃.

This research aims to find an environmental alternative for durable concrete by adding calcium lactate in bioconcrete. The calcium lactate are added in several different concentrations. The engineering concrete properties and enhancement of self-healing are studied.
1.3 Objective

The objectives of this study are:

(i) To determine the optimum concentration of calcium lactate which are added into concrete.

(ii) To investigate the effect of calcium lactate together with *Enterococcus faecalis* and *Bacillus sp* towards the engineering concrete properties in unsterillized condition.

(iii) To study the effect of the addition of calcium lactate with *Enterococcus faecalis* and *Bacillus sp* on the self healing of concrete in unsterillized condition.

1.4 Scope of study

This study mainly focused on laboratory works, where all laboratory work are conducted in University Tun Hussein Onn Malaysia (UTHM). The growth of *Enterococcus faecalis* and *Bacillus sp* is conducted in the environmental laboratory, whereas the casting and testing of concrete is conducted in structural laboratory. The percentage of *Enterococcus faecalis* used is 3% whereas the 5% *Bacillus sp* is used in this study. The different percentages used is based on previous study (Irwan *et al.*, 2015). In the previous study, optimum percentage was tested in order to determine the best percentage that yielded the best results in improving the properties of concrete. The optimum amount of percentage that is added into the concrete which achieved the best result is used in this study.

The *Enterococcus faecalis* and *Bacillus sp* are both regrown based on the optimum number of days by the bacteria growth curve. Apart from that, calcium lactate is added as an additional calcium source to increase precipitation of calcium carbonate. The calcium lactate added acts as a catalyst for the bacteria to precipitate calcium carbonate. Calcium lactate is chosen as the calcium nutrient source due to the popularity as an organic nutrient source used by Xu *et al.*, (2015), Xu and Yao (2014) and Jonkers and Erik (2008) in bioconcrete research. Calcium lactate can
easily be found in milk and cheese, thus making the use of calcium lactate and bacteria in concrete innocuous. Several concentrations of calcium lactate (0.22 g/L, 1.09 g/L and 2.18 g/L) are added to determine the optimum percentage which optimizes the engineering concrete properties in term of compressive strength, tensile strength, flexural strength and water penetration. Microstructure, chemical composition and elemental analysis are conducted on concrete samples. Self healing of concrete with bacteria and calcium lactate is analyzed using Ultrasonic pulse velocity (UPV) and Stereomicroscope.

1.5 Importance of study

As the world is getting more and more crowded due to the surge of human population, constant and ever rapid construction should take place to make way for this growing population. Hence, houses, condominiums, apartments, schools, University, shopping complexes, shop-lots and so much more are constantly being built to sustain this growth.

With the ever-growing construction industry, the use and production of concrete is absolute. Apart from producing concrete for the growing industry, concrete is also produced for the re-pair and re-built of a demolished building that has reached the end of its lifespan. The lifespan of a building is due to the concrete durability which in current standard is only 50 years. Thus, after 50 years the buildings are either demolished or abandoned due to safety reasons.

Mehta (2002) had stated that during the second half of the twentieth century, most of the structures that was built were prone to premature deterioration of concrete, especially for structures that are exposed to industrial and urban environments. The degradation of concrete was mostly associated with the corrosion of steel reinforcement due to alkali-aggregate reaction or sulfate attack. The reinforced concrete structures begin to deteriorate earlier than the design lifespan of the structure due to the cracks, microcracks which resulted in penetration of water and therefore cause durability problems.

Present day concrete mixes contain high reactive Portland cement in order to induce early strength for the purpose of high speed construction. Early strength concrete undergoes high drying shrinkage and high thermal contraction. This process
causes cracks to occur and thus durability problems in concrete. The search for an environmentally-friendly alternative to cope with the concrete problem is important more than ever. Natural resources are being depleted faster than it has the chance to recover due to the chemicals being produced and natural resources being use to construct new building. The alternative is to build structure that can stand the test of time, by building structure that are less prone to deterioration or could sustain itself. Bioconcrete is the solution to this problem, as bacteria with calcium lactate in the concrete has the ability to produce calcium carbonate which in turns improves engineering concrete properties. The reduction of water penetration lessen the chance of steel reinforcement being corroded thus improving the durability of concrete. Self healing of concrete micro-cracks is also possible by production of calcium carbonate. Thus, the importance of this study is to determine the effect of Enterococcus faecalis and Bacillus sp with calcium lactate on the engineering concrete properties and analyze the potential of these particular bacterium on self healing of concrete micro-cracks.

