MICROBIAL TREATMENT OF CEMENT KILN DUST FOR UTILIZATION IN CONCRETE

A THESIS

SUBMITTED IN FULFILLMENT OF THE REQUIREMENT
FOR THE AWARD OF THE DEGREE OF

DOCTOR OF PHILOSOPHY

IN

BIOTECHNOLOGY

BY

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2014

CERTIFICATE

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DECLARATION

I hereby declare that the research work presented in this thesis entitled **"Microbial Treatment of Cement Kiln Dust for Utilization in Concrete"** submitted for the award of the degree of Doctor of Philosophy in the Department of Biotechnology, Thapar University, Patiala is an authenticated record of my own research work carried out under the supervision of Prof. (Dr.) Rafat Siddique (Department of Civil Engineering) and Dr. Anita Rajor (School of Energy & Environment) and refers other researcher's work are duly listed in the reference section.

The matter presented in this thesis has not previously been submitted in part or full to any other university or institution for the award of any degree in India or abroad.

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ACKNOWLEDGEMENT

The real spirit of achieving a goal is through the way of excellence and austere discipline. I thank the almighty whose blessings have enabled me to accomplish my dissertation work successfully. This acknowledgement is intended to be thanks giving to all those people who have been involved directly or indirectly with my dissertation work.

First and foremost, I would like to express my thanks and indebtedness to my guide(s) Prof. (Dr.) Rafat Siddique, Sr. Professor of Civil Engineering, Thapar University, Patiala and Dr. Anita Rajor, Associate Professor, School of Energy and Environment, Thapar University, Patiala for their deep involvement, invaluable and continuous motivation throughout this work. I am highly obliged to them for being there always whenever I needed them.

I would like to thank Dr. Dinesh Goyal, Head, Department of Biotechnology, Thapar University, Patiala and Dr. Naveen Kwatra, Head, Department of Civil Engineering, Thapar University, Patiala who has been a constant source of inspiration for me and providing research facilities throughout this work.

I do not find enough words with which I can express my feeling of thanks to Dr. N. Tejo Prakash and Dr. H. Bhunia and entire faculty of both departments, and Dr. (Mrs.) Poonam Sharma, Sr. Microbiologist, Deptt. of Plant Breeding & Genetics, PAU, Ludhiana (Punjab) for their help, inspiration and moral support, which went a long way in successful completion of my thesis.

The cheerful support of my friend and colleagues Dr. Raghavendra Aminedi, Harpreet Singh Bhangu, Dr. Purnima Khanna, Teena Sharma, S. Malkit Singh, Mahipal Joshi and Jaswinder Singh is sincerely appreciated. Special words of appreciation go to Sh. Varinder Jain (Office Superintendent, CMS, TU), Sh. Ram Nawal, Smt. Lalita Pandit, Sh. Ram Sumiran, Sh. Amarjit Singh, Sh. Varinder Sharma, Sh. Phool Chand, Surinder, Mohd. Suhail, Gurpreet Singh and other laboratory staff, who helped me in my experiment work.

I am also thankful to Sh. Anirudh Handa (Office Superintendent), Sh. Prem Lal, office staff of Department of Biotechnology and Ms. Prabhpreet Kaur, office In-charge, School of Energy & Environment for providing necessary administrative support. I also acknowledge the support of Central Research Facility IIT Ropar, Institute Instrumentation Center IIT Roorkee and SAI Labs Thapar University for SEM and XRD analysis.

I want to dedicate my doctorate degree to my parents, sister and my guide(s) whose inspiration, advice, good wishes, encouragement and affection acted as a premium to my achievements.

I am thankful to SERB, Department of Science and Technology, Govt. of India for providing financial support for this research work.

(Kunal)

LIST OF PUBLICATIONS FROM PRESENT WORK

A. Peer- Review Journal Paper(s)

i. Kunal, Rafat Siddique and Anita Rajor (2014). Strength and Microstructure Analysis of Bacterial Treated Cement Kiln Dust Mortar. Construction and Building Materials **(Elsevier)**, 63: 49-55. (IF 2013: 2.265)

ii. Kunal, Rafat Siddique and Anita Rajor (2014). Influence of bacterial treated cement kiln dust on the properties of concrete. Construction and Building Materials **(Elsevier)**, 52: 42-51. (IF 2013: 2.265)

iii. Kunal, Anita Rajor and Rafat Siddique (2013). Biological treatment of alkaline cement kiln dust by using alkalitolerant bacteria. Journal of Pure and Applied Microbiology, 7: 1933-1942. (IF 2013: 0.073)

iv. Kunal, Rafat Siddique and Anita Rajor (2012). Use of cement kiln dust in cement concrete and its leachate characteristics. Resources, Conservation and Recycling **(Elsevier)**, 61: 59-68. (IF 2013: 2.692)

B. Conference Paper(s)

i. Kunal, Rafat Siddique and Anita Rajor (2014). Re-using of Cement Kiln Dust in Concrete after Bacterial Treatment. In Proceedings of Third International Conference on Recycling and Reuse of Materials (ICRM 2014) by IIUCNN, MG University, Kottayam, Kerala and Wroclaw University of Technology, Poland at Kottyam (Kerala) **India**, 11-13 April 2014. pp. 28 (Abstract); Full Paper in CD ROM.

ii. Kunal, Rafat Siddique and Anita Rajor (2014). Utilization of bacterial treated cement kiln dust in concrete. In Proceedings of the 29th International Conference on Solid Waste Technology and Management by the Journal of Solid Waste Technology and Management & Widener University at Philadelphia, PA, **USA**, March 30-April 02, 2014. Full paper pp. 889-902.

iii. Kunal, Anita Rajor and Rafat Siddique (2013). Biological Remediation of alkaline cement kiln dust for sustainable environment. In Proceedings of the V International Conference on Environmental, Industrial and Applied Microbiology (BioMicroWorld2013) by Formatex Research Centre at Faculty of Medicine - Complutense University, Madrid, **Spain**, 02-04 October 2013. pp. 599.

iv. Kunal, Anita Rajor and Rafat Siddique (2013). Treatment of Alkaline Cement Kiln Dust with *Bacillus* sp. for Sustainable Environment Protection. In Proceedings of the 28th International Conference on Solid Waste Technology and Management by The Journal of Solid Waste Technology and Management & Widener University at Philadelphia, PA, **USA**, 10-13 March 2013. (Full Paper) pp. 85-93.

v. Kunal, Rafat Siddique and Anita Rajor (2013). Influence of Microbial Treatment on Alkalinity of Cement Kiln Dust. In Proceedings of the International UKIERI Concrete Congress: Innovations in Concrete Construction at Dr. B.R. Ambedkar National Institute of Technology, Jalandhar, **India**, 05-08 March 2013. pp. 129 (Abstract); Full Paper in CD ROM by Excel India Publishers, New Delhi. pp. 1050-1059.

ABSTRACT

Rapid industrialization and urbanization increases the demand of building and construction material for infrastructure development. Generation of cement kiln dust (CKD) during cement clinker manufacturing has become one of the major environmental and economical issues. Globally, cement industries generate 700 million tons of cement kiln dust every year as a measure to control product quality and to ensure uninterrupted operation of the plant due to high alkalinity and heavy metal content, and major part of it is landfilled. In order to reduce disposal costs utilization of CKD as construction material has become an attractive alternative to its disposal.

Cement kiln dust is highly alkaline in nature which restricts its utilization in mortar and concrete as it causes crack, deformities and reduces the strength and quality of cement and concrete. Also, the alkaline leachate generated from landfilled CKD may contaminate soil, surface and ground water. So, it is necessary to monitor the alkalinity of CKD before utilization. This can be achieved by biological methods using microorganisms that requires low energy inputs, and does not produce hazardous by-products.

This study presents the utilization of bacterial treated cement kiln dust in mortar and concrete, and its leachate behavior. In this study, the effect of bacterial (alkali-tolerant *Bacillus* strain KG1) treated cement kiln dust (after reducing the alkalinity) as partial replacement of Portland cement (5, 10, 15, 20 and 30% w/w) on the normal consistency, setting times and hydration process of blended cement pastes, and on compressive strength at 7, 28 and 91 days of curing of blended cement mortar and concrete was investigated. Several properties such as split tensile strength, ultrasonic pulse velocity, RCPT and porosity of concrete was also studied at the age of 7, 28 and 91 days. Bacterial (isolate KG1, KG4, KG5 and KGMD1) inoculums were optimized at 0.8 O.D. for 20 days of incubation. Isolate KG1 showed maximum reduction in alkalinity and thus used for the studying the mortar and concrete properties. EDX and XRD analysis confirmed the reduction in alkalinity of powdered CKD after bacterial treatment. All the isolates were identified as member of group six (alkaliphile) of the genus *Bacillus* using morphological, biochemical and physiological characteristics and molecular characterization of 16S rRNA sequences.

Increased water consistency, hydration and decrease in setting time was observed in 10% CKD blended cement paste, above which hydration decreases and setting time increases due to reduced alkali content in blended cement pastes. The increase in hydration at later curing ages (91 days) responsible for increase of 19.54% compressive strength in 10% bacterial treated CKD mortar compared with 0% CKD mortar.

Increase of 26.6% compressive strength in 10% bacterial treated CKD concrete was observed compared with 0% CKD concrete at 91 days of curing. Similarly, increase in split tensile strength was also observed in 10% bacterial treated CKD concrete with curing age. Ultrasonic pulse velocity test results revealed that pulse velocity of concrete specimens were within the range 3500-4500 m/s and termed “good” quality of concrete. Maximum pulse velocity was observed in 10% CKD concrete specimens (both untreated and treated). The chloride permeability of concrete specimens containing untreated and bacterial treated CKD showed “very low” permeability with increasing curing period from 7 to 91 days except 20 and 30% CKD concrete which falls in low chloride permeability range. Results of water absorption and porosity showed decrease of 20% and 12.35%, respectively, in 10% bacterial treated CKD concrete compared to 10% untreated CKD concrete at 91 days of curing. Leachate analysis of fresh concrete mix showed 34-92% reduction in metal content (Ag, Cd, Cr, Cu, Fe, Mo, Ni, Pb and Zn) by different isolates. Leaching of silver (Ag) is maximally inhibited by bacterial treatment followed by Cr, Cu, Ni and Zn. It was also observed that with increasing untreated CKD in concrete the metal content increases whereas with addition of bacterial treated CKD in concrete, reduction in metal content was non- significant due to presence of metals in Portland cement.

Scanning electron microscopy (SEM) results exhibits formation of non-expansive ettringite in pores and increased calcium silicate hydrate (CSH) which dense the mortar and concrete structure, and thus, increases the compressive strength in bacterial treated CKD mortar and concrete. XRD analysis confirmed the formation of CSH and non expansive ettringite within the matrix of treated mortar and concrete specimens responsible for improvement in the strength of the material.

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ABBREVIATIONS

Abbreviations		Word(s)
ACI	-	American Concrete Institute
ANOVA	-	Analysis of Variance
APHA	-	American Public Health Association
ASTM	-	American Society for Testing and Materials
BLAST	-	Basic Local Alignment Search Tool
BTU	-	British Thermal Units
CASH	-	Calcium Aluminum Silicate Hydrate
CBA	-	Coal Bottom Ash
CBPD	-	Cement By-Pass Dust
CH	-	Calcium Hydroxide
CKD	-	Cement Kiln Dust
CL	-	Calcium Lime
CLSM	-	Controlled Low Strength Material
CMC	-	Carboxy Methyl Cellulose
CNFs	-	Carbon Nano Fibres
CNTs	-	Carbon Nano Tubes
CPCB	-	Central Pollution Control Board
CS	-	Copper Slag
CSH	-	Calcium Silicate Hydrate
CTM	-	Compression Testing Machine
DNA	-	Deoxy Ribonucleic Acid

EDTA	-	Ethylene Diamine Tetra Acetic Acid
EDX	-	Energy Dispersive X-ray spectroscopy
EPA	-	Environmental Protection Agency
FA	-	Fly Ash
GGBFS	-	Ground Granulated Blast Furnace Slag
GHGs	-	Green House Gases
HRWRA	-	High Range Water Reducing Admixtures
ICDD	-	International Center for Diffraction Data
ICPMS	-	Inductively Coupled Plasma Mass Spectrometry
ICP OES	-	Inductive Coupled Plasma Optical Emission Spectroscopy
IEA	-	International Energy Agency
IS	-	Indian Standard
ISAT	-	Initial Surface Absorption Test
ISI	-	Indian Standards Institute
ITZ	-	Inter Transition Zone
JCPDS	-	Joint Committee on Powder Diffraction Standards
MCL	-	Maximum Contaminant Level
MEP	-	Multiple Extraction Procedure
MK	-	Metakaolin
MM	-	Minimal Medium
MR	-	Methyl Red
MSWI	-	Municipal Solid Waste Incinerator
NCBI	-	National Centre for Biotechnology

		Information
NCIM	-	National Collection of Industrial Microorganisms
NCL	-	National Chemical Laboratory
NP	-	Natural Pozzolan
NRA	-	National Rivers Authority
O.D.	-	Optical Density
OPC	-	Ordinary Portland Cement
PAHs	-	Polycyclic Aromatic Hydrocarbons
PC	-	Portland Cement
PCA	-	Portland Cement Association
PCE	-	Polycarboxylic Ether
PCR	-	Polymerase Chain reaction
PFA	-	Pulverized Fuel Ash
RCPT	-	Rapid Chloride Permeability Test
RCRA	-	Resource Conversation and Recovery Act
RNA	-	Ribonucleic Acid
SCC	-	Self Consolidating Materials
SCM	-	Supplementary Cementitious Material
SEM	-	Scanning Electron Microscopy
SF	-	Silica Fume
SPLP	-	Synthetic Precipitation Leaching Procedure
SSA	-	Sewage Sludge Ash
TAE	-	Tris Acetate EDTA

TCLP	-	Toxicity Characteristic Leaching Procedure
TF	-	Titanium Fume
TGA	-	Thermo Gravimetric Analysis
UHP	-	Ultra High Performance
UPV	-	Ultrasonic Pulse Velocity
USEPA	-	United States Environmental Protection Agency
UTM	-	Universal Testing Machine
UV	-	Ultra Violet
VA	-	Volcanic Ash
VP	-	Voges Proskauer
w/c	-	Water to cement ratio
w/b	-	Water to binder ratio
WET	-	Water Extraction Test
WHO	-	World Health Organization
XRD	-	X- Ray Diffraction

LIST OF UNITS

Units		**Word(s)**
kg	-	Kilogram
g	-	Gram
mg	-	milligram
µg	-	Microgram
ng	-	Nanogram
°C	-	Degree celsius
%	-	Percent
rpm	-	Revolutions per minute
m	-	Meter
cm	-	Centimeter
mm	-	Millimeter
µm	-	Micrometer
nm	-	Nanometer
cm^2/g	-	Centimeter square per gram
N	-	Normal
M	-	Molar
MPa	-	Mega Pascal
kg/m^3	-	Kilogram per cubic meter
N/mm^2	-	Newton per square millimeter
kN	-	Kilo Newton
h	-	Hour

min	-	Minutes
sec	-	Seconds
kcal/kg	-	Kilocalorie per kilogram
L	-	Liter
mL	-	Milliliter
µL	-	Microliter
g/L	-	Gram per liter
mg/L	-	Milligram per liter
mg/mL	-	Milligram per milliliter
me/L	-	Milli-equivalent per liter
V	-	Volts
kV	-	Kilovolts
pmole	-	Pico moles
kb	-	Kilobases
bp	-	Basepair
m/s	-	Meter per second
θ	-	Theta
α	-	Degree of hydration

CHAPTER -1

INTRODUCTION

This chapter deals the production of cement clinker, generation and properties of cement kiln dust (CKD), its utilization in concrete and global production in brief. It also includes leachate and its generation, role of alkali-tolerant microbes in bioleaching and the objectives of the present work.

Solid waste management has become one of the major global environmental issues due to continuous increase in industrial globalization and generation of waste.

Rapid industrialization and urbanization increases the demand of building and construction material for infrastructure development. Concrete is one of the most durable and widely used construction material in the world. Acording to European Concrete Platform ASBL (2009), its annual consumption estimated between 21 and 31 billion tons in year 2006. Concrete is made from cement, aggregates (gravel and sand), water and admixtures. Portland cement plays an important role in the production of mortar and concrete due to its binding properties, and thus acts as a major constructional material of choice in building and structures. Increasing urbanization continuously driving the cement industry to keep growing. According to Oss (2014), 4000 million tons of cement (3400 million tons of clinker) was generated in 2013 worldwide, whereas in India 280 million tons of clinker was generated. There is 5.26% and 11.11% increase in cement production was observed in year 2013 compared to year 2012 and 2011, respectively. From the last few years, the increasing cement generation has arisen certain challenges such as energy and resource conservation, cost of production, green house gas emission, etc. Above all cement kiln dust (CKD) generation during cement clinker manufacturing has become one of the major environmental and economical issues.

1.1 Cement Kiln Dust (CKD)

Cement Kiln Dust (CKD), a bypass dust, is generated in large quantities during the manufacturing of Portland cement. It is regarded as a waste material and is responsible for a significant loss to the cement industry in terms of the value of raw materials, processing, energy usage, dust collection and, above all, disposal and storage. The production of Portland cement is generally a four step process from the acquisition of the raw materials to the grinding of the clinker into Portland cement.

Based on the preparation of the feed material (a composite of different raw materials) prior to calcination, cement kilns are classified as either wet process kilns or dry process kilns. Wet process kilns are generally simpler but less energy efficient and feed is prepared in the form of slurry containing 30% to 40% water (Mehta, 1993). Modern cement plants favor the dry process over the wet process and accept the feed materials in dry form. An efficient dry-kiln will consume only about 60% of the energy required to produce a ton of cement in a typical wet-process kiln (PCA, 1992). A tremendous advancement in dry cement kiln technology is the preheater/precalciner kiln, where fuels are combusted in the preheater system just upstream of the rotary kiln. Here the moving feed material powder is dispersed in a stream of hot gas (heating to about 800 ^{o}C) coming from the kiln and are fed into the upper end of cylindrical rotary kilns which rotates at 1-4 revolutions per minute (rpm). The kiln is slightly inclined (3-4%) and rotates about its longitudinal axis. As the raw mix travels down the kiln, it is gradually heated to 1450 °C in the burning or clinkering zone. Successive reactions in different regions of the kiln produce hard pellets called clinker by partial fusion of raw materials, typically 3-20 mm in diameter, which when ground with gypsum and other additives produce the fine powder called Portland cement.

During heating, the raw materials move down slowly with the controlled rotational speed of the kiln. The oxygen in the form of air for combustion of the fuel is provided which moves against the flow of raw material. The combustion gases released from the fuels entraps the particulate matter and the volatilized material generated from the burning of feed material and carrying them up the kiln. The entrapped particulate matter and precipitates constitutes cement kiln dust (CKD) and can be collected or removed from the dust collection system (baghouses or electrostatic precipitators) close to the kiln or the quantity of the dust generated can be recycled or discarded.

Cement industries generate cement kiln dust to maintain the product quality (low alkali clinker from high alkali raw materials) and to ensure uninterrupted operation of the plant due to its high alklainity. According to USEPA (1993), the generation of CKD has been estimated to be 15 to 20% of clinker or cement production. In year 2013, around 600 to 800 million tons of CKD were generated worldwide whereas the Indian production was estimated at 42 to 56 million tons. The major factor preventing return of more dust to the kilns is the high concentration of alkalis in the dust that would cause the alkali content of the clinker to exceed the allowable value. Additionally, the high concentrations of volatiles develop deposits on the walls of the kiln which can result in frequent shut down of the plant. Hence cement plants generate CKD as a means of removing volatile alkalis, chlorides and sulfates from the kiln system. Though CKD is generated from the same raw materials as Portland cement, a significant variation in physical and chemical properties has been observed for CKDs obtained from different cement plants.

1.2 PHYSICAL PROPERTIES OF CKD

Cement kiln dust is a fine powdery material with uniform size and of grey to tan in color. The specific gravity of CKD ranges from 2.70 to 3.00 less than that of Portland cement (Gs- 3.15). CKD is slightly soluble in water (0.1% - 1.0%). The typical physical properties of CKD are given in Table 1.1. Particle size distribution is an important physical characteristic of CKD. Corish and Coleman (1995) reported that the alkali concentration in CKD depends mainly upon the particle size fraction i.e. finer the CKD particle higher the alkali content (Table 1.2).

Table 1.1: Typical physical properties of CKD (Collins and Emery, 1983)

Property	Value
Gradation (75 % passing)	0.030 mm (no. 450 sieve)
Maximum particle size	0.300 mm (no. 50 sieve)
Specific surface (cm^2/g)	4600-14000
Specific gravity	2.6-2.8

Table 1.2: Effect of particle size on alkali content of CKD (Corish and Coleman, 1995).

Size (microns)	Mass (%)	Na_2O (%)	K_2O (%)
46-68	0.3	0.3	3.6
34-48	0.4	0.3	3.5
24-34	0.7	0.4	4.5
17-24	1.8	0.4	5.1
12-17	5.1	0.4	5.2
6-12	27.3	0.3	5.4
0-6	64.4	0.4	10.7

Table 1.3: Typical chemical composition of ordinary Portland cement and cement kiln dust

Constituents (%)	Ordinary Portland Cement (Taha et al., 2004)	Cement Kiln Dust		
		Maslehuddin et al. (2008)	Taha et al. (2004)	Udoeyo and Hyee (2002)
SiO_2	20.85	17.1	15.84	2.16
Al_2O_3	4.78	4.24	3.57	1.09
Fe_2O_3	3.51	2.89	2.76	0.54
CaO	63.06	49.3	63.76	52.72
MgO	2.32	1.14	1.93	0.68
SO_3	2.48	3.56	1.65	0.05
K_2O	0.55	2.18	2.99	0.11
Na_2O	0.24	3.84	0.33	--
TiO_2	0.25	--	0.48	--
Mn_2O_3	0.05	--	0.07	--
Cl	0.01	--	1.09	--
Loss on Ignition	1.75	15.8	5.38	42.39

1.3 CHEMICAL COMPOSITION OF CKD

Chemically, Cement kiln dust has a composition similar to that of the ordinary Portland cement. Its chemical composition depends upon the raw materials used and the cement manufacturing processes. Compounds of lime, silica, and alumina are the major constituents of cement dust. Table 1.3 lists the typical chemical composition of the ordinary Portland cement and CKD (Maslehuddin et al., 2008; Taha et al., 2004; Udeyo and Hee, 2002). CKD is typically characterized by higher alkali content as compared to Portland cement, particularly in potassium and sulfur content.

The pH of CKD water mixture is typically about 12 and consequently is considered to be caustic. If CKD used in concrete, the alkali content needs to be monitored to avoid alkali-aggregate reaction. Certain trace metals such as cadmium, lead, selenium, and radionuclides are generally found in cement kiln dust.

1.4 BENEFICIAL USES OF CKD

Cement Kiln Dust is one of such industrial waste or by product which is progressively significant environmental concern related to its emission and disposal. Due to highly alkaline in nature and heavy metal content, its reuse in cement kiln is restricted as raw feed material to maintain the quality of cement, thus, major part of its is landfilled. Due to lack of landfilling space and ever increasing disposal cost, utilization of CKD has become an attractive alternative to its disposal.

In 1912, the first commercial use of CKD was made as a potassium fertilizer for citrus and other crops by Riverside Portland Cement Company in Crestmore. Cement kiln dust has the potential for reuse in many different ways, and the most common beneficial uses of CKD are cement replacement, soil stabilization, waste treatment, asphalt pavement and other uses. As a stabilizing agent for wastes, the absorptive capacity and alkaline properties of CKD can reduce the moisture content, increasing the bearing capacity, and therefore providing an alkaline environment for waste materials. Below are some practical applications where CKD is used and its long term performance can be assessed:

- **Blended Cements:** A great deal of work has been carried out by using CKD in blended cements. Addition of up to 15% kiln dust improved the compressive strength and accelerated the hydration.

- **Soil Stabilization:** CKD, due to presence of free lime content, has been extensively used in the stabilization of sandy and clay soils, in order to improve strength and plasticity indices.

- **Waste Stabilization/Solidification:** Highly alkaline nature of CKD makes it effective for waste stabilization by reducing the mobility of hazardous substances and contaminants, so as to make it suitable for land disposal or for other beneficial uses.

- **Mine Reclamation:** CKD, due to its fine particle size and high alkalinity, is also used to neutralize the acidic media and effluent from mining and mineral industries. Increased surface area of CKD particles allows more space for metal adsorption; and makes it potential neutralizing agent and sorbent.

- **Activator for Pozzolans:** Pozzolanic materials such as slag or fly ash due to low pH inhibit the hydration of blended cement pastes. CKD acts as activator for such pozzolanic materials required for further hydration due to high alkali and sulfate content that are least soluble and more effective.

- **Highway Bases and Subbases:** The effectiveness of CKD in substituting the hydrated lime in lime-fly ash- aggregate road base mixtures has been extensively studied. Similar strength development and freeze-thaw durability results were reported for both CKD-fly ash mixtures and lime-fly ash mixtures.

- **Agricultural Amendment:** CKD is used as soil amendment or fertilizer due to high lime and potassium content. It also acts as neutralizing agent for acidic soils. The composition of CKD varies with each cement plant, so the level of trace metals. The movement of trace metals should be determined before using CKD as soil amendment.

1.5 IMPACT OF CKD

***On Re-utilization*:** Generally, the cement kiln dust is alkaline in nature (pH 12) and is considered to be caustic. If used in concrete, the alkaline nature needs to be monitored to avoid expansive reaction between alkalis and certain aggregates, which leads to cracking and causes deterioration (Juenger and Jennings, 2001). The loss of strength was attributed to the alkalies in the dust. The American Society for Testing and Materials (ASTM) specifies that alkalin content of Pertland cement should not be more than 0.6 percent (Wilson and Anable, 1986). Reuse of high alkaline cement dust in the kiln causes the formation of free CaO and, potassium and sodium containing compound that leads to a reduction in the strength of cement and causes crack, deformities and reduce the quality of cement and concrete (Rehsi and Garg, 1986).

***On Landfilling*:** CKD's on the average are typically characterized by high alkali, sulfate and heavy metal content. Major part of unutilized dust is dumped in open land as landfill material and poses threat to environment in terms of soil, surface and ground water contamination through leachate. CKD when comes in contact with water, hydroxides, sulfates and silicates of Ca, Mg, Na, K, and Al are formed that impair natural water alkalinity, hardness and causes respiratory and gastrointestinal diseases. CKD also contains trace amount of metals such as arsenic, barium, lead, zinc, magnesium, titanium, manganese and aluminium which can potentially cause detrimental effects to receiving environment by leachate generated from CKD disposal sites (Duchesne and Reardon, 1998).

1.6 LEACHATE AND ITS COMPOSITION

Leachate is the liquid that drains from a landfill when comes in contact with water. It contains both suspended and dissolved materials. The composition of leachate depends upon the type of waste and the age of landfill. Leachate is a strongly odoured yellow or dark brown colored acidic liquid because of hydrogen, nitrogen and sulfur rich organic species such as mercaptans.

In a landfill when percolating water comes in contact with decomposing solid waste it gets contaminated and and if it then flows out from waste material ermed as leachate. Percolation of water through the waste promotes the process of decomposition by fungi and bacteria which creates an anoxic environment by readily utilizing the available oxygen. During the decomposition process several by- products (such as organic acids) are released which increases the temperature, reduces the pH and dissolved the metal ions in the developing leachate. Landfill leachate, generated form mixture of municipal, commercial and mixed industrial wastes, is generally characterized by the presence of four groups of contaminants such as dissolved organic matter, inorganic macro components such as cations and anions, heavy metals and xenobiotic organic compounds such as halogenated organics.

1.7 ROLE OF MICROORGANISMS

Rapidly increasing world population and improvement in industrial development has raised the problem of waste generation by many folds. Waste management is associated with the proper disposal and utilization of waste materials containing variety of heavy metals. Metals are ubiquitous in nature and constitute about 75% of the known elements in the ecosphere. They are vital to our industry, infrastructure and daily life, but if improperly dealt with can poses threat to human health and the environment. According to Sparks (2005), thirteen trace metals and metalloids such as Ag, As, Be, Cd, Cr, Cu, Hg, Ni, Pb, Sb, Se, Tl and Zn are considered as priority pollutants. Metals are significant natural components of soil and many acts as essential nutrients for plants and microbes.

Microorganisms involved in waste treatment have been a major subject of microbial ecology and several studies have been published to achieve growing attention towards the bioremediation of metal pollution. Metals are directly or indirectly involved in all aspects of microbial growth and metabolism; and the metabolic activity thus influences the solubility, mobility, bioavailability and toxicity of the metal speciation (Gadd, 2010). Metals exhibit a range of toxicities towards microbes. Toxic metals, their chemical derivatives, metalloids and organometals can have significant effect on microbial population and may affect the microbial activity. Metal toxicity can be affected by the physico-chemical nature of environment and the chemical behavior of the metal speciation may be affected. Despite apparent toxicity, microbes have the ability to grow and flourish in metal polluted locations due to variety of mechanisms such as solubilization (bioleaching), mobilization and immobilization processes that contribute to microbial resistance towards toxic metals and detoxify the metal speciation (Fig. 1.1).

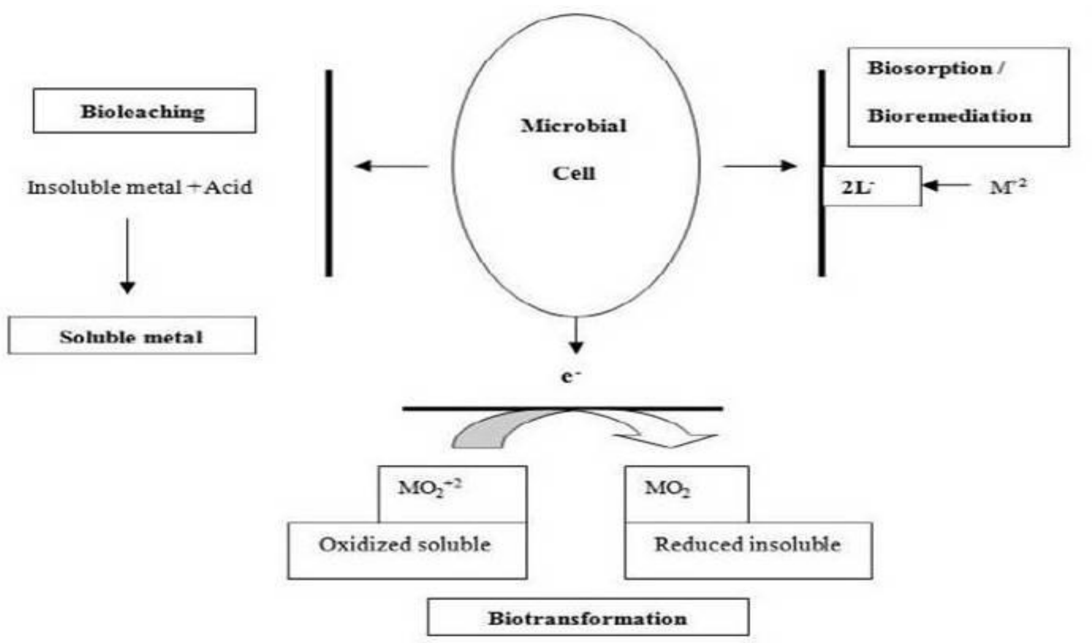

Fig. 1.1: Microbe-metal interaction mechanism (Mishra and Rhee, 2010)

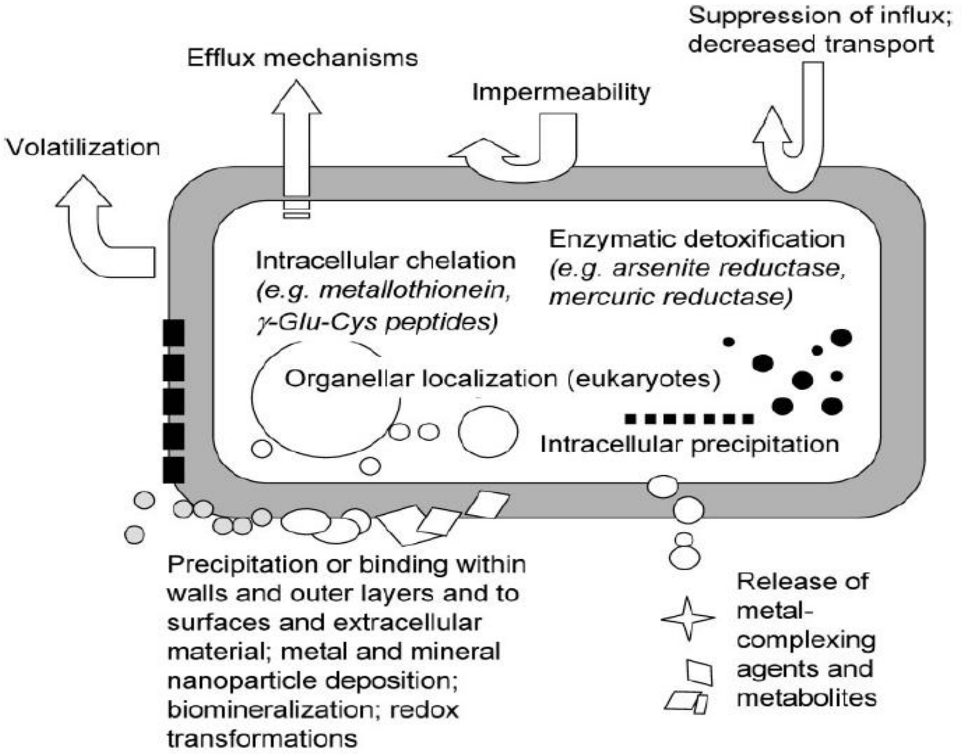

Fig. 1.2: Specific or non-specific mechanisms involved in the remediation and transformation of metals affects redox transformations, intracellular chelation and precipitation mediated by the microbes (Gadd, 2010)

1.7.1 Bioleaching

Bioleaching, or biological leaching, is an interaction between metals and microorganisms which result in the solubilization of metals. The process of bioleaching is based on the ability of microorganisms to transform solid compounds, resulting in soluble and extractable elements which could be recovered (Krebs et al., 1997). This biological process has received increased attention, since it represents a ‘clean technology’ and is able to leach metals from waste that are otherwise not able to be recovered by conventional methods (Ehrlich, 1997). Conventional methods are not economically feasible as the overall process cost is influenced by several factors such as concentration of metal in solution, operational mode of equipment, need for secondary treatment, disposal of sludge generated, etc. The biological process is a suitable process that requires low energy inputs, and does not produce hazardous by-products. Metal extraction by bioleaching is due to the production of chelating agents, complexing compounds and organic acids excreted by microbes into the environment and these days this technique is getting priority in application for metal recovery and detoxification of soil, sewage sludge and industrial wastes contaminated with heavy metals.

