3.1 Introduction

The experimental investigation has been conducted mainly at the concrete laboratory of Civil Engineering Department and Department of Physics, Jadavpur University, Kolkata, India. Some studies have been also made at University Science and Instrument Center (USIC), Geology Department of Jadavpur University and Central Glass and Ceramic Research Institute (CGCRI), kolkata, India.

3.2 Objectives

The main objectives of the experimental investigation are as follows

(i) To study the strength behavior of mortar/concrete incorporating a facultative anaerobic, thermophilic iron reducing microorganisms and to compare with the control concrete (without microorganism).

(ii) To study the effect of cell concentration of such microorganisms on the strength of mortar/ concrete with ages.

(iii) The effect of water-cement ratio on the compressive strength of mortar/concrete with / without microorganisms has also been incorporated.

(iv) To study the strength behavior of mortar/concrete incorporating *Escherichia coli* (*E-coli*) microorganism for comparison.

(v) To study the microstructure of mortar and concrete with and without microorganisms through Scanning Electron Microscope (SEM), X-ray Diffraction (XRD) analysis and Image analysis.
(vi) To study the pore size distribution within the mortar/concrete matrix with and without microorganism by Mercury Intrusion Porosimetry test.

(vii) To study the Ultrasonic Pulse Velocity and Water Absorption within the mortar/concrete with and without microorganism.

### 3.3 Materials

#### 3.3.1 Cement

The cement used was of grade 53 Ordinary Portland Cement conforming to IS 12269:1987 manufactured and marketed by Grasim India Ltd. The physical and chemical properties of cement samples are shown in table-3.1 and table-3.2.

#### Table -3.1: Physical Properties of Cement

<table>
<thead>
<tr>
<th>Type</th>
<th>Specific surface area (m²/kg)</th>
<th>Specific gravity</th>
<th>Setting time</th>
<th>Compressive strength MPa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Initial (minutes)</td>
<td>Final (minutes)</td>
</tr>
<tr>
<td>53 grade</td>
<td>310</td>
<td>3.10</td>
<td>106</td>
<td>330</td>
</tr>
</tbody>
</table>

#### 3.3.2 Sand

Natural sand passing through 4.75mm sieve having angular shaped particles was used. The different properties of sand are presented in table 3.3 and table-3.4. The sand samples are washed before use in mortar/concrete and it conform the requirements as per IS: 383-1970.
### Table -3.2: Chemical Properties of Cement

<table>
<thead>
<tr>
<th>Particulars</th>
<th>Results in percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Oxide composition</strong></td>
<td></td>
</tr>
<tr>
<td>CaO</td>
<td>59.3</td>
</tr>
<tr>
<td>SiO$_2$</td>
<td>20.0</td>
</tr>
<tr>
<td>Al$_2$O$_3$</td>
<td>6.3</td>
</tr>
<tr>
<td>MgO</td>
<td>2.5</td>
</tr>
<tr>
<td>SO$_3$</td>
<td>2.0</td>
</tr>
<tr>
<td>K$_2$O/Na$_2$O</td>
<td>0.5</td>
</tr>
<tr>
<td>Loss of ignition</td>
<td>1.9</td>
</tr>
<tr>
<td>Insoluble residue</td>
<td>0.7</td>
</tr>
<tr>
<td>Others</td>
<td>1.9</td>
</tr>
</tbody>
</table>

### Table-3.3: Sieve Analysis of Sand

<table>
<thead>
<tr>
<th>Type</th>
<th>IS sieve designation</th>
<th>Percent finer</th>
<th>Permissible limit of zone-II as per 383-1970</th>
<th>Fineness modulus</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium Sand</td>
<td>4.75 mm</td>
<td>100</td>
<td>90-100</td>
<td>2.89</td>
<td>Zone-II as per IS:383-1970</td>
</tr>
<tr>
<td></td>
<td>2.36 mm</td>
<td>98.60</td>
<td>75-100</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.18 mm</td>
<td>85.20</td>
<td>55-90</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>600 µm</td>
<td>20.80</td>
<td>35-59</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>300 µm</td>
<td>4.60</td>
<td>8-30</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>150 µm</td>
<td>1.00</td>
<td>0-10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pan</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
### Table-3.4: Properties of Sand

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Physical Properties</th>
<th>Test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Specific gravity</td>
<td>2.5</td>
</tr>
<tr>
<td>2</td>
<td>Water absorption (%)</td>
<td>1.0</td>
</tr>
<tr>
<td>3</td>
<td>Moisture content (%)</td>
<td>0.05</td>
</tr>
<tr>
<td>4</td>
<td>Silt Content (%)</td>
<td>2.5</td>
</tr>
</tbody>
</table>

#### 3.3.3 Coarse Aggregate

10 mm down crushed rock was used as coarse aggregate. The properties of coarse aggregate are shown in table- 3.5 and table- 3.6.