1.6 Organization of thesis

This thesis aims to investigate the effect of different concentrations of calcim lactate and bacteria towards the engineering concrete properties and self-healing of concrete under unsterrilized condition. Overall, this thesis consists of five chapters with each chapter focusing on a different subject matter as follow.

Chapter 1 is the introduction for the whole thesis which gives an overview of introduction, problem statement, objectives, scope of study and importance of study. It covers a brief overview on the current problems faced by the industry. In addition, this chapter briefly explains the current method in solving issue with concrete and suggest a more environmentally-friendly method to solve these issue.

Chapter 2 presents the review of literature related to this study, which includes the introduction of the type of concrete, the idea of bioconcrete, type of bacteria used by researchers, the effect of adding calcium source, the enzymatic pathway of these
bacteria and the tests conducted by researchers to validate the effect of bacteria towards concrete.

Chapter 3 focuses on the material used in conducting this research, process of identifying the bacteria, preparation of concrete samples and procedure for tests conducted. Furthermore, this chapter also includes the details for preparing samples for self-healing, analysis for self-healing and analysis of concrete samples.

Chapter 4 presents the findings of this research. This includes the identification of bacteria, effect of bacteria with different concentration of calcium lactate on engineering concrete properties and self-healing of concrete. In addition, findings of Microstructure, chemical composition and elemental analysis is presented and discussed in this chapter.

The last chapter in this thesis-Chapter 5 gives a summary of the whole result as an integral part of the study. The recommendations for future works or further works are also provided in this chapter.
CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Developments of concrete have been rapid during recent years; this is due to the demand of the industry. Various types of concrete were developed to cater with the different structure and environment. Among the types of concrete developed are foam concrete, self-compacting concrete, geo-polymer concrete and bioconcrete.

Foam concrete is a lightweight concrete that is created using Portland cement paste or cement filler matrix (mortar) with a uniformly distributed pore structure produced by adding forming agent. It was created to produce a concrete that is light but with substantial strength (Ramamurthy et al., 2009).

Self-compacting concrete (SCC) was design and created to ease the concrete casting in small or hard to reach places. Self-compacting concrete is quite suitable to be used in structural elements which have dense reinforcement. This characteristic also enables easier workability, little workmanship and reduces the duration of the concrete construction stage. Apart from self-compacting concrete (SCC), a non-combustible and non-flammable concrete that was created is Geo-polymer concrete. Geo-polymer concrete utilizes the waste material from industries such as fly ash, silica fume, biomass material and many more to replace cement in concrete. This resulted in a much greener product for the industrial with the same or better mechanical properties.

As brilliant as the concrete innovations is, it is not without flaw. The main flaw of concrete is the proneness to cracks. Therefore, development of new concrete to withstand or self-repair crack is necessary. (Siddique and Chahal, 2011). Presently to
reach optimal levels of sustainability, several investigations are being made to reduce the environmental impact of concrete such as replacing Portland clinker with alternative cements and increasing concrete durability (Gonsalves, 2011). The use of bacteria in concrete remediation is a new approach to an old idea that a microbial mineral deposit constantly occurs in natural environments. Specifically, microbiologically-induced calcite is environmentally innocuous, compared to synthetic polymers currently used for concrete repairs (Ramachandran et al., 2001).

2.2 Bioconcrete

The term bioconcrete is a combination of biology (micro-organism) and concrete, Bioconcrete. Thus, this study focus on an environmental approach in improving engineering concrete properties and enhancement of self-healing by inducing micro-organisms mainly Enterococcus faecalis and Bacillus sp.

Ramachandran et al., (2001) stated the bacterial concrete refers to a new type of concrete in which selective cementation of porous media by microbiologically-induced CaCO$_3$ has been introduced for remediation of damaged structural formation or micro cracks.

The field of using bacterial to improve concrete appears to be more beneficial as bacterial concrete appears to produce more substantially crack plugging minerals than control specimens (without bacteria). Microbial carbonate precipitation (biodeposition) decreases the permeation properties of concrete. Hence, a deposited layer of calcium carbonate on the surface of concrete resulted in the decrease of water absorption and porosity. The highest decrement of permeation after 140 hours is 24% compared to control (Siddique and Chahal, 2011). The presence of bacteria which was the precipitated calcium carbonate by bacteria has resulted in significant decrease of water uptake of up to 85% compared to concrete without any addition of bacteria (Muynck et al., 2008).