1.7.2 Metal Mobilization

A variety of microorganisms are able to mediate the mobilization and leaching of various metals from solid materials. These microbes include autotrophic bacteria, heterotrophic bacteria and fungi. The leaching ability of the microorganisms is based on redox processes, methylation, and chelation by metabolites and siderophores; and by autotrophic and heterotrophic leaching. Other excreted metabolites such as amino acids, phenolic compounds and organic acids may play a role in dissolution of insoluble metal compounds and minerals (Fig. 1.2). Microorganisms can manage the solubilization not only interacting with solid phases directly but also oxidation of Fe^{2+} to Fe^{3+} as an electron acceptor indirectly (Bosecker, 1997). To date, research on bioleaching has been directed largely at autotrophic microorganisms (especially *Thiobacillus*), whereas on the other side, heterotrophic microorganisms are important for alkali materials due to their ability to grow at high pH or extreme conditions.

Autotrophic leaching is carried out by chemolithotrophic, acidophilic bacteria which fixes carbon dioxide and obtains energy from the reduction of sulfur and oxidation of ferrous iron compounds causes solubilization of metals by the production of Fe^{3+} and sulfuric acid (Gadd, 2010). The autotrophic production of sulfuric acid by genera *Thiobacillus* and *Leptospirillum* decreased the pH of their immediate environment resulting in the solubilization of metals compounds from sewage sludge, waste leachate and soils, etc.

Microorganisms can acidify their environment by secreting low molecular mass carboxylic acids that form stable complexes with large number of metals by supplying protons and metal complexing anions. The microbial production of organic acids by heterotrophic bacteria is of developing interest in bioremediation and metal recovery than autotrophic bacteria as the heterotrophic bioleaching process is appropriate for wastes having high pH as *Thiobacilli* solubilize the metal speciation only at low pH and is not effective above pH 5.5. Bacterial production of siderophore is the largest class of known compounds that binds and shuttle iron. Siderophores are primarily excreted to aid iron assimilation but are also bind to other metals such as magnesium, manganese and chromium resulted in a complete decrease in the bioavailability of metal compounds.

Microorganisms can also mobilize metals, metalloids and organic compounds by oxido-reduction (redox) processes. Solubility of metal compounds increased on reduction of Fe^{3+} to Fe^{2+} and Mn^{4+} to Mn^{2+}. Increase in solubility of Fe and Mn components upon reduction decrease the solubility of other metals resulting in immobilization (Gadd, 2010). Under anaerobic conditions most iron reduction is carried out by anaerobic bacteria that convert Fe^{3+} to Fe^{2+} with suitable electron donor. Several microbially produced metal chelators such as oxalate, citrate, humic acids and tannins also enhance the microbial reduction process to solubilize the metal compounds.

1.7.3 Metal Immobilization

Immobilization of metals reduces the external free metal species in solution. Microorganisms are capable of accumulating the metals by binding them as cations to the cell surface, such as adsorption, and termed as biosorption. Microbial biomass, either live or dead cells can bind metal ions by the process of biosorption to cell walls, precipitation of metal compounds in and around the cell and to fungal hyphae, extracellular polysaccharides and intracellular accumulation, depending upon the metabolism (Fig. 1.2). Leaching of one or more metal constituents also influenced by metabolic products such as organic or inorganic acids, ligands or other metabolites produced by living cells.

Biosorption can also provide nucleation sites for the sorption of some metal cationic species that can be accumulated with in cells through membrane transport system of varying affinity and specificity. There are certain types of microorganisms which make their significant contributions towards the binding, accumulation and concentration of heavy metals (Table 1.4) from the aqueous solutions.

Table 1.4: Toxic heavy metals accumulating microorganisms

Organism	Heavy Metal (s)
Citrobacter sp.	Lead, Cadmium
Thiobacillus ferrooxidans	Silver
Bacillus cereus	Cadmium
Bacillus subtilis	Chromium
Pseudomonas aeruginosa	Uranium
Micrococcus luteus	Strontium
Rhisopus arrhizus	Mercury
Aspergillus niger	Thorium
Saccharomyces cerevisiae	Uranium

Fig. 1.3 shows a schematic process of biosorption for heavy metal removal. In the desorption process the adsorbed metal is removed and the biosorbant can be reused. It provides a basis for a new approach in which heavy metals even at low concentration can be removed from the solution (Volesky, 1990) and thus, acts as easier and economical process for the recovery of the waste material.

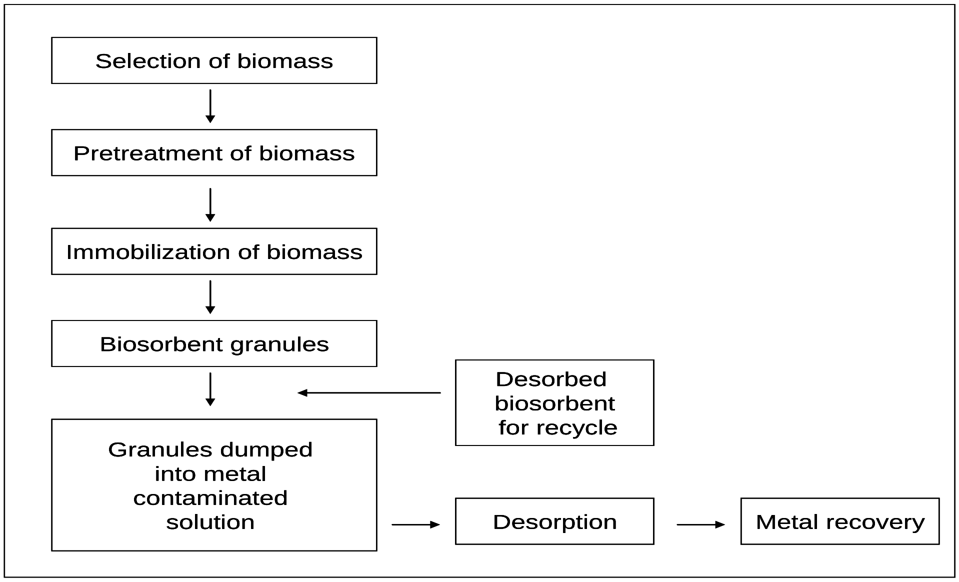

Fig. 1.3: Schematic representation of biosorption procedure (Alluri et al., 2007)

1.7.4 Alkaliphilic/Alkali-tolerant Microorganisms

Microorganisms have been isolated from variety of environments. The most abundant occurrences of microorganisms are generally observed in moderate environments. Several environments can be regarded as extreme and includes low or high pH, low or high temperature, high salinity, presence of organic solvents, heavy metals etc. Microorganisms which inhabit extreme environment are termed as “extremophiles”. Extremophiles are gaining lot of momentum in research due to their ability to grow under extreme conditions and to abate environmental pollution.

Alkaliphilic microorganisms can play a pivotal role as potential biotechnological applications due to enzymes viz. proteases, cellulases, xylanases that are stable at high pH (>9.5) and temperature (>50 °C) (Ghosh et al., 2007). The enzymatic activities enable them to gain resistance against alkaline environments to biodegrade certain xenobiotics, to produce several metabolites and to detoxify detrimental metal compounds. Cell surface of the alkaliphile can maintain the intracellular pH values near neutral in alkaline environment of pH 10-13. The presence of Na^+ ions in the growth medium played a pivotal role in adaptation of alkaliphilic *Bacillus* species at high pH values.

1.8 SIGNIGICANCE AND OBJECTIVES OF THE RESEARCH

1.8.1 Significance in Research Area

India, being an industrializing country, has the significant contribution to the generation of hazardous wastes from a wide spectrum of industries. The management of these wastes poses many challenges for the industry. With increasing urbanization, the demand of cement in the world increases, so does the production of cement, which leads to the generation of huge quantities of cement kiln dust. The problem of disposing of unused CKD places a considerable burden on the cement industry in terms of cost of disposing, diminishing landfilled space and environmental hazards. The alkalinity of CKD affects the natural buffering capacity of surface waters and soils. CKD may contain metals, which are generally considered as toxic heavy metals and in high concentration they may be hazardous to human health and the environment.

The utilization of CKD as replacement of a portion of cement in concrete makes it significant and economical both in reducing the amount of expensive cement employed and in utilizing the waste material which is costly to dispose off. Due to high alkalinity of CKD, its use in the cement/concrete has not been fully explored. No work has been reported on the alkalinity of CKD and the quality of leachate from concrete made from CKD. Therefore, it is essential to study the alkalinity and quality of CKD incorporated concrete to further improve it.

1.8.2 Gap in the Research Area

Portland cement is the most widely used building material in world with about 4 billion tons produced in 2013. India has got a huge cement industry with a total capacity of 280 million tons, (Oss, 2014). The generation of CKD, a by-product of cement manufacturing, has been estimated to be 15 to 20% of clinker production (USEPA, 1993) which put CKD generation by India at an estimated 42 to 56 million short tons for 2013. Though the amount of CKD that is reused beneficially has increased by over 35%, there is still a large amount of kiln dust generated (Adaska and Taubert, 2008).

Cement kiln dust is a potential material for use in concrete (Siddique, 2006; Peethamparan et al., 2008; Pavia and Regan, 2010) but the alkaline nature and leachate from it may contain hazardous compounds, which may possibly affect the human health and environment. High alkali content of CKD leads to reduction in the quality and strength of the cement and concrete (Ghanem et al., 2010; Rehsi and Garg, 1986); and also affects the hydration and microstructure of cement paste (Juenger and Jennings, 2001). As not much work has been reported on the alkaline nature and quality of leachate from CKD and concrete made with CKD, it is proposed through this research to investigate the influence of microbes (bacteria or actinomycetes) on the properties of mortar and concrete, and leachate from concrete using cement kiln dust.

1.8.3 Objectives of the Research Work

Keeping in the mind, the gap in the research area, objectives are as follows:

- Screening and optimization of microbial inoculum for controlling alkalinity of CKD.
- Studies on compressive strength, water absorption and porosity of concrete supplemented with treated CKD.
- Studies on relationship of alkalinity and leachate with reference to compressive strength, water absorption and porosity of concrete.

1.9 ORGANIZATION OF THE THESIS

The thesis consists in five chapters:

Chapter 1 introduces utilization of cement kiln dust (CKD) in concrete, its properties and production in brief. It also includes leachate and its composition, role of microorganisms in bioleaching and the objectives of the present work.

Chapter 2 reviews the existing literature on the typical properties of CKD, its application in mortar and concrete, mechanical and durability properties of mortar and concrete using CKD, its role in reducing carbon dioxide emission, leachate analysis of CKD, leachate test methods, bioleaching and role of alkali-tolerant microorganisms.

Chapter 3 details the materials used with their properties, isolation and screening procedure along with molecular characterization for bacterial isolates, bacterial treatment of CKD, mortar and concrete mix design, casting of specimens, study of various properties, methodology adopted for testing of different properties, SEM and XRD analysis.

Chapter 4 presents the results, and their analysis for isolation and screening of alkali-tolerant bacterial isolates, characterization of cement kiln dust, optimization of bacterial treatment of cement kiln dust, identification and molecular characterization of bacterial isolates. It also presents the mortar and concrete properties including compressive strength, split tensile strength, ultrasonic pulse velocity, rapid chloride permeability, water absorption and porosity of control concrete, untreated CKD containing concrete and bacterial treated CKD concrete along with the SEM and XRD studies to analyze the microstructure and presence of various phases of compounds of the mortar and concrete made with cement kiln dust (untreated and bacterial treated) in varying percentages replacement of cement. Leachate analysis of control, untreated and bacterial treated CKD containing concrete mix for alkalinity, chloride and metals.

Chapter 5 summarizes and concludes the findings of the study.

References are placed at the end.

CHAPTER – 2

LITERATURE REVIEW

This chapter reviews the existing literature on supplementary cementitious materials, utilization of CKD in cement mortar and concrete, effect of CKD components on hydration and other properties of CKD blends, leachate test methods, leachate analysis of CKD, bioleaching by alkalitolerant/alkaliphilic bacteria.

2.1 SUPPLEMENTARY CEMENTITIOUS MATERIALS

Advancement in concrete technology in recent years is generally focused on the strength and durability of structures during the design of mortar and concrete. Concrete is made from coarse aggregates (gravel or crushed stone), fine aggregates (sand), water, cement and admixtures with annual consumption estimated between 21 and 31 billion tons in 2006 (European Concrete Platform ASBL, 2009). Portland cement plays an important role in the production of mortar and concrete due to its binding properties, and thus acts as a major constructional material of choice. In year 2013, approximately 4000 million tons of cement were generated worldwide compared to 3800 million tons in year 2012 (Oss, 2014). This increase in cement production carries with it great environmental issues such as energy and resource conservation, cost of production and green house gas emission. The cement industry alone is responsible for about 7% of all CO_2 generated worldwide and it is estimated that for 1 ton of cement production around 1 ton of CO_2 and other green house gases (GHGs) released into the atmosphere. This shows that the cement and concrete industry is facing tremendous challenge for sustainable development of building and construction materials.

Supplementary cementitious materials (SCMs) are pozzolanic materials (natural, industrial wastes and by-products) recognized worldwide to be efficiently utilized as partial cement replacement to improve the strength and durability of cement mortars and concrete. Portland cement contributes about 7-8% of global CO_2 greenhouse emissions. Thus to reduce these emissions, the use of pozzolanic materials need to be deployed to improve the properties of constructional materials. The utilization of SCMs in mortar and concrete not only reduces the disposal problems but also makes it economical. The leachate generated from the landfilling of such materials may contain hazardous components, which affects the environment in terms of surface, ground water and soil contamination. So, it is important to know the quality of leachate generated, disposal of wastes, their environmental impact, and beneficial utilization of SCMs for potential development towards solid waste management. Utilization of SCMs in cement/concrete not only prevents them from being land-filled but also enhances the mechanical properties and durability of mortar and concrete in the fresh and hardened states.

SCMs are either natural, industrial by-products or man-made materials (nano-materials), and are listed as follows:

i. Cement kiln dust
ii. Metakaolin
iii. Volcanic ash
iv. Silica fume
v. Sewage sludge ash
vi. Fly ash
vii. Lime
viii. Ground granulated blast furnace slag
ix. Coal bottom ash
x. Natural pozzolans
xi. Nano-materials

2.1.1 Cement Kiln Dust

Cement kiln dust (CKD), a uniform fine powdery material, is generated in large quantities during the production of cement clinker from air pollution control devices (e.g. cyclone, bag house, or electrostatic precipitator). Cement kiln dust has the potential for reuse in many different ways such as cement replacement, soil stabilization, waste treatment, asphalt pavement etc., but the cement making process is the best way to reuse this by-product material. The pozzolanic properties of CKD, similar to cement, also makes it a potential replacement for Portland cement in utilization in mortar and concrete. El-Aleem et al. (2005) investigated the compressive strength of CKD - mortar (0, 2, 4, 6, 8, and 10% replacement to cement) at the curing ages of 3, 7, 28, and 90 days. It was observed that replacement of cement up to 6% by CKD slightly decreases the compressive strength where as with increase in CKD content, the compressive strength decreased sharply.

Maslehuddin et al. (2009) studied that replacement of Type I and Type V Portland cement with CKD at 0, 5, 10, and 15% by mass showed decrease in compressive strength (>5%) of concrete mixes containing 10 and 15% CKD after curing period of 3, 7, 14, 28, 56, and 90 days compared to control. Wang et al. (2002) found increased compressive strength in 15% CKD-cement blends (47.8 MPa) in comparison to cement alone (46.3 MPa) after 28 days of curing having the w/b ratio of 0.50. Siddique (2006) also discussed the effect of CKD on the cement pastes/mortars/concrete and controlled low strength materials (CLSM) properties such as setting time, electrical conductivity, compressive strength, tensile strength, and leachate analysis, and found reduced workability and strength in cements containing only CKD .

2.1.2 Metakaolin

Metakaolin, a pozzolanic material obtained from the thermal activation of kaolin clay, is fine white clay mainly used for the manufacture of porcelain. The dehydroxylation of kaoline at 500-800 °C (calcinations process) removes water and above this temperature a two dimensional crystal structure formed termed as metakaolin. Due to its fine particle size and pozzolanic nature, it reacts rapidly with calcium hydroxide during hydration of cement to produce calcium silicate hydrate and aluminosilicate hydrate and proves a promising material for manufacturing of mortar and concrete. Nicolas et al. (2014) studied the performance and durability of concrete containing metakaolin used as partial cement replacement. Replacement of 25% of Portland cement CEM 1 with metakaolin and addition of super plasticizer improved the workability of concrete. Reduced porosity was also observed measured by gas permeability and migration of chloride ions resulted in increased durability. This is due to the formation of increased amount of calcium silicate hydrate and calcium alumino-silicate hydrate (CASH) by utilizing portlandite present in highly pozzolanic metakaolin.

Paiva et al. (2012) studied the effect of metakaolin (MK) on the workability and strength of concrete with water or high range water reducer admixture (HRWRA). They observed that with increasing MK content by replacing cement the workability decreases due to high surface area of MK particle. Thus in order to keep workability constant, water or HRWRA content must be increased. Addition of metakaolin in concrete reduces the strength by grouping of particles (agglomerate) in the concrete mix due to fine particle size with high surface energy. These grouping of particles retained air inside and acts as large particles thus reduce the strength. Increase in strength was achieved by adding HRWRA which disperses the particles randomly leading to lower values of porosity and improved microstructure of MK containing concrete. Several studies also reported that addition of metakaolin increased the cement hydration due to its pozzolanic nature. The formation of CSH gel densifies the inter transition zone (ITZ) between binder and aggregate promoting the porosity refinement, improved mechanical strength, chemical resistance and durability (Wild and Khatib 1996; Khatib and Clay 2004).

Wild and Khatib (1997) observed the portlandite content in cement mortars and pastes containing 5, 10 and 15% MK with water binder ratio of 0.55. It was observed that during early ages the removal of Portlandite was maximum due to pozzolanic reaction corresponds to increased strength. Increasing the MK content decreases the CH content in mortar pastes whereas in cement pastes inclusion of MK increases the CH content gradually with time and significantly decline the strength.

2.1.3 Volcanic Ash

Hamidi et al. (2013) evaluated the pozzolanic activity of natural and thermal treated andesite (fine particles volcanic rock) using Fratinni test, X-ray diffraction (XRD), thermo gravimetric analysis (TGA), saturated lime test etc. Mortars containing 20 and 40% andesite were analysed by XRD and TGA, and found decease in portlandite content during hardening of the mortar whereas at later ages, the increased formation of water bound hydrates (CSH/CASH) confirmed the pozzolanic activity of andesite. Hamidi et al. (2013) also observed that the thermal treatment of andesite at 800 °C improved the pozzolanic activity and 10% substitution of which preserved the mechanical properties of mortars whereas at higher substitution level, acomparable loss in compressive strength was observed.

Olawuyi and Olusola (2010) studied the effect of volcanic ash (VA) as partial cement replacement (0 – 30 %) and laterite as sand replacement (0 – 30 %) on the compressive strength of concrete for 7, 14, 21, and 28 days. The results showed that with increase in volcanic ash content the density and compressive strength of concrete decreased by 4.4% and 28%, respectively. After 28 day curing, density of the concrete reduced from 2390 kg/m^3 to 2285 kg/m^3 whereas reduction in compressive strength from25.08 N/mm^2 to 17.98 N/mm^2 was observed for 0-30% variation of ash content only. It was also observed that the compressive strength decreased with increase in laterite content but increased with increase in curing age.

2.1.4 Silica Fume

Silica fume is generated during the smelting process of silicon and ferrosilicon industry. High silica content and fineness makes it effective pozzolanic material for utilization in mortar and concrete. Incorporation of silica fume influences the degree of orientation of CH crystals and thickness of transition phase in mortar and concrete resulted in accelerated hydration of cement, reduced chloride ion diffusion, improves corrosion resistance and alkali silica expansion by transforming the bigger pores into smaller ones, and thus, influences the durability. Cwirzen and Penttala (2005) investigated the influence of cement paste-aggregate interfacial transition zone (ITZ) on the durability of silica fume incorporated high performance concrete having water binder ratio of 0.30, 0.35 and 0.42 with varying concentration of silica fume. It was observed that during freezing and thawing cycles the ITZ accelerates the damaging mechanism by increasing the movement of the pore solution in 3% silica fume concrete with water binder ratio of 0.42. They concluded that addition of silica fume in moderate quantity densifies the ITZ resulted in increased durability of concrete.

Khan and Lynsdale (2002) developed high performance concrete using binary and ternary blends of cementitious materials such as ordinary Portland cement (OPC), pulverized fuel ash (PFA) and silica fume (SF). Mixes were prepared using PFA up to 40% and blended with 0, 5, 10, and 15% SF as partial cement replacements with water binder ratio of 0.27, 0.40 and 0.50. Properties such as compressive strength, tensile strength, oxygen permeability and carbonation were studied. Increasing PFA content decreased the compressive strength of concrete at all ages of 7, 28, 90 and 180 days. Blending of PFA with 10% SF increased the strength whereas above 10%

no significant improvement in strength was observed at 28 days of curing. Similar observations were also observed in tensile strength. A modest improvement in compressive strength and tensile strength was observed at 90 and 180 days. Addition of 10% silica fume in PFA mix reduced the oxygen permeability and increased the carbonation depth. It was concluded that incorporation of 8-12% SF in PFA mixes yielded optimum strength and permeability values.

Cwirzen et al. (2008) studied the mechanical properties, frost durability and bond strength of ultra high performance (UHP) concrete and mortar characterized by low water to cement ratio, use of secondary binders and superplasticizers. The UHP concrete prepared by using carbon free undensified silica fume showed high workability and strength. The heat treated (90 °C) specimens showed compressive strength varied between 170-202 MPa, whereas non-heat treated (20 °C) showed130-150 MPa compressive strength at 28 days of curing. Similar increase in strength was also observed in UHP mortar. No significant difference in microstructure of UHP mortar and concrete were observed whereas decrease in shrinkage was observed in UHP concrete specimens. Similar findings were also observed by Scott and Singh (2011) in high performance silica fume concrete.

ACI Committee 234 (2006) reported that addition of silica fume accelerates the hydration of cement during early stages due to fine particle size. According to Gleize et al. (2003) and Wolseifer (1984), silica fume acts mainly at the interface of cement paste-aggregate in mortars and concrete, and thus, produces high strength and low permeability. Sakr (2006) concluded that concrete containing 15% of silica fume as partial replacement to cement had better compressive strength and resistance to sulfate attack.

2.1.5 Sewage Sludge Ash

Cyr et al. (2006) assessed the recycling of industrial waste material in cement based materials used for construction purposes. It was observed that the utilization of 25% of various waste materials such as sewage sludge ash (SSA), treated municipal solid waste incinerator fly ash (MSWI FA), treated polluted sediments (Sed), baggase fly ash (Bag) and titanium fume (TF) as cement replacement showed 60-90% of the strength values compared to reference strength. It was concluded that these materials be reused in some applications for example, in SCC for trench filling, where high compressive strength is not required.

Cyr et al. (2006) studied the quantitative impact of waste residues (SSA, MSWI FA, Sed, Bag and TF) on environment. Utilization of such waste materials in cement based materials stabilizes the harmful waste components necessary for sustainable development and to ensure the ecological compatibility of wastes materials. Leaching test carried out according to European standard EN 12457 reported that with 12.5% cement replacement a significant quantity of chromium, zinc and lead was leached compared with the reference material (cement).

Cyr et al. (2007) observed that high specific surface area of fine sewage sludge ash particles leads to a significantly high water demand and delayed the cement hydration. Mortars containing 25% and 50% of SSA showed lower compressive strength values than control mortars but had a long-term positive effect due to pozzolanic activity. It was also observed that mortars containing SSA leached out higher amount of elements than from the control mortar.

Cyr et al. (2007) also reported the use of sewage sludge ash (SSA), waste material obtained from the incineration of waste water sludge, cement or sand replacement in cement based materials. Addition of SSA in cement reduces the workability of fresh mortar and increases the setting time of cement. This is due to the irregular shape of ash grains with higher surface area leading to higher water demand. It was observed that addition of 25 and 50% SSA in mortars showed 92 and 84% of strength, respectively, at 91 days of curing compared to reference mortars.

2.1.6 Fly Ash

Shi C and Qian J (2003) formulated an effective method to increase the utilization of fly ash in concrete by chemical activation of reactivity of fly ash. Khatib (2008) studied the influence of fly ash (FA, 0-80%) as a partial cement replacement on the properties of self compacting concrete (SCC). It was observed that increased FA content produces high strength and low shrinkage SCC. Up to 60% cement replacement with FA increased strength (40 N/mm^2) was observed whereas SCC with 80% FA showed absorption values below 2% and reduction in shrinkage by two third compared to reference at 56 days of curing.

The compressive strength of concrete containing fly ash is readily determined by the ratio of addition of fly ash and the particle size. It was observed that the early age compressive strength of concrete increased due to the addition of fly ash (Maslehuddin, 1989). Siddique (2003a; b;

2004) reported drastic improvement in strength properties of concrete containing fly ash after 28 days of curing and proposed that with the increase in percentage of fly ash as partial replacement to cement, the compressive strength also increased. Saraswathy et al. (2003) observed improved compressive strength and durability of concrete when fly ash was used as partial replacement to cement (10% and 20%). Erdogdu and Turker (1998) and Chindaprasirt et al. (2007) reported that use of fly ash in concrete reduced the water demand but increased the strength due to fine particle size.

2.1.7 Lime

Velosa and Cachim (2009) tested concrete specimens containing different percentages of hydraulic lime and a pozzolanic material. Mechanical strength of concrete specimens and a pozzolanic index was determined to evaluate the effectiveness of the pozzolanic material. Schmidt et al. (2011) studied the influence of environmental temperatures on the performance of polymeric stabilizing agent in fresh cementitious materials.

Makhloufi et al. (2012) studied the durability of limestone mortars containing mineral admixtures such as blast furnace slag, lime stone filler and natural pozzolana. The specimens were immersed in demineralized water and lime water to study degradation subjected to two protocols. The first consists of continuous immersion for 180 days at 23±1 °C, while the second protocol consists of seven drying-immersion and heating-cooling cycles for 42 days followed by continuous immersion up to 180 days. The compressive strength of mortars containing 30% limestone content and 10% slag and natural pozzolana each, continues to increase with curing period, immersed in demineralized water, where in other treatments the strength increased up to 12 days and beyond this age the strength decreases. The strength gain of mortars containing limestone (10%) cured in demineralized water is lower than that of mortars cured in lime water. This is attributed that the portlandite was consumed by slag and pozzolana which reduces the pozzolanic reaction in mortars compared to the mortars cured in lime water. It was also observed that during drying-immersion and heating-cooling cycles the degradation was higher in control and in limestone (30%) containing mortars due to rapid leaching of portlandite in demineralized water. Specimens containing slag (30%) and pozzolana (30%) were less sensitive to degradation as they contain less portlandite, which was consumed for pozzolanic activity.

2.1.8 Ground Granulated Blast Furnace Slag

Ground granulated blast furnace slag (GGBFS) is a by-product of iron and steel industries when mixture of coke, iron-ore, limestone are fed in to blast furnaces at a temperature of around 1450-1550 °C. GGBFS is used as cement replacement for up to 85% depending upon the applications. Li and Zhao (2003) determined the compressive strength of high-strength concrete containing GGBS (15%) and fly ash (25%) and observed increase in strength with age up to 365 days but only with slightly lower values during early ages (before 28 days). Hooton and Titherington (2004) and Cheng et al. (2005) concluded that addition of GGBS alone or in blend with silica fume improved chloride penetration resistance due to denser structure compared to plain concrete.

The influence of ground granulated blast furnace slag (GGBFS, 0-80%) and metakaolin (MK, 0-20%) as partial replacement to cement on concrete was investigated by Khatib and Hibbert (2005). The incorporation of GGBFS alone in concrete reduces the strength at early ages whereas increase in strength was observed with addition of metakaolin. Addition of up to 60% GGBFS in concrete improved the long term strength whereas addition of up to 20% MK contributes to early strength due to pozzolanic nature of MK. The decrease in early age compressive strength of concrete containing GGBFS alone can be compensated by inclusion of MK (up to 10%). Above 10% addition no improvement in strength was observed. Similar results were also observed in tensile strength of concrete containing 60% GGBFS and 10% MK. Thus, it was concluded that the strength of GGBFS concrete can be improved by incorporating MK content of less than 10%.

2.1.9 Coal Bottom Ash

Coal bottom ash (CBA), an incombustible part of coal, accumulates at the bottom of the dry boiler furnace during the burning of coal at thermal power plants. As the consumption of coal by power plants increases, so does the production of coal ash. The problem of disposing of unused coal ash is associated with the cost of disposing, diminishing landfilled space and environmental hazards and places a considerable burden on the power industry. Pozzolanic nature of coal bottom ash makes it suitable for use in mortar and concrete and is significant both in reducing the large amount of cement production and in utilizing the waste material which is costly to dispose off.

Canpolat et al. (2004) studied the effect of zeolite, coal bottom ash and fly ash on the properties of cement as partial cement replacement. They observed increase in compressive strength by replacing up to 15% cement with zeolite but when fly ash was used in combination decrease in strength was observed. Kurama and Kaya (2008) reported that replacement of Portland cement with CBA up to 10% could improve the mechanical properties of concrete, whereas Kizgut et al. (2010) observed that addition of 25% of coal bottom ash resulted in compressive strength corresponds to 93.82% of the strength of control obtained at 90 days curing time. Chun et al (2008) studied the compressive, tensile and flexural strength of pond ash concrete as a replacement for fine aggregate (0-30%) and observed that slump decreased with increase in CBA content but there was increase in strength properties compared to normal concrete.

Aggarwal et al. (2007) studied the compressive strength, splitting tensile strength and flexural strength of concrete containing bottom ash (0-50%) as fine aggregate replacement. The compressive strength of cubes (150 mm size), splitting tensile strength of cylinders (150 x 300 mm) and flexural strength of concrete beams (101.4 x 101.4 x 508 mm) were performed at 7, 28, 56 and 90 days of curing period. Test results reported that water demand increases with increase in bottom ash content to maintain the required workability of mix. This was due to the fineness of bottom ash which increased the specific surface thus, reduced the workability. It was observed that with curing age the strength of the specimen increases but with increasing bottom ash content the strength decreases. In different mixes, the strength of bottom ash concrete at 90 days was varied from 56-65%, 75-85% and 86-90% at 7, 28 and 56 days of curing, respectively. Similar trend of reduction in splitting tensile and flexural strength was also observed with addition of bottom ash. Replacement of 20% fine aggregate with bottom ash showed enhanced splitting tensile strength whereas with 50% replacement the strength was minimum. Flexural strength test results showed that concrete containing 40% bottom ash attained comparable strength at 90 days of curing and can be used for pavement application. The reduction in strength of concrete specimens was attributed to the poor interlocking between ash and aggregates, as the ash particles were spherical in nature.

2.1.10 Natural Pozzolans

Haral et al. (2013) studied the role of natural pozzolans (NP) as an alternative to SCMs on the fresh properties such as flowability and flow rate of self compacting concrete with optimized dosages of superplasticizer (polycarboxylic ether; PCE). A negligible change in the flowability was observed up to 20% cement replacement with NP whereas apparent reduction in flowability was observed with increase in replacement levels of 30 and 40%. Thus to achieve required flowability additional PCE required. It was concluded that up to 20% of NP can be used as active binder to provide required flowability without using superplasticizer.

Cachim et al. (2014) reported the use of industrial waste materials as cement or aggregate substitution in concrete production for safe and sustainable environment. Natural pozzolans (NP) such as calcined clays and volcanic ash may have an important role in improving the strength and durability of cement mortars and concrete when used as cement replacement. Use of NP by replacing cement up to 20% (in mass) achieved minimum strength of 60 MPa at 28 days and leads to reduction in chloride ion migration, corrosion and concrete degradation. This is attributed to the decrease in pore size and pozzolanic action of natural pozzolans with lower heat of hydration and higher setting time resulted in increased long term strength. They concluded that the increasing concern with environment and sustainable issues, the incorporation of waste materials in mortar and concrete is today's need and their use will have an undoubtful increment in the near future.

2.1.11 Nano-materials

Cwirzen (2010) showed the effect of nano-materials on physical properties of cementations matrixes. The mechanical properties such as compressive and flexural strength can be increased up to 50% by addition of 0.23 wt% of carbon nano-tubes. Frost resistance was improved by carbon nano-tubes and carbon nano-fibres and/or nano-silica. After addition of carbon nano-fibres, other properties such as autogenous shrinkage decreased significantly. Nano-silica enabled an immense densification of the hydrated binder matrix, which in turn improved for instance the durability and mechanical properties.

Nasibulin et al. (2009) developed a novel cement based hybrid material containing carbon nanotubes (CNTs) and carbon nanofibres (CNFs) that showed enhanced tensile strength, modulus of elasticity, electrical and thermal conductivity. Instead of adding, the CNTs and CNFs were attached to the cement particles for proper dispersion in the matrix and this was confirmed by SEM analysis which showed that the CNTs and CNFs were nicely imbedded into the CSH gel. This significantly improves the compressive strength by 2 times and 40 times increase in electrical conductivity of paste made of cement based hybrid material after 28 days of water curing.

Mudimela et al. (2009) proposed a method of adhering CNTs and CNFs on surface of silica fume particles impregnated by iron salt and iron oxide. CNTs having diameter of 10-15 nm and 12-20 nm were synthesized on silica particles at 600 °C and 750 °C, respectively, whereas CNFs with diameter 30 nm were grown on cement particles using acetylene as carbon source. The produced carbon nano-materials provide good bonding between carbon and matrix materials by homogenously dispersed in the matrix resulted in enhancement of concrete mechanical properties

2.2 CEMENT KILN DUST

Cement kiln dust, an industrial waste by-product, is generated in large quantities during the manufacturing of Portland cement. In year 2013, over 4000 million tons of cement was generated throughout the world (Oss, 2014). India, has got a huge cement industry of about 20 cement companies with the total capacity of 280 million tons which makes it second largest cement producer in the world after China (2300 million tons) tones, (Oss, 2014). Worldwide CKD generation in year 2013 was approximately 700 million tons whereas Indian production was at 42 to 56 million tons, estimated to be 15 to 20% of cement clinker production (USEPA, 1993).

The physical and chemical properties of CKD depend upon the type of cement kiln operation and raw materials used. CKDs are derived from same process and raw materials as cement clinker but still composition of CKD differ from Portland cement because CKDs are partially burnt whereas clinker is fully burnt. The particle size of a CKD also greatly influences the properties of mortar and concrete.

2.2.1 Physical Properties

Portland cement is angular in shape and average particle size is approximately 15 µm. Peethamparan et al. (2008) studied the particle size distribution of four different CKDs obtained from long dry pyroprocess, precalciner, preheater and wet pyroprocess; and a Type I Portland cement. CKD obtained from long dry pyroprocess had the finest particle size whereas CKD from precalciner pyroprocess had the coarsest particle size. The particle size of preheater and wet pyroprocess CKD had similar to that Type I Portland cement and varied between 10 and 20 µm. fineness of the CKD have been typically reported in terms of Blaine fineness, relative density and particle size distribution. Konsta-Gdoutos and Shah (2003) observed that the Blaine fineness of the CKD varies between 318-1400 m^2/kg whereas relative density typically between 2.6-2.8, less than 3.15 of Portland cement. Lachemi et al. (2008) and Ravindrarajah (1982) concluded that Blaine fineness of the studied CKDs were finer than Portland cement.

2.2.2 Chemical Properties

CKD's chemical composition depends upon the raw materials used and the cement manufacturing processes. Studies conducted by Sreekrishnavilasam et al. (2006) on chemical composition of CKDs obtained from 63 published datasets from different cement plants and by Tennis and Bhatty (2006) on chemical composition of Portland cement obtained from 123 cement plants indicated that oxides of calcium and silica are the major constituents of CKD similar to cement, although the values are lower than the values for Portland cement.