### Table 3.5: Sieve Analysis of Coarse Aggregate.

<table>
<thead>
<tr>
<th>Type</th>
<th>IS sieve designation</th>
<th>Cumulative percentage retained</th>
<th>Cumulative percentage passing</th>
<th>Fineness modulus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crushed rock</td>
<td>80mm</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40mm</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20mm</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td></td>
<td>10 mm</td>
<td>40</td>
<td>60</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.75 mm</td>
<td>90</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.36 mm</td>
<td>100</td>
<td>00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.18 mm</td>
<td>100</td>
<td>00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>600 micron</td>
<td>100</td>
<td>00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>300 micron</td>
<td>100</td>
<td>00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>150 micron</td>
<td>100</td>
<td>00</td>
<td>6.30</td>
</tr>
</tbody>
</table>

### Table-3.6: Properties of Coarse Aggregate

<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Physical properties</th>
<th>Test result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Specific Gravity</td>
<td>2.46</td>
</tr>
<tr>
<td>2</td>
<td>Water absorption (%)</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>Moisture content (%)</td>
<td>1.0</td>
</tr>
</tbody>
</table>
3.3.4 Water

Water used for mixing and curing was clean and free from oils, acids, alkalis, salts, sugar, organic materials or other substances that may be deleterious to concrete. Distilled water was used for mortar as well as concrete throughout this experiment.

3.3.5 Microorganisms

The bacterial diversity of a hot spring in Bakreshwar, India, was investigated by a culture-independent approach. 16S ribosomal DNA clones derived from the sediment samples were found to be associated with gamma-proteobacteria, cyan bacteria, and green nonsulfur and low-GC gram-positive microorganism. The first of the above phylotypes cobraunches with *shewanella*, a well-known iron reducer. This phylogenetic correlation has been exploited to develop culture conditions for facultative thermophilic iron reducing. This novel microorganism was characterized in the Department of Life Science and Biotechnology of Jadavpur University, Kolkata, India. Based on 16S ribosomal DNA sequence homology, these microorganisms belong to *Shewanella* species⁹ [(S. alga u 91544)-sequence assigned by RDP]. This microorganism is cultured anaerobically in a modified medium and added to the mortar/ concrete at different concentration to study its effect on strength improvement of mortar /concrete¹².

Preparation of media

A number of *Shewanella* strains are known to use for anaerobic respiration a wide variety of electron acceptors including metal oxides and hydroxides. The bacterium used is water grown hot spring bacterium that has some unique requirement of oxidizing agent and in the Semi Synthetic media, iron (0.2M) was used in +III state, which accepts electron and itself goes to +II state. Final medium was prepared by mixing media 1 and media 2 in 1: 9 ratios and pH was kept at 7.5. Media 1 contains iron in +III state as FeOOH and media 2 contained sodium di-hydrogen phosphate (0.6 gram/1000 ml), potassium chloride (0.33 gram/1000 ml), sodium carbonate (2.5 gram/1000 ml), yeast extract (0.02 %) and peptone (0.5%).
**Media 1 composition**

\[
\text{FeCl}_3 + 3\text{NaOH} \rightarrow \text{Fe(OH)}_3 + 3\text{NaCl} \\
\text{FeCl}_3 + 3\text{NaOH} \rightarrow \text{FeO(OH)} + \text{H}_2\text{O} + \text{NaOH}
\]

Growth condition- the bacteria being anaerobic, was grown in sealed gas-pressure vials (100 ml of the vial contains 30 ml growth medium). Air content in the sealed vial was replaced totally by carbon dioxide using syringe-needle system before inoculation of bacterial cultural. The inoculated cultures were kept incubation at 65°C for 6 to 8 days. The details of the process of bacteria culture in the laboratory are presented through a flow chart as shown in figure- 3.1.