Bacterial protein bioremediase is bacteria in powdered form. This form of bacteria was used in the fabrication of concrete mortar and beams. Overall, positive results in terms of compressive strength, flexural strength of beams and self-healing of cracks had been found in this study (Chattopadhyay et al., 2011). The addition of bioremediase in Portland pozzolanic cement has found to have an increase of
compressive strength of up to 39.4% as compared to control cube and an increase of 33% of flexural strength of beam as compared to beams without bacteria. It is also found to have a resistance to environmental pollutants such as water absorption and sulfate ions. This bioremediase is non-harmful to human beings and is eco-friendly as well. Apart from that, the use of bioremediase in concrete has shown significant self-healing properties on concrete cracks. (Chattopadhyay et al., 2011).

Xu and Yao (2014) and Abo-El-Enein et al., (2012), both have similar finding in which adding calcium source into bioconcrete indeed increase the amount of precipitated calcium carbonate through the bacteria enzymatic pathway. This increment of calcium carbonate resulted in improvement of concrete properties. However, different type of calcium showed different results. It is concluded that organic calcium source such as calcium lactate is more suited to produce high amount of calcium carbonate than inorganic calcium source such as calcium chloride and calcium acetate.

Based on the works of previous researchers, bioconcrete has been extensively studied by researchers conducting study on bacteria in concrete. The addition of bacteria in concrete has positive results in terms of improving engineering concrete properties and self healing. A direct method of using bacteria culture during the concrete mixing process is unpopular because most bacteria are acidic in nature and do not mix well in alkaline environment such as concrete. Apart from that, most bacteria are aerobic bacteria thus require oxygen to survive. Concrete has little to no oxygen, thus different method of adding bacteria into concrete are device such as encapsulation, bacteria powder and lightweight aggregate in order to sustain the life of the bacteria. Based on previous study, addition of calcium source increases precipitation of calcium carbonate significantly, depending on the type of calcium source (inorganic or organic). In this study, a direct method of adding Enterococcus faecalis and Bacillus sp with different concentrations of calcium lactate in concrete is study. The effect towards engineering concrete properties and enhancement in self-healing is conducted.

### 2.3 Bacteria

Siddique and Chahal (2011) has define bacteria as unicellular (single cell) microorganisms. Bacteria are normally found everywhere and anywhere on earth, growing
in soil, acidic hot springs, water and even deep in the Earth’s crust. Bacteria can also be found in organic matter such as in live bodies of plants and animals. Averagely, there are 40 million bacterial cells in a gram of soil and a million bacterial cells in a millimeter of fresh water. The approximate number of bacteria on Earth is five nonillion \( (5 \times 10^{30}) \) which forms much of the world’s biomass.

According to Chahal et al., (2010) bacteria are able to promote the precipitation of calcium carbonate in the form of calcite. Calcium carbonate precipitation occurs as a by-product of a common microbial metabolic process which increases the alkalinity and produce microbial calcite precipitation. Figure 2.1, shows the image of bacteria with calcium carbonate formation.

![Figure 2.1: Image of bacteria with calcium carbonate formation of the cell wall (Siddique and Chahal, 2013)](image)

Siddique and Chahal (2013) have stated that there are four phases of bacteria growth:

Phase 1: Lag phase

This is when the bacteria have only begun to adapt to a high nutrient environment and is preparing for rapid growth. During this phase, proteins necessary for the growth of bacteria are produced, thus has high biosynthesis rates.
Phase 2: Logarithmic Phase (Log Phase)

This phase of the bacterial growth is marked by rapid exponential phase, the growth rate of this phase is also known as growth rate (k) and the time taken from bacteria to multiply is known as the generation time (g). During this phase of growth, nutrients are being depleted by the bacteria due to the rapid growth, after which limiting growth occurs due to the depleted nutrient.

Phase 3: Stationary Phase

This phase is known as stationary phase due to the stop of growth of bacteria which is caused by the depletion of nutrient during log phase.

Phase 4: Death Phase

This phase is known as the death phase in which the bacteria starts dying off due to depleted nutrient.

In this research, the bacteria are used when it reaches log phase, which is when the bacteria reached optimum growth (highest number of bacteria count). This is determined through optical density and growth curve of the bacteria. Apart from that, the bacteria used in this study are Gram positive. Bacteria are either Gram positive or Gram negative. This is determined by conducting Gram staining tests. Bacteria without cell walls could not retain stains therefore are Gram negative. The presence of cell walls has a positive influence towards the compressive strength of concrete. Based on Pei et al., (2013) study, which compares compressive strength between samples which contains bacteria with cell walls and without. It was found that bacteria with cell walls significantly improve the compressive strength of concrete.