2.2.3 Applications of CKD

Researchers have investigated the use of CKD in waste water treatment (Morgan et al. 1984), as a soil stabilizer (Miller and Zaman, 2000; Corish and Coleman, 1995), , an anti-stripping agent in asphalt, partial replacement of soda in glass production, an activator for other industrial by-products as mineral admixture in cement and concrete and as a component of blended cements and masonary products (Klemn, 1980, Bhatty, 1995 and Bhatty et al., 1996). Most engineering applications and research to date have focused on combining CKD with an eminently hydraulic binder such as Portland cement. Studies of number of researchers have shown that CKD could be used in making cement pastes/mortars/concrete. Siddique (2006) discussed the influence of CKD on the cement pastes/mortars/concrete and CLSM properties like compressive strength, tensile strength, setting time, sorptivity, electrical conductivity and leachate analysis. CKD was used as a partial substitute of blast furnace slag cement in cement industry and building products by Abo-El-Enien (1997) and a partial replacement of cement in concrete by Sri Ravindrarajah (1982). High degree of hydration, reduced workability and strength was observed with various percentages of cement replaced by CKD. But replacement of cement up to 15% with CKD showed a non-significant difference in strength loss.

The possibility of utilizing the CKD as an activator for various industrial waste materials was investigated by several researchers. Salem and Ragai (2001) investigated the variation in electrical conductivity of granulated slag- CKD- silica fume blended cements with hydration time. The use of copper slag (CS) and CKD as partial replacement of Portland cement and their blends in mortar mixtures was investigated by Taha et al. (2004) and concluded that CKD as partial replacement or in blends with CS improved the strength of cement mortars at all curing ages. Similar results were also observed by Konsta-Gdoutos and Shah (2003) by utilizing CKD as an activator for granulated blast furnace slag.

Substitution of copper slag alone (5%) and in combination of cement by-pass dust (1.5% slag and 13.5% cement dust) for Portland cement in concrete was studied by Al-Jabri et al. (2006). In 5% copper slag substitution no significant change in compressive strength was observed while lower strength values were obtained in higher copper slag replacement (13.5%). The use of CKD as an replacement to Portland cement has been evaluated by several researchers and studied its use in blended with Portland cement as well as ground granulated blast furnace slag, fly ash, and calcium-lime (Adaska and Taubert, 2008; Pavia and Regan, 2010). It is believed that the addition of fly ash and/or slag and/or calcium-lime to the cement-CKD blends produced higher strengths and durability than the blends containing no fly ash and/or slag and/or calcium-lime.

2.2.4 Utilization of CKD in Cement Mortar/Concrete

Cement kiln dust has the potential for reuse in waste treatment, soil stabilization, cement replacement, asphalt pavement and other uses. Due to alkaline properties and absorptive capacity, CKD acts as a stabilizing agent for waste materials by increasing the bearing capacity and reducing the moisture content. The most common beneficial uses of CKD are in cement making process. In United States approximately 60–67 percent (8–8.4 million tons) of the total CKD generated is used in this way (Adaska and Taubert, 2008). CKD's cement like pozzolanic properties makes it a potential replacement for cement in flowable slurry, concrete, etc. Several studies have been reported on different aspects of the utilization of cement kiln dust in cement paste, mortar/concrete (Taylor, 1997; Dyer et al., 1999; Shoaib et al., 2000; Heikal et al., 2002; Batis et al., 2002; Konsta-Gdoutos and Shah, 2003; Wang et al., 2004; Taha et al., 2004; Maslehuddin et al., 2008; 2009).

2.2.4.1 Compressive strength

Pavia and Regan (2010) investigated the properties of mortars containing cement kiln dust (CKD) and calcium lime (CL), and found that with increasing CKD content compressive strength was increased with the exception of 5% CKD mortar. Increase of 1.6, 7.3 and 8.3% strength, respectively, was observed in 10, 15 and 20% CKD mortars. Taha et al. (2004) evaluated the usefulness of copper slag (CS) and CKD as a partial replacement for Portland cement (PC) in mortar mixes with water to binder ratio of 0.4. Improvement in compressive strength of cement mortars was observed at all ages when CKD was used as a partial replacement of PC or as an activator for CS but when using lime as an activator or CS alone, cement mortars showed reduced strength. Maslehuddin et al. (2009) and El-Sayed et al. (1991) investigated the corrosion behavior of embedded reinforcement and compressive strength of cement paste containing CKD. It was observed that replacement of cement with 5% CKD by weight showed no significant reduction on reinforcement passivity and in the strength of the cement paste. Similar findings were also observed by Batis et al. (2002) when CKD and blast furnace slag were added in proper ratio in ordinary Portland cement. This indicated that CKD can be used as a potential material for replacement of Portland cement in mortar and concrete.

Maslehuddin et al. (2009) studied the compressive strength of concrete containing containing 0, 5, 10, and 15% CKD as partial replacement to Portland cement type I and type V after 3, 7, 14, 28, 56, and 90 days of curing period. Compared to control mixes where PC type I and type V alone were used, a decrease in compressive strength (>5%) was observed at all ages in mixes containing 10 and 15 CKD. The authors concluded that up to 5% CKD could be used without compromising the compressive strength of concrete. El-Aleem et al. (2005) studied the compressive strength of mortars containing CKD at 0 2, 4, 6, 8, and 10% Portland cement replacement at the curing period of 3, 7, 28, and 90 days. It was observed that up to 6% CKD replacement compressive strength was slightly decreases where as above 6%, sharp decrease in compressive strength was observed. Thus, the authors concluded that CKD up to 6% by weight of cement can successfully be used in hardened mortar without compromising the strength. These results were in concomitant with the findings of Shoaib et al. (2000).

Udoeyo and Hyee (2002) studied the effect of CKD (20 - 100% replacement with 20% intervals) as PC replacement on the compressive strength of concrete at the curing ages of 1, 3, 7, and 28 days with w/b ratio of 0.65. The authors reported that at high replacement levels of PC the strength decreased with an increase in CKD content. Wang et al. (2002) also studied the compressive strength of mortars having the w/b ratio of 0.50 containing CKD at partial replacement levels (0, 15 and 25%) of PC at the curing age of 28 days. The authors found that the compressive strength of mortar containing 15% CKD content was increased from 46.3 MPa to 47.8 MPa compared to cement alone which is due to an appropriate alkalinity that increases the dissolution of silicate species and increased formation of hydration products such as calcium silicate hydrates (CSH). Above this percentage (25% CKD) decrease in compressive strength (39.4 MPa) was observed due to the low pozzolanic property of CKD. Batis et al. (1996) tested the 90 day compressive strength of concrete containing 6% CKD (CKD A and B) at the w/b ratio of 0.65 and 0.75. At lower w/b ratio (0.65) the compressive strength of the specimens with CKD A was the same as the plain cement specimen while 35% reduction in compressive strength of concrete at higher w/b ratio (0.75) was observed with CKD A compared to CKD B concrete and plain cement specimens. It was also observed that replacement of PC up to 6% CKD reduced the porosity of concrete by 4% compared to the control and exhibited good performance.

Daous (2004) observed compressive and tensile strength properties of the cement blends containing waste materials such as fly ash, CKD in different ratios. It was found that blends containing 90% cement and 4% fly ash showed satisfactory mechanical strength of 94% of strength of ordinary Portland cement whereas blends containing 30% CKD only showed 80% of compression strength of Portland cement. Al-Harthy et al. (2003) observed that with increase in CKD content (0-30% replacement of cement with 5% intervals) the compressive strength of concrete decreased at all curing ages of 3, 7 and 28 days with w/b ratio of 0.50-0.70. It was found that with increasing w/b ratio the strength decreased maximum in 0.70 followed by 0.60 and 0.50 at 28 days of curing. Up to 8% and 13% reduction in strength was observed in concrete mixes containing5 and 10% CKD at w/b ratio of 0.70, whereas, for 0.60 and 0.50 w/b ratio this decrease was 12 and 1.8% for 5% CKD and 18 and 4.5% for 10% CKD substitution levels, respectively. At maximum level (30%) of CKD substitution the reductions in strengths were 31, 29, and 22%, at w/b ratios of 0.70, 0.60, and 0.50, respectively.

CKD acts as an excellent activator for non pozzolanic materials due to high alkali and sulfate content. Konsta-Gdoutos and Shah (2003) used CKD for ground granulated blast furnace slag (GGBFS) as an activator to create non-conventional cementitious binders for concrete. Compressive strength of cylindrical mortars (75×150 mm size) containing CKD-slag blends (GGBFS and CKD E, P, A, X) was determined at the ages of 7, 28, and 56 days. Compressive strength was increased with increase in curing time for all the CKD–slag blends compared to cement-slag blends indicating slag activation and the formation, precipitation, and accumulation of CSH as products of hydration. Higher compressive strength was observed in CKD (A)-slag blends at the age of 56 due to the activation of slag by CKD (A) as it was fine and uniform in size which provided higher specific surface and promoted intense pozzolanic reaction. Shi (2001) also observed similar results and concluded that the increased strength of the blends with curing time was due to the availability of Ca^{2+} ions provided by the free lime content.

Wang et al. (2004 and 2007) investigated the strength development of non-clinker cements made with 50% CKD and 50% fly ash (FA) using NaOH (addition of 2 and 5%) and elevated curing temperatures of 38 and 50 °C as different activation methods. The results concluded that at early ages all activation methods significantly improved the binder strength than at the later ages. These results were confirmed by the findings of Gebauer (1981) where no improvement in the strength of CKD-FA pastes observed with increasing curing temperature. This may be due to the effect of hydration temperature on the microstructure of binder pastes (Kjellsen et al., 1992; Verbeck and Helmuth, 1968). Bhatty (1984a; b; c; 1986) also observed that the addition of FA into a CKD–OPC system lowered the alkali content and resulted in improved strength.

2.2.4.2 Tensile and flexural strength

Al-Harthy et al. (2003) studied the flexural strength of concrete containing CKD as partial replacement of cement (0, 5, 10, 15, 20, 25 and 30% w/w) at the ages of 3, 7, and 28 days with w/b ratios of 0.50, 0.60 and 0.70. Control mix (0% CKD) showed flexural strengths in the range of 4.70 to 3.80 MPa with all water-to-binder ratios whereas with increase in CKD replacement for cement flexural strength and toughness decreases. No-significant decrease in flexural strength was observed at 5 and 10% cement replacement levels and this was due to reduction in the cement content as the amount of CKD increased in the concrete.

Wang et al. (2002) found increase in flexural strength of mortar from 8.2 MPa to 8.5 MPa up to 15% CKD replacement of cement compared to cement alone which drastically reduced as the CKD content increases. The increase in strength may be due to an appropriate alkalinity resulted in increased formation of calcium silicate hydrate (CSH) and decrease in pore size. Shoaib et al. (2000) investigated the effect of cement kiln dust on splitting tensile strength of concrete partial to replacement of cement (0, 10, 20, 30, and 40% w/w) at the ages of 1, 3 and 6 months with water-to binder ratio of 0.50. Gradual decrease in splitting tensile strength due to poor bond strength between aggregate and cement was observed in all the concrete mixes as the amount of CKD increases. Similar results were also observed by Udoeyo and Hyee (2002) while investigating the split tensile strength and modulus of rupture effects of replacing cement with CKD at 20, 40, 60, 80 and 100% replacements in concrete.

2.2.4.3 Hydration

Several authors were investigated the hydration properties of CKD and Portland cement. Taylor (1997) reported that chemical and hydrothermal activations in class F fly ash (FA) are often used to increase the hydration (pozzolanic reaction) of fly ash blended cements. At low temperature, class F FA reacts slowly due to low chemical reaction which caused the high degree of polymerization of tetrahedral silicate with oxygen. This study indicates that the blends of CKD and fly ash may create a cementitious material for proper utilization in cement and concrete, and this was due to activation of hydration in fly ash by alkalis from CKD.

Amin et al. (1995) also studied the effect of calcinate temperature and kiln dust content on the hydration and observed increased activation of slag with increasing calcination temperature of CKD. El-Didamony et al. (1997) reported that calcination of kiln dust at 1300 °C with 15% anhydrite is suitable for the production of super sulfated cement. Bhatty (1984a; b; c; 1986) used class C and F fly ash, five different types CKD's, slag for the production of binary, ternary and quaternary mixes using ordinary Portland cement (OPC) and observed reduced workability, setting time and strength of CKD blended cements.

Dyer et al. (1999) also reported that high alkali and sulfate content in CKD makes it an excellent activator for pozzolanic materials. Ternary blends with two types of CKD, pulverized fuel ash (PFA) and OPC were prepared and observed accelerated binder hydration in blends due to CKD. Konsta-Gdoutos and Shah (2003) used CKD as an activator for GGBFS in concrete and studied the effect of CKD on heat of hydration. Four different types of CKDs ('E', 'P', 'A' and 'X') and observed that CKD 'A' was the finest particle with specific surface of 8270 cm^2/g and a mean size of 9 µm. The mean particle size of CKD 'P' and 'X' were in the range between 1 and 45 µm whereas of CKD 'E' was 60 µm and found the coarsest one The heat of hydration of CKD slag pastes was measured by DSS Q drum colorimeter whereas the phases present in hydration products were identified by X-ray diffraction (XRD) technique . XRD results indicated that carbonate particles were inert and they did not participate in hydration reaction. Peaks of $Ca(OH)_2$ were not observed after 28 days of hydration as whole of available lime was consumed. Slag blends containing 50% mixture of CKD 'E' and 'P' showed similar heat evaluation curve to that of the Portland cement–slag blends.

Shi et al. (2002) studied the degree of hydration of the cement pastes blended with CKD and fly ash (FA) by X-ray diffraction (XRD) and thermo gravimetric analysis (TGA) and found ettringite as the major crystalline hydration product of binders. Wang et al. (2004) investigated the hydration of CKD-fly ash paste cured at 24, 38 and 50 °C with 0, 2 and 5% of NaOH additions. They observed that a major crystalline hydration product of CKD-FA binder was ettringite. Presence of high sulfate and aluminate content formed the ettringite in a CKD-FA system that was quite stable even at 120 days. Addition of alkali (NaOH) formed ettringites at low temperature of curing whereas when temperature increased from 38 to 50 °C reduction in ettringite formation was observed. It was concluded that addition of 2% NaOH with CKD-FA at

50% significantly improves the strength of CKD-FA binder cured at elevated temperature (38 °C).

Taylor (1997) studies the progression of hydration using isothermal conduction calorimetry by monitoring the rate of heat liberation from the cement paste. It was indicated that hydration process takes place in a sequence of stages as (1) initial reaction, (2) induction, (3) acceleration, (4) deceleration, and (5) slow continued reaction. During hydration, C_3S and C_2S in Portland cement react with water to form calcium silicate hydrate (CSH) and calcium hydroxide (CH). The C_3S hydration occurs more rapidly than C_2S hydration and provides most of the strength developed by Portland cement in early ages while C_2S contributes mostly to the later age strength (Gartner et al., 2002). Nonat (1994) observed that lime is playing an important role in the initial hydration and is the most important parameter during C_3S hydration.

2.2.4.4 Setting time

Maslehuddin et al. (2008) observed that CKD replacement with Portland cement (PC) at 0, 5 and 10% by mass increases the water demand thus, decreases the setting time and concluded that decrease of both initial and final setting times is almost linear as function of CKD replacement (EI-Aleem et al., 2005). Maslehuddin et al. (2008) and EI-Aleem et al. (2005) observed that with 10% CKD replacement of PC initial set time decreased to 65 min from 135 min (control without CKD) whereas the final set time decreased approx. to 110 min from 230 min. This was due to the high amounts of lime and alkalis in CKD which increases the water demand and retards the setting time.

Ramakrishnan (1986) studied concrete properties made with 5% cement kiln dust (CKD) with 95% by weight of regular Type I cement and found delayed setting times of cement and fresh concrete properties. Heikal et al. (2002) investigated setting times, electrical conductivity and fluidity of blends of slag cement, having composition of 70/30, 50/50, and 30/70% by mass of, which then was mixed with 2.5, 5.0, 7.5 and 10% by mass of CKD. It was observed with the addition of 2.5% CKD by mass to mixes 70/30 and 50/50 Portland cement clinker and granulated slag (setting time was reduced whereas in mix 30/70 PC slag with 2.5% CKD, increased final setting time was reported. Konsta-Gdoutos and Shah (2003) also evaluated the initial setting time of CKD–slag pastes and observed shorter setting time for the blend with CKD (A) having high amount of sulfate (17%), due to the formation of ettringite and also in ‘E’, ‘P’ and ‘X’ type CKDs.

Bhatty (1984a; b; c; 1986) published a series of reports on the addition of CKD, PC along with FA and slag with variable results and reported reduced strength, setting time and workability in blends containing CKD alone due to the presence of high alkali content. The addition of slag and fly ash with CKD lowered the alkali content and resulted in improved strength and setting. Daous (2004) reported increased initial setting time of the blends higher percentage of waste materials in the cement blend. Blend containing 70% of Portland cement exhibited 220% increase in setting time compared to specimens prepared with 100% cement.

Udoeyo and Hyee (2002) stated that the value of the initial and final setting time were within the relevant BS and ASTM standards, but did not suggest possible mechanisms for the increased setting time. Bhatty (1984b) stated that time of initial set was always shorter compared to cement for any blends containing 10% CKD. In case if CKD characterized by high sulfate, high free lime and low chloride (0.26%) contents then 20% blend with CKD have shorter time of initial set.

2.2.4.5 Durability of concrete containing CKD

Relative dynamic modulus, absorption coefficient and durability factor methods along with air contents, weight loss, resonant transverse frequency, chloride permeability, freezing and thawing, and pulse velocity were employed to investigated the durability behavior of the concretes. Ramakrishnan and Balaguru (1987) studied freezing and thawing durability of concretes containing 5% CKD as replacement for three types of cements (Type I, II, and III). It was observed that the air contents of the CKD-concrete mixtures ranged from 3.1% to 8.4% and concluded that 5% CKD replacement of cement does not affect the freeze–thaw durability of concrete. Wang and Ramakrishnan (1990) reported that concrete specimens with 5% CKD showed resistance to freeze-thaw with little loss in mass where as significantly less resistance to freeze-thaw was observed by Batis et al. (1996) in specimens containing 6% CKD compared to control specimens

Bhatty (1984c) investigated the effect of three different CKDs with Type I cement on alkali aggregate reaction in mortars. Lower compressive strengths were achieved in CKD concrete than Portland cement concretes due to varying amounts of alkalis, sulfates, and chlorides in CKD, which may lead to durability problems because of the deterioration caused by alkali-aggregate reactions. Bhatty (1986) and Ramakrishnan (1986) found that the loss in durability may be overcome by blending pozzolanic material such as fly ash with CKD (Bhatty, 1986). Maslehuddin et al. (2009) observed significant decrease in electrical resisitivity in PC Type I and Type IV - concrete mixes containing 15% CKD. Increase in chloride content with increasing CKD content in concrete mix was observed which leads to decrease in electrical resistivity and significantly accelerates the corrosion process. Durability can also be measured by determining the permeability of the mortar and concrete mixtures.

Al-Harthy et al. (2003) used the initial surface absorption test (ISAT) and sorptivity to measure the permeability characteristics of CKD mortar samples. It was observed that incorporation of CKD decreased the sorptivity of the mortar i.e. improved the absorption properties, decreased the permeability and, thereby, enhanced the durability and corrosion resistance. Konsta-Gdoutos et al. (2001) observed that 15% CKD-PC blends accelerates corrosion due to the introduction of chloride ions in the mix by CKD. Batis et al. (1996) tested steel corrosion of CKD concrete and reported accelerated corrosion rate whereas fineness and relatively higher alkalinity acts as barrier for corrosion. El-Sayed et al. (1991) observed that addition of 5% CKD in cement mortars provides corrosion protection from aggressive sulfate and chloride ions in the mix to the high hydroxide (OH^-) content that develops during hydration as a result of the CKD. This OH^- content helped in maintaining the passive oxide layer that protected the steel.

The mechanical properties and the durability of mortar and concrete are largely depends upon the the chemistry of the cement concrete system. Extensive studies are still needed to completely understand the variability of CKD chemistry, its physical and chemical parameters and its effects on mortar and concrete properties that will provide acceptable performance.

2.3 UTILIZATION OF CKD FOR REDUCING CARBON DIOXIDE EMISSION

Concrete is the most economical and durable construction material in the world. Cement, a main constituent of concrete, plays an important role due to its binding properties. Accounting for over 90% of total energy use and virtually all of the fuel use, clinker production considered as the most energy-intensive stage in cement production,. Cement manufacturing process contributes approximately 5% of global carbon emissions and out of which roughly half of the CO_2 is emitted during calcinations process while the remaining from energy usage during the production process (Hendriks et al., 2000; USEPA, 2004). Malhotra (2004) reported that approximately one ton of green-house gases (GHGs) are generated during production of one ton of Portland cement that requires 1.6 tons of raw materials (limestone and clay).

Cement and concrete industries estimated to require about 850 to 1600 kcal/kg heat energy for different processes of cement production (CPAI, 2004) and is considered the third energy consumer after aluminium and steel industry (Nehdi, 2001). . It is estimated that for one ton of cement production approximately 111 kWh of electricity and 768,544 BTU (British Thermal Units) of energy is required (IEA, 2007; Wilson, 2011). As a result, natural resources and energies (fuel or electrical) are depleting gradually and is great warning for us as well as the future generation for the preservation of these. Therefore, it is the crucial time to find an alternative way of concrete production or to search another sustainable binder (such as CKD, silica fume, etc.) that can be used as cement supplement for the production of sustainable concrete. Utilization of cement kiln dust in cement and concrete manufacturing could be another solution. Consumption of CKD and other waste materials in cement replacement and concrete production could reduce the energy demand as well as CO_2 emission rate by decreasing the demand and production of cement (Malhotra, 2004).

The amount of clinker needed to produce a given amount of cement can be reduced by the use of supplementary cementitious materials (SCMs) such as cement kiln dust, coal fly ash, slag, and natural pozzolans like rice husk ash and volcanic ashes (Neuwald, 2004). The addition of these SCMs into concrete generates blended concrete which not only reduces the amount of material landfilled but also. Supplementary cementing material (SCM) is one of the most sustainable construction materials because when blended with concrete it reduces Portland cement content in concrete, improves durability of concrete, avoids disposal of industrial byproducts; and, reduces the amount of clinker required for cement production, decreased emission of greenhouse gases and use of natural raw materials (Karim et al., 2011).

This may offer reduction in environmental impacts and material costs of construction. Mihelcic (2007) observed that the use of natural pozzolans could save contractors up to 25% per bag of cement, which can provide an economic benefit to the building of new infrastructure. Bremner (2001) suggested that by replacing only 18.5% of the cement with slag or fly ash per year can reduce up to 300 million tons of CO_2 in the world. Replacing 15% of cement worldwide by other SCMs will reduce CO_2 emissions by 250 million tons while replacing 50% of cement by other SCMs will reduce CO_2 emissions by 800 million tons. This is equal to removing ¼ of all automobiles in the world (Malhotra, 2004). The use of blended cements is a particularly attractive efficiency option since the intergrinding of clinker with other additives not only allows for a reduction in the energy used (and reduced GHG emissions) in clinker production, but also directly corresponds to a reduction in carbon dioxide emissions in calcinations as well (Worrell and Galtisky, 2004). Furthermore, for the concerns in concrete construction we all have the responsibilities to preserve our natural resources and reduce the CO_2 emission by utilizing more wastes in cement and concrete manufacturing to achieve the sustainable concrete as well as sustainable development.

2.4 LEACHATE ANALYSIS OF CKD

Leaching represents the source term for release of potentially hazardous substances, both dissolved and suspended materials, when landfilled waste comes in contact with water. Leaching of waste generates leachate and its composition varies widely depending upon the age and the waste contained landfill material. The risks of leachate generation can be mitigated by properly designed and engineered landfill sites to minimize the risk of solid wastes on human health and the environment.

Cement kiln dust is not considered to be a hazardous material under the Environmental Protection Agency (EPA)'s Resource Conversation and Recovery Act (RCRA) regulations but the leachate generated from landfilled CKD and waste piles may possess hazardous characteristics. In order for CKD to remain a safe material, it has to be handled properly to prevent environmental contamination and the toxicity of cement kiln dust must be determined on a case-by-case basis (Haynes and Kramer, 1982). The composition of CKD depends upon the raw materials and kiln processes from which it is generated. EPA (1993) reported aluminum, calcium, iron, magnesium, potassium, silicon, sodium, titanium, manganese, sulfur, and chloride as bulk constituents found in CKD . CKD is mainly composed of oxides, alumino-silicates, sulfates and chlorides such as lime (CaO), arcanite (K_2SO_4) and sylvite (KCl) that are unstable or highly soluble at earth surface conditions. These phases will either dissolve completely or more stable when CKD contacts water, and less soluble secondary phases precipitates (Eary et al., 1990).

Representative ranges and median trace element concentration for CKD are presented in Table 2.1. Lower concentrations of arsenic, barium, chromium, nickel and vanadium in CKD were observed whereas concentration of cadmium, lead, selenium, and silver concentrations were higher. EPA’s review of results from Toxicity Characteristic Leaching Procedure (TCLP) analyses on CKD samples concluded that trace metal constituents rarely exceed RCRA toxicity limits (EPA, 1995). CKD wastes are generally alkaline and have very low concentrations of organic compounds. The pH values for CKD leachate range from 6.11 to 12.98 standard units (EPA, 1993).

Test results were analyzed by Siemens D5000 X-ray diffractometer and concluded that CKD leachate contained extremely high concentrations of leachable potassium, sulfate, caustic alkalinity and Cr and Mo. Even for the high dilution leach test (20:1, water/CKD), Cr concentrations were more than 40 times EPA’s maximum contaminant level (MCL) value of 0.05 mg/L. CKD leach results at different water–solid ratios provided evidence that there was no solubility control for the concentrations of Na, Cl, Cr, Mo, Se, and in most instances, K in solution while evidence for solubility control was found for Si, Ca, SO_4, Mg, Al, Zn, Ti, Sr, and Ba concentrations.

The Portland Cement Association (PCA, 1992) analyzed metal contaminants in cement kiln dust (79 plants in the United States and 10 in Canada) using the EPA’s toxicity characteristic leaching procedure (TCLP). CKD samples were tested for concentrations of trace metals including mercury, selenium, thallium, cadmium, lead, antimony, silver, arsenic, nickel, barium, beryllium, and chromium and observed that CKD from only two plants had concentrations of any materials over an acceptable limit. Duchesne and Reardon (1998) observed high concentrations of potassium, sulfate and caustic alkalies in leachate when CKD was brought in contact with water. Series of batch leaching experiments were performed by reacting samples of CKD at water:solid ratios of 3:1, 6:1, and 20:1 in 60 mL high density polyethylene containers.

Table 2.1: Trace metal concentration in CKD

Element[#]	CKD (EPA 1994)		PCA Survey[a]		PCA Report (1992)		Haynes and Kramer (1982)	
	Range	Median	Range	Median	Range	Median	Range	Median
Antimony	1.77-2.72	6.2	0.53	0.53	0.083-3.43	0.21	0.701-70	0.83
Arsenic	2.1-20.3	4.9	3.7-53	46.2	1.323-159	9.07	1.3-518	10.0
Barium	11-779	103	150	150	35-1402	133	-	-
Beryllium	0.158-1.6	0.59	0.509-0.523	0.521	0.032-3.54	0.539	-	-
Cadmium	0.89-80.7	4.6	3.0-12.1	9.05	0.008-59.6	3.27	0.687-352	7.6
Chromium	11.5-81.7	18.1	32.7-49	35.2	8.25-293	29.1	11-172	35.0
Lead	5.1-1490	287	151-270	21.	33.5-7390	188	11.335-1750	148
Mercury	0.005-14.4	0.11	0.100-0.107	0.106	1-60	14	-	-
Nickel	6.9-39	15.9	10-23.8	21.1	0.001-25.5	0.045	5.421-91	16.0
Selenium	2.5-109	11.3	6.5	6.5	0.227-0.307	7.23	-	-
Silver	1.1-22.6	3.7	0.504	0.504	3.549-40.7	9.28	1.291-17	4.7
Thallium	0.99-108	3.5	4.616	4.616	0.109-776	8.96	-	-
Vanadium	6.6-204	25.9	23-39.2	38.3	-	-	-	-

[a] Data from the 1991 PCA Survey of U.S. cement plants; [#] All values are in mg/L

2.5 LEACHATE TEST METHOD

The leaching behavior of all types of materials is related to several critical factors, including specific element solubility and availability or release potential. The leachant methods vary in the amount and particle size of leached sample, the type and volume of leachant solution, the leachant delivery method, and time. Leaching methods are categorized in number of ways. The first deals with whether the leaching fluid is a single addition (static extraction tests) or is renewed (dynamic tests). Methods can also be classified as (1) column or flow through systems, (2) bulk and flow around test systems for monolithic samples, and (3) batch leaching in which the sample is placed in a given volume of leachant solution. Results are generally reported as a concentration, sometimes as the concentration in the leachant solution (mg/L) or as the leached concentration from the solid (mg/kg).

2.5.1. Column Methods

Column or lysimeter test has been used for simulation of leaching from waste. This test is performed on granular material, with the leachant continuously or intermittently flowing through the material, to measure contaminant leaching under advective conditions. The flow of the leaching solution may be in either down-flow or up-flow direction, and continuous or intermittent. A basic assumption in column leaching is that the distribution of the leaching solution is uniform and that all particles are exposed equally to the leachate solution under continuous flux. Therefore, this test is also called as dynamic test and may be more representative of field conditions. Typical column test includes the ASTM Column Extraction Method (ASTM D4874-95, 2002), NEN 7343 (NNI, 1995a) and the Nord test Column Method (NORD TEST, 1995) and the European Standard Column Test (prEN 14405, 2002).

2.5.2. Bulk and Flow around Test Methods

Bulk leaching generally refers to leaching large samples, either in a large column or in heaps. They are either hydro-metallurgical systems or are used in a research setting to leach a non homogeneous sample with a large particle size (Dalverny et al., 1996). Flow around tests systems are generally performed on monolithic samples (massive solid, such as cement), and generally used to characterize the release of pollutants (leachate) from stabilized waste materials.

Typical bulk and flow around test systems include the ANSI/ANS 16.1 test (ANS, 1986), NEN 7345 (NNI, 1995b) and the tank leaching test Dutch NVN 5432 (Groot and van der Sloot, 1992).

2.5.3. Batch Methods

Several batch leaching methods have been developed to simulate the leaching processes of waste materials in landfill. The most commonly used batch leaching methods are the Toxicity Characteristic Leaching Procedure (TCLP), Synthetic Precipitation Leaching Procedure (SPLP), Standard Test Method for Shake Extraction of Solid Waste with Water (ASTM D3987, 2006) and Waste Extraction Test (WET; California Code of Regulations, 1985).

2.5.3.1 Toxicity characteristic leaching procedure (TCLP). The TCLP test is the USEPA (2003a) commonly used standard batch leaching procedure for determining the leaching characteristics of hazardous waste. This test was designed by USEPA as a rapid regulatory compliance test for determining whether a waste is suitable for disposal in a landfill with municipal waste and to simulate leaching in a sanitary landfill under co-disposal conditions. Many state regulatory agencies have adopted the TCLP for characterization of solid wastes which are not federally regulated. The extracts generated by TCLP were analyzed by ICP-OES (Inductive Coupled Plasma Optical Emission Spectroscopy) and ICP-MS (Inductive Coupled Plasma Mass Spectroscopy) (Douglas, 2003).

2.5.3.2 Synthetic precipitation leaching procedure (SPLP). This method was developed as an alternative to the TCLP by USEPA (2003b) and designed by Hageman and Briggs (2000) to evaluate the impact of contaminated soils on groundwater. The extraction fluid consists of slightly acidified de-ionized water that is formulated to simulate natural precipitation. The SPLP extraction method studied by Hageman and Briggs (2000) was meant only for 100% solids.

2.5.3.3 Synthetic ground-water leaching procedure (SGLP). The Synthetic Groundwater Leaching Procedure test is a draft of ASTM procedure for long-term leaching (Hassett et al., 2005). In this procedure, leachate having pH of 11.9 was decanted and filtered through membrane filters (Millipore GS of 0.22 µm). Finally, aliquots of filtered solution with 1% HNO_3 (v/v) and 10 mg/mL of indium as the internal standard were prepared.

2.5.3.4 Shake extraction method of solid waste with water. This method (ASTM D3987-06, 2006) is a standard compliance-type test first issued in 1981 and last revised in 2006. The intention of the test is to provide a rapid extraction procedure for industry, but not to simulate site-specific conditions (van der Sloot et al., 1997).

2.5.3.5 Waste Extraction Test (WET). The WET test is similar to the TCLP in that it uses a buffered organic acid solution as the extraction fluid. This test uses a pH buffered citrate acid solution with sodium hydroxide (NaOH), a 10:1 L/S ratio, and a 48 h testing period. The WET extraction solution is prepared with a combination of 0.2 M citric acid solution and 4.0 N NaOH to pH 5.0 ± 0.1. One liter of this solution is added to a 100 g sample and rotated for 48 hours. After rotation, the final pH is measured, and the sample is filtered and analyzed.

2.5.3.6 The National Rivers Authority (NRA) leaching test. This method was developed and recommended by the National Rivers Authority for the purposes of general assessment of the leachability of mainly inorganic contaminants from contaminated land in the UK (Lewin et al., 1994). This method was developed as an alternative to more aggressive tests such as the TCLP.

In serial batch methods, a sample of waste is leached successively with fresh aliquots of the same leaching fluid. This method is intended to eliminate the effect of concentration on solubility and to simulate long-term exposure to the leachant solution. These methods include EPA’s Multiple Extraction Procedure (MEP, 1986), the Standard Test Method for Sequential Batch Extraction of Waste with Acidic Extraction Fluid (ASTM D 5284, 1999), and the Standard Test Method for Sequential Batch Extraction of Waste with Water (ASTM D 4793, 1999).

Sequential leaching tests use a single sample that is leached by a series of different leaching fluids. The Availability Test for Granular Materials (NEN 7341, 1993), a Dutch Standard leaching test, leaches a material at pH 4 and at pH 8; the two pH’s are intended to bracket the range found naturally in the environment. A three-step sequential extraction procedure (Quevauviller et al., 1997) developed by the commission of the European Communities Bureau of Reference has also been modified to include aqua regia digestion of the residual material (Raksasataya et al., 1996). A Short Sequential Procedure uses two steps to assess the lability of heavy metals in soil particles (Maiz et al., 2000).

2.6 BIOLEACHING

Landfill leachate is a combination of physical, chemical, and microbial processes in the waste generated from the waste material by transferring the pollutants to the percolating water (Christensen and Kjeldsen, 1989). The risk of groundwater pollution is probably the most severe environmental impact from landfills because historically most landfills were built without engineered liners and leachate collection systems. Decomposition of landfilled waste is a complex series of biological and chemical reactions occurs in four phases such as an initial aerobic phase, anaerobic acid phase, initial methanogenic phase, and a stable methanogenic phase.