Preparation of standard curve (optical density vs. cell count), Bacterial culture (1 ml; 4-5 days old) was taken in a sealed vial and diluted to 10 times by adding 9 ml of sterilized growth media. From this diluted cell culture 10 times dilution was made again and so on. In this way different cell concentrations were prepared. Optical density of each cell concentration was then measured against the blank media at 620 nm in colorimeter (ERMA INC, AE 11M). Then by counting the cell number in Hemocytometer of each cell concentration, standard graph of cell number vs. optical density was prepared. This graph was used to determine the cell concentration of any culture under study just by observing the optical density at 620 nm of that culture.

**Scanning Electron Micrograph**

For Scanning Electron Microscopy, bacterial cells were fixed with 2.5% (v/v) gluteraldehyde in culture medium for about 24 hour at room temperature. Samples were dehydrated by incubation for 15 min in each of a graded aqueous acetone series (20, 40, 60, 80 and 100% acetone). The samples were air dried and transferred onto SEM alumina support and sputtered with gold by a coater of Blazers (type 07120-A). Photomicrographs of bacterial cells were taken at different magnification in SEM (Model: Jeol JSM 5200) as shown in figure 3.2. This study was done in the University Science and Instrument center (USIC), Jadavpur University, Kolkata, India.
Add microorganisms with cell concentration $10^7$/ml

Mix FeCl$_3$ + NaOH

Centrifuge the solution

Add Salts & others*

Autoclave the Solution

Make the media anaerobic

Incubation of cell and media

Ready to use active cell of concentration $10^7$/ml media

* 1. Sodium hypophosphate (NaH$_2$PO$_4$)
   2. Potassium chloride (KCl)
   3. Sodium carbonate (Na$_2$CO$_3$)
   4. Yeast extract
   5. Peptone.

Figure -3.1: Flow Chart for the Preparation of Microorganism.
Figure-3.2: SEM Views of Microorganisms at Different Concentration. Figure-(A) cell concentration of $10^7$/ml, figure-(B) cell concentration of $10^6$/ml, figure-(C) cell concentration of $10^5$/ml cell, figure-(D) cell concentration of $10^4$/ml, figure-(E) cell concentration of $10^3$/ml, figure- (F) cell concentration of $10^2$/ml of water.
3.4 Mix Proportion and Test Specimens

Mortar

The mixture proportion of cement and sand for both with and without microorganism was fixed at 1:3 (by weight). Three different water cement ratios (0.40, 0.42 and 0.45) was taken. The standard specimen size of 70.7 mm × 70.7 mm × 70.7 mm was taken as per IS 4031-1988. Two different sets of samples were prepared for each water cement ratio. In the first set, facultative anaerobic microorganism was incorporated and in the second set E.coli microorganism was added. The cell concentration of 0, 10, $10^2$, $10^3$, $10^4$, $10^5$, $10^6$ and $10^7$ per ml of water was used in both sets. The total experiment was repeated.

Concrete

For concrete, cube specimen of size 100 mm × 100 mm × 100 mm and cylinder specimen of size 100 mm × 200 mm were used. The size of cubes and cylinder are reduced to control the amount of microorganism culture in the laboratory. The proportion of ingredient (cement : fine aggregate : coarse aggregate) was 1: 1.5:3 (by weight). Three different water cement ratio (0.45, 0.48 and 0.5) was taken. Same concentration of microorganism was used in the concrete mixture. Some companion cubes are also made for water absorption and Ultrasonic Pulse Velocity test.

3.5 Mixing Procedure and Testing at Fresh State

For mortar, sand and cement was taken as per ratio and mixed properly by hand. Distilled water was taken as per requirement and the required amount of microorganisms were added to this water and mixed. The prepared solution was added to the dry cement sand mixture and mixed properly. Different specimens were cast and compacted by vibration machine. Similar method was employed for mixtures with E.coli microorganism.
Table-3.7: Details of Specimens