2.3.1 Types of bacteria used in concrete

There are various species of bacteria that are used in bioconcrete research such as in Table 2.1. Where, different species of bacteria in bioconcrete are studied through different applications such as surface treatment and concrete properties.
Table 2.1: Various bacteria used in concrete

<table>
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<tr>
<th>Researcher</th>
<th>Title</th>
<th>Bacteria species</th>
<th>Application</th>
<th>Research result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramachandra et al.,  (2001)</td>
<td>Remediation of concrete using microorganisms</td>
<td>(1) &lt;em&gt;Bacillus Pasteurii&lt;/em&gt; (2)&lt;em&gt;Pseudomonas Aeruginosa&lt;/em&gt;</td>
<td>Microbial mortar cracks healer and to increase the compressive strength of mortar</td>
<td>This study shows that live bacteria has higher increment of compressive strength compared to dead bacteria.</td>
</tr>
<tr>
<td>Ghosh and Mandal (2006)</td>
<td>Development of bioconcrete material using an enrichment culture of novel thermophilic anaerobic bacteria</td>
<td>&lt;em&gt;Escherichia coli&lt;/em&gt;</td>
<td>To Increase Strength in Concrete Mixture</td>
<td>The bacteria used contributed to the increment of strength.</td>
</tr>
<tr>
<td>Tittelboom et al., (2010)</td>
<td>Use of bacteria to repair cracks in concrete</td>
<td>&lt;em&gt;Bacillus Sphaericus&lt;/em&gt;</td>
<td>Crack repair in concrete</td>
<td>Bacteria protected in silica gel healed cracks in concrete of up to 10mm and 20mm deep.</td>
</tr>
<tr>
<td>Arunachala et al., (2010)</td>
<td>Studies on the characterization of Biosealant properties of &lt;em&gt;Bacillus Sphaericus&lt;/em&gt;</td>
<td>&lt;em&gt;Bacillus Sphaericus&lt;/em&gt;</td>
<td>Microbial Concrete as Surface Treatment</td>
<td>A maximum calcium carbonate concentration by 6µM concentration of bacteria was found in this study</td>
</tr>
<tr>
<td>Jonkers (2011)</td>
<td>Bacteria-based self-healing concrete</td>
<td>&lt;em&gt;Bacillus Cohnii&lt;/em&gt;</td>
<td>Self-healing concrete</td>
<td>The bio-chemically mediated process resulted in efficient sealing of sub-milimeter sized cracks (under 0.15mm width)</td>
</tr>
</tbody>
</table>

There are many types of bacteria that are used by researchers in concrete to test on concrete properties and self-healing of concrete cracks. These bacteria used by
researchers are of various sources, some of soil, hot spring and many more. Nevertheless, all these bacteria added into concrete could yield positive results not only in terms of concrete properties, but in the increase of concrete durability.

Raman Chandran et al., (2001) studies the use of Bacillus Pasteurii and Pseudomonas Aeruginosa which are endospore-forming soil micro-organism. From this study, it can be concluded that the type of bacteria and amount added into the mortar mixture influences the compressive strength of the mortar. The range concentration used for Bacillus Pasteurii are 3.8x10^{3}, 3.8x10^{5}, 3.8x10^{7} and 7.6x10^{3}, 7.6x10^{5}, 7.6x10^{7}. While the concentration of bacteria used for Pseudomonas Aeruginosa are 3.8x10^{3}, 3.8x10^{5}, 3.8x10^{7}. Both bacteria were used as live and killed bacteria culture. It was found that, the used of live bacteria increases the mortar strength by 6.15% compared to dead bacteria. Bacillus Pasteurii added in lower concentrations of bacteria, achieved higher compressive strength of up to 15.4% compared to higher concentration. The bacteria used in this study are more dependent on oxygen. Therefore, it was found that the shallow cracks healed due to the availability of oxygen whereas the deeper cracks remained crack.