Aiyathiti et al. (2009) analyzed the composition of cement kiln dust by using energy dispersive X-ray fluorescence (EDXRF) technique and reported that Si, Ca, Ti, K, Al and Zn were the prominent elements present in CKD while Ba, S, Cl, Fe, P, Ni, Cu, Mn, Sr, Zr, Br, As, Tl, Pb, Rb, Cd and Y were present as minor components. CKD contains insignificant amount of trace metals and are not usually a concern for most applications. PCA (1992) evaluated for the presence of trace metals such as antimony, arsenic, barium, beryllium, cadmium, chromium, lead, mercury, nickel, selenium, silver and thallium in CKD from 79 cement plants from United States and 10 plants from Canada found that average levels of trace metals were significantly below the regulation limits. However, this does not mean that cement kiln dust does not contain anything that could pose a hazard to the environment. Number of factors that affect the level of contaminants in cement kiln dust includes, raw material content, concentration of alkalis and sulfates, waste fuels and recirculation of dust into kiln.

Guo and Eckert (1996) found that cement kilns co-fired with hazardous wastes contains 68, 10, 72, 18 and 68 times higher concentration of arsenic, beryllium, cadmium, chromium and lead in cement and CKD compared with kilns co-fired with non-hazardous wastes. Naik et al. (2001) conducted ASTM (ASTM D 3987) leach method on various samples to study the leachability of controlled low strength material (CLSM) mixtures because it simulates leaching of substances in CLSM that can occur through permeation of water under field conditions. , Leachate may cause harmful effects on the ground and surface water surrounding the landfill site if it does not returned to the environment in a carefully controlled manner. Christensen et al. (2001) reviewed the characteristics of leachate plumes down gradient of landfills. Surface water pollution caused by leachate has also been observed, although relatively few cases have been described in the literature. The major potential effects of a leachate release to surface water are expected to be oxygen depletion in part of the surface water body, changes in the stream bottom fauna and flora and ammonia toxicity. During the stabilization of landfilled waste, most organic materials and metal compounds are converted into simpler compounds or water soluble forms by aerobic and anaerobic microorganisms, leading to the formation of landfill gas and leachate termed as bioleaching.

Bioleaching methods are being considered as economical over conventional methods for metal recovery from low-grade ores and concentrate, thus reduces the demand for resources such as ores, energy or landfill space (Krebs et al., 1977). The leaching effectiveness depends largely and on the chemical and mineralogical composition of the solid materials to be leached and efficiency of the microorganisms. Some important factors that affect the bioleaching process are nutrient culture medium, pH, temperature, mineral substrate, pulp density and metal resistance of the microorganisms (Bosecker, 1997).

2.6.1 Microorganisms in Bioleaching

Four classes of microorganisms are involved in bioleaching; these include chemolithoautotrophic bacteria, heterotrophic bacteria, chemolithoautotrophic archea and heterotrophic fungi (Table 2.2). These microorganisms are capable of growing in the presence of toxic metals present in solid waste and extracting the metals therein by the formation of organic or inorganic acids (acidolysis), excretion of complexing agents (complexolysis) and oxidation and reduction reactions (redoxolysis). Among the chemolithoautotrophic bacteria, the two most commonly used species are *Thiobacillus thiooxidans* and *Thiobacillus ferrooxidans*, which use carbon dioxide from the atmosphere as their carbon source and derive energy from the oxidation of reduced or partially reduced sulfur compounds. Sulfuric acid is the main inorganic acid. A series of inorganic acids (sulfuric acid) and organic acids (formed by bacterial and fungal metabolism) are found in leaching environments resulting in acidolysis, complex and chelate formation (Berthelin, 1983).

Table 2.2: Examples of microorganisms used in bioleaching processes (Krebs et al., 1997)

Example of organisms	Main leaching agent	pH range	Temperature range (^oC)
Chemolithoautotrophic bacteria			
Thiobacillus ferrooxidans	Ferric ion, sulphuric acid	1.4-6.0	28-35
Thiobacillus thiooxidans	Sulphuric acid	0.5-6.0	10-37
Leptospirillum ferrooxidans	Ferric ion	2.5-3.0	30-37
Heterotrophic bacteria			
Bacillus megaterium	Citrate, amino acids	-	-
Pseudomonas putida	Citrate, gluconate	-	-
Chemolithotrophic archaea			
Sulfobacillus themosulfidooxidans	Ferric ion, sulphuric acid	Extremely acidophilic	50
Sulfolobus acidocaladarius	Ferric ion, sulphuric acid	0.9-5.8	55-85
Heterotrophic fungi			
Aspegillus niger	Citrate gluconate, oxalate	-	-
Penicillium simplicissimum	Citrate gluconate, oxalate	-	-
Rhizopus sp.	Lactate, fumarate, gluconate	-	-

Shen et al. (2003) carried out an investigation on bioleaching of trivalent chromium (Cr^{3+}) and other metals from tannery sludge by sulphur-oxidizing bacteria. It was observed that with sludge solid concentration of 40 g/L at pH 2, 73% Al, 87% Cr^{3+}, 72% Fe, 62% Mg and 73% Zn was leached. For efficient bioleaching, an attempt was made for optimization of the ferrous sulphate concentration as well as sludge solid concentration by Pathak et al. (2009) and observed that using 10 g/L ferrous sulphate, indigenous iron-oxidizing bacteria can bring down pH to a value needed for significant solubilization of metals.

Sukla et al. (1995) carried out bioleaching of copper converter slag using strain of fungus (*Aspergillus niger*) to determine the level of leaching and role of acids (oxalic, acetic & succinic acid) in leaching out of metals. The maximum leaching of Cu, Co and Ni was found to be 47, 50 & 23% respectively and succinic acid was better in leaching out of metals than oxalic and acetic acid. Leaching of Cu, Co & Ni from copper converter slag by using *Thiobacillus ferrooxidans* (Mehta et al., 1999) and *Aspergillus niger* (Sukla et al., 1992) were also assessed and concluded that the microorganisms oxidized the iron available in the solution which accelerated the dissolution of the valuable metals by indirect mechanism. Several researches have assessed the bioleaching of Nickel (Pogaku and Kodali, 2006), copper (Duncan and Teather, 1966; McElroy and Duncan, 1974; Urgya et al., 2004), sulphide mineral concentrates (Sharp et al., 2001) from ores, zinc and aluminium from industrial waste sludge (Solisio et al., 2002), cobalt and arsenic from mine (Urgya et al., 2004) by using *Thiobacillus ferrooxidans*. Salo-Zieman et al. (2005) studied the bioleaching of nickel from nickel ore by using co-culture of *Acidithiobacillus ferrooxidans* and *A. thiooxidans* as well as *Sulfolobus* and observed that 86-100% of nickel was recovered. Dorbot and Lechaveliar (1981) studied the recovery of metals in water insoluble form from industrial waste water by using fungus *Cladosporium*, *Penicillium*, *Trichoderma*, Black mycelium and Aureobasidium. Among the filamentous fungi, the genera of *Aspergillus* and *Penicillium* have been reported to have a high ability to accumulate heavy metals and radionuclides (bioaccumulation) from their external environment (Volesky, 1991).

Over the past decade, the potential of metal biosorption by microbial biomass has been well established. Biosorption is the sorption (accumulation) and /or complexation of dissolved metals by the activity of microorganisms or microbial biomass. It provides an alternative for the potentially economically attractive treatment for the metal removal and recovery particularly suited for industries generating wastes containing heavy metals. Among toxic substances reaching hazardous levels are heavy metals which include lead, mercury, chromium, arsenic, nickel, selenium, zinc, cadmium, silver, gold and uranium (Ahalya et al., 2003). Heavy metal pollution in the ground water and in aquatic system has become a serious threat today and of great environmental concern as they are non-biodegradable and thus persistent which cause toxicity to humans and other life forms. Biosorption is the economical and efficient method alternative to the conventional waste treatment methods, to remove the toxic heavy metals from wastes before their disposal.

Biosorption, using biomaterials such as fungi, bacteria, yeast and algae is regarded as cost effective treatment for heavy metal removal is either specific or non-specific for certain types of metals (Hosea et al., 1986; Volesky and Kuyucak, 1988). The metal-binding or uptake capacity of several microbes like fungi (*Rhizopus*, *Absidia*, *Penicillium*, *Aspergillus*, *Trichoderma* etc), bacteria (*Bacillus*, *E. coli*, *Pseudomonas* etc.), brown algae (*Sargassum*, *Ascophyllum*, *Vaucheria* etc.) and yeast (*Saccharomyces*, *Candida*, *Streptomyces*, *Pichia* and *Kluyveromyces*) has been discussed in detail by Volesky and Holan (1995), Podgroskii et al. (2004), Wang and Chen (2006) and Alluri et al. (2007). Breierover et al. (2002) investigated the absorption ability of cadmium (Cd^{2+}) ions into cells and the role of exo-polymers to adapt the toxic environment of Cd^{2+} metal by eight different yeast species and observed that all yeast strains were capable of accumulating the cadmium metal to different extent by transforming the metal into complex polymeric component which is not toxic for cells.

2.7 ROLE OF ALKALIPHILIC/ ALKALI-TOLERANT MICROORGANISMS

The most diverse occurrences of organisms are generally observed in moderate environments. Certain environmental conditions such as pH, temperature and salinity conditions are extremely high or low on earth which was thought to prevent the existence of life, and such environments are regarded as extreme environments. Extreme environments are populated by certain groups of organisms adapted to these conditions which are usually referred to as alkaliphiles, halophiles, acidophiles and thermophiles, reflecting the particular type of extreme environment which they inhabit. Microorganisms which inhabit such extreme environments are also termed as “extremophiles”. Extremophiles are gaining a lot of research interest because of their remarkable conversion potential and their capability to abate environmental pollution under stressful conditions (Aislabie et al., 2006; Cronin and Lo, 2006; Ferrer et al., 2007).

Most of the alkaliphiles have been reported from neutral environmentas compared to acidic soil samples (Horikoshi, 1999). The frequency of alkaliphiles in the environment especially in the ordinary neutral soil sample has been shown to be 10^2-10^5 per g of soil, which corresponds to 1/10 – 1/100 of the population of the neutrophiles. For isolating alkaliphilic microorganisms *in vitro,* the sample could be enriched with different substrates such as peptones, glucose, bile salts, casamino acids and caseine (Duckworth et al., 1996). An alkaline media has to be used by adjusting the pH of the medium to around 10 with sodium carbonate (Na_2CO_3) and/or Borax-NaOH, Na_2HPO_4/NaOH, KCl/NaOH buffer systems (buffering capacity over the range of pH 9-12 in various media).

Cell surface of the alkaliphile can maintain the intracellular pH values near neutral in alkaline environment of pH 10-13. The presence of Na^+ ions in the growth medium played a pivotal role in adaptation of alkaliphilic *Bacillus* species at high pH values. Various studies reported that *Bacillus* strains shows variation in ease of NaCl requirement due to species specific differences in the halotolerance (Yumoto et al., 2005). Guffanti et al. (1986) observed that the total membrane cytochrome content was considerably higher in cells grown at pH 10.5 than at pH 7.5 which exhibited the largest pH dependent differences. Growth pH also revealed different pattern of protein profiles.

In recent years, there has been increasing interest in alkaliphilic bacteria due to their potential industrial application that require enzymes such as proteases, pectinases, xylanases, cellulases, etc.), that are stable at high pH values (pH > 9.5) and at temperature above 50 °C (Horikoshi, 1971; Horikoshi et al., 1971; Engle et al., 1995; Gupta et al., 2008; Nagar et al., 2010). Alkaliphilic bacteria have been studied for their adaptation to high pH, isolated from variety of environments and most of the isolates belong to genus *Bacillus*. Studies on alkaliphilic *Bacillus* strains have shown that the characteristics of these bacteria differ depending on the strain (Yumoto et al., 2005). At least 26 alkaliphilic and alkalitolerant *Bacillus* species have been identified to date (Denizci *et al.*, 2010) that constitute the sixth rRNA group in the genus *Bacillus* (Nielsen *et al.*, 1994), and many of them have attracted much attention with regard to industrial, basic research and biotechnological applications (Ghosh et al., 2007; Lee et al., 2008).

Sanghi et al. (2008 and 2009) isolated xylanase enzyme from alkaliphilc *Bacillus subtilis* ASH strain using agricultural waste residue such as wheat straw, wheat bran, saw dust etc. in solid state and submerged fermentation. Production of xylanase was observed highest at 37 °C and neutral pH after 72 h of incubation, whereas the production was also observed at alkaline pH

11.0 and temperature 60 °C. This study revealed the importance of alkaline xylanase from alkaliphilic *Bacillus* sp. in the treatment of pulp and paper during bleaching process over the use of toxic chlorinated compounds.

Effluent discharge from textile industries also have high pH or alkaline as the dyeing process of fabric involves different chemicals and dyes such as basic dyes. So, the environment around the discharge outlet also alkaline in nature and may acts as the habitat for alkaliphiles, thus, attracted the considerable attention of alkaline xylanases, pectinases or other enzymes in different industrial and biotechnological applications (Nagar et al, 2010; Gupta et al., 2008) . Kulshreshtha et al. (2010) reported that the enzymatic activities of alkaliphiles and alkali-tolerants enable them to gain resistance against alkaline environments to biodegrade certain xenobiotics, to produce several metabolites and to detoxify detrimental metal compounds.

2.8 IMPACT OF CKD

CKD’s on the average are typically characterized by high alkali, sulfate and heavy metal content that is responsible for the removal of dust from the kilns as its reutilization as raw feed in cement production may reduce the quality of cement and strength of concrete. Major part of unutilized dust is dumped in open land as landfill material and poses threat to environment in terms of soil, surface and ground water contamination through leachate. In recent years, there has been an increase in the utilization of CKD for beneficial applications such as cement replacement, waste treatment, soil stabilization and other uses. CKD’s cement like properties also makes it a potential replacement for Portland cement in cement paste, mortar/concrete.

The alkalinity of CKD needs to be monitored to avoid expansive alkali aggregate reaction which causes crack, deformities and reduce the quality of cement and concrete (Juenger and Jennings 2001; Ghanem et al. 2010; Davis and Hooks 1975). The loss of strength was attributed to the alkalies in the dust. The American Society for Testing and Materials (ASTM) specifies a limit of 0.6 percent alkali in Portland cement (Wilson and Anable, 1986). If the amount of alkali in the kiln dust is high, its reusing in the kiln causes the formation of potassium and sodium containing compound and free CaO, leading ultimately to a reduction in the strength of cement and causes crack, deformities and reduce the quality of cement and concrete (Rehsi and Garg, 1986). Bhatty

(1984a; b; 1986) found that due to high alkalinity in the dust, CKD blended cement had reduced workability, setting times and strength.

Generally, the pH of water-CKD mixture or slurry is usually around 12.5 and is considered to be alkaline and corrosive as per Resource Conservation and Recovery Act (RCRA). Exposure to alkaline CKD also irritates the mucous membrane, increased risk of lever abnormalities, pulmonary disorder and reduction of ventilator capacity (Zeleke et al. 2010; Al-Neaimi et al. 2001; Adak et al. 2007). Mishra (1991) reported that as CKD comes in contact with water; hydroxides are formed that impair natural water alkalinity. The addition of Ca, Mg, Na, K, and Al as hydroxides, sulfates and silicates affects the hardness of the water and causes respiratory and gastrointestinal diseases. CKD also contains trace amount of metals such as arsenic, barium, lead, zinc, magnesium, titanium, manganese and aluminum which can potentially cause detrimental effects to receiving environment by leachate generated from CKD disposal sites (Duchesne and Reardon, 1998).

EPA’s analysis of cement dust showed that it contains toxic constituents including metals such as arsenic, thallium, antimony, lead and chromium, and organic by-products like total 2,3,7,8-substituted dioxins and total hexachloro-dibenzodioxins in leachate. The Portland Cement Association (PCA, 1992) analyzed the metal content (Hg, Se, Tl, Cr, Pb, Ag, As, Ni, Ba, Be and Cd) in CKD (from 79 plants in US and 10 in Canada) using toxicity characteristic leaching procedure (TCLP) method and observed that CKD from only two plants has concentration of any material over an acceptable limit. EPA (1993) concludes that several metals such as antimony, barium, lead, manganese, strontium, thallium and zinc, are found in CKD but at variable levels. The concentration of barium and manganese was found to be lower in CKD whereas concentration of chromium and nickel was higher. The elevated level of some metals in the CKD samples is one of the main reasons to prevent its re-utilization in cement kiln and cement-concrete systems. The metals from CKD could be released into the ground water or water streams through leachate and potentially resulting in the deleterious effects to human health and environment.

Microorganisms play a major role in bioremediation process of toxic metal elements converting them to less toxic or non-toxic elements. Alkaliphilic and halophilic microorganisms are potential candidates as they have ability to tolerate high concentration of alkalinity and salinity (anions and cations) necessary for their growth. Several studies have been conducted on the bioremediation of industrial effluents or alkaline waste water using various microorganisms (Kulshreshtha et al., 2012; Mangaiyarkarasi et al., 2011; Ibrahim et al., 2011and 2012; Solanki and Kothari, 2012). Kaur et al. (2013) studied the metal leachate analysis of waste foundry sand after treated with fungal isolate and its utilization in concrete. But, no such study has been made on the metal leachate analysis of alkaline solid industrial waste such as cement kiln dust using alkaliphilic or alkalitolerant bacteria.

Therefore, the generation of large quantities of CKD is responsible for a significant financial loss to the cement industry in terms of the value of the raw materials, processing, energy usage and, above all, its disposal and storage. Due to the cost associated with the production and disposal of CKD and the strong and strict environmental regulations on the proper management of CKD, the cement industries now showing a keen interest in finding the proper utilization of CKD in a manner that protects human health and the environment. Keeping this in mind, the present study has been made on the bacterial treatment of cement kiln dust by alkali-tolerant bacterial strain for reducing the alkalinity and analyzing the metal leachability from untreated and bacterial treated CKD. The treated cement dust then utilized in the production of cement mortar and concrete and studied their different properties such as hydration, compressive strength, split tensile strength, ultrasonic pulse velocity, chloride permeability, water absorption and porosity.

CHAPTER – 3

EXPERIMENTAL PROGRAM

This chapter deals with the details of experimental programs for the isolation and screening of bacterial isolates, physical & chemical analysis of cement kiln dust, bacterial treatment of cement kiln dust and identification of bacterial isolates. It includes the details for the measurements of water consistency, setting times, hydration of cement blended pastes, compressive strength of mortar and concrete, setting time, ultrasonic pulse velocity, rapid chloride permeability, water absorption and porosity of concrete containing (untreated and bacterial treated) CKD with varying percentages (0, 5, 10, 15, 20 and 30%). It also presents the leachate analysis (alkalinity, chloride and metal) obtained from fresh concrete mixes containing untreated and bacterial treated CKD. It also describes the microstructure analysis which includes SEM to study the surface morphology in hardened concrete made with CKD (untreated and bacterial treated), XRD technique to study the various phases formed in hardened concrete.

3.1 ISOLATION OF ALKALI-TOLERANT BACTERIA

3.1.1 Preparation of Growth Media

Enrichment medium was used for the growth of alkali-tolerant bacterial cultures. The composition of enrichment medium is given in Table 3.1. pH was adjusted to 10.5 with 1 N NaOH (measured by pH meter Cyberscan pH 510). All the components of growth medium (except glucose) was sterilized at 121 °C, 15 psi for 15 min. Sterile glucose solution (10%) was added to the medium aseptically.

Table 3.1: Composition of enrichment medium

Composition	g/L
Glucose	10
Peptone	10
Yeast extract	5
Di-potassium phosphate (K_2HPO_4)	1
Bacteriological agar type I	15
pH	10.5 ± 0.1

Minimal medium (M9) was used for the acclimatization of isolated cultures for screening of their tolerance to high pH. The composition of minimal medium is given in Table 3.2. The high pH of the medium was adjusted to 11 and 12 with KCl-NaOH buffer (alkaline buffer; pH 12-13). KCl-NaOH buffer was sterilized separately and then added to the medium aseptically before pouring the agar into pertriplates (or while preparing the liquid broth for liquid culture). The procedure for preparation of KCl-NaOH buffer is given below:

Salt: Potassium chloride (KCl)
Base: Sodium hydroxide (NaOH)

Stock solutions:

A: 0.2 M solution of potassium chloride (14.9 g KCl in 1 L distilled H_2O)

B: 0.2 M solution of sodium hydroxide (8 g NaOH in 1 L distilled H_2O)

Mix 25 mL of A and given volume of B and dilute to a volume of 100 mL with distilled water.

pH	12.0	12.2	12.4	12.6	12.8	13.0
B (mL)	6.0	10.2	16.2	25.6	41.2	66.0

Table 3.2: Composition of minimal medium (M9)

Composition	g/L
Sucrose	10
Di-potassium phosphate (K_2HPO_4)	2.5
Potassium dihydrogen phosphate (KH_2PO_4)	2.5
Diammonium phosphate dibasic ($(NH_4)_2HPO_4$)	1
Magnesium sulfate heptahydrate ($MgSO_4.7H_2O$)	2
Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$)	0.01
Manganous sulfate ($MnSO_4.4H_2O$)	0.007
pH (maintained by KCl-NaOH buffer)	11 and 12

3.1.2 Preparation of Petriplates

Poured the medium in sterilized Petri plates (U.V. light sterilized; Tarsons) and allowed to solidify for 24 h under aseptic conditions in laminar air flow bench. Once the agar was solidified, agar plates were kept in an inverted position for 24 h at room temperature for sterility test.

3.1.3 Isolation of Alkali-tolerant Bacterial Isolates

The alkali-tolerant bacterial strains were isolated from the rhizospheric soil and textile industry dye waste water using serial dilution spread plating technique (Cappuccino and Sherman, 1989) on enrichment medium agar plates having pH 10.5 as per the procedure given below:

Medium used: Enrichment agar and its composition is given in Table 3.1.

Materials used: Petriplates, 9 mL sterile water test tubes, spreader, rhizospheric soil sample, waste water sample, laminar air flow chamber, spirit, blotting paper, etc.

Procedure

1. One g of soil (or 1 mL of waste water sample) was added to 10 mL sterile water blank and shaken well.
2. After shaking the test tube was put in a rack for 5-10 min so that the particles or precipitates settled down and labeled it 10^{-1} dilution.
3. One mL of the supernatant was then added to a test tube containing 9 mL water using sterile micro tip making a dilution corresponding to 10^{-2}.
4. Further dilutions were prepared in a similar way up to 10^{-8}.
5. Aliquot of 0.1 mL (or 100 µL) was taken from dilution 10^{-6}, 10^{-7} and 10^{-8} using sterile micro tips and transferred to the respective agar petri plates. The aliquot was spread with the help of a sterilised spreader.
6. The respective dilution agar plates were incubated at 35 ± 2 ºC for 48 h in an inverted position.
7. For each dilution three petriplates were used.

On the basis of colony morphology and color microbial colonies were selected. The single isolated colonies were then picked and re-streaked (using streak plate method) on same agar medium plates till pure colonies were obtained, and stored at 4 ºC for further use.

3.1.4 Acclimatization of Alkali-tolerant Bacterial Isolates at Higher pH (11 and 12)

After the isolation on enrichment medium, pure isolates were then screened for their tolerance to pH 11 and 12 and this was performed on a minimal medium (M9), using spread plating technique, instead of enrichment medium as the components of the enrichment medium gets precipitated at such high pH. Medium of pH 11 and 12 was prepared using appropriate biological KCl-NaOH buffer (preparation given in 3.1.1) instead of NaOH due to precipitation or turbidity of the medium with NaOH. The cultures isolated on enrichment medium were then further sub-cultured and acclimatized on M9 medium (pH 11-12) till isolates were adapted to grow at high pH. The selected isolates were maintained at pH 12 and stored at 4 ˚C for further experimentation.

3.1.5 Screening of Acid Production by Bacterial Isolates

3.1.5.1 By pH meter

Screening of isolates was done on the basis for their potential to reduce the pH of the alkaline liquid minimal (M9) and enrichment medium (pH 12). The media flasks containing bacterial isolates were incubated at 35±2 °C at 120 rpm. The acid production by microbial isolates was monitored for decrease in pH at regular intervals for up to 5 days by calibrated pH meter (Cyberscan pH 510). Each treatment had three replications.

3.1.5.2 By phenolphthalein indicator test

The reduction of the pH in the minimal (M9) medium (pH 12) by isolates was also observed by adding phenolphthalein pH indicator to the medium. At high pH (>10), the indicator gives bright pink color to the alkaline solution while at low pH (i.e. pH ≤ 8) it is colorless. The medium was inoculated with respective bacterial isolate which had an OD value of 1.0. The media flasks containing bacterial isolates were incubated at 35±2 °C at 120 rpm. Change in color from pink to colorless was the indicator of the acid production by the microbial isolates. After every sampling at regular intervals from the medium the broth was centrifuged at 10,000 rpm for 5 min to palletize the cells so that cells might not interfere in analyzing the optical density of the supernatant with spectrophotometer at 554 nm wavelength. All the treatments had three replications.

3.2 CHARACTERIZATION OF CEMENT KILN DUST

Cement kiln dust (CKD) was obtained from the cement plant located at Himachal Pradesh, India (name of the cement plant is not disclosed due to official concerns). CKD was collected from electrostatic precipitators during the production of cement clinker.

3.2.1 Physical Analysis of CKD

Physical analysis including fineness and specific gravity was determined as per BIS: 4031(part 1) 1996 and BIS: 2720 (part4) 1980, respectively.

3.2.1.1 Fineness

Fineness of CKD was determined by using sieve analysis method (BIS:4031 part 1-1996). The sample of CKD tested was free from agglomerates. Accurately 100 g of CKD was taken and carefully placed on 90 µm sieve to avoid any loss. Sieve was closed by lid, and agitated by swirling, planetary and linear movement until no more fine material passed through it. After agitation the lid was removed and the residue over 90 µm sieve was weighed. The fineness of the sample was then calculated by using formula given below and expressed in percent mass retained on 90 µm sieve.

$$\text{Fineness (\% mass retained on 90 µm sieve)} = \frac{\text{Weight of sample retained on sieve}}{\text{Total weight of the sample taken}} \text{ x } 100$$

3.2.1.2 Specific gravity

Specific gravity of CKD was determined by using specific gravity bottle as per BIS: 2720 part 4 (1980) method. The specific gravity bottle with lid was oven dried to remove any water adhering to the walls of the bottle. The bottle with its lid was air cooled and weighed (W1). The bottle was then filled with water up to the graduated mark and weighed (W2) again. After weighing the water was decanted off and the bottle was refilled with kerosene till it flushed with the graduated mark. Weight of bottle with kerosene was recorded and denoted as W3. Fifteen gram (15 g) of CKD sample was taken (W5), placed in to the bottle and filled with kerosene with constant stirring to remove entrapped air. The bottle containing CKD and kerosene was wiped dry and weighed (W4). The specific gravity was calculated by using formula:

$$\text{Specific gravity of CKD} = \frac{\mathrm{W5(W3-W1)}}{\mathrm{(W5+W3-W4)(W2-W1)}}$$

3.2.1.3 pH

The pH of the cement dust was determined by using pH meter (Cyberscan pH 510). The CKD was mixed with double distilled water in the ratio 1:10 in a conical flask. The flask was then put on orbital shaker @ 130 rpm for 1 h. The resultant supernatant was filtered and the pH of the aliquot was measured.

3.2.2 Chemical Analysis of CKD

Chemical analysis of CKD was carried out by using energy dispersive X-ray spectroscopy (EDX; JEOL JSM-6510 LV, USA). The powdered sample of CKD was mounted on brass stubs, gold coated and percent oxide forms of elements such as calcium oxide (CaO), silica (SiO_2), alumina (Al_2O_3), magnesium oxide (MgO), sulphur trioxide (SO_3), sodium Oxide (Na_2O), potassium Oxide (K_2O), iron Oxide (Fe_2 O_3), cupric oxide (CuO) and zinc oxide (ZnO) were obtained.

3.3 BACTERIAL TREATMENT OF CEMENT KILN DUST

For bacterial treatment, CKD was spread in the plastic trays. The CKD was mixed with bacterial culture in the ratio 4:1 (CKD: culture). The incubation temperature was 35±2 °C. For the reduction in alkalinity and chloride by bacterial isolates, time (in days) and bacterial inoculum was optimized. The confirmatory analysis for alkalinity reduction was done by using EDX and XRD (X-ray diffraction) technique.

The alkalinity and chloride in leachate/water sample was determined by APHA (2005) method as per the procedure given below:

3.3.1 Alkalinity

Alkalinity of the sample is the way of measuring the capacity to neutralize acids and is considered to be mainly due to presence of hydroxides, carbonates and bicarbonates. Total alkalinity of leachate/water sample was determined by titrating the sample with standard solution of strong acid using methyl orange as an indicator. Pictorial representation of alkalinity estimation is shown in Figure 3.1.

Reagents:

(a) Methyl orange indicator solution: 50 mg methyl orange powder was dissolved in 100 mL distilled water.

(b) Standard Sulphuric acid solution (0.02N): 200 mL 0.1 N sulphuric acid (H_2SO_4) was diluted to 1000 mL with distilled water.

Procedure:

100 mL sample was taken in a 500 mL conical flask and 2-3 drops of methyl orange indicator was added. Sample was titrated against standard sulphuric acid solution (0.02N) and end point was noted when color changed from yellow to orange. Initial and final volume of titrant used was recorded and total alkalinity was calculates by using the following formula:

$$\text{Total Alkalinity } (\text{mg/L CaCO}_3) = \frac{\text{A x B x 50}}{\text{mL of sample taken}} \text{ x 1000}$$

A = Volume of titrant (H_2SO_4) used; B= Normality of the titrant (0.02 N); 50 = factor used to convert me/L to mg/L (equivalent weight of $CaCO_3$)

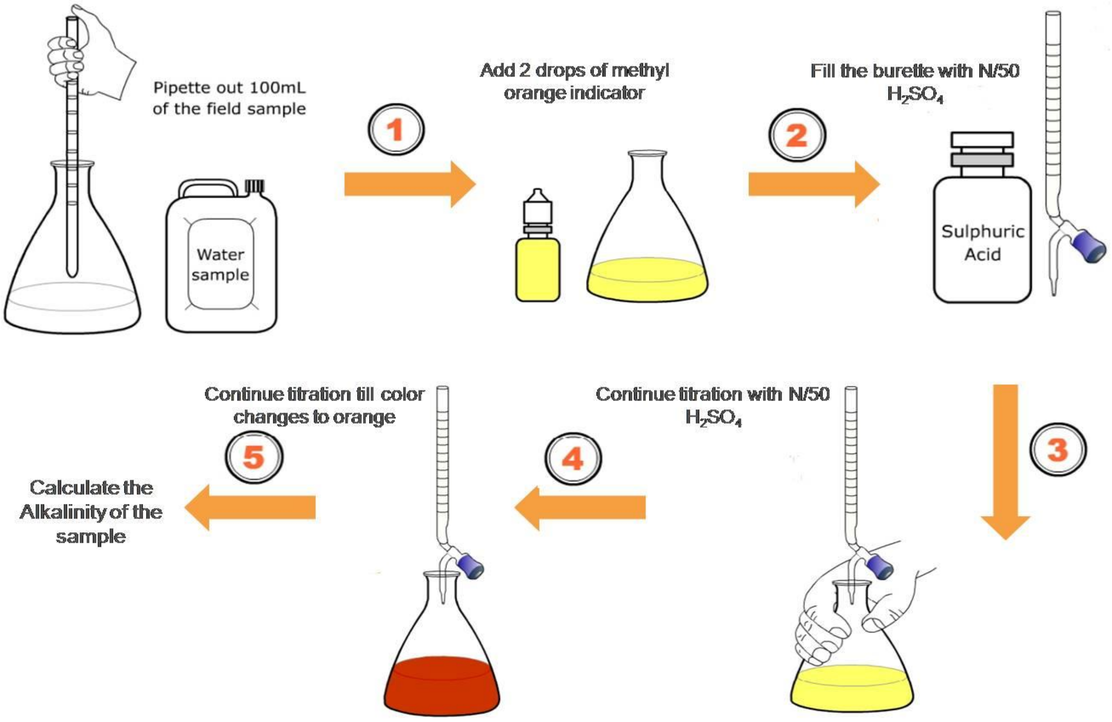

Figure 3.1: Pictorial representation of alkalinity estimation

3.3.2 Chloride

Chloride is one of the major inorganic anion in water, waste water or industrial waste leachate and present in the form of chloride (Cl^-) ions. High chloride content may harm metallic pipes and structures, as well as growing plants. Concentration of 250 mg/L chloride ion in water causes salty taste if the cation is sodium, but if the concentration is around 1000 mg/L and the salty taste is absent then the predominant cations are calcium and magnesium. Chloride content of leachate/water sample was determined by titrating the sample with standard silver nitrate using potassium chromate as indicator. Pictorial representation of alkalinity estimation is shown in Figure 3.2.

Reagents:

(a) Potassium chromate indicator solution: 50 g potassium chromate powder was dissolved in little amount of water and allowed to stand for 12 h. The solution was filtered and volume was made to 100 mL by distilled water.

(b) Standard silver nitrate solution (0.0141 N): 2.395 g silver nitrate ($AgNO_3$) was dissolved in distilled water and diluted to 1000 mL.

Procedure:

100 mL leachate or water sample was taken in 500 mL conical flask and 2-3 drops of potassium chromate indicator was added. Sample was titrated against standard silver nitrate solution (N/71 or 0.0141 N) and end point was noted when color changed from fluorescent yellow to brick red. Initial and final volume of titrant used was recorded and chloride was calculates by using the following formula:

$$\text{Chloride}\ (\text{mg/L}\,\text{CaCO}_3) = \frac{\text{A x B x 35.45}}{\text{mL of sample taken}}\text{x 1000}$$

A = Volume of titrant ($AgNO_3$) used; B= Normality of the titrant (0.0141 N); 35.45 = Atomic mass of chlorine

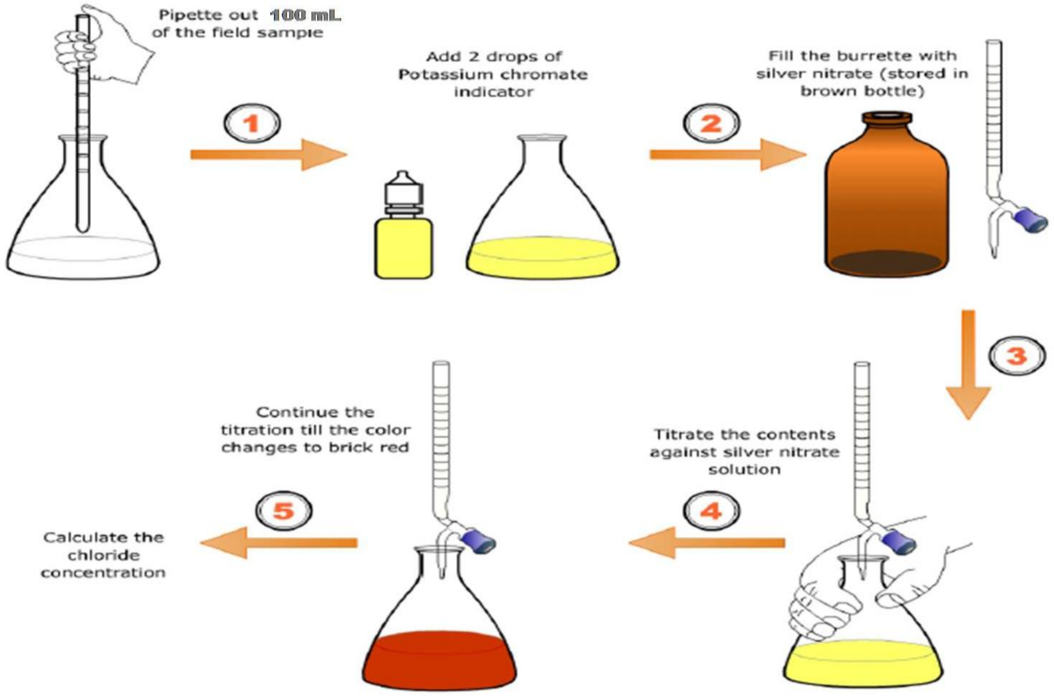

Figure 3.2: Pictorial representation of chloride estimation

3.3.3 Optimization of Time

The cement kiln dust was mixed with bacterial isolates had an O.D. value of 1.0 (measured by spectrophotometer @ 600 nm wavelength). The treatment mixture was incubated at 35±2 °C for 25 days in three replications and moisture was maintained in the form of water for the growth of isolate. The addition of water to CKD was such that water provides only moisture to CKD, i.e., CKD should be in the form of moist powder not the slurry or in the liquid mixture. After 5 days of incubation, sucrose solution (10%) was added only once during treatment to provide carbon source for the growth of bacterial strain. A control CKD without bacteria was also incubated at similar conditions as for bacterial treated samples. After every 5 days, samples from different treatments were removed and mixed with water (1:10) in conical flask with shaking (@ 130 rpm for 1 h) to generate leachate which then analyzed for the alkalinity along with control treatment as per APHA (2005) method as shown in section 3.3. The pH of the leachate samples were also measured during each sampling by pH meter.