<table>
<thead>
<tr>
<th>Specimen</th>
<th>Size</th>
<th>Type</th>
<th>Type of microorganism</th>
<th>Concentration (cells/ml of water)</th>
<th>No of samples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 days</td>
</tr>
<tr>
<td>Mortar</td>
<td>Cube (70.7 × 70.7 × 70.7) mm</td>
<td>Control</td>
<td>nil</td>
<td>Nil</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>With microorganism</td>
<td><em>Shewanella Sp.</em></td>
<td>$10^7 - 10^9$</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td><em>E-coli</em></td>
<td>$10^7 - 10^9$</td>
<td>72</td>
</tr>
<tr>
<td>Concrete</td>
<td>Cube (100 × 100 × 100) mm</td>
<td>Control</td>
<td>nil</td>
<td>Nil</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>With microorganism</td>
<td><em>Shewanella Sp.</em></td>
<td>$10^7 - 10^9$</td>
<td>72</td>
</tr>
<tr>
<td></td>
<td>Cylinder 100 mm dia × 200 mm ht.</td>
<td>Control</td>
<td>nil</td>
<td>Nil</td>
<td>9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>With microorganism</td>
<td><em>Shewanella Sp.</em></td>
<td>$10^7 - 10^9$</td>
<td>72</td>
</tr>
</tbody>
</table>

For concrete, cement sand and coarse aggregate ratio was taken as per ratio and mixed properly by mixture machine in dry condition. Distilled water was taken as per ratio and the required amount of microorganisms were added to this water and mixed. Finally water and microorganisms were added to dry mix and mixed properly. All specimens were cast and compacted properly through vibration machine.

3.6 Curing

All the samples were remolded after 24 hour. Almost all the samples are cured at the concrete laboratory under distilled water at $30 \pm 5 \degree C$ till testing. Distilled water has been used to reduce the effect of others microorganism present in the water.
3.7 Testing Procedure

The details of the different testing procedure on mortar and concrete are presented herewith. All the tests were conducted in accordance with the Indian Standard Specification. American Society for Testing of Materials (ASTM) standard was used for some special tests.

3.7.1 Compressive strength

It is customary to determine the compressive strength of mortar/concrete, since many properties of mortar/concrete are related to the compressive strength. In this study the development of compressive strength with ages was determined up to the age of 28 days. A typical testing of mortar samples are shown in figure-3.3. At each age, six identical samples were tested in accordance with  IS:516-1965 and the mean values are reported

3.7.2 Split tensile strength

Indirect tensile strength of concrete was determined in accordance with the procedure detailed in  ASTM C 496 using 100mm diameter by 200mm long cylinders. As per American Society for Testing and Materials (ASTM C 496) - split tensile strength of concrete may be calculated in the following way.

\[ T = \frac{2P}{\pi ld} \]

Where

- \( T \) = splitting tensile strength in KPa.
- \( P \) = Maximum applied load indicated by the testing machine (KN).
- \( l \) = Length in meter.
- \( d \) = Diameter in meter.
Figure-3.3: Compressive Strength Testing Apparatus
3.7.3 Scanning Electron Microscope (SEM)

Scanning Electron Micrograph study cannot be used to make quantitative assessment but can be used for qualitative purpose. SEM specimens were dried, then gold coated and stored in the desiccators prior to examination using a Jeol JSM 5200.(refer figure- 3.4)

Theory

Interaction of the scanning electron microscope electron beam with the specimen surface causes a range of effects where electrons and X-rays are emitted of that generated from the specimen under the beam. The beam is composed of primary electrons, which collide with electrons in orbit around the nuclei of elements present in the specimen. Primary electrons have energy values (up to 40 KeV), and can cause displacement of these secondary electrons, that may subsequently leave the specimen surface and be detected. Secondary electrons do not have high-energy values, compared to backscattered electrons; conventionally bellow 50eV, whereas backscattered electrons have energies ranging from 50eV to that of the primary electrons. Backscattered electrons can therefore be a mixture of high energy secondary electrons knocked out of position by higher energy primary electrons, or primary electrons that have re-emerged from the specimen surface after colliding with and being re-directed by, in-situ electrons that have not been displaced. If an electron has been knocked out of orbit around an atom’s nucleus, and is subsequently replaced by another electron from a lower energy electron shell of the atom, an X-ray can be generated that is characteristic of the element from which it came. The interaction of the beam with the specimen is known as the interaction volume, and is the volume below the surface of the material, in which, primary electrons interact with electrons orbiting around atoms of the material, in which primary material. Secondary electrons are detected from a near surface region of this volume, while backscattered electron can be detected from this and much deeper parts of the specimen. Many electrons escaping from this region are absorbed into the material or not detected at all, as is the case with a small number of x-rays. The range of detected electrons and X-rays is similar in size to the interaction volume as the material less easily absorbs X-rays.
Secondary Electron Imaging (SEI)