Jonkers (2011) studies of the use of Bacillus Cohnii in the self-healing of concrete were found to be quite positive as all specimens were 100% healed with the addition of bacteria. Relatively to the control cubes where it was found that 2 out of 6 control specimens healed. The control cubes could heal because of autonomous healing where the 2nd hydration of concrete matrix could self-heal the concrete cube without the addition of bacteria. In this study, the bacteria used was immobilized in porous expanded clay particles prior to the concrete mix, this is to protect the bacteria during the casting and hardening of the concrete. Apart from that, in this study it was found that the bacteria added into the concrete mix in expanded clay particles were found to last longer than direct casting.

In the case of Tittelboom et al., (2010), the use of active (live) and autoclaved (dead) bacteria was used in concrete to compare the effect toward crack sealing. Both active and autoclaved bacteria were added in concrete with the protection of silica gel, the silica gel is used to protect the bacteria from the high alkalinity of concrete, as bacteria naturally are acidic. The autoclaved and active bacteria can seal cracks. By having active bacteria in the concrete, precipitation of calcium carbonate is seen and therefore enhances the durability of concrete.
The bacteria used in Ghosh and Mandal (2006) was obtained from Jadavpur University. The bacteria, *Escherichia coli* is environmentally innocuous and therefore it is easy to handle. In this study, it was found that the enrichment culture of these particular bacteria was able to grow inside the matrix of the concrete and the precipitation process of calcium carbonate resulted in compressive strength improvement. The anaerobic bacteria can sustain and grow inside the concrete matrix without the supply of oxygen or food.

Arunachalam *et al.*, (2010) study of *Bacillus Sphaericus* depicts that the amount of calcium carbonate that this bacterium can precipitate depends on the pH level of the medium. This bacterium optimum pH that precipitate optimum calcium carbonate is pH 8. The result from this study showed that the use of this bacteria as a crack healer is highly positive as the concrete samples with the use of bacteria were found to have fully healed.

Overall, many different bacteria are used in bioconcrete study. Most of which comes from the *Bacillus* family. Most researchers who study bioconcrete either encapsulate or immobilize the bacteria to protect from the roughness of concrete mixing and the high alkaline environment of the concrete. In this study, *Enterococcus faecalis* and *Bacillus sp* were used due to their ability to produce calcium carbonate. According to Mayur and Jayeshkumar (2013), bacteria generally are able to produce calcium carbonate through either Sulphur cycle or nitrogen cycle. *Enterococcus faecalis* bacterium is isolated from fresh urine. Human urine contains plenty of urea and thus the bacteria isolated generally can produce calcium carbonate through the nitrogen cycle. Apart from that, *Bacillus sp* is isolated from acid mine water which generally contains plenty of Sulphur. Bacteria isolated from this environment would produce calcium carbonate through the Sulphur cycle.

Both bacterium was enriched to suit the concrete environment by removing oxygen and increasing the pH of the bacterium. Based on the Biosafety Guideline (2010) both bacteria used in this study is in risk group 1 (RG1) which stated that organisms in this group are unlikely to cause human disease or animal disease. Therefore, both bacteria are not harmful and are safe to use for study.
2.3.2 Enterococcus faecalis

Bacteria generally come in many shapes and sizes such as spherical, rod and comma shaped. These shapes and sizes of bacteria are commonly known as bacteria morphology. The sizes of bacteria vary by species; bacteria are commonly 0.5-5.0 µm in length. Many bacterial species are either spherical (Cocci) or rod-shaped (Bacillus). Rod-shaped bacteria, known as vibrio can be slightly curved or comma-shaped. The variety of shapes is determined by the bacterial cell wall and cytoskeleton. It is important because the shape of the bacteria can influence the acquirement of nutrients by bacteria (Siddique and Chahal, 2011). The scientific names of bacteria normally point out to the shape of the bacteria species. Therefore, Enterococcus faecalis have a coccus shape. Enterococcus faecalis is Gram positive bacteria occurring in single, pair or in short chains (Holzapfel and Wood, 2014). The morphology of the bacteria is confirmed through Gram staining.

A similar species as Enterococcus faecalis is Ureolytic bacteria. This bacterium is a commonly used in many bioconcrete studies (Dick et al., 2006; Muynck et al., 2008; Tittelboom et al., 2010; Siddique and Chahal., 2011; Aiko et al., 2011; Wang et al., 2012). Chahal et al., (2010) had stated that ureolytic bacteria can influence the precipitation of calcium carbonate by the production of urease enzyme. The production of enzymes catalyzes the hydrolysis of urea to CO₂ and ammonia, which resulted in an increase of pH and carbonate concentration in the bacterial environment. The increase of the pH of bacteria is vital as the pH of concrete is high. Therefore, the increase of pH of ureolytic bacteria enables the bacteria to sustain life in the concrete matrix.