3.3.4 Optimization of Culture Inoculum

After the optimization of time, the bacterial inoculum was optimized by mixing the bacterial isolate with CKD in different concentrations (O.D. of 0.6, 0.7, 0.8, 0.9 and 1.0; measured by spectrophotometer @ 600 nm wavelength). The treatment mixture was incubated at 35±2 °C for 20 days (optimized time) in three replications and moisture was maintained in the form of water for the growth of isolate. Similarly as in section 3.3.1, after 5 days of incubation, sucrose solution (10%) was added only once during treatment to provide carbon source for the growth of bacterial strain. A control CKD without bacteria was also incubated at similar conditions as for bacterial treated samples. After 20 days of incubation, samples from different treatments were removed and mixed with water (1:10) in conical flask with shaking (@ 130 rpm for 1 h) to generate leachate which then analyzed for the alkalinity and chloride along with control treatment as per APHA (2005) method as shown in section 3.3.

3.3.5 EDX and XRD Analysis of Treated CKD

To confirm the decrease in alkalinity, bacterial treated CKD was analyzed with energy dispersive X-ray spectrometry (EDX) for change in chemical composition and with X-ray diffraction (XRD) for change in alkali phases (such as arcanite peak; K_2SO_4) in CKD. All CKD samples were air dried and grounded to make fine powder before EDX and XRD analysis.

The samples analyzed with EDX were mounted on brass stubs using carbon tape and then determined for the change in chemical composition. The XRD analysis was carried out by using PANalytical X'pert Pro (Netherlands) system. The random powder mount specimens were scanned from 10 to 60° 2Ɵ. Interpretation of the X-ray patterns was carried out by matching the peaks with the reported literature and by using standard JCPDS cards, a database of X-ray powder diffraction patterns maintained by the International Center for Diffraction Data (ICDD).

3.4 IDENTIFICATION OF ALKALI-TOLERANT BACTERIAL ISOLATES

Alkali-tolerant bacterial isolates were identified on the basis of morphological, biochemical, physiological characterization and phylogenetic analysis using 16S rRNA sequencing.

3.4.1 Morphological Characterization

The bacterial isolates were morphologically identified on the basis of colony color, form, margin, elevation on agar medium and type of surface growth, clouding and sedimentation in broth medium.

3.4.1.1 Bacterial colony characteristics

(i) On agar plate: The pure culture of isolate grown in liquid minimal medium (M9) was spread on M9 agar plates by diluting the culture using serial dilution technique. After spreading the culture, agar plates were incubated at 37 °C in inverted position for 24-48 h. After incubation following macroscopic characteristics were observed and recorded:

- *(a) Form/shape:* The colony shape on agar plate was observed in terms of: circular, irregular, filamentous or spindle as shown in Fig. 3.3.
- *(b) Colony color:* Color of the colony was observed in terms of white, orange, yellow or translucent, opaque, shiny etc.
- *(c) Margin:* The outer edge of colony was observed as smooth/entire, lobate, undulate or filamentous as shown in Fig. 3.3.
- *(d) Elevation:* The elevation of the colony was observed by holding plate to the side as flat, raised, convex etc. as shown in Fig. 3.3.

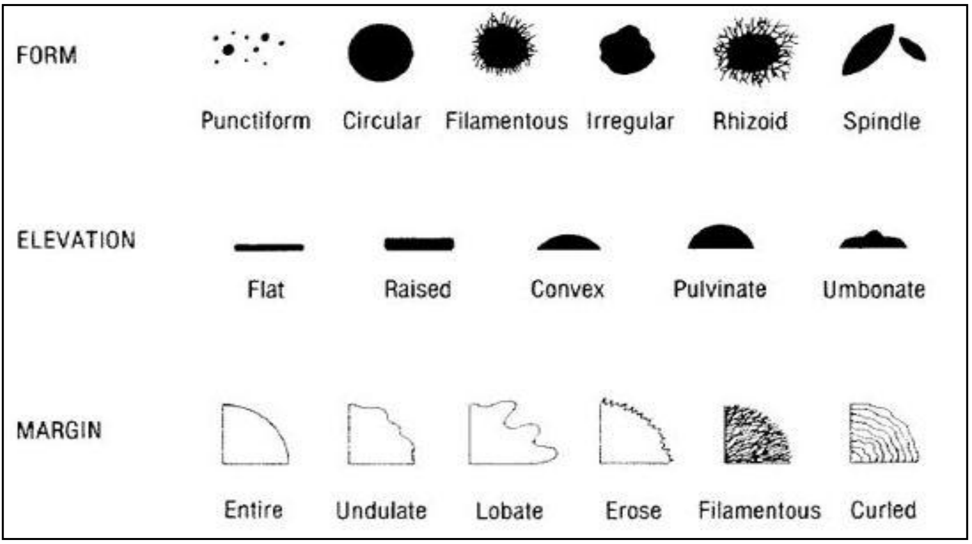

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Fig. 3.3: Terms used to describe colony morphology (Pelczar, 1957)

(ii) In liquid medium (broth): The pure culture of the isolate from agar plate was inoculated in M9 broth using inoculation needle/loop and incubated at 37 °C for 24-48 h @130 rpm on orbital shaker. After growth the following characteristics were observed and recorded:

(a) *Surface growth:* Observation was carried out to determine the type of growth on the surface of the broth or deposition of culture on the walls of flask in the form of ring or not.

(b) *Clouding*: In liquid medium, observation was carried out to determine the uniform growth of bacteria in terms of turbidity of medium as turbid, heavy turbid or moderate.

(c) *Sedimentation*: Sedimentation of bacterial culture in liquid medium tends to entangle and settling at the bottom forming granular deposits. Settlement of cells during growth was observed in terms of moderate sediment, scanty, flocculent or flaky.

3.4.1.2 Microscopic characteristics

Microscopic analysis was done by using Gram staining to determine the shape and type of stain taken by the isolate. Gram staining was done according to method described by Cappuccino and Sherman (1989).

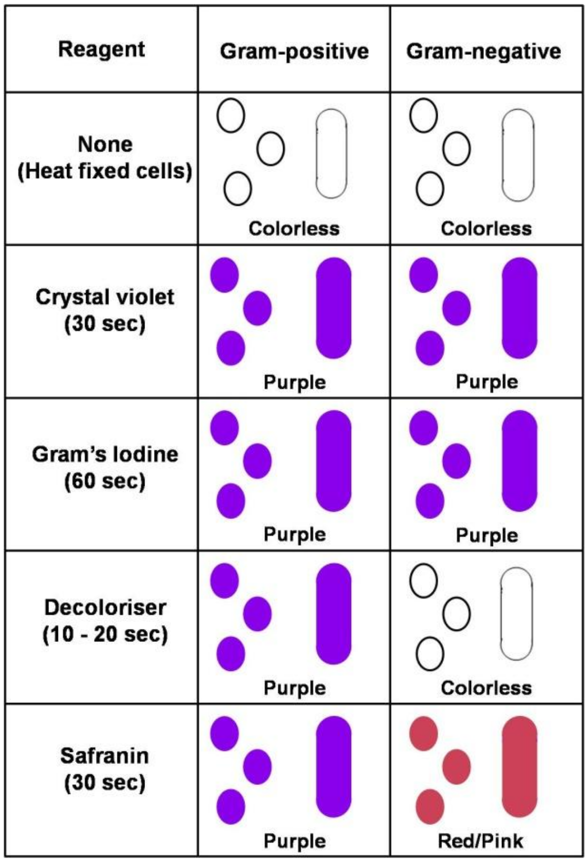
Gram Staining

Gram staining is the first step towards identifying an unknown bacterial isolate phenotypically. Gram's procedure differentiates vast array of bacterial organisms into two broad groups according to cell wall structure. Gram-positive cells appear purple and have a thick peptidoglycan layer, while Gram-negative cells appear pink and have a thin peptidoglycan layer. Fig. 3.4 represents the color changes that occur in bacterial cell during Gram staining process.

Procedure:

1. A thin smear of bacterial culture was made on clean glass slide.
2. Smear was air dried and heat fixed.
3. Smear was then covered with crystal violet (primary stain) for 30 sec and then washed gently with distilled water.
4. Gram's iodine (mordant) was applied on the smear for 30 sec and washed with distilled water.

5. Slides were flooded with decolorizer (95% ethyl alcohol) drop wise until no more purple color was flown from the smear (approximately for 10-20 sec). After that the slide was washed with distilled water.
6. Smear was flooded with safranin (counter stain) for 30 sec and washed with distilled water.
7. Extra water from stained slides was dried by blotting paper and, then slide was air dried and examined microscopically under 40X and 100X for cell morphology and color.



Reagent	Gram-positive	Gram-negative
None (Heat fixed cells)	Colorless	Colorless
Crystal violet (30 sec)	Purple	Purple
Gram's Iodine (60 sec)	Purple	Purple
Decoloriser (10 - 20 sec)	Purple	Colorless
Safranin (30 sec)	Purple	Red/Pink

Fig. 3.4: Color change occurs in bacterial cell during Gram staining process

3.4.2 Biochemical Characterization

The biochemical characteristics of bacterial isolates were determined as per methods described by Aneja (2008). Several tests such as hydrogen sulfide production, motility, methyl red-Voges Proskauer, citrate, starch, gelatin hydrolysis, catalase, lipase, casein, pectin, cellulose, carbohydrate and amino acid utilization were performed. The positive test denoted the growth of the isolate in respective medium; whereas negative test denoted the absence of growth of isolate. Some bacterial isolates showed weak positive results denoted by w/+.

3.4.2.1 Hydrogen sulfide production test

This test determines the reduction (hydrogenation) of sulphur containing amino acids (such as cystine, cysteine and methionine) or inorganic compounds such as thiosulfates ($S_2O_3^{2-}$), sulfites (SO_3^{2-}) or sulfates (SO_4^{2-}) during the process of metabolism by bacteria. The H_2S production can be detected by incorporating H_2S indicator such as ferrous (Fe^{2+}) or lead (Pb^{2+}) containing metal salts to the culture medium. If H_2S gas is produced, it reacts with metal salt forming visible black precipitates.

Table 3.3: Composition of SIM agar medium

Composition	g/L
Peptone	30.0
Beef extract	3.0
Ferrous ammonium sulfate	0.2
Sodium thiosulfate	0.025
Agar	3.0
pH	7.3

Procedure

The hydrogen sulfide test was done on sulfide indole motility (SIM) agar medium (Table 3.3). The components of SIM medium were dissolved in distilled water, poured in test tubes and autoclaved. The culture was inoculated into the agar test tube by means of stab inoculation (approximately 1-2 cm deep in agar). The inoculated tubes were then incubated at 35±2 °C for 48 h. After incubation the tubes were observed for the presence or absence of black coloration along the line of stab inoculation. The positive test for H_2S production was indicated by the presence of black color due to the reaction between H_2S gas and the Fe^{2+} metal ion present in the medium resulted in formation of black colored insoluble compound, ferrous sulfide. Fig. 3.5 shows the schematic representation of hydrogen sulfide test.

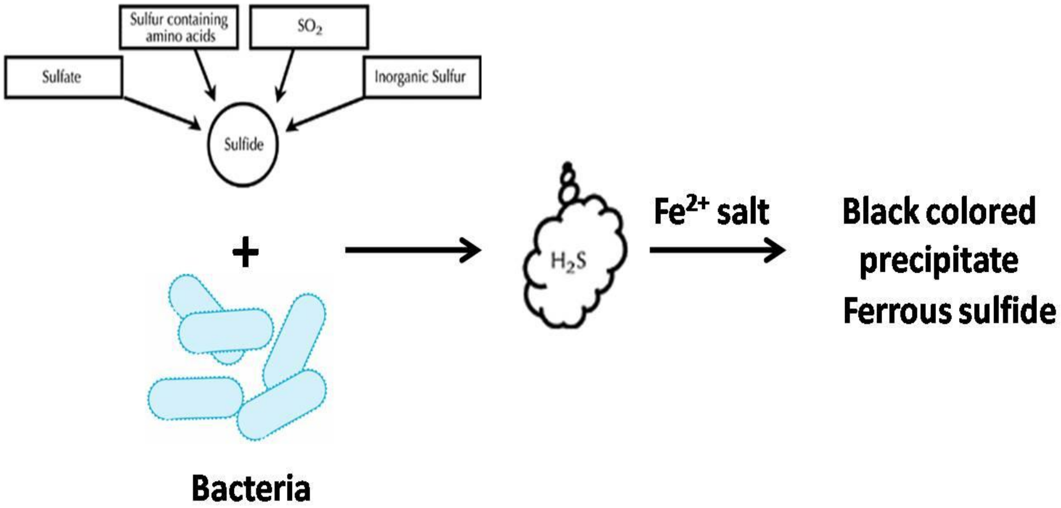


Fig. 3.5: Schematic representation of hydrogen sulfide test

3.4.2.2 Motility test

This test determines the mobility of bacteria through liquid or semi solid medium by means of locomotory organs such as flagella, cilia, pseudopodia or by special fibrils that provide gliding form of motility.

Procedure

The motility test was done on sulfide indole motility (SIM) agar medium (Table 3.3). The components of SIM medium were dissolved in distilled water, poured in test tubes and autoclaved. The culture was inoculated into the agar test tube by means of stab inoculation (approximately 1-2 cm deep in agar). The inoculated tubes were then incubated at 35±2 °C for 48 h. After incubation the tubes were observed for the growth of culture along the stab inoculation deep inside the agar or dispersion of the culture away from the stabbed area. The negative result was indicated by the growth of culture in a distinct zone along the stab.

3.4.2.3 Methy red-Voges Proskauer (MR-VP) test

This test differentiates two types of bacteria, one that produces large amount of organic acid (stable end product) such as formic, acetic, lactic, succinic, etc. and other produces ethanol or acetoin (non-acidic end product). Organism that produces acid in the medium retains red color (a positive test) upon adding of methyl red indicator where as other shows yellow color (a negative test) due to increase in pH of the medium. The VP reagent includes KOH and naphthol solution which reacts with acetoin and produces red color (a positive test) whereas no change in color indicates negative test.

Table 3.4: Composition of MR-VP broth

Composition	g/L
Peptone	7.0
Glucose	5.0
Potassium phosphate	5.0
pH	6.9

Procedure

Methy red-Voges Proskauer test was performed by inoculating the bacterial culture in two tubes containing MR-VP broth (one for MR test and other for VP test). Table 3.4 shows the composition of MRVP broth. The inoculated tubes were then incubated at 35±2 °C for 48 h. After incubation the 5 drops of methyl red pH indicator was added to the one of MRVP broth tube whereas in other tube VP reagent, mixture of 12 drops of VP-I reagent (5% α-naphthol in ethanol) and 2-3 drops of VP-II reagent (40% KOH in distilled water), was added. After 15-20 min the tubes were observed for change in color. The positive result of acid production by bacterial in MRVP broth was indicated by presence of red color in tube containing methyl red whereas no change in color in tube containing VP reagent. Opposite results were obtained for MRVP test, i.e. MR+, VP- or MR-, VP+.

3.4.2.4 Citrate test

This test is used to screen the bacterial isolate for its ability to utilize/ferment citrate as carbon and energy source. The utilization of citrate depends upon the presence of citrase enzyme produced by bacteria that breaks down the citrate into oxaloacetic and acetic acid. This changes the pH of the medium and indicated by change in color of the growth medium containing bromothymol blue pH indicator. The positive test for citrate utilization is indicated by the blue color of the medium whereas no change (green color) in the color of the medium indicates negative test.

Procedure

The citrate test was done on Simmon's citrate agar medium (Table 3.5). The components of the medium were dissolved in distilled water, poured in test tubes and autoclaved. After autoclaving the tubes were put in an inclined position to form agar slants. The culture was inoculated into the agar test tube by means streaking (zig zag position). The inoculated tubes were then incubated at 35±2 °C for 48 h. After incubation the tubes were observed for change in color of the medium. The tubes where the bacteria utilizes citrate showed blue color (a positive test) while no change in color (green color) indicated negative test.

Table 3.5: Composition of Simmon's citrate agar medium

Composition	g/L
Ammonium dihydrogen phosphate ($NH_4H_2PO_4$)	1.0
Dipotassium phosphate (K_2HPO_4)	1.0
Sodium chloride (NaCl)	5.0
Sodium citrate	2.0
Magnesium sulfate ($MgSO_4.7H_2O$)	0.2
Agar	15.0
Bromothymol blue	0.8
pH	6.9

3.4.2.5 Starch hydrolysis test

This test was used to screen the bacterial isolate on this ability to degrade starch by producing amylase enzyme. Starch is a complex polysaccharide composed two constituents – amylose and amylopectin. Starch is too large to pass through cell membrane, thus action of amylase enzyme hydrolyses starch into smaller fragments (disaccharides and monosaccharides) that enter into the cytoplasm and used up by endoenzymes to form individual glucose subunits. Bacterial isolate that produces amylase enzyme hydrolyze the starch when grown on starch agar medium and determine by using iodine solution (as an indicator). Iodine reacts with starch to produce dark blue coloration in the medium, whereas clear zone around the bacterial growth indicates amylolytic activity.

Procedure

Starch hydrolysis test was carried out on starch agar medium (Table 3.6). Starch agar plates were prepared and bacterial culture was inoculated by spread plating or streak plating technique. The inoculated agar plates were then incubated at 35±2 °C for 48 h. After incubation bacterial growth was observed and the agar plates were flodded with iodine solution (Gram's iodine). The area where bacterial growth was observed showed a clear zone indicated the amylolytic activity (a positive test) whereas no clear zone (blue color of the medium) indicated negative test.

Table 3.6: Composition of starch agar medium

Composition	g/L
Starch (soluble)	20.0
Peptone	5.0
Beef extract	3.0
Agar	15.0
pH	7.0

3.4.2.6 Gelatin hydrolysis test

This test was used to screen the hydrolysis of gelatin (a protein) by bacterial isolate as a carbon and energy source for growth. Hydrolysis of gelatin to amino acids is brought by proteolytic exoenzyme, known as gelatinase, produced by bacteria when grown on semi-solid medium containing gelatin. Gelatin serves as solidifying agent in the growth medium when cooled below 25 °C, and exists as a liquid above this temperature. After the bacterial growth the agar tubes showing liquid medium even after cooling at 4 °C gives positive test, whereas the solidification of medium after cooling gives negative test.

Table 3.7: Composition of gelatin agar medium

Composition	g/L
Gelatin	40.0
Peptone	20.0
Sodium chloride (NaCl)	5.0
Dipotassium phosphate (K_2HPO_4)	2.5
pH	6.8 ± 0.2

Procedure

Gelatin hydrolysis test was performed on gelatin agar medium (Table 3.7) and for this agar tubes were prepared. The tubes were inoculated by bacterial isolate using stab inoculation and incubated at 35±2 °C for 48 h. One uninoculated tube was kept as a control. After incubation the tubes (inoculated and uninoculated) were placed in refrigerator (4 °C) for 15-30 min. Liquefaction of the gelatin agar medium indicated the positive test of gelatin hydrolysis due to the production of gelatinase enzyme by bacterial culture, whereas solidification of the medium indicated negative test. Fig. 3.6 shows the schematic representation of gelatin hydrolysis test.

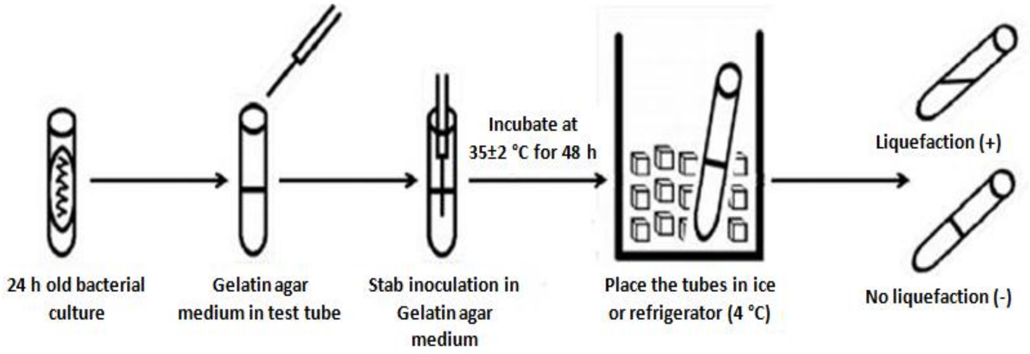


Fig. 3.6: Schematic representation of gelatin hydrolysis test

3.4.2.7 Catalase test

This test was used to detect the enzyme catalase produced by bacteria that protects the cell from the toxic by-products of oxygen metabolism i.e. hydrogen peroxide (H_2O_2), a potent oxidizing agent. Catalase enzyme breaks down the H_2O_2 into water (H_2O) and oxygen (O_2). This test was done by flooding the culture with 3% H_2O_2 solution and the bubble formation due to evolution of O_2 gives positive test for catalase.

Table 3.8: Composition of trypticase soy agar medium

Composition	g/L
Trypticase	15.0
Phytone	5.0
Sodium chloride (NaCl)	5.0
Agar	15.0
pH	7.3

Procedure

Catalase test was performed by growing the culture on trypticase soy agar slants (Table 3.8). The agar slants were inoculated by bacterial isolate using streak inoculation and incubated at 35±2 °C for 48 h. One uninoculated tube was kept as a control. After incubation the agar slants (inoculated and uninoculated) were filled (2 cm above the slant) with 3% H_2O_2 solution. Extensive bubble formation indicated the positive test whereas no bubble formation indicated the negative test.

3.4.2.8 Casein hydrolysis test

Casein is a milk protein composed of amino acids linked by peptide bonds. This test was performed to detect the proteinase (caseinase) producing bacteria that degrade the casein protein. Casein gives white color to the milk. Growth medium containing casein is white in color but when the bacterial growth occurs, the caseinase enzyme hydrolyzes the casein and a clear zone around the growth gives the positive test.

Procedure

Skim milk agar (Table 3.9) was used for the casein hydrolysis test. The ingredients of the medium were dissolved in distilled water, autoclaved and poured in petriplates. The agar plates were inoculated with the culture using spot inoculation or streak inoculation and incubated at 35±2 °C for 48 h in an inverted position. After incubation the agar plates were observed for the presence or absence of clear zone around the bacterial growth.

Table 3.9: Composition of skim milk agar medium

Composition	g/L
Skim milk powder	100.0
Peptone	5.0
Agar	15.0
pH	7.2

3.4.2.9 Pectin hydrolysis test

Pectic substances are the primary constituents of plant tissues and composed of complex carbohydrates (galacturonic acid). This test was performed to detect the pectinase (pectin hydrolyzing enzyme) producing bacteria on solid agar medium containing pectin as carbon source for the growth of bacteria. The hydrolysis of the pectin was determined by using 1% hexadecyltrimethyl ammonium bromide solution that precipitates the unhydrolyzed pectin and clear zone around the bacterial growth indicates the positive test.

Procedure

Pectin hydrolysis test was performed on pectin agar medium (Table 3.10). The ingredients of the medium were dissolved in distilled water, autoclaved and poured in petriplates. The agar plates were inoculated with the culture using spot inoculation or streak inoculation and incubated at 35±2 °C for 48 h in an inverted position. After incubation the agar plates were flooded with 1% hexadecyltrimethyl ammonium bromide reagent. The reagent precipitated the unhydrolyzed pectin gave white opaque color to the medium. A clear zone around the bacterial growth was observed indicating the pectin hydrolysis by pectinase enzyme.

Table 3.10: Composition of pectin agar medium

Composition	g/L
Pectin	10.0
Sodium nitrate ($NaNO_3$)	2.0
Potassium chloride (KCl)	0.5
Magnesium sulfate ($MgSO_4.7H_2O$)	0.5
Dipotassium phosphate (K_2HPO_4)	1.0
Ferrous sulfate ($FeSO_4$. $7H_2O$)	0.01
Agar	20.0
pH	6.0

3.4.2.10 Cellulose degradation test

Cellulose is the most abundant organic compound and the major constituent of higher plants. It is a polysaccharide unit composed of liner chain glucose units linked by β-1,4glycosidic bonds. Degradation of cellulose is brought about by the release of extracellular enzyme, cellulose, by bacteria, fungi and actinomycetes. The degradation of cellulose was detected in solid agar medium containing carboxymethyl cellulose (CMC) as carbon source using 1% hexadecyltrimethyl ammonium bromide solution. Similarly, as in pectin hydrolysis, the reagent precipitates the intact CMC and the clear zone around the bacterial growth indicated the degradation of cellulose (a positive test).

Table 3.11: Composition of Czapek – mineral salt agar medium

Composition	g/L
Carboxymethyl cellulose (CMC)*	5.0
Sodium nitrate ($NaNO_3$)	2.0
Potassium chloride (KCl)	0.5
Magnesium sulfate ($MgSO_4.7H_2O$)	0.5
Dipotassium phosphate (K_2HPO_4)	1.0
Peptone	2.0
Agar	20.0
pH	6.5

* Dissolve CMC in water with heat till it dissolves.

Procedure

This test was performed on modified Czapek –mineral salt medium containing CMC (Table 3.11). Czapek –mineral salt agar petriplates were prepared and inoculated with bacteria culture by streak inoculation and incubated at 35±2 °C for 48 h in an inverted position. After incubation the agar plates were flooded with 1% hexadecyltrimethyl ammonium bromide reagent. The reagent precipitated the unhydrolyzed cellulose (CMC) gave white opaque color to the medium.

A clear zone around the bacterial growth was observed indicating the cellulose degradation by cellulase enzyme.

3.4.2.11 Carbohydrate utilization test

Carbohydrates are the organic molecules composed of carbon, hydrogen and oxygen. Carbohydrates act as carbon and energy source for the growth of large number of bacteria, fungi and yeasts by metabolizing them by the process of catabolism. Organisms utilize different types of carbohydrates depending upon their enzyme complement, and are used as method for the biochemical differentiation of microorganisms. The growth of the bacterial isolate on different carbohydrates was assessed using minimal medium with pH 12 as growth medium.

Table 3.12: Composition of carbohydrate minimal medium

Composition	g/L
Carbohydrate*	10
Di-potassium phosphate (K_2HPO_4)	2.5
Potassium dihydrogen phosphate (KH_2PO_4)	2.5
Diammonium phosphate dibasic ($(NH_4)_2HPO_4$)	1
Magnesium sulfate heptahydrate ($MgSO_4.7H_2O$)	2
Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$)	0.01
Manganous sulfate ($MnSO_4.4H_2O$)	0.007
pH (maintained by KCl-NaOH buffer)	12

* Carbohydrate: Glucose, Lactose, Mannitol, Xylose, Fructose, Maltose

Procedure

Carbohydrate utilization test was performed on minimal medium having pH 12 (Table 3.12). The carbohydrate in this medium (i.e. sucrose) was replaced by different available carbohydrates (such as glucose, lactose, mannitol, xylose, fructose, maltose). The liquid medium (broth) flasks (containing different carbohydrates) were prepared and inoculated with bacterial culture. The inoculated flasks along with respective carbohydrate control (uninoculated) were incubated at 35±2 °C @ 130 rpm for 48 h. The presence (denoted as +), absence (denoted as -) and weak growth (denoted by w/+) was assessed by comparative analyzing the inoculated tubes with control (uninoculated) tubes.

3.4.2.12 Amino acid utilization test

Amino acids are the smallest unit and building blocks of peptides and proteins. Amino acids act as carbon and nitrogen source for the growth of large number of bacteria, fungi and yeasts by metabolizing them by the process of catabolism. Organisms utilize different types of amino acids depending upon their enzyme complement, and are also used as method for the biochemical differentiation of microorganisms. The growth of the bacterial isolate on different amino acids was assessed using minimal medium with pH 12 as growth medium.

Table 3.13: Composition of amino acid minimal medium

Composition	g/L
Amino acid*	10
Di-potassium phosphate (K_2HPO_4)	2.5
Potassium dihydrogen phosphate (KH_2PO_4)	2.5
Diammonium phosphate dibasic ($(NH_4)_2HPO_4$)	1
Magnesium sulfate heptahydrate ($MgSO_4.7H_2O$)	2
Ferrous sulfate heptahydrate ($FeSO_4.7H_2O$)	0.01
Manganous sulfate ($MnSO_4.4H_2O$)	0.007
pH (maintained by KCl-NaOH buffer)	12

*Amino acid: Glycine, Tyrosine, Valine, Arginine, Serine, Tryptophan, Alanine, Leucine and Threonine

Procedure

Amino acid utilization test was performed on minimal (M9) medium having pH 12 (Table 3.13). The carbon source in this medium (i.e. sucrose) was replaced by different available amino acids (such as glycine, tyrosine, valine, arginine, serine, tryptophan, alanine, leucine and threonine). The liquid medium (broth) flasks (containing different carbohydrates) were prepared and inoculated with bacterial culture. The inoculated flasks along with respective amino acid control (uninoculated) were incubated at 35±2 °C @ 130 rpm for 48 h. The presence (denoted as +), absence (denoted as -) and weak growth (denoted by w/+) was assessed by comparative analyzing the inoculated tubes with control (uninoculated) tubes.

3.4.3 Physiological Characterization

Physiological characterization included the growth of bacterial isolate under saline conditions (0-20% NaCl content), different temperature (20-55 °C) and pH (5-12) in both enrichment (Table 3.1) and minimal medium (Table 3.2). The growths of isolates under different conditions were measured in terms of optical density by spectrophotometer @ 600 nm wavelength. Incubation conditions for salinity and pH was 35±2 °C @ 130 rpm for 48 h.

Salinity range: 0, 2, 4, 6, 8, 10, 12, 14, 16, 18, 20% NaCl

Temperature range: 20, 25, 28, 35, 37, 45, 50, 55 °C

pH range: 5, 6, 7, 8, 9, 10, 11, 12

The efficient acid producing alkaliphilic/alkali-tolerant bacterial strains were then presumptively identified as per Sneath (1986), Cowman and Steel’s manual (Barrow and Feltham, 1993) and study conducted by Yoon et al. (2005).

3.4.4 Phylogenetic Analysis

The 16S rRNA gene sequencing was performed by Xcelris Labs Limited, Ahmedabad (India). DNA was isolated from bacterial strain KG1, KG4, KG5 and KGMD1 by using QIAamp DNA Purification Kit (Qiagen).

3.4.4.1 Quantitation and quality assessment of DNA

After isolation, the DNA stock samples were quantified using Nanodrop spectrophotometer at 260 and 280 nm (1 O.D. @ 260 nm wavelength = 50 µg DNA per mL). Purity of DNA was judged on the basis of optical density ratio at 260:280 nm. The DNA having ratio between 1.8 to 2.0 was considered to be of good purity. Concentration of DNA was estimated using the formula.

$$\text{Concentration of DNA (mg/mL)} = \text{OD@ } 260 \text{ nm} * 50 * \text{dilution factor}$$

Quality and purity of DNA were checked by agarose gel electrophoresis containing agarose 0.8% (w/v) in 0.5X TAE (pH 8.0) buffer. Ethidium bromide (1%; Et Br) was added @ 10µL/100mL. The wells were charged with 5µL of DNA preparations mixed with 1µl gel loading dye (6X). Electrophoresis was carried out at 80V for 30 min at room temperature. DNA from the isolates was visualized under UV using gel documentation system (Fig. 3.7) and the DNA was used further for PCR.

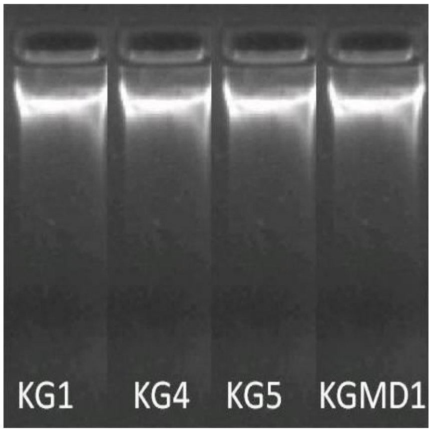


Fig. 3.7: Agarose gel image of genomic DNA isolated from bacterial isolates

3.4.4.2 Polymerase chain reaction (PCR)

The 16S rRNA gene fragment was amplified by PCR method from genomic DNA using universal bacterial primers 8F (AGA GTT TGA TCC TGG CTC AG) and 1492R (ACG GCT ACC TTG TTA CGA CTT). Table 3.14 shows the composition of reaction mixture for PCR and the reaction was carried out in Eppendorf Thermal Cycler for 30 cycles (Table 3.15).

Table 3.14: Composition of reaction mixture for PCR

Component	Quantity (µL)	Final concentration
DNase-RNase free water	7.50	--
2X PCR master mix (Fermentas)	12.50	1X
Forward primer (10 pmole/ µL)	1.00	10 pmole
Reverse primer (10 pmole/ µL)	1.00	10 pmole
Diluted DNA (30 ng/µL)	3.0	--
Total	25.00	--

Table 3.15: Steps and conditions for PCR

Steps	Temperature (°C)	Time (sec)	Cycles
Initial denaturation	95	120	1
Final denaturation	94	30	30
Annealing	52	30	
Extension	72	90	
Final extension	72 °C	600	1

Amplified PCR product was checked by using gel electrophoresis. PCR product (5 µL) of each isolate was mixed with 1 µL of 6X gel loading dye and electrophoresed on 1.2 % agarose gel containing ethidium bromide (1% solution @10 µL/100 mL) at constant 5V/cm for 30 min in 0.5 X TAE buffer (Fig. 3.8). PCR bands of isolates showed 1.5 kb size compared with 1 kb gene marker (M). Amplified PCR product was purified using Qiagen Mini elute Gel extraction kit according to the manufacturer's protocol.