Secondary electrons escape from material surfaces with low energies and are very abundant. Images formed from secondary electrons are used to obtain morphological details about specimens, and give a feeling of depth to the image, specially if captured as stereo pairs. In this respect they are especially useful in the image of fracture surface and crystallization products of cement particle hydration where surface morphology can reveal much detail about the specimen. Specimens require little preparation apart from drying and coating (usually gold or carbon) before being placed in the microscopes specimen chamber.

Backscattered Electron Imaging (BEI)

Polished surfaces of concrete are well suited to backscatter electron imaging (BEI) by the scanning electron microscope, and has become a very popular method used in the investigation of concrete or cementitious materials. Phases, both hydration and aggregate, are imaged based on the atomic number of the elements that constitute those phases. Interaction of the high-energy electron beam with specimen surfaces causes some high-energy electron to be reflected or emitted back from the surface, where they are detected and used to form an image. The images are 8-bit grey-scale and composed of 256 separate shades from 0 for black and 255 for white (this depends on calibration of the signal and sometimes the scale is reversed depending on machine set-up). In the scanning electron microscope, the beam is scanned across the specimen surface in a raster, dwelling on points on the specimen surface for a pre-determined period of time (usually measured in microseconds). Each point corresponds to one picture element or pixel in the image, which is made up of rows of pixels. The image dimension is therefore measured in pixel dimensions along the x and y axes, typically being of $512 \times 512$ for lower resolution images, or for example, $1024 \times 756$ for higher resolution images. Again this depends on individual equipment design and set up.

The Images of concrete can be obtained at magnification factors of several thousand times before effective resolution of feature is lost. In these images, the brightest phases are usually of anhydrous cement particles unless steel or metal bearing aggregates are included, this is because anhydrous particles are composed of phases (i.e. $C_3S$, $C_2S$, $C_4AF$).
C\textsubscript{3}A and C\textsubscript{4}AF) that are of a higher atomic number than hydration phases. Natural aggregates can often have inclusion of various forms of metal ore and also appear brighter. Areas impregnated with epoxy resin appear almost black and provides an easy identification of cracks, air voids, and porosity content of the concrete.

**Sample preparation**

SEM examination was made on a cylindrical sample of 10mm diameter and 10mm height approximately that are prepared from the broken samples of 28 days cubes and immediately gold coated to avoid further chemical reactions. The specimens are stored in the desiccators. So, it is expected that the carbonation effect is insignificant. Also, as all the specimens are prepared in similar condition such effect must be identical in all specimens. The Backscattered Electron Imaging technique has been used for SEM analysis of mortar and concrete samples. The micrographs are obtained at different magnification for the justification of improvement in microstructure of mortar/concrete samples with microorganism compared to control mortar/concrete. This test was done in the University Science and Instrument Center (USIC), Jadavpur University, Kolkata, India.

![Figure-3.4: JEOL JSM 5200 SEM](image)

Figure-3.4: JEOL JSM 5200 SEM
3.7.4 Image Analysis

Image analysis has been also performed to determine the micro structural view of each particle of the concrete sample in qualitatively at lower magnification. Critical interpretation has been made from the image of the concrete sample with/without microorganism. The details of the testing method are discussed below.

Theory

Having captured images, colour or grayscale, a natural progression would be to attempt to analyse the images in some way such that quantified or semi-quantified data can be extracted from them. In this way, images of materials become more than just pictures providing purely qualitative information. There are four main stages to image analysis:

1. Image capture
2. Image processing
3. Image feature measurement
4. Results generation and interpretation

Image captures

Each of these stages requires considerable expertise and knowledge of the way in which the image data is manipulated, and each should be regarded as being equally important, with the potential to seriously distort output data if the effect of each stage of data manipulation is not fully understood. The primary purpose of a microscope is to generate an image. This image is usually magnified and is controlled by the resolving power of the microscope. For image capture purposes using optical microscopes, the image needs to be well illuminated in terms of brilliance of the image and evenness of the illuminating light, it needs to be at an appropriate magnification where features of interest are adequately resolved, and it needs to be in perfect focus.
**Image processing**