2.3.3 Bacillus sp

The genus Bacillus includes a wide variety of saprobic bacteria which are widely distributed in the Earth’s habitats. Saprobic bacteria are bacteria that derived nutrients from non-living or decaying organic matter. The bacillus species are Gram positive, rod-shaped bacteria with an average size of 0.5-5 µm and are known for its versatility in degrading complex macromolecules, Bacillus is also a common source of antibiotic.
This species is commonly found in soil, spores are continuously dispersed by means of dust in water (Talaro and Chess, 2012).

According to Emilio et al., (2008), sulphate reduction bacteria which is a similar bacterium as *Bacillus sp* can live in an anaerobic state, which means that this bacterium is capable of living in environments that has no oxygen, perfect for concrete which contains little to no oxygen within its concrete matrix. Apart from that, the average pH range for this bacterium is pH 5.5 to 9, which is not sufficient for concrete as concrete has high alkalinity. Therefore, for this bacterium to survive in concrete, the level of pH was slowly raised through adding chemicals. The sulphate reduction bacteria oxidize the sulphate existing in the water and transform it into hydrogen sulfides in gaseous state.

Bacteria from the *Bacillus* species family is commonly used in bioconcrete studies (Ramakrishnan et al., 2005; Kantha et al., 2010; Wiktor and Jonkers, 2011; Pei et al., 2013). Ramakrishnan et al., (2005), stated that the ability of this common soil bacterium to continuously precipitate calcium carbonate is an advantage to concrete as the impermeable calcite layer produced improves the concrete.

### 2.3.4 Function of bacteria in concrete

Muynck et al., (2008) and Bang et al., (2001) have both stated in their studies that the function of the bacteria in the concrete plays an important role by producing urease which causes hydrolysis of urea to ammonia and carbon dioxide. The ammonia causes the increment of pH and the hydrolysis process induces calcite precipitation. Naturally, precipitation of calcium carbonate occurs in concrete as well, but is a lengthy process. Thus, micro-biologically induce calcium carbonate precipitation is preferred for a quick and effective precipitation. Salwa, (2011) argues that microbial precipitation is faster compared to chemical precipitation.

Chemical precipitation of calcium carbonate could be controlled by the increased or decreased of calcium ions, carbonate concentration, pH value and the presence of nucleation sites. In terms of micro biological precipitation of calcium carbonate the nucleation site is irrelevant as the bacteria acts as a nucleation site.

The production of the urea level of bacteria varies according to the condition in which the bacteria are grown. When *Bacillus Subtilis* cells is grown in nitrogen poor
medium, the production of urea increases 20 to 25 times the original. This causes rapid precipitation of calcium carbonate (Salwa, 2011). Apart from nitrogen poor environment, Varenyam et al., (2013) studies the effect of using Bacillus pasteurii and Pseudomonas aeruginosa in aerobic condition. It was shown to have improved compressive strength by 18% as compared to control. In Ghosh et al., (2006) studies on the use of an anaerobic thermophilic microorganism instead of using aerobic microorganism, it was found to have a profound impact on the compressive strength of up to 25% increment as compared to the control. The bacteria work by depositing on the microorganism cell surfaces and within the pores of the cement-sand matrix thus plugging the gaps in the matrix.

Nemati and Voordouw (2003) states that microbial cell secretes an insoluble organic compound such as exopolysaccharides which contribute to the cementation and plugging in natural settings. In the natural setting, the bacterium produces this organic compound to heal cracks in highly permeable rock formations. This biomineralization occurs through either active precipitation of carbonate microorganism or passively by the bacteria induced changes in the chemistry of the system. The control biological formation of calcium carbonate in the oil reserve industry is achieved by the decomposition of urea by the catalytic action of urea enzyme, which is achieved by adding bacteria to produce urea. The effect of temperature toward the production of calcium carbonate was studied and was found that temperature has no effect with the production of calcium carbonate. Whereas, it is the concentration of urease that has an effect towards the production of calcium carbonate. With high concentration of urea, the plugging was quite rapid and the permeable as compared to the low concentration of urea.

In Xu and Yao (2014) study, the bacteria added into the concrete for self-healing were coupled with organic calcium salts such as calcium lactate and calcium glutamate as precursors to calcium carbonate. In this study, 2 methods were used to determine the most effective self-healing which are either by external applied treatment or a 2 component self-healing with G-Ca or L-Ca. From the result of this study, the external applied self-healing is the most effective. L-Ca produced significantly good results by 20% compared to control.