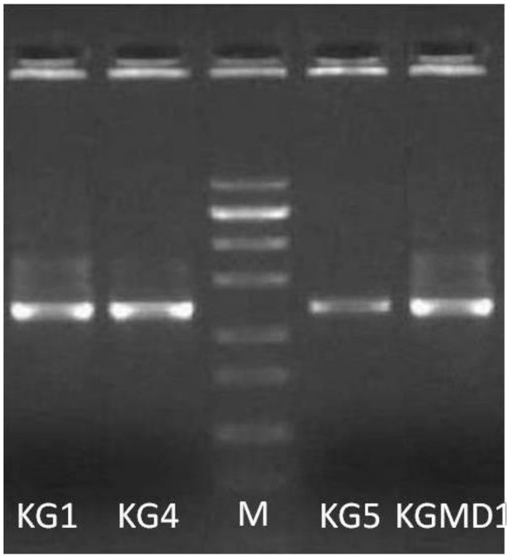


Fig. 3.8: Agarose gel image of amplified PCR product; M = 1kb gene marker

3.4.4.3 Sequencing of purified 16S rDNA (or rRNA) gene segment

Sequencing of the purified 16S rRNA gene was performed using BigDye® Terminator v3.1 Cycle sequencing kit (Applied Biosystems, USA) as recommended by manufacturer. The purified sequencing reaction mixtures were electrophoresed automatically using ABI 3730xl Genetic Analyzer (Applied Biosystem, USA).

3.4.4.4 Sequence analysis

The 16S rRNA gene sequence of the bacterial strains (KG1, KG4, KG5 and KGMD1) were processed manually, analyzed at NCBI (National Centre for Biotechnology Information) server (http://www.ncbi.nlm.nih.gov) using BLAST tool and compared to the corresponding neighbour sequences from the GenBank-NCBI database. Multiple alignment of all the strains were performed with related *Bacillus* species (from GenBank-NCBI database) using Multalin program (Corpet, 1988) and phylogenetic tree was constructed by the neighbor joining method (Saitou and Nei, 1987). Evolutionary distance matrices for the neighbor joining method were calculated using the algorithm of Kimura's two-parameter model (Kimura, 1980). The topology of the phylogenetic tree was evaluated by performing a bootstrap analysis with 1000 replicates. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequence of the bacterial strains and related *Bacillus* species were collected and phylogenetic tree were constructed in MEGA 5. The 16S rRNA sequences were submitted to GenBank-NCBI and provided with the accession numbers. The bacterial strains were also deposited to National Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory (NCL), Pune (India).

3.5 STUDY OF MORTAR AND CONCRETE PROPERTIES

3.5.1 Materials Used

3.5.1.1 Cement

Cement, a fine grey powder of IS mark 43 grade Portland pozzolana cement (Brand- UltraTech) was used for all mixes. The cement used was fresh and without any lumps. Testing of cement was done as per BIS-8112 (1989) and the results are reported in Table 3.16. The chemical composition of cement in the oxide form was analyzed by EDX is given in section 3.2.2.

Table 3.16: Physical properties of Portland pozzolana cement

Properties	**Values**	**Standard Values**
Initial setting time (min)	120	Not less than 60
Final setting time (min)	240	Not greater than 600
Fineness (% mass retained on 90 µm sieve)	1.0	<10
Specific gravity	3.03	--
Standard consistency (%)	34	--
pH	13.25	--

3.5.1.2 Fine and Coarse aggregate

Natural sand with 4.75 mm maximum size was used as fine aggregate after sieved through 4.75 mm sieve to remove any particle greater than 4.75 mm and removed the dust. Locally available garvel having the size of 12.5 mm were used as coarse aggregates in this work. Testing of fine and coarse aggregates was done as per BIS: 383-1970 (1970). Properties of the coarse and fine aggregates used are shown in Table 3.17.

Table 3.17: Physical properties of fine and coarse aggregate

Characteristics	Coarse Aggregate	Fine Aggregate
Specific gravity	2.62	2.57
Water absorption (%)	0.80	1.02
Fineness modulus	2.0	2.28
Size (mm)	12.5 max.	4.75 max.
Moisture content (%)	Nil	0.16

3.5.1.3 Cement kiln dust

Cement kiln dust (CKD) is fine powdery material of grey-black in color and relatively uniform in size. The physical and chemical properties of CKD were determines as per the procedure given in section 3.2. As CKD is generated from same raw materials as cement clinker, instead of having similar chemical composition to that of ordinary Portland cement, significant variation in physical and chemical properties of CKDs obtained from different cement plants has been observed.

3.5.1.4 Water

Water used for casting specimens conformed to the requirements of BIS- 456 (2000). Test results are given in Table 3.18.

Table 3.18: Properties of water

Properties	Observed value
pH	7.88
Dissolved Solids (mg/L)	280
Suspended Solids	Nil
Chlorides (mg/L)	20
Sulphates (mg/L)	68
MPN Value/100 mL.	Nil

3.5.2 Mortar Properties

The untreated and bacterial treated CKD was used for the mortar mixtures along with series of control mixtures containing untreated CKD. Cement was replaced with CKD in proportion of 5, 10, 15, 20 and 30% by weight. For control mortar (without CKD), the mix proportion was prepared using cement and fine aggregate in the ratio (1:3). Cement-mortar properties were studied in three replications. The water for normal consistency, setting times (initial and final) and hydration of cement-CKD paste, and compressive strength of CKD blended cement mortar was determined.

3.5.2.1 Water for normal consistency

Normal consistency is defined as the amount of water required to prepare cement paste or cement blended paste with required degree of plasticity. Normal consistency was determined by the penetration of Vicat's needle attached to Vicat's apparatus as per BIS: 4031 part4 -1996 (or ASTM C 187 e11) method for control cement paste (0% CKD) and CKD blended cement pastes (5, 10, 15, 20 and 30%).

3.5.2.2 Setting time

Setting time is used to evaluate the time lapsed to undergo the normal hydration reaction by cement paste. Initial setting time determines the time lapses from the moment water is added until the cement paste ceases to be in plastic state whereas final setting time determines the time lapses from the moment of water is added until the cement paste is hardened. Initial and final setting time of the control cement paste (0% CKD) and CKD blended cement pastes (5, 10, 15, 20 and 30%) were determined as per BIS:4031 part 5 – 1996 (or ASTM C 191-08) method using Vicat's apparatus (Fig. 3.9). Initial setting time was recorded when the Vicat's needle fails to pierce the cement or blended cement paste block to a point 5.0 ± 0.5mm measured from the bottom of the mould from the time of water is added. Final setting time was recorded when the Vicat's needle with annular attachment makes an impression on the surface of cement paste block instead of penetration into the paste block.

Fig. 3.9: Vicat's Apparatus

3.5.2.3 Hydration

Hydration of cement or blended cement paste can be defined as the amount of water that has been chemically combined to form hydration products and can be determined by measuring the "non-evaporable water". The hydration of the cement paste is denoted by "α", which varies between 0 and 1 (complete hydration of paste) and estimated by the method given by Powers (1947). A small sample of hardened paste (cement or blended cement) was dried in an oven at

100 °C to remove “evaporable” water and then weigh. After weighing, the sample was ignited to 1050 °C in a furnace and weighed again. At 1050 °C temperature, the hydration products decompose and the “non-evaporable” water was driven off. The hydration was estimated at the age of 7, 28 and 91 days of curing by the formula:

$$\alpha = \frac{\text{wt. of dry sample} - \text{wt. of ignited sample}}{\text{wt. of ignited sample}}$$

3.5.2.4 Compressive strength

The casting of mortar cubes, containing untreated and bacterial treated CKD, of size 5000 mm^2 prepared for compressive strength was in accordance with Indian Standard BIS: 4031 part 6 - 1996 (1996). After casting, the specimens were allowed to remain in iron molds for first 24 h at room temperature (27 ± 2°C), then demolded and placed in the water tank at room temperature for curing. The mortar cubes were cured for 7, 28 and 91 days. Specimens were tested for compressive strength using universal testing machine (UTM; HungTa instruments) with load bearing capacity of 1000 kN (Fig. 3.10). The compressive strength was calculated by the formula given below and then compared with the mortar made without CKD.

$$\text{Compressive strength} = \frac{\text{Load in Newton (N)}}{\text{Area in mm}^2}$$

Fig. 3.10: Universal testing machine (UTM) for compressive strength testing of mortar

3.5.3 Concrete Properties

Concrete cubes of M20 grade having size 150 mm x150 mm x 150 mm and cylinders having 100 x 200 mm size were casted in triplicates using untreated and bacterial treated cement kiln dust as partial replacement of cement (0, 5, 10, 15, 20 and 30%).

3.5.3.1 Mixture preparation for cube casting

Ingredients of M20 grade concrete mix and their mix ratio are given in Table 3.19. Mixture proportions of concrete M20 is shown in Table 3.20. This mix proportion was used both for untreated and bacterial treated CKD concrete.

Table 3.19: Mix proportion (M20)

Unit of Batch	Water (L)	Cement (kg)	Fine Aggregate (kg)	Coarse Aggregate (kg)
Cubic meter content	195	390	569	1165
Ratio of ingredients	0.5	1	1.45	2.98

Table 3.20: Mix proportions of concrete

Materials	Cement kiln dust (%)					
	0	5	10	15	20	30
Cement (kg/m^3)	390	370.5	351	331.5	312	273
Fine Aggregate (kg/m^3)	568.87	568.87	568.87	568.87	568.87	568.87
Coarse Aggregate (kg/m^3)	1164.12	1164.12	1164.12	1164.12	1164.12	1164.12
Water (kg/m^3)	185	185	185	185	185	185
CKD (kg/m^3)	-	19.5	39.0	58.5	78.0	117.0

3.5.3.2 Preparation of cubes and cylinders

Concrete cubes and cylinders were made (Fig. 3.11) using concrete mixture without CKD and mixture containing untreated and bacterial treated CKD as partial replacement of cement (5, 10, 15, 20 and 30%). After casting, the specimens were stored at room temperature and demolded after 24 hours. After demolding, test specimens were put into a water-curing tank for 7, 28 and 91 days at room temperature.

Fig 3.11: Casting of concrete cubes and cylinders (M20)

3.5.3.3 Sample collection for leachate characterization

During fresh concrete mix, 10g of concrete mixture (control, with untreated and bacterial treated CKD) samples were collected randomly for leachate characterization (ASTM D 3987).

3.5.3.4 Compressive strength

The casting of concrete cubes, containing untreated and bacterial treated CKD, of size 150 mm^3 prepared for compressive strength was in accordance with Indian Standard BIS: 516 - 1959 (1959). The cubes were cured for 7, 28 and 91 days and studied in triplicate. Specimens were tested for compressive strength using compression testing machine (CTM; AIMIL Ltd.) with load bearing capacity of 3000 kN (Fig. 3.12). The compressive strength was calculated by the formula given below and then compared with the concrete made without CKD.

$$\text{Compressive strength} = \frac{\text{Load in Newton (N)}}{\text{Area in mm}^2}$$

Fig. 3.12: Compression testing machine (CTM) for compressive strength testing of concrete

3.5.3.5 Split tensile strength

The split tensile strength of concrete cylinders (size 100 x 200 mm) was conducted according to ASTM C 496-11 (2011) using universal testing machine (Hung Ta Instruments, Taiwan) (Fig. 3.13). The average reading of three cylinders was recorded for each treatment at the age of 7, 28 and 91 days for all mixtures. Split tensile strength was calculated by the formula:

$F_t = 2P/\Pi DL$

Where, F_t = Split tensile strength (N/mm^2)

P = Load applied (N)

D = Diameter of specimen (mm)

L = Length of specimen (mm)

Fig. 3.13: Universal testing machine (UTM) for split tensile strength testing of concrete

3.5.3.6 Ultrasonic pulse velocity (UPV)

It is a non-destructive method uses mechanical waves resulting in no damage to the concrete. The UPV test was done to assess the quality of concrete as per BIS: 13311 (part 1) – 1992 using TICO ultrasonic instrument (Fig. 3.14). The propagation of pulse waves depends upon the elastic properties and density of the medium. High pulse velocity denotes the good quality of concrete. Concrete cylinders of size 100 mm x 200 mm were casted and analyzed after 7, 28 and 91 days of curing. Table 3.21 shows the grading of concrete specimens on the basis of standard pulse velocity values.

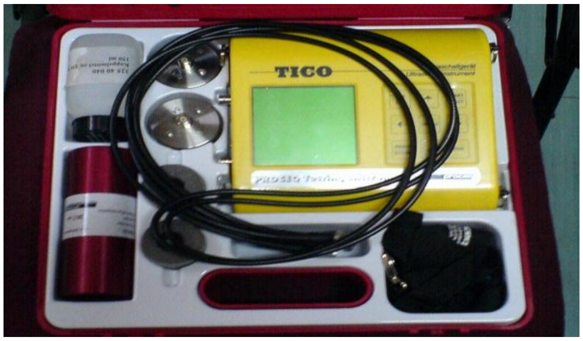


Fig. 3.14: Ultrasonic pulse velocity apparatus (TICO instruments)

Table 3.21: Classification of the quality of concrete on the basis of ultrasonic pulse velocity

Longitudinal Pulse Velocity (m/s)	Quality of Concrete (Grading)
>4500	Excellent
3500-4500	Good
3000-3500	Doubtful
2000-3000	Poor
<2000	Very Poor

3.5.3.7 Rapid chloride permeability test (RCPT)

RCPT has been developed as a quick method to measure the rate of transport of chloride ions in concrete as per ASTM C 1202 (2012) method for determining the durability of concrete structures to prevent corrosion. Cylindrical samples of 100 mm diameter and 200 mm height were prepared. After curing for 7, 28 and 91 days, the samples were cut into 50 mm thick slices and were tested with the RCPT (PROOVE'it, Germann Instruments) by applying 60 V (Fig. 3.15). The current passing through the specimen in 6 h was measured and interpreted on the basis of total charge (Coulomb number) transferred from NaCl (3%) to NaOH (0.3 N) in the form of chloride ions. The relationship between chloride penetrating rate and the charge passed in coulombs is given in Table 3.22.

Fig. 3.15: Set up of rapid chloride permeability test (PROOVE'it, Germann Instruments)

Table 3.22: Chloride ion penetrability based on charge passed (ASTM C1202 –12)

Charge Passed (coulombs)	Chloride Ion Penetrability
>4000	High
2000-4000	Moderate
1000-2000	Low
10-1000	Very Low
<100	Negligible

3.5.3.8 Water absorption and porosity

Concrete cubes of size 150 mm were used to determine for the water absorption and porosity by using the ASTM C 642 (1997) after 7, 28 and 91 days of curing.

Procedure

(i) Oven-dry mass: After curing the mass of specimens of different treatments were determined and dried in an oven at a temperature of 100 to 110°C for 24 h. After oven drying the specimens were allowed to cool at room temperature (20 to 25°C) and the mass of the specimen was taken. The oven dried mass was taken three times for period of 24 h interval to confirmed the dryness of the specimen. The mass of the specimen was designated as A.

(ii) Saturated mass after immersion: After oven drying the specimens were immersed in water at approximately 21°C for 48 h. The mass of specimens were determined after 48 h by removing the surface moisture with paper towel and designated as B.

(iii) Saturated mass after boiling: The specimes weighed after immersesd in water for 48 h were then placed in a water bath, covered with tap water and boiled for 5 h. The specimens were then allowed to cool overnight (approx. 14 h) by natural loss of heat at room temperature (20 to 25 °C). The mass of the specimens were then determined after removing the surface moisture and designated as C.

(iv) Immersed apparent mass: The apparent mass of the specimens after immersion and boiling, were taken by suspending the specimens with wire in the water tank. The apparent mass in water was designated as D.

Calculation

Absorption after immersion, % = [(B-A)/A] * 100

Absorption after immersion and boiling, % = [(C-A)/A] * 100

Bulk density, dry = [A/(C-D)] *ρ = g_1

Bulk density after immersion = [B/(C-D)] .ρ

Bulk density after immersion and boiling = [C/(C-D)] .ρ

Apparent density = [A/(A-D)] .ρ = g_2

Volume of permeable pore space (voids), % = $(g_2 - g_1)/g_2 * 100$

where,

A = mass of oven-dried specimen, g

B = mass of surface- dried specimen after immersion, g

C = mass of surface- dried specimen after immersion and boiling, g

D = apparent mass of specimen in water after immersion and boiling, g

g_1 = bulk density, dry Mg/m^3

g_2 = apparent density, Mg/m^3

ρ = density of water =1 Mg/m^3 = $1 g/cm^3$

3.6 CHARACTERIZATION OF LEACHATE FROM CKD AND CONCRETE MIXTURE

Samples were taken from each treatment of fresh concrete mix and leachate was obtained by using shake extraction of solid waste method (ASTM D 3987- 2012). Leachate obtained from M20 concrete made with untreated and bacterial treated CKD as partial replacement of cement (5, 10, 15, 20 and 30%) was characterized for alkalinity, chloride and metal concentration. Cement kiln dust was also analyzed for metal content after bacterial treatment (KG1, KG4, KG5 and KGMD1) and compared with untreated CKD.

3.6.1 Alkalinity and Chloride Analysis of Fresh Concrete Leachate

Leachate generated during fresh concrete mixing of different treatments was analyzed for alkalinity and chloride as per APHA (2005) method (Section 3.3). Total alkalinity was estimated by using methyl orange indicator and N/50 H_2SO_4. Ten mL of leachate sample was taken in a 50 mL conical flask. Few drops (2-3) of methyl orange indicator was added which turned the color of the leachate colorless to yellow. Then the leachate sample was titrated with N/50 H_2SO_4 with gentle shaking till the orange color appeared. The initial and final volume of consumed N/50 H_2SO_4 was noted and alkalinity was calculated as per equation:

$$\text{Alkalinity (mg/L CaCO}_3) = \frac{\text{Final reading} - \text{Initial reading}}{\text{Volume of sample taken (mL)}} \text{ x } 1000$$

Chloride content was estimated by using potassium chromate indicator and N/35.5 $AgNO_3$ solution (Section 3.3). Ten mL of leachate sample was taken in a 50 mL conical flask. Few drops (2-3) of potassium chromate indicator was added which turned the color of the leachate colorless to fluorescent yellow. Then the leachate sample was titrated with N/35.5 $AgNO_3$ with gentle shaking till the brick red color appeared. The initial and final volume of consumed N/35.5 $AgNO_3$ was noted and chloride was calculated as per equation:

$$\text{Chloride (mg/L CaCO}_3) = \frac{\text{Final reading} - \text{Initial reading}}{\text{Volume of sample taken (mL)}} \text{ x } 1000$$

3.6.2 Metal analysis of CKD and Fresh Concrete Leachate

For metal analysis, samples from control mix, untreated and bacterial treated CKD were taken after 20 days of treatment. For leachate extraction, 1 g of the sample was added to the Milli Q water (1:20) and agitated at about 20 ˚C for 18 h (ASTM D 3987-2012). The leachate extracts were then centrifuged for 10 min at 3000 g for the settling of large particles and the resulting aqueous phase was filtration through a 0.22 µm filter. The filtered extract was then acid digested and analyzed for various metals by using inductive coupled plasma mass spectrometer (ICP-MS) (Zarcinas et al. 1987). The digested leachate was analyzed for 10 metals including Chromium (Cr), Cobalt (Co), Iron (Fe), Lead (Pb), Silver (Ag), Cadmium (Cd), Nickel (Ni), Zinc (Zn), Copper (Cu) and Molybdenum (Mo). The multi-element metal standard was procured from Sigma-Aldrich.

The leachate analysis of fresh concrete mixture of different treatments (0, 5, 10, 15, 20 and 30% CKD) was also performed for metal content and analyzed as per the method describing above by taking 10 g of fresh concrete mixture in 100 mL of water.

3.6.2.1 Acid digestion of samples for metal analysis

The digestion vessels and volumetric ware were acid washed and rinsed with double distilled water. Two mL of sample was added to digestion vessel, and 5± 0.1 mL of 70% nitric acid (HNO_3) and 1 ± 0.1 mL of concentrated hydrochloric acid (HCl) was also added to the vessel in a fume hood (or digestion chamber). The temperature of the digestion vessels were maintained at 110 °C throughout the digestion. The samples were removed from the digestion block periodically to cool until the foam dissolved. The samples were digested continuously with nitric acid until the brown NO_2 fumes were barely visible. Two ml of 72% perchloric acid ($HClO_4$) was added to the vessel. The digestion was continued at 110 °C for 16 h. The digestate appeared water white. It was then cooled to room temperature. The final volume of the digestate was brought to 20 mL using Milli-Q water.

3.6.2.2 Metal analysis by inductively coupled plasma mass spectrometry (ICP-MS)

The acid digested samples were filtered through a coarse filter paper and then through 0.45 μm membrane filter. The filtrate was analyzed for metal content (Cr, Co, Fe, Pb, Ag, Cd, Ni, Zn, Cu and Mo) by using ICP-MS (Inductively Coupled Plasma- Mass Spectrometry) at IIT Roorkee (Fig. 3.16).

Fig. 3.16: Inductively coupled plasma mass spectrometry (ICP-MS) instrument for metal analysis

3.7 MICROSTRUCTURE ANALYSIS

Mortar and concrete specimens were subjected to microstructure analysis which includes scanning electron microscopy (SEM) and X-ray diffraction studies (XRD).

3.7.1 SEM Analysis

Scanning electron microscopy (SEM) has been developed for the analysis of complex microstructure of cement and concrete, and verifies the mechanism predicted by compressive strength. After each curing period (7, 28 and 91 days), the broken mortar and concrete specimens were taken from inner core of the matrix after compressive strength testing, and were dried at 80 °C for 24 h. Before SEM analysis, the samples were placed in desiccators for over-night for removal of moisture. The small broken specimens (with and without bacterial treated CKD) of mortar and concrete mounted on brass stubs using carbon tape, gold coated and then microscopic structure was analyzed at 15 kV by scanning electron microscope (JEOL JSM 6510 LV, USA) (Fig. 3.17).

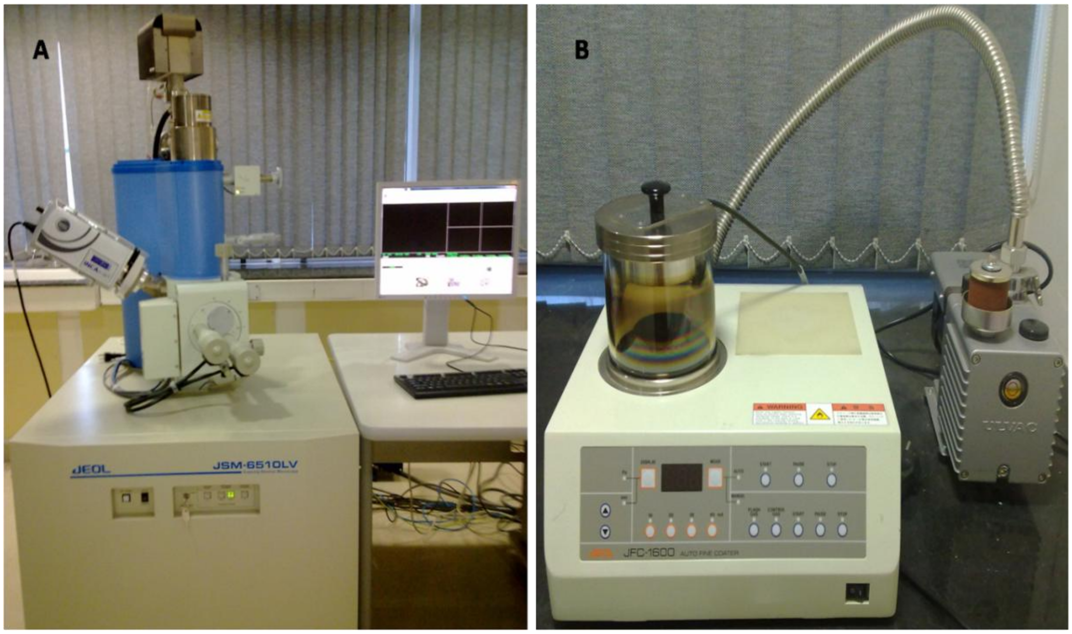


Fig. 3.17: Scanning electron microscope (SEM) with gold coating equipment (B)

3.7.2 XRD Analysis

X-ray diffraction (XRD) has been developed as the most prominent technique used for unraveling the hydration phases of the materials produced during hydration process. X-ray diffraction is based on the fact that, in a mixture, the measured intensity of a diffraction peak is directly proportional to the content of the substance producing it. Concrete samples from each mix (after 7, 28 and 91 days of curing) was taken from inner core of the matrix (crushed into fine powder by pestle-mortar) and analyzed in powder X-ray diffraction (XRD; PANalytical X'Pro) (Fig. 3.18). The XRD spectrum was taken from 2 h = 5 θ to 2 h = 60 θ. The peaks in the new positions of the spectrum were marked, compared and identified from the Joint Committee on Powder Diffraction Standards (JCPDS) data file and from the published literature.

Fig. 3.18: X-Ray diffraction (XRD) instrument

3.8 STATISTICAL ANALYSIS

Statistical analysis (t-test and analysis of variance; ANOVA) was calculated for the data by using GraphPad Prism (version 5) software and the significant results were represented at $P<0.05$ or $P<0.0001$ significant level. The standard error of mean values was analyzed for leachate samples estimated for alkalinity, chloride and metal content.

CHAPTER – 4

RESULTS AND DISCUSSION

In this chapter, the findings of an experimental investigation are presented. It includes the results for the isolation and screening of alkali-tolerant bacteria producing acid, physical & chemical characterization of cement kiln dust (CKD), optimization of parameters (time and inoculum) for bacterial treatment of CKD, identification of bacterial isolates by morphological, biochemical, physiological characterization and phylogenetic analysis. Results regarding water consistency, setting time, hydration of cement blended pastes, compressive strength of mortar and concrete, split tensile strength, ultrasonic pulse velocity, RCPT, water absorption and porosity of concrete containing (untreated and bacterial treated) CKD (0, 5, 10, 15, 20 and 30%) are presented in this chapter. Also included are the results of alkalinity, chloride analysis and metal analysis of CKD leachate before and after bacterial treatment, metal leachate analysis of fresh concrete mix, SEM and XRD of hardened concrete cubes containing untreated and bacterial treated CKD with varying percentages.

4.1 ISOLATION OF ALKALI-TOLERANT BACTERIA

Alkaliphiles have been isolated mainly from neutral environments, sometimes even from acidic soil samples. The frequency of alkaliphiles in the environment especially in the ordinary neutral soil sample has been shown to be 10^2-10^5 per g of soil, which corresponds to 1/10 – 1/100 of the population of the neutrophiles. In the media used to isolate alkaliphilic microorganisms, the sample could be enriched with different substrates such as peptones, glucose, bile salts, casamino acids and caseine (Duckworth et al., 1996; Horikoshi, 1999). An alkaline media has to be used by adjusting the pH of the medium to around 10 with sodium carbonate (Na_2CO_3) and/or borax-NaOH, Na_2CO_3/$NaHCO_3$, Na_2HPO_4/NaOH, KCl/NaOH buffer systems having buffering capacity over the range of pH 9-12 in various media (Maeda and Taga, 1980; Guffanti et al., 1986; Horikoshi, 1999; Gupta et al., 2008). Out of a number of microorganisms based on colony morphology, color and screening for acid production, four alkali-tolerant bacterial cultures designated as KG1, KG4, and KG5 were isolated from rhizospheric soil of citrus plant, whereas KGMD1 was isolated from textile industry waste water on enrichment medium agar plates having pH 10.5. The selected isolates (KG1, KG4, KG5 and KGMD1) were isolated from the rhizospheric soil and waste water as such these acts as habitat of abundant different types of microorganisms and the cultures isolated were able to tolerate and grew at higher pH values by adjusting their cell metabolism essential for survival (Kumar et al., 2011). Initially, the strains were isolated on enrichment medium having pH 10.5 then sub-cultured number of times at pH 11 and then 12 in solid and liquid (broth) minimal medium for acclimatization of the culture at such high pH values. Few studies have been reported on the use of buffer systems such as phosphate buffer (Chislett and Kushner, 1961) and carbonate buffer (Guffanti et al., 1986; Kumar et al., 2011) to increase the pH of the growth medium but no such work has been reported on the use of KCl-NaOH buffer as buffer system for increasing pH of the medium. The pH of the KCl-NaOH buffer system is around 13 so can be used for preparing medium of high pH value such as 12. The isolates were then maintained on minimal medium (pH 12) for further use.

4.1.1 Screening of Isolates for Acid Production

Acid production by the isolates KG1, KG4, KG5 and KGMD1 was observed in both minimal medium and enrichment medium having high pH (Fig. 4.1 and 4.2) and demonstrated highly significant ($p<0.0001$) reduction in pH. The screening of the isolates for acid production was done by measuring the pH of the alkaline medium using (i) pH meter, and (ii) phenolphthalein indicator.

4.1.1.1 By pH meter

In minimal medium, all the isolates reduced the pH of the alkaline medium after an incubation period of 3 days. The pH of the control medium was 12 and after three days of inoculation by respective bacterial isolates (KG1, KG4, KG5 and KGMD1) the overall change in pH of 3.97, 3.89, 3.87 and 3.49 units, respectively, was observed (Fig. 4.1). At 3 days of incubation the pH was significantly lowered by all the isolates whereas at 4 and 5 days of incubation change in

0.03-0.34 pH units was observed which seems to be negligible when compared with change in pH up to 3 days of incubation (Table 4.1).

Table 4.1: Reduction of pH in the alkaline minimal medium by *Bacillus* sp. strain KG1, KG4, KG5 and KGMD1

Isolate	Initial pH	Minimal Medium M9							
		pH of the medium							
		2 day	Δ pH	3 day	Δ pH	4 day	Δ pH	5 day	Δ pH
KG1	12.00	10.18	1.82	8.03	2.15	8.00	0.03	7.82	0.18
KG4	12.00	10.24	1.76	8.11	2.13	8.01	0.10	7.83	0.18
KG5	12.00	10.5	1.50	8.13	2.37	8.03	0.10	7.93	0.10
KGMD1	12.00	10.63	1.37	8.51	2.12	8.27	0.24	7.93	0.34

Δ pH = change in pH after each incubation day

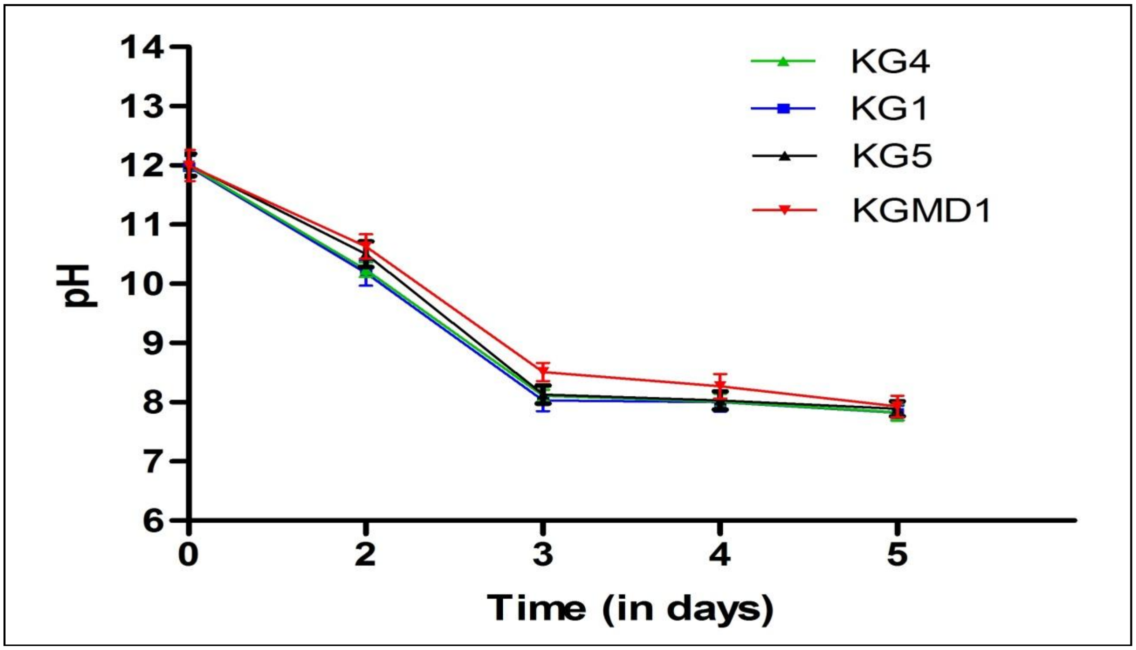


Fig. 4.1: Reduction of pH in the alkaline minimal medium by *Bacillus* sp. strain KG1, KG4, KG5 and KGMD1

Similar trend was also observed in enrichment medium (pH 11.88). Decrease in pH was mainly observed after 3 days of incubation. The results showed that at 3 days of incubation the maximum reduction in pH 2.91, 3.19, 3.16 and 2.56 units by isolate KG1, KG4, KG5 and KGMD1, respectively which corresponds to the acid production by the microbial isolates. At 4 and 5 days of incubation change in pH up to 0.90 units was observed. In minimal medium sucrose was the sole carbon source; microbial isolates efficiently utilized the carbon source and produced acids which eventually decrease the pH of the medium (Horikoshi, 1999).

Table 4.2: Reduction of pH in the alkaline enrichment medium by *Bacillus* sp. strain KG1, KG4, KG5 and KGMD1

Isolate	Initial pH	Enrichment medium							
		pH of the medium							
		2 day	Δ pH	3 day	Δ pH	4 day	Δ pH	5 day	Δ pH
KG1	11.83	11.00	0.83	8.92	2.08	8.02	0.90	7.71	0.31
KG4	11.88	11.80	0.08	8.69	3.11	8.67	0.02	8.67	0.00
KG5	11.88	11.81	0.07	8.72	3.09	8.63	0.09	8.63	0.00
KGMD1	11.88	11.83	0.05	9.32	2.51	8.82	0.50	8.69	0.13

Δ pH = change in pH after each incubation day

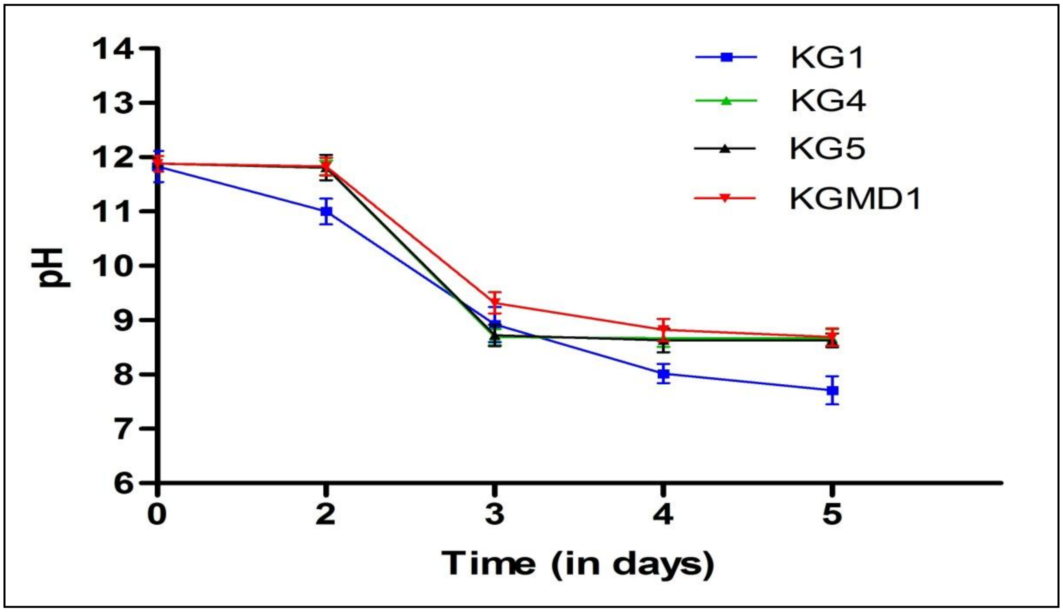


Fig. 4.2: Reduction of pH in the alkaline enrichment medium by *Bacillus* sp. strain KG1, KG4, KG5 and KGMD1

4.1.1.2 Phenolphthalein indicator test

An alternative method was also performed to screen the acid production potential of the isolates *Bacillus* sp. strain KG1, KG4, KG5 and KGMD1 by using phenolphthalein indicator. Decrease in the optical density (O.D. @ 554 nm) of the M9 medium with respect to change in color of phenolphthalein also indicated acid production by the isolate. Initially at high pH (~12) the M9 medium found bright pink in color and after inoculating the strain the decrease in pH was indicated by fading of the color from pink to colorless with growth. Fig. 4.3 shows the color fading of the M9 broth (in flasks) containing phenolphthalein.