This stage covers a wide range of possible image enhancement operations, including removal of artifacts, shading corrections, contrast/brightness optimizing, filtering, thresholding/segmenting, feature identification, feature reconstruction, arithmetic and logical image operations and mask generation. The main objective of this is to generate or prepare an image that is ready for quantified measurement routines during the following stage. This often means that a binary image is produced which has a bit depth of only two, i.e. it consists of areas/features of either black or white. Colour images can also be prepared for measurement, but their sensitivity to colour variation is very high given that true-colour images (24 bit or more) can cover a possible digital spectrum of several millions of colours. The significance of this is that segmentation either at this stage or during measurement stages can include pixels not related to a Region of Interest (ROI) or exclude pixels of an ROI, or both.

**Image feature measurement**

Image feature are represented on the system by ROI’s. These ROI’s can be recognized by the system based on their colour depth (either black or white). If grayscale or colour image ROI’s are selected, densitometric calculations can also be performed, i.e. average shade of grey or colour within the ROI. Having identified a ROI, the system is able to calculate geometrical and mathematical parameters of the ROI based on pre-defined criteria, such as total area, perimeter, shape factor, length measurements (feret ratios), and relative orientation and so on.

**Results generation and interpretation**

The data obtained from each of the measured ROI’s within an image frame can then be output to a database for export to spreadsheet software and further analysis, or presented in the form of graphics. Typically the results of such analysis can be incorporated into reports or presentations to provide feedback from the investigation. Image data should be interpreted with care. It should always be remembered that statistically, the small area analyzed by a microscope is almost certainly not representative of the bulk nature of the material/structure under investigation, and that
many images should be analyzed before any degree of confidence for results findings can be achieved. Images are also 2D in nature and do not therefore, in themselves, represent the bulk nature of the material.

Sample preparation

A thin section of thickness about 0.3mm has been prepared for concrete samples having different concentration of microorganisms. The thin section is then mounted on glass slide of size 3.5 cm ×7.5 cm and placed under the camera fitted LEICA microscope for Image analysis(Refer figure 3.5). This test was done in the Department of Geological Science, Jadavpur University, Kolkata, India.

Figure-3.5: LEICA Microscope which was Attach Camera with QWIN Software
3.7.5 Mercury Intrusion Porosion Test

Mercury Intrusion Porosimetry (MIP) is a technique used to measure pore size distribution, and has an advantage in that it is able to span the measurement of pore sizes ranging from a few nanometers, to several hundred micrometers. From distribution of pore sizes of concrete ranging from sub-nanometer to many millimeters, MIP has formed an important tool in the characterization of pore size distribution and total volume of porosity. The details of the theory and testing procedure are explained as follows.

Theory

Mercury is a non-wetting liquid for almost all substances and consequently it has to be forced into the pores of these materials. Submerging the sample under a confined quantity of mercury and then increasing the pressure of the mercury hydraulically accomplish pore size and volume quantification. The detection of the free mercury diminution in the penetrometer stem is based on a capacitance system and is equal to that filling the pores. As the applied pressure is increased the radius of the pores, which can be filled with mercury, decreases and consequently the total amount of mercury intruded increases. The data obtained give the pore volume distribution directly and with the aid of a pore physical model, permit a simple calculation of the dimensional distribution of the pore size. Determination of the pore size by mercury penetration is based on the behaviour of non-wetting liquids in capillaries. A liquid cannot spontaneously enter a small pore, which has a wetting angle of more than 90 degrees because of the surface tension (capillary depression), however, this resistance may be overcome by exerting a certain external pressure. The pressure required is a function of the pore size. The relationship between pore sizes exerted when the pore is considered to be cylindrical is expressed as:

\[ Pr = 2SCos (q) \] ………..(1)

Where

- \( r \) = pore radius
- \( S \) = surface tension of mercury
- \( q \) = contact angle (wetting angle)
- \( p \) = absolute pressure exerted.
The relationship is commonly known as the Washburn equation. Although in almost any porous substance, there are no cylindrical pores, this equation is generally used to calculate a pore size distribution from mercury porosimetry data. The Washburn equation is derived from the following considerations: in a capillary with a circular section, the surface tension of the liquid is exerted in the area of contact over a length equal to the pore circumference. This force, \(2prq\), is perpendicular to the plane of the contact surface.