Although promising results are reported regarding the use of bacteria in self-healing of concrete, Schalangen and Sangadj (2013) stated in their study that bacteria and compounds needed for mineral precipitation are applied externally after crack
formation occurred. This is due to the limited lifetime of the enzymatic activity of the applied bacteria species. In terms of realistic approach, having applied bacteria externally to self-heal concrete naturally is not the most effective on approach. As some cracks, may form in hard to reach places or hidden from plain-sight.

Studies have reported that bacteria functions to precipitate calcium carbonate through enzymatic pathway. This pathway enables the bacteria to utilize the calcium content within the concrete. Various calcium sources and bacteria are studied to find the ultimate mix to improve engineering concrete properties and self-healing of concrete. However, the method in which the bacterium is added into the concrete and introduction of different concentrations of calcium source is different from researcher to researcher in the bioconcrete community. The calcium lactate acts as a catalyst and provides bacteria the additional calcium source to precipitate calcium carbonate. Therefore, the importance of this study is to determine the optimum concentration needed to have improvement in terms of engineering concrete properties and self-healing.

2.4 The importance of calcium carbonate in concrete

Calcium carbonate formation by bacteria is a natural process, this formation aids in the improvement of concrete. The addition of calcium lactate in bioconcrete is to facilitate in precipitation of calcium carbonate by bacteria. Calcium carbonate is a common mineral on earth, calcium carbonate precipitation occurs naturally to form natural rocks such as limestones, fossiliferous and exists in many different environments (Salwa, 2011). Limestones are used as part of the ingredient in the production of cement along with other mixtures. The composition of concrete consists of various materials of natural origin, such as sand, aggregate and limestone.

According to Stuckrath et al., (2014), calcium carbonate is the focus on autogenous or self-healing of concrete due to the fact that it can be intentionally engineered to improve the self-healing capacity of the concrete matrix. There are several autogenous healings that concrete possesses to close small cracks or normally cracks less than 0.05 mm wide. These are either by swelling of cement paste, hydration of remaining unhydrated cement, precipitation of calcium carbonate crystals and crack filling by impurities in the water or by debris from crack surface. Therefore, many
studies that aims to improve self-healing of concrete focuses on precipitation of calcium carbonate.

Faiz and Steve (2014) study the effect of adding nano-CaCO$_3$ on the compressive strength. It was found that small percentage of nano-CaCO$_3$ could improve the compressive strength of the concrete and led to a denser microstructure which changed the formation of hydration products. This contributed to early strength and higher durability.

Calcium carbonate is formed during the process of carbonation where calcium hydroxide chemically reacts with carbon dioxide in the air. Calcium hydroxide is found within the concrete matrix and is brought up when moisture migrates to the surface. Carbonation does not only occur at the surface, but also deep within the concrete matrix. Apart from that, fresh cement paste has normally high pH value of 12.5 to 13, which is beneficial to the structure as it protects the reinforced steel from rusting (Bjorn, 2006).

Naturally, the production of calcium carbonate in concrete lowers the pH thus eventually causing corrosion of steel reinforcement. This in turn lowers the structural integrity of the building. Steel reinforcement corrosion is usually due to water seeping into cracks that forms due to loading. However, adding bacteria in concrete lessen the chance for structural deterioration due to the bacteria pore plugging ability which reduces the water seepage and produces calcium carbonate without reducing the pH of the concrete. As the bacteria used in this study is enriched in alkaline condition to suit the concrete environment.