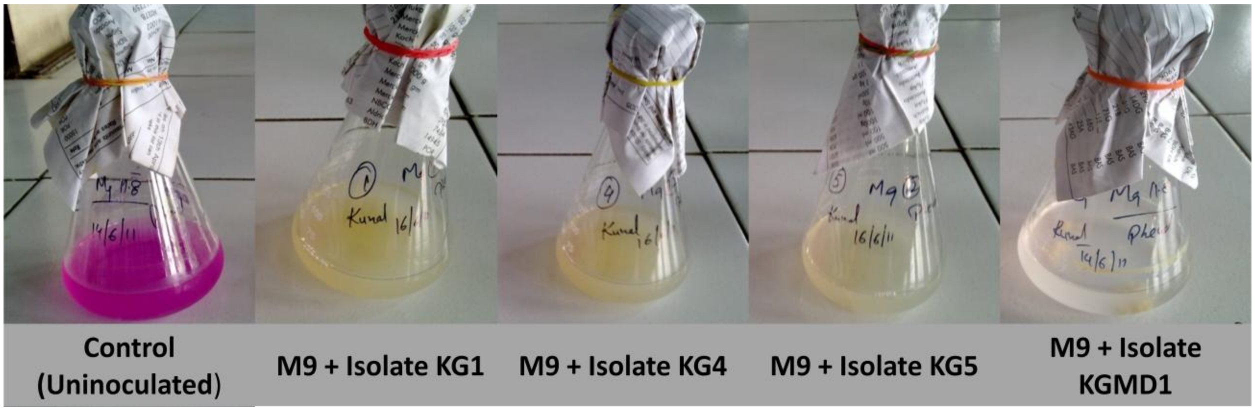


Fig. 4.3: Change in color of the minimal (M9) medium containing phenolphthalein before inoculation (control) and after inoculation with strain KG1, KG4, KG5 and KGMD1

The change in color of the M9 medium containing phenolphthalein with time was also determined using spectrophotometer before and after bacterial inoculation. Fig. 4.4 shows the significant (p = <0.0001) decrease in the optical density of the colored medium with time and observed that strain KG1 and KG4 decreased the pH of the medium (fading of the color of the medium) within 30 h of inoculation followed by KGMD1 and KG5 after 36 and 42 h, respectively. The change in pH possibly is attributed to the production of organic acids in the medium by alkaliphilic *Bacillus* sp. in the presence of carbohydrates and these results were in agreement with the observation of Paavilainen et al. (1994). By comparing these two media compositions for microbial growth, minimal media was observed better media compared to enrichment media as decrease in pH in minimal medium was observed within 2 days of incubation while no such reduction in pH was observed in enrichment medium. Also, the minimal medium is an economical and defined media compared to enrichment medium; so, minimal media was used for the sub culturing process of the isolates for this study.

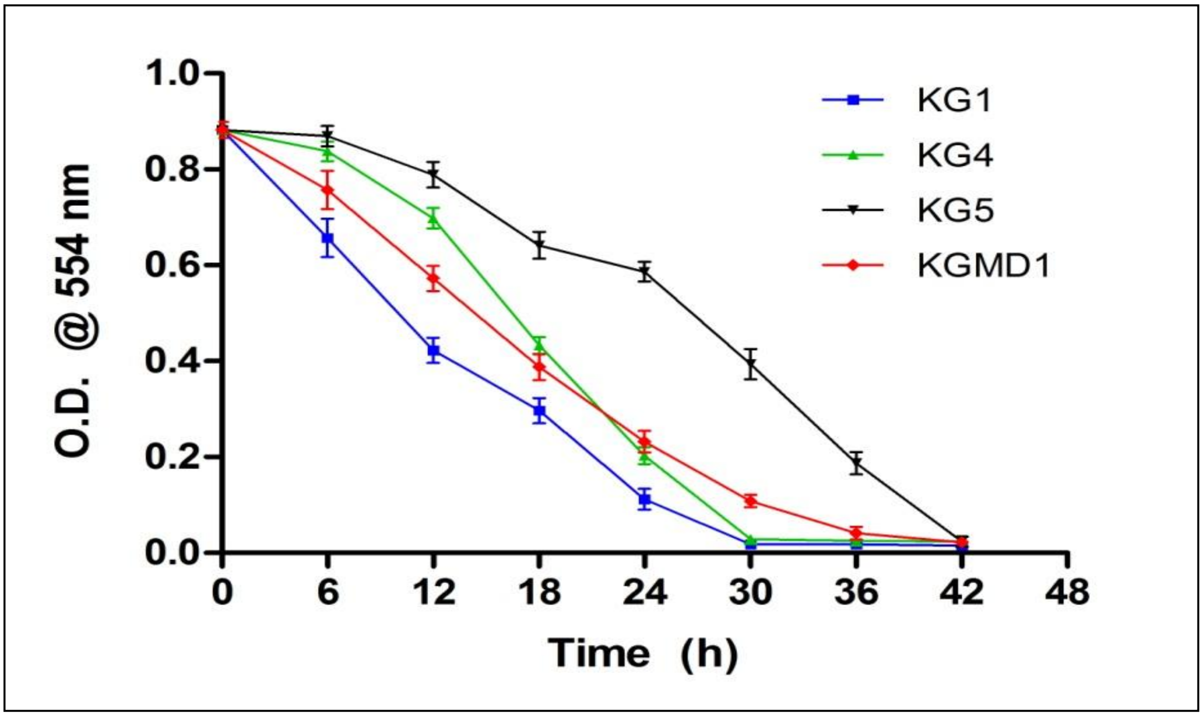


Fig. 4.4: Decrease in the optical density of the centrifuged colored medium (M9 + phenolphthalein) with time after inoculation with isolate KG1, KG4, KG5 and KGMD1

4.2 CHARACTERIZATION OF CEMENT KILN DUST

4.2.1 Physical Properties

Cement kiln dust (CKD) is fine powdery material of grey-black in color and relatively uniform in size. Table 4.3 presents the physical properties of cement kiln dust. Fineness of CKD was observed as 22.5% (percent mass retained on 90 µm sieve). CKD was somewhat coarser than Portland cement (fineness = 1%) but finer than fine aggregate. Specific gravity of CKD was 2.39 and pH was 12.15. The average particle size of CKD was below 90 µm and moisture content was 0.02%. Due to pozzolanic nature, high alkalinity and derived from same raw materials as cement clinker, CKD was used as partial replacement to cement in this study.

The typical specific gravity of CKD varies between 2.4-2.8 (Collins and Emery, 1983; Taha et al., 2004) and less than the value (3.15) assigned to Portland cement (Konsta-Gdoutos and Shah, 2003). Peethamparan et al. (2008) investigated the particle size of four different CKDs and reported that the average particle size of the CKD obtained from preheater and wet pyroporcess of cement kiln ranges between 10-20 µm. Sreekrishnavilasam et al. (2006) observed the particle size of five fresh CKDs and one landfilled CKD. The author stated significant variation in mean particle size of different fresh CKDs ranges from 2.8-55 µm. It was also observed that CKDs may have greater percentages of particles smaller than Portland cement whereas the particle size of landfilled CKD may go beyond 100 µm.

Table 4.3: Physical properties of cement kiln dust

Physical properties	Values
Color	Grey-black
Specific gravity	2.39
Fineness (% mass retained on 90 µm sieve)	22.5
pH	12.15
Moisture content (%)	0.02

4.2.2 Chemical Properties

Cement kiln dust is generated during the manufacturing of cement clinker from the same raw materials and thus, has a composition similar to that of the ordinary Portland cement. But, significant variation in different CKDs has been observed depending upon type of raw material used, burning fuel and cement manufacturing processes. Cement kiln dust was analyzed for the presence of elements in oxide form. Table 4.4 presents the chemical composition of CKD used in the study. CKD is mainly composed of lime (CaO; 55.78%), silica (SiO_2; 13.17%), alumina (Al_2O_3; 2.38%) and iron (Fe_2O_3; 2.62%) followed by alkali (K_2O; 1.12%) and sulfur (SO_3;

1.13%). Absence of sodium (Na_2O) and chloride was observed with small amounts of copper (CuO; 0.89%), magnesium (MgO; 0.69%) and zinc (ZnO; 0.66%) in CKD whereas sodium (Na_2O; 0.32%) is present in cement. CKD is typically characterized by higher alkali content as compared to Portland cement, particularly in potassium (K_2O) and sulfur (SO_3) content and is responsible for alkalinity of cement kiln dust (Udeyo and Hee, 2002; Taha et al., 2004; Maslehuddin et al., 2008). The typical chemical composition of the ordinary Portland cement and cement kiln dust reported in different literatures is already shown in Table 1.3.

Sreekrishnavilasam et al. (2006) and Tennis and Bhatty (2006) studied the chemical composition of CKDs and Portland cement obtained from 63 and 123, respectively, published datasets from different cement plants, and indicated that oxides of calcium and silica are the major constituents of CKD similar to cement, although the values are lower than the values for Portland cement.

Table 4.4: Chemical properties of cement kiln dust

Constituents	Values (%)
Calcium oxide (CaO)	55.78
Silica (SiO_2)	13.17
Alumina (Al_2O_3)	2.38
Magnesium oxide (MgO)	0.69
Sulphur trioxide (SO_3)	1.13
Potassium oxide (K_2O)	1.12
Sodium oxide (Na_2O)	--
Iron oxide ($Fe_2 O_3$)	2.62
Cupric oxide (CuO)	0.89
Zinc oxide (ZnO)	0.66
Loss of Ignition (LOI)	--

4.3 BACTERIAL TREATMENT OF CEMENT KILN DUST

Several environment authorities such as EPA (Environmental Protection Agency in US) and CPCB (Central Pollution Control Board in India) have set guidelines to monitor the pH or alkalinity of industrial wastes before discharging to environment. According to the guidelines the pH of the effluent or waste discharged by the industries should be in the range of 6.5-8.5 (i.e. neutral) as this is the important parameter to check surface and ground water pollution. Although several chemicals such as hydrochloric acid (Cronin and Lo, 1998), sulphuric acid (Prisciandaro et al., 2005), phosphoric acid (Baccella et al., 2000) and carbon dioxide (Gebhardt, 2001; Rao et al., 2007; Mohamed and El-Gamal, 2009) are available to neutralize the high alkaline industrial waste by-products and waste water but these chemicals are difficult to handle as they are hazardous, corrosive, and large quantities of acid would required which is economically not feasible and having dangerous effect on the health of the workers as well as on the industrial processes.

The removal of the alkalinity of CKD by using alkaliphilic/alkali-tolerant bacterial strain is an eco-friendly method and was the challenging task for this study as till date no study was performed in treating the alkaline solid industrial wastes with biological organisms. Cement kiln dust was treated with different isolates in the ratio CKD to culture 4:1. The bacterial treatment was optimized on the basis of time (days) of incubation and the culture inoculum (optical density; O.D. @ 600 nm wavelength).

4.3.1 Optimization of Time

The incubation time (in terms of days) for the treatment of cement kiln dust with *Bacillus* sp. strain KG1, KG4, KG5 and KGMD1 was optimized by inoculating the cultures had an O.D. of 1.0. Fig. 4.5 shows the reduction in alkalinity of CKD in different treatments with respect to incubation time. The treatment was incubated for 25 days and analysis for alkalinity was performed after regular interval of 5 days. The alkalinity of control CKD was 1467±26.58 mg/L on $CaCO_3$ scale. After 5 days of incubation the alkalinity was reduced to 690±32.97, 630±13.89, 640±13.89 and 640±13.92 mg/L $CaCO_3$ in treatments inoculated with strain KG1, KG4, KG5 and KGMD1, respectively. The maximum reduction in alkalinity of 72.7 (400±28.31), 70.7 (430±16.46), 72.0 (410±15.04) and 70.0% (440±23.18) was observed in strain KG1, KG4, KG5 and KGMD1, respectively, treated CKD after 20 days of incubation. The results exhibited significant ($p<0.05$; t-test at 95% confidence limits) reduction in the alkalinity of the CKD in leachate as compared to control. Thus, in all treatments the optimum incubation time of 20 days was observed.

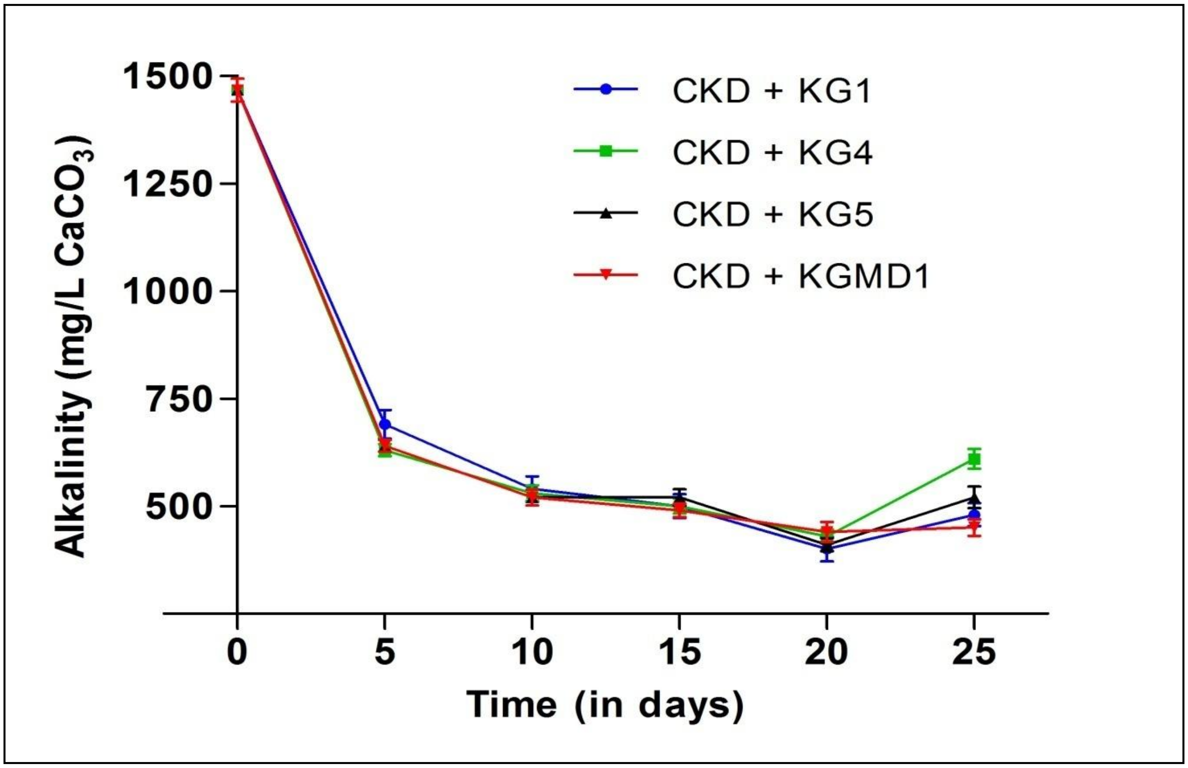


Fig. 4.5: Alkalinity reduction of the cement kiln dust after treatment with *Bacillus* sp. strain KG1, KG4, KG5 and KGMD1 at different incubation time

The leachate of bacterial (strain KG1, KG4, KG5 and KGMD1) treated samples at regular interval of 5 days up to 20 days was also analyzed for pH reduction (Table 4.5). The pH of the control CKD leachate was 12.15 which after bacterial treatment at 20 days of incubation reduced to 8.57, 10.20, 9.72 and 10.49, respectively. From the Table 4.5, it is clear that strain KG1 showed maximum reduction in pH of CKD after 20 days of incubation followed by KG5, KG4 and KGMD1.

Table 4.5: Reduction in pH of cement kiln dust leachate after treatment with *Bacillus* sp. strain KG1, KG4, KG5 and KGMD1 at different incubation time

Days → Treatment	5 d	10 d	15 d	20 d	Δ pH @ 20 d
Control CKD	12.15±0.15				--
CKD + KG1	11.22±0.07	11.07±0.08	10.13±0.09	8.57±0.07	3.58±0.08
CKD + KG4	11.21±0.07	11.19±0.10	11.15±0.08	10.20±0.14	1.95±0.01
CKD + KG5	11.08±0.09	10.85±0.09	10.13±0.07	9.72±0.10	2.43±0.05
CKD + KGMD1	11.17±0.13	11.14±0.14	11.04±0.11	10.49±0.13	1.66±0.02

Δ pH @ 20 d = change in pH at 20 days of incubation compared with control

4.3.2 Optimization of Culture Inoculum

The bacterial inoculum (in terms of O.D. @ 600nm) for the treatment of cement kiln dust with *Bacillus* sp. strain KG1, KG4, KG5 and KGMD1 was optimized by inoculating the cultures had an O.D. in the range of 0.6 - 1.0 with 0.1 intervals for 20 days. Fig. 4.6 shows the alkalinity reduction of the cement kiln dust after treatment with *Bacillus* sp. strain KG1, KG4, KG5 and KGMD1 having different cell density (O.D. 0.6 to 1.0). The alkalinity of the control CKD in leachate was 1467 mg/L whereas after treatment with strain KG5, 91.82 % (120±25.24 mg/L $CaCO_3$) alkalinity was reduced with cell density having O.D. value 0.8 followed by KG1 (83.64%), KG4 (79.52%) and KGMD1 (71.37%), respectively. The results exhibited significant ($p<0.05$; t-test at 95% confidence limits) reduction in the alkalinity of the CKD in leachate as compared to control. Thus, in all treatments the optimum inoculum size of 0.8 O.D. at 20 days of incubation was observed.

Alkalinity and pH are two different measurable parameters of leachate or water analysis and they are often confused as to their relationship and interpretation. The measurement of pH indicates intensity of acidity, alkalinity or neutrality of leachate or water while measurement of alkalinity gives capacity of H^+ and OH^- ions present i.e. how much alkalinity is present in sample as alkalinity ranges from above pH 4.0 to 14.0 and determines the form of alkalinity as being bicarbonate, carbonate or hydrate alkalinity.

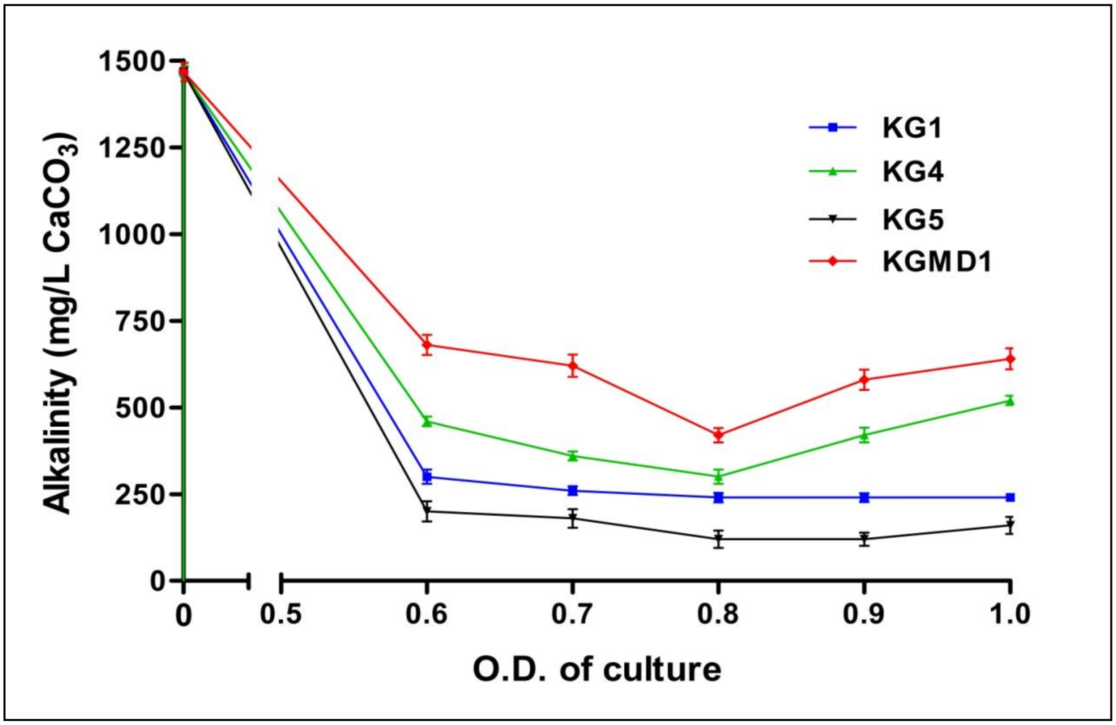


Fig. 4.6: Alkalinity reduction of the cement kiln dust after treatment with *Bacillus* sp. strain KG1, KG4, KG5 and KGMD1 with different cell density (O.D. 0.6-1.0 @ 600 nm)

If pH is above 8.3 then alkalinity is due to hydroxides and carbonates whereas if pH is above 4.3 then the alkalinity is due to bicarbonates. As per WHO, US EPA and Indian Standards Institute (ISI), the pH and alkalinity of the drinking water should be in the range from 6.5-8.5 and 200-600 mg/L, respectively. After treating the CKD with bacterial isolates the pH was ranged from 8.57-10.49 which is slightly on higher side in KG1 treated CKD whereas the alkalinity was ranged from 120-420 mg/L and within the desirable limits of drinking water guidelines of WHO, US EPA and ISI. Thus, after bacterial treatment CKD can be easily used in landfilling or reutilized in different applications to reduce surface and ground water pollution.

The chloride content in the control CKD leachate was 460 mg/L which is higher than the maximum permissible limits (250 mg/L) given by USEPA and WHO standards for drinking water. CKD treated with strain KG1 with 0.8 O.D. registered significant ($p<0.05$; t-test at 95%

confidence limits) reduction of 86.96% (60±6.66 mg/L) in chloride content in leachate compared to control followed by other three strains KG4, KG5 and KGMD1 with 82.61% reduction (80±17.04 mg/L). Chlorides generally are present in ground water as sodium chloride or calcium chlorides.

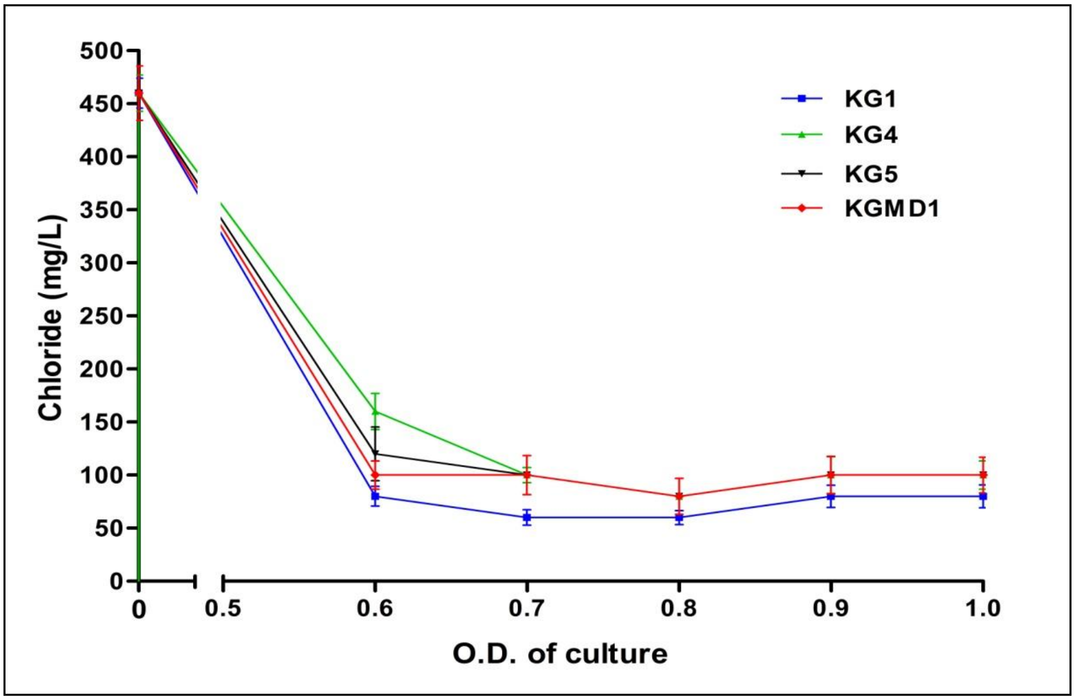


Fig. 4.7: Chloride reduction of the cement kiln dust after treatment with *Bacillus* sp. strain KG1, KG4, KG5 and KGMD1 with different cell density (O.D. 0.6-1.0 @ 600 nm)

The chloride in drinking water is not of any health concern but high chloride and magnesium content may affect the acceptability of drinking water by affecting the taste. Excessive chloride concentration increases the hardness and the rate of corrosion of metals in the water distribution system which leads to increased metal concentration in the ground water. Water with chloride in excess of 2000 mg/L is not recommended for many construction purposes as it affects the solidity and strength of concrete. Water with high chloride content also not suitable for irrigation as evapo-transpiration tends to increase the chloride and salinity at the root zone of plants and difficult for crops to take up water due to difference in osmotic pressure between the water outside plant and within the plant cell. Therefore, the analysis of the chloride content in the CKD sample before and after bacterial treatment is necessary to know whether the sample (water or leachate) is suitable for construction, irrigation or other industrial purposes or not. As CKD contains only inorganic mineral salts mainly in the form of oxides (CaO, SiO_2, Al_2O_3, Na_2O, K_2O etc.) thus, for the growth of isolates in CKD, carbon source should be added so that whole environment itself acts as the growth medium for the isolate.

Preliminary studies in this work conducted to check the growth of isolate in CKD leachate containing 2% glucose solution as carbon source. A cell density (OD) of 0.27 was observed compared to control medium (without inoculation) which showed that the above said medium supports the growth of isolate to some extent. The maximum reduction of alkalinity in CKD + culture (OD 0.8) was due to the proper concentration of cell for the treatment. Low concentration of cells affects the efficiency of the reaction whereas high concentration generates the unfavourable conditions for growth as there was limited nutrition for large number of cells. Thus, it was found that treatment of CKD with cell density of 0.8 O.D. gives the promising results.

4.3.3 EDX and XRD Analysis for Confirmation of Alkalinity Reduction

The EDX and XRD analysis of the powdered CKD sample treated with bacterial culture (O.D. 0.8 of KG1, KG4, KG5 and KGMD1) was also performed to confirm the decrease in the alkalinity.

4.3.3.1 EDX analysis

The EDX spectrum of cement kiln dust revealed the presence of calcite ($CaCO_3$), and quartz (SiO_2) as the major components along with peaks for Al, Mg, K, S and O (Fig. 4.8). Analysis of CKD showed absence of Na and Cl with small amount of Fe, Cu and Zn (Table 4.6). The alkalinity was mainly due to presence of K (K_2O) and S (SO_3) in CKD. The compound percent composition of K_2O and SO_3 in control CKD treatment was 1.12 and 1.13, respectively. After treatment with strain KG1, K_2O and SO_3 content was reduced to 0.59 and 0.82, respectively which corresponds to 47.32% reduction compared to control CKD (no bacterial treatment) where as reduction in 24.43% SO_3 content and 47.30% equivalent alkali was observed with EDX. The reduction of 17.86, 34.82 and 24.11% of K_2O in KG4, KG5 and KGMD1 treated CKD, and reduction of 8.85% SO_3 content in both KG5 and KGMD1 treated CKD was observed. In KG4 treatment SO_3 content was not detected. EDX pattern of control CKD compared with different bacterial treatments is depicted in Fig. 4.8 - 4.12. The reduction in peak height of K (correspond to K_2O) in treated CKD compared to control CKD was observed and this confirmed significant ($p<0.0001$; two way ANOVA) reduction in alkalinity.

Table 4.6: Chemical composition of cement kiln dust without and with bacterial treatment analyzed by EDX. (Values are ± S.D, n=3)

Chemical Composition (compound %)	CKD control	CKD + KG1	CKD + KG4	CKD + KG5	CKD + KGMD1
CaO (Ca)	55.78±0.10	48.19±0.47	53.66±0.14	57.16±1.10	48.24±0.12
MgO (Mg)	0.69±0.09	0.58±0.16	0.54±0.07	0.59±0.10	0.59±0.08
Al_2O_3 (Al)	2.38±0.09	2.19±0.22	1.89±0.09	2.66±0.18	2.14±0.09
SiO_2 (Si)	13.17±0.08	10.97±0.21	10.86±0.10	11.44±0.21	11.05±0.09
SO_3 (S)	1.13±0.07	0.82±0.09	N.D.	1.03±0.12	1.03±0.07
K_2O (K)	1.12±0.08	0.59±0.15	0.92±0.06	0.73±0.12	0.85±0.07
Fe_2O_3 (Fe)	2.62±0.06	2.41±0.11	2.62±0.10	3.12±0.14	2.55±0.11
CuO (Cu)	0.89±0.09	0.80±0.11	1.13±0.07	0.51±0.11	0.82±0.06
ZnO (Zn)	0.66±0.07	0.64±0.03	0.99±0.08	N.D.	0.93±0.05

N.D. = Not detected

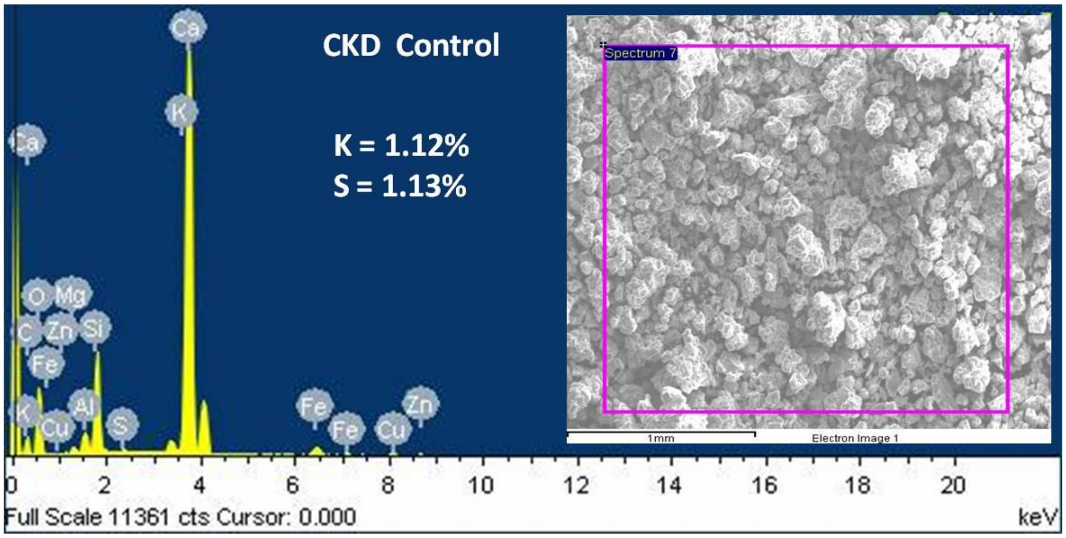


Fig. 4.8. EDX of untreated cement kiln dust (control)