The force tending to push the liquid out of the capillary is:

\[
2prs \cos(q)
\]

Against this force, the external pressure will be exerted over the area within the contact circumference, equal to:

\[
pr^2P
\]

When equilibrium is reached, these two forces have the same value:

\[
2prs \cos(q) = pr^2P
\]

Equation (1) therefore shows the pore radius is inversely proportional to the pressure:

\[
r = \frac{[2s\cos(q)]}{p}
\]

when using mercury (taking the surface tension as 480mN/m\(^2\) and wetting angle as 141.3\(^0\) and assuming that all pores are cylindrical, the following relationship is obtained.

\[
r = \frac{7500}{p} \quad (2)
\]

Where

- \(r\) is the pore radius in nm
- \(p\) is the absolute applied pressure in kg/sq.cm

for irregular shaped pores the ratio between the pore cross-section (related to the pressure exerted) and the pore circumference (related to the surface tension) is not proportional to the radius and depending on the pore shape, equation(2) will give lower values. The wetting angle (taken as 141.3\(^0\)) depends on the nature of the sample, and should therefore be considered as an average value only. Surface tension should also be considered as a
variable value. At $25^\circ$C it is 484.2 dynes/cm$^2$, while at $50^\circ$C it is 472 dynes/cm$^2$; 480 dynes/cm$^2$ has been taken as an average value. Specimens prepared for Mercury Porosion Intrusion testing were dried to remove all moisture from the pore structure. They are then placed into sealed penetrometers which are weighed both before and after being loaded with the specimen. The penetrometers are placed into the machine where they are evacuated and then filled with mercury. The pressurized testing then commences and the machine calculates and records how much mercury is being forced into the pore structure based on the above equation.

**Sample preparation**

Preparation of sample for the testing of Mercury Intrusion Porosimetry cast mortar first and then after 28 days prepare a cylindrical sample of about 10mm diameter and 20mm height was made from the cured mortar cubes through diamond cutter. This test has done in the Central Glass and Ceramic Research Institute (CGCRI) Kolkata, India (Refer figure- 3.6).
3.7.6 XRD-Analysis

The X-ray diffraction (XRD) technique offers a convenient way to determine the mineralogical analysis of crystalline solids. If a crystalline mineral is exposed to X-rays of a particular wavelength, the layers of atoms diffract the rays and produce a pattern of peaks, which is characteristic of the mineral. The horizontal scale (diffraction angle) of a typical XRD pattern gives the crystal lattice spacing, and the vertical scale (peak height) gives the intensity of the diffracted ray. When the powder specimen being X-rayed contains more than one mineral the intensity of characteristic peaks from the individual minerals are proportional to their amount. Use BRUKER D8 ADVANCE instrument in the Physics Department of Jadavpur University Kolkata, India.

Figure -3. 7: A photographs of X-ray Diffractometre
3.7.7 Ultrasonic Pulse Velocity Method

Ultrasonic Pulse Velocity method consists of measuring the time of travel of an ultrasonic pulse, passing through the mortar and concrete\textsuperscript{38}. The concrete cube specimens having dimension 100mm × 100mm × 100mm were used in this study. Direct transmission of pulse was employed for the study. Test has performed on both mortar/concrete samples (with/without microorganism). Although the pulse velocity is affected by a number of factors, the most important parameter is the porosity of mortar/concrete. It can also be related to strength and dynamic modulus of elasticity.

3.7.8 Water Absorption

Water Absorption of both, mortar and concrete cubes has been tested as ASTM C-642\textsuperscript{37}. Sample having varying cell concentration were cast and cured under water for 28 days. The mass was determined after drying in an oven at a temperature of 100-110\degree C for not less than 24 hours and subsequent cooling. The samples were than immersed in water for 30 minutes and the mass was determined after surface dry condition. Finally water absorption has been calculated from the above two masses.

3.8 Summary

The experimental program was aimed to characterize the beneficial effect of this microorganism when added in mortar/concrete with appropriate amount. The micro structural study has been made to identify the cause of strength improvement in mortar/concrete with microorganisms. All the test results are presented in the chapter-4.