2.5 Additional calcium nutrient source

Naturally, concrete precipitates calcium carbonate through carbonation process which occurs over time (Bjorn, 2006). To speed up the precipitated calcium carbonate, bacteria and calcium nutrient source is introduced in concrete. Precipitation of calcium carbonate is one of the four elements in concrete which can be manipulated to achieve self-healing of concrete (Stuckrath et al., 2014). Calcium nutrient source acts as an additional food source for the bacteria to precipitate calcium carbonate at a higher rate. This higher rate of precipitation increases the strength of concrete and aids in self-healing of micro-cracks.
Several different calcium sources were used together with bacteria by other researchers such as Xu et al., (2015), Abo-El-Enein et al., (2013) and Xu and Yao (2014). Abo-El-Enein et al., (2013) studied the use of several different calcium sources such as calcium chloride, calcium acetate and calcium nitrate. An improvement in physio-mechanical properties and mortar crack remediation is found with samples containing calcium than without any calcium source addition. Whereas Xu and Yao (2014) studied the difference between using organic and inorganic calcium sources. It was found that the type of calcium source has a profound impact on the crystal, form, size and morphology of CaCO₃. Calcium sources that were used were calcium glutamate and calcium lactate which are organic calcium source and calcium chloride which is an inorganic calcium source. It was found that organic calcium source has a better result in calcium carbonate precipitation compared to inorganic calcium source. Apart from that, limited amount of durability in terms of surface treatment is achieved with the aid of bacteria alone. The addition of calcium source resulted in higher amount of precipitated calcium carbonate, which led to a higher decrease in capillary water absorption and carbonation.

In Jonkers (2011), expanded clay particle was used as a partial replacement in concrete. The bacteria and calcium lactate were embedded within the expanded clay particles. The expanded clay particles served as a partial replacement of aggregate in normal concrete. The replacement tested in this study was a 50% replacement of aggregate with expanded clay particle. The huge replacement caused a decrease of compressive strength after 28th day of curing compared to control (expanded clay particles with bacteria only) but the self-healing capacity with calcium lactate is better.

Calcium lactate is an organic calcium source. It is used in many milk, cheese and food products. It is innocuous to human which is important as people are surrounded in concrete environment almost 24 hours a day. Therefore, it is important to create a durable concrete which do not have any negative effect towards the environment and human health. Apart from that, Mayur and Jayeshkumar (2013) have stated that the presence of calcium lactate added to the additional calcium ions which are needed to precipitate calcium carbonate. The amount of calcium lactate in this study was adopted from Xu et al., (2015) which used 0.025 mol/L calcium lactate. This concentration can produce significant amount of calcium carbonate within a short period of time. Calcium lactate used in this study are in 0.001 mol/L, 0.05 mol/L and 0.01 mol/L. The effect towards the engineering concrete properties and enhancement
in self-healing of concrete is studied. Calcium lactate is in the formed of liquid. It is added as a supplementary in the water used for concrete mixing. The bacteria liquid culture and calcium lactate is added directly to the concrete mix.

### 2.6 Enzymatic pathway of bacteria

Bacteria can precipitate calcium carbonate. However, different types of bacteria and abiotic factors such as salinity and composition of medium contributes to different ways to precipitate calcium carbonate. According to Mayur and Jayeshkumar (2013), there are two pathways for precipitation of calcium carbonate by bacteria. The first pathway usually involves sulphur cycle and particular sulphate reduction. This pathway is usually carried out by sulphate reducing bacteria under anoxic condition. Whereas, the second pathway involved nitrogen cycle which is usually carried out by ureolytic bacteria. Bosak (2005) had stated that sulphate reduction bacteria could precipitate calcium carbonate by an increase in alkalinity such as the equation below:

\[
\text{SO}_4^{2-} + 2\text{CH}_2\text{O} \rightarrow \text{HS}^- + 2\text{HCO}_3^- + \text{H}^+ \quad (2.1)
\]

\[
\text{Ca}^{2+} + \text{HCO}_3^- \rightarrow \text{CaCO}_3 + \text{H}^+ \quad (2.2)
\]

The precipitation of calcium carbonate by sulphate reduction bacteria increases the pH. Sulphate reduction bacteria reacts with calcium lactate which aids in increment of calcium carbonate precipitation (Braissant et al., 2007). The enzymatic pathway of the bacteria is the chemical reaction which occurs when bacteria is added into concrete. It describes what the bacterium does in the concrete and the process in which calcium carbonate is produce as a by-product of the bacteria. According to Tugba and Debora (2014), Microbially-induced precipitation of calcium carbonate is a chemical reaction commonly facilitated by microorganisms and is associated with sulfate reduction, urea hydrolysis and iron reduction. The urea hydrolysis is the most studied and the stoichiometries reactions are given as:

\[
\text{H}_2\text{NCONH}_2 (\text{Urea}) + \text{H}_2\text{O} \rightarrow 2\text{NH}_3 + \text{CO}_2 \quad (2.3)
\]

\[
2\text{NH}_3 + \text{CO}_2 + \text{H}_2\text{O} \leftrightarrow 2\text{NH}_4^+ + \text{CO}_3^- \quad (2.4)
\]
REFERENCES