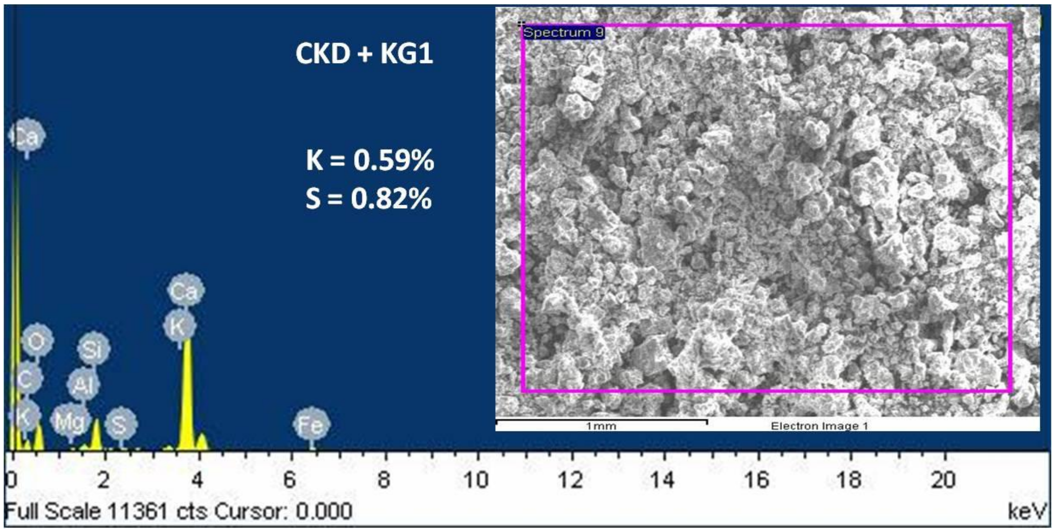


Fig. 4.9. EDX of cement kiln dust treated with strain KG1

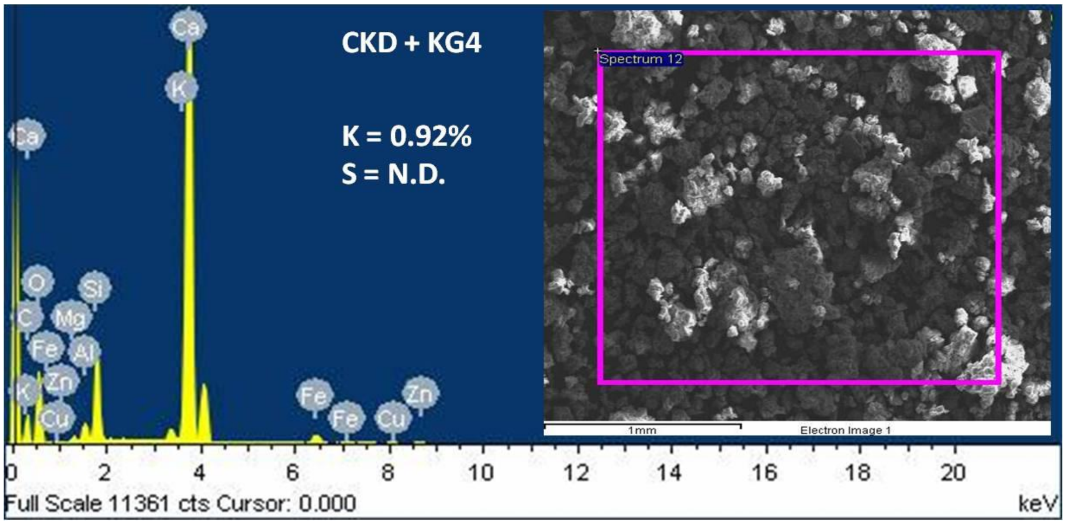


Fig. 4.10. EDX of cement kiln dust treated with strain KG4

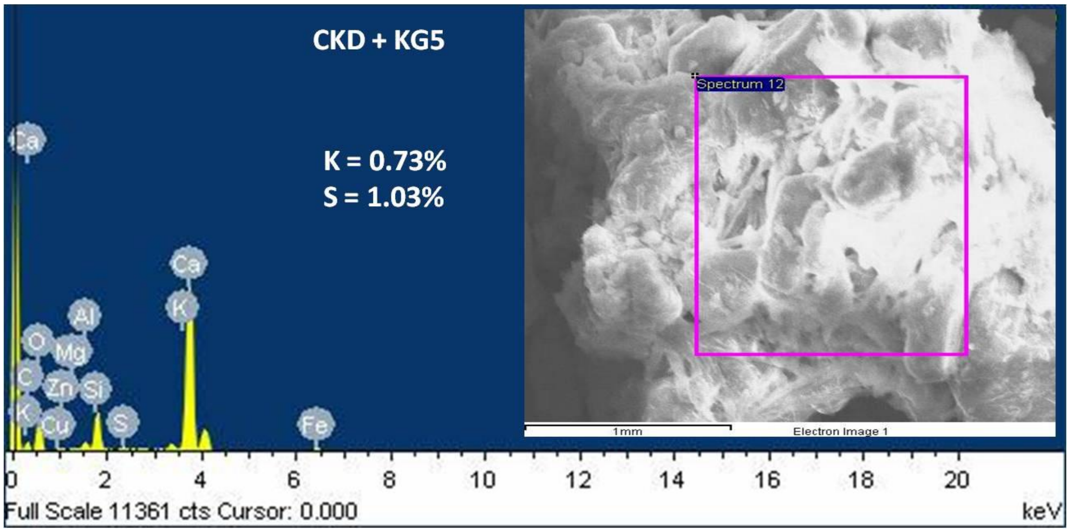


Fig. 4.11. EDX of cement kiln dust treated with strain KG5

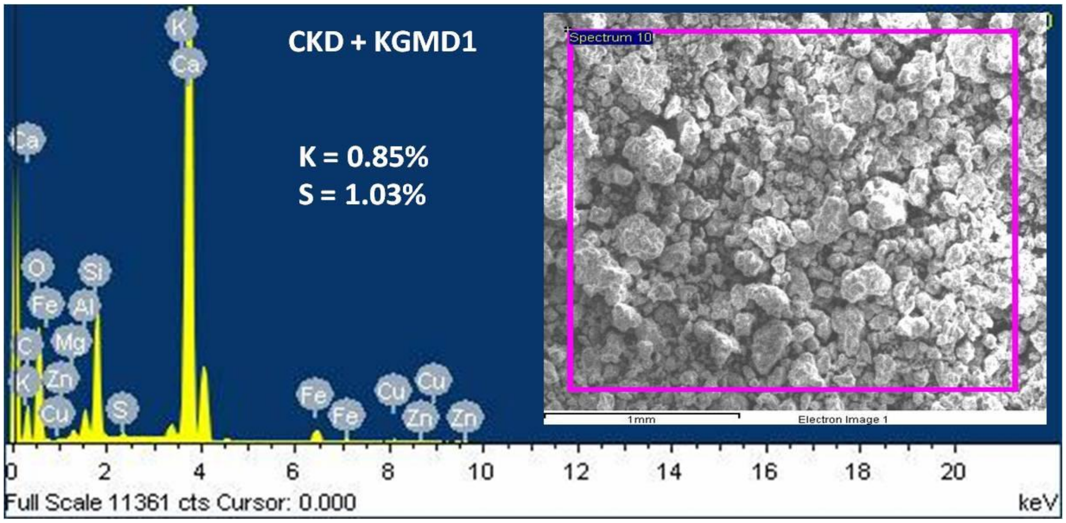


Fig. 4.12. EDX of cement kiln dust treated with strain KGMD1

4.3.3.2 XRD analysis

Fig. 4.13 shows the XRD analysis of Portland cement and CKD used in the study. The XRD analysis (Fig. 4.13) revealed that the CKD mainly consists of calcite ($CaCO_3$), anhydrite ($CaSO_4$), dolomite ($CaMg(CO_3)_2$) and free lime (CaO). Traces of crystalline alkali sulfate phases such as arcanites (K_2SO_4), and the hydration product phases such as syngenite ($K_2(CaSO4)_2$), ettringite ($Ca_6Al_2(SO_4)_3(OH)_{12}{\cdot}26H_2O$) and calcium hydroxide ($Ca(OH)_2$) were also observed. After bacterial treatment absence of arcanite phase peak (at 31˚ 2Θ) was observed in all the treatments that confirmed the alkalinity reduction of CKD compared to control (Fig. 4.14).

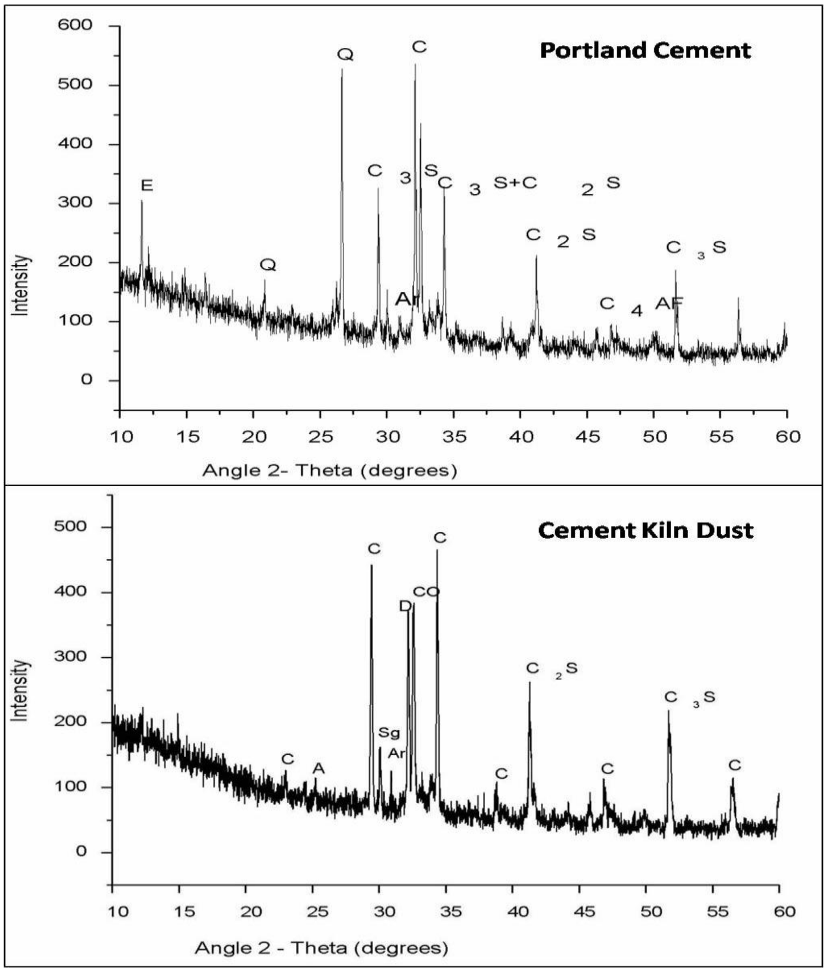


Fig. 4.13: Diffraction pattern along with crystalline phases in Portland cement and untreated CKD. C-calcite ($CaCO_3$), D-dolomite ($CaMg(CO_3)_2$), A-anhydrite ($CaSO_4$), CO-calcium oxide (CaO), Sg-syngenite ($K_2Ca(SO_4)_2$), Ar-arcanite (K_2SO_4), E-ettringite ($Ca_6Al_2(SO_4)_3(OH)_{12}{\cdot}26H_2O$) C_2S-dicalcium silicate, C_3S-tricalcium silicate

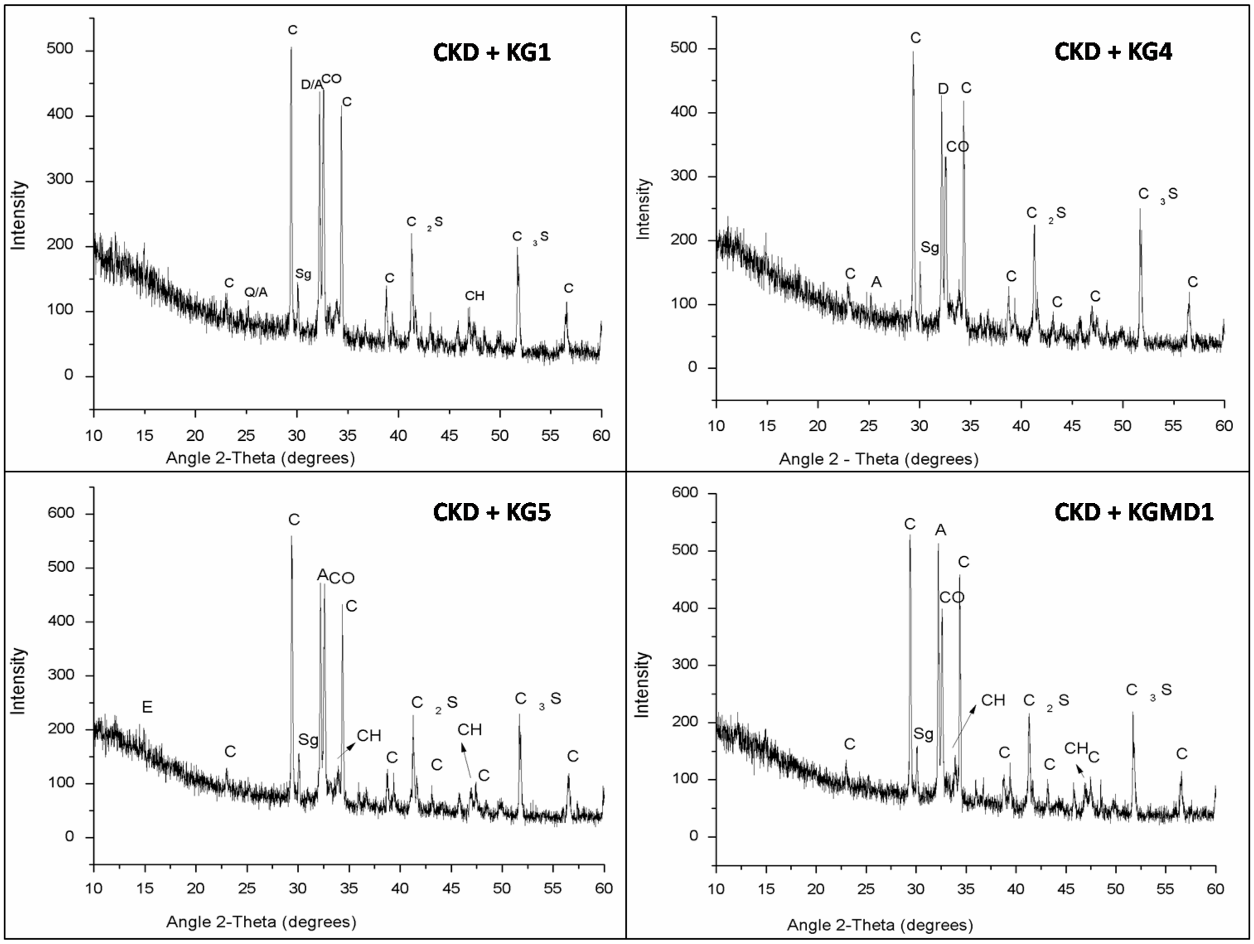


Fig. 4.14: Diffraction pattern along with crystalline phases in CKD treated with bacterial isolates KG1, KG4, KG5 and KGMD1. Absence of arcanite phase peak at 31° angle 2-theta

EDX and XRD analysis supported the results in terms of absence of sulfate and crystalline alkaline sulfate phases in treated powdered CKD compared to control. The free sulfates and K_2O content in the control CKD on treatment converted into bound form such as anhydrites or ettringite (calcium aluminium sulfate phase) or syngenite (potassium calcium sulfate phase) which reduced the free sulfate content in CKD and thus, reduced the alkalinity. The fact behind these results can be supported by the fact that microorganisms utilize carbohydrates usually by fermentation and respiration process; and acid production may have been due to the carbohydrate metabolism. Bacterial isolates are highly diverse in the metabolism and channel the diversity of materials into different central pathways (glycolysis, Kreb's cycle, etc.) of cell metabolism for complete oxidation, thereby producing an array of organic acids (Kumar et al., 2011).

Preliminary investigations in this work showed that the direct addition of organic and inorganic acids in neutralization of cement kiln dust is not feasible as large amount of these acids would be needed which is not economical. Use of inorganic acids such as hydrochloric acid, sulfuric acid, etc. in neutralization process can be hazardous and corrosive and may cause health problems to the workers. Chloride also increases the alkalinity in the CKD. Reduction in chloride content also marks an important parameter to study as its high concentration in ground water through leachate affects the taste of the ground water and increases its alkalinity also. Excess chloride causes cardiovascular diseases. High chloride content corrodes steel and imparts permanent hardness to ground water. In agriculture, chloride increases salinity of soil through irrigation. In this study approximately 86.96% chloride was reduced which showed that CKD on bacterial treatment can safely be reused and dispose off to landfill. Thus, use of biological tool for the neutralization of alkaline solid waste acts as a better alternative to conventional chemical methods. Growth of bacterium on minimal medium also shows its economic effectiveness and acts as cheap growth medium compared to ready to prepare enriched growth medium such as nutrient broth, etc.

4.4 IDENTIFICATION OF ALKALI-TOLERANT BACTERIAL CULTURES

4.4.1 Morphological Characterization

The bacterial isolates were morphologically identified on the basis of macroscopic (colony color, form, margin, elevation on agar medium and type of surface growth, clouding and sedimentation in broth medium) and microscopic (Gram staining) characteristics. All the isolates were Gram positive (G +ve) and rod shaped. In microscopic studies, all the bacterial isolates (KG1, KG4, KG5 and KGMD1) were observed as purple color cells with isolate KG4 as rod shaped while others were observed as small rod shaped cells (Fig. 4.15).

On the agar plate the isolates were observed as irregular in form with lobate and undulate margins. Isolate KG1 and KG5 showed flat elevation whereas slightly raised and convex shaped was observed in isolate KG4 and KGMD1 (Table 4.7).

Table 4.7: Morphological characteristics of bacterial isolate KG1, KG4, KG5 and KGMD1

Morphological Test	Isolate KG1	Isolate KG4	Isolate KG5	Isolate KGMD1
Gram Staining	G +ve	G +ve	G +ve	G +ve
Shape	Small rods	Rods	Small rods	Small rods
Form	Irregular	Rhizoid or irregular	Rough or round initially then irregular	Slight irregular
Margin	Lobate	Undulate	Lobate or undulate	Lobate
Elevation	Flat	Flat or convex	Flat	Raised or flat
Surface	Rough	Rough or wrinkled	Rough	Smooth
Colonies	White opaque	White glistening shiny	White opaque; over growth shows white glistening colony	White opaque
Growth in broth				
Surface growth	No ring	Slight ring	Ring	Slight ring
Clouding	Heavy turbid	Heavy turbid	Moderate turbid	Heavy turbid
Sediment	Flocculent	Flocculent or flaky	Moderate sediment	Sediment scanty

All the colonies of the isolates were white opaque in color with rough surface except KGMD1 which exhibited smooth surface. In broth, all the isolates formed ring structure on the walls of flask except KG1 (no ring) with turbid growth, less sedimentation and flaky appearance (KG4). Sanghi et al. (2008) reported the morphological characters of alkalophilic *Bacillus subtilis* ASH isolated from soil. *B. subtilis* ASH was Gram positive rod shaped alkalophile capable to grow at pH value up to 11.0. On agar plate strain showed cream colored colonies with round margin, convex elevation and rough surface. Table 4.7 shows the morphological characteristics of the isolates in agar and broth medium. Fig. 4.15 and 4.16 shows the results of Gram staining and growth of bacterial colonies on agar plates.

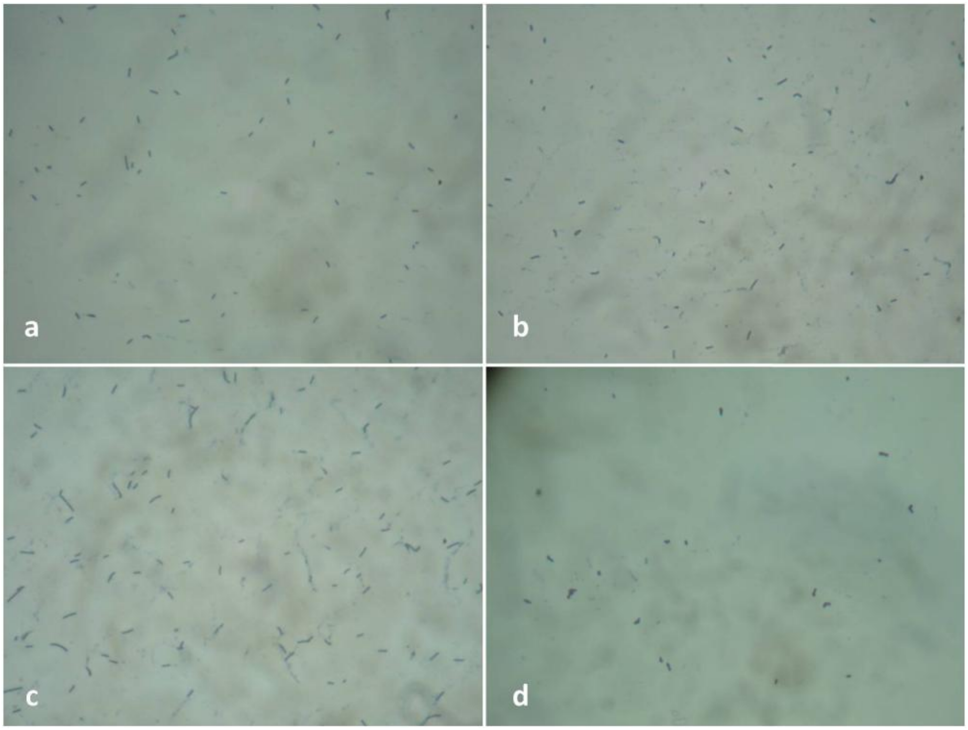


Fig. 4.15: Gram staining of isolate (a) KG1, (b) KG4, (c) KG5 and (d) KGMD1 (40X magnification)

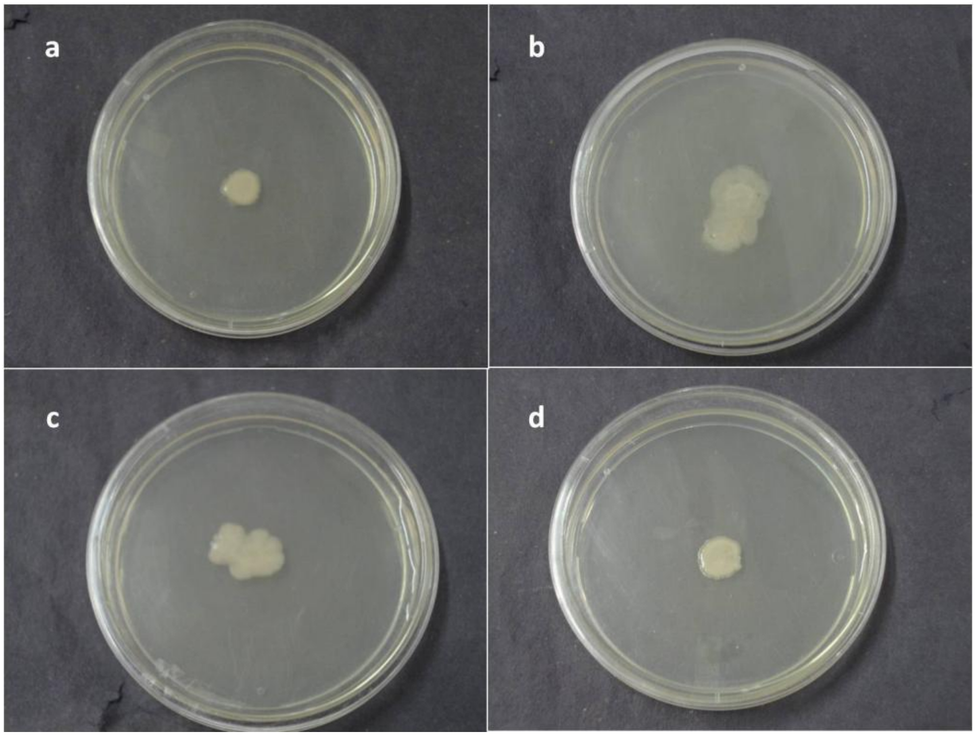


Fig. 4.16: Agar plate morphology of isolate (a) KG1, (b) KG4, (c) KG5 and (d) KGMD1

4.4.2 Biochemical Characterization

The biochemical characteristics of the isolate KG1, KG4, KG5 and KGMD1 are shown in Table 4.8 - 4.10. All the isolates were able to hydrolyze starch, gelatin, pectin and also exhibited the catalase and citrate test. Isolate KG4 showed weak positive result for casein and cellulase test (Table 4.8).

Table 4.8: Biochemical characteristics of bacterial isolate KG1, KG4, KG5 and KGMD1

Biochemical Test	Isolate KG1	Isolate KG4	Isolate KG5	Isolate KGMD1
H_2S Production Test	-	-	-	-
Motility Test	-	-	-	-
Methyl Red Test	-	-	-	+
Voges-Proskauer Test	+	+	+	-
Citrate Utilization Test	+	+	+	+
Starch Hydrolysis Test	+	+	+	+
Gelatin Test	+	+	+	+
Catalase Test	+	+	+	+
Lipase Test	-	-	-	-
Casein Test	-	w/+	-	-
Cellulase Test	-	w/+	-	-

(+) – positive growth; (-) – negative growth; (w/+) – weak growth

Fig. 4.17 - 4.19 shows the characteristic results of starch, pectin hydrolysis and citrate utilization, respectively, by isolate KG1, KG4, KG5 and KGMD1. In starch and pectin hydrolysis, the clear zone around the culture growth shows positive test that denotes that all the isolates had amylase and pectinase enzyme for the degradation or hydrolysis of starch and pectin (Fig. 4.17 and 4.18). Similarly, Fig. 4.19 reveals the citrate utilization by all the isolates when grown on Simmon's

citrate agar medium. The growth of the isolates on agar medium changed the color of the medium to blue from green whereas no change in color was observed in control (unioculated) agar tube. Presence of enzyme pectinases, amylases and xylanases in Bacillus sp. and their role in treatment of alkaline pectic waste water and pulp and paper effluent was also reported by Sanghi et al. (2008) and Gupta et al. (2008). Sanghi et al. (2008) reported that B. subtilis ASH strain showed positive test for citrate, starch, gelatin, catalase and Voges Proskauer while negative for H_2S production. Similar results were observed for isolate KG1, KG4, KG5 and KGMD1 which shows that these isolates belong to genus *Bacillus*.

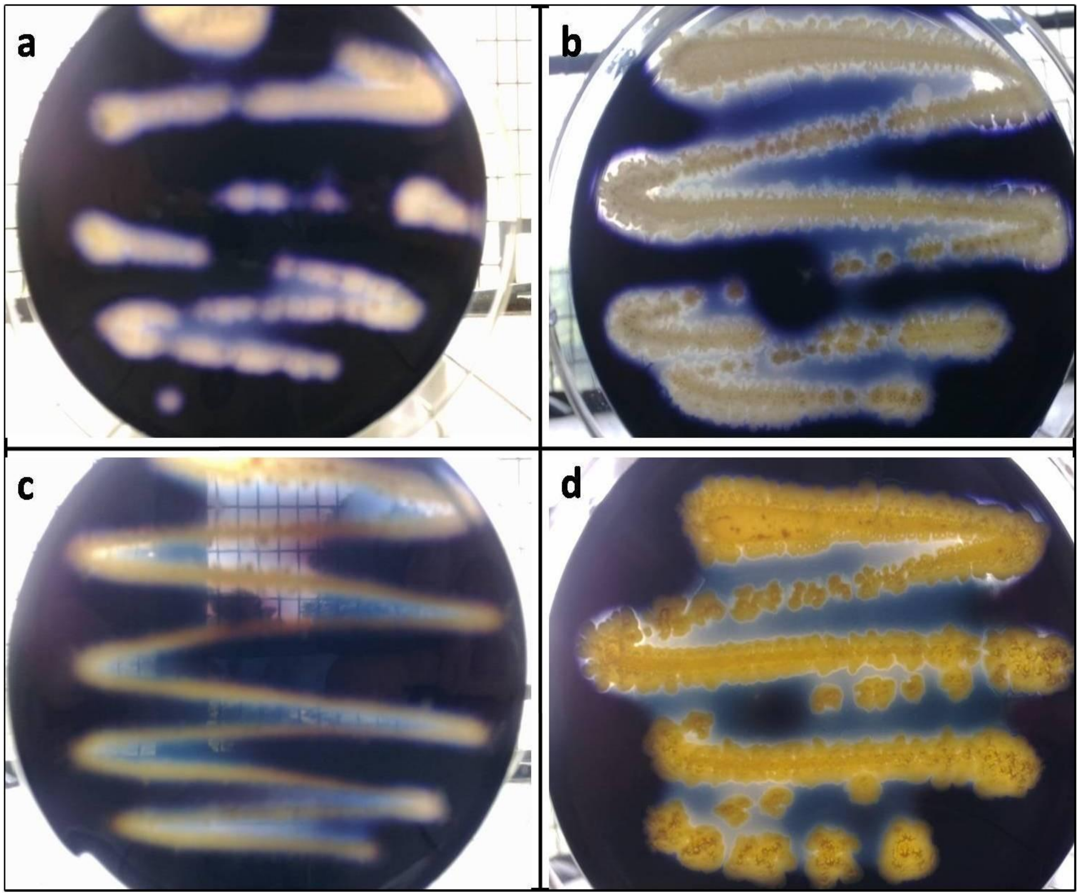


Fig. 4.17: Starch hydrolysis test of isolate (a) KG1, (b) KG4, (c) KG5 and (d) KGMD1

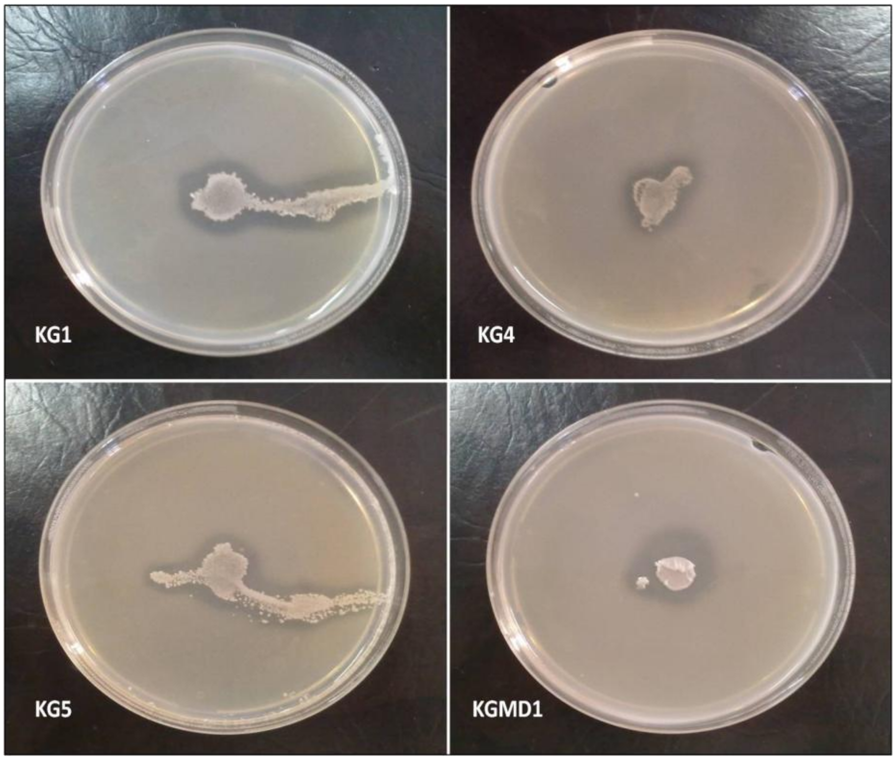


Fig. 4.18: Pectin hydrolysis test of isolate KG1, KG4, KG5 and KGMD1

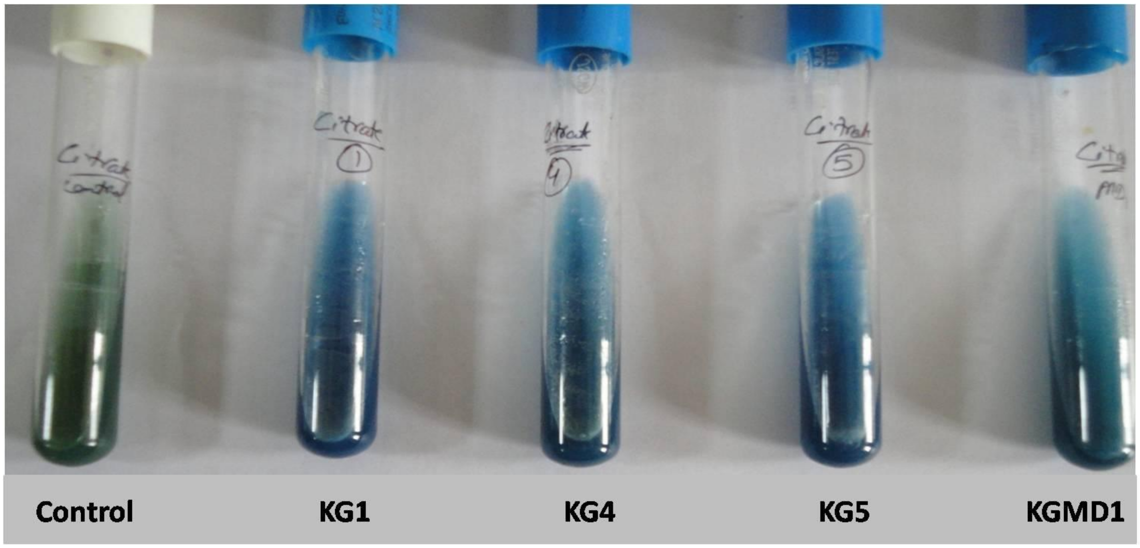


Fig. 4.19: Citrate utilization test of isolate KG1, KG4, KG5 and KGMD1

Carbohydrate utilization test reveals that all the isolates were able to utilize glucose, sucrose, fructose and maltose as carbon source (Table 4.9) except KG5 and KGMD1 for mannitol and KGMD1 for lactose and xylose. Amino acid utilization by isolate KG1, KG4, KG5 and KGMD1 showed positive test for tyrosine, valine, arginine, serine, alanine, leucine and threonine. From the Table 4.10, it is observed that isolate KGMD1 had efficiently utilized the glycine and tryptophan as carbon source compared to other isolates.

Table 4.9: Carbohydrate utilization test of bacterial isolate KG1, KG4, KG5 and KGMD1

Carbohydrate utilization	Isolate KG1	Isolate KG4	Isolate KG5	Isolate KGMD1
Glucose	+	+	+	+
Sucrose	+	+	+	+
Lactose	+	+	+	-
Mannitol	+	+	-	-
Xylose	+	+	-	+
Fructose	+	+	+	+
Maltose	+	+	+	+

(+) – positive growth; (-) – negative growth; (w/+) – weak growth

Table 4.10: Amino acid utilization test of bacterial isolate KG1, KG4, KG5 and KGMD1

Amino acid Utilization	Isolate KG1	Isolate KG4	Isolate KG5	Isolate KGMD1
Tyrosine	+	+	w/+	+
Glycine	-	-	-	+
Valine	+	+	+	+
Arginine	+	+	+	+
Serine	w/+	+	w/+	+
Tryptophan	-	-	w/+	+
Alanine	+	+	+	+
Leucine	+	+	+	+
Threonine	+	+	+	+

(+) – positive growth; (-) – negative growth; (w/+) – weak growth

4.4.3 Physiological Characterization

Physiological characterization of the bacterial isolates was performed by culturing on both enrichment and minimal medium at different salt (NaCl) concentration (0-20%), temperature (20-55 °C) and pH (5-12). It was observed that isolate KG1 and KG4 tolerate up to 8% and 16% salinity in minimal and enrichment medium, respectively whereas isolate KG5 and KGMD1 tolerate up to 4% (in minimal medium) and 14% (in enrichment medium) salinity. Isolate KG1 and KG4 shows good growth in the temperature range of 28-50 °C whereas isolate KG5 and KGMD1 grows well in the range 30-45 °C and 35-45 °C, respectively. Similarly, the optimum pH range for isolate KG1 is 5-12 while for KG4, KG5 is 6-12 and for KGMD1 the optimum pH range is 8-12 (Table 4.11). The results showed that all the isolates are able to grow at high salinity and pH (8-10) which denotes the halo-tolerant and alkali-tolerant nature of the isolates. Nagar et al. (2010) observed that *B. pumilus* SV 85S strain grows well at temperature range 10-50 °C, pH 5-10 and tolerate salinity in the range 2-10%.

Thus, the results of morphological, biochemical and physiological characteristics of isolate KG1, KG4, KG5 and KGMD1 showed that these strains are the member of group six (alkaliphile) of the genus *Bacillus* (Nielsen et al., 1994). The characteristics of the isolates were then presumptively identified on the basis of the characters of *Bacillus* sp. reported by Sneath (1986), Barrow and Feltham (1993) and Yoon et al. (2005), and designated as *Bacillus* sp. KG1, KG4, KG5 and KGMD1.

Table 4.11. Physiological characteristics of bacterial isolate KG1, KG4, KG5 and KGMD1

Character	Isolate KG1	Isolate KG4	Isolate KG5	Isolate KGMD1
Salinity tolerance (% NaCl)	<8% in minimal medium <16% in enrichment medium	<8% in minimal medium <16% in enrichment medium	<4% in minimal medium <14% in enrichment medium	<6% in minimal medium <14% in enrichment medium
Temperature range (°C)	28-50	28-50	30-45	35-45
pH range	5-12	6-12	6-12	8-12

4.4.4 Phylogenetic Analysis

The phylogenetic analysis of the alkali-tolerant bacterial isolates was done by molecular identification using 16S rRNA technique and phylogenetic tree construction. Sequences of 16S rRNA gene were generated by using forward (8F) and reverse (1492R) universal primers. The resulting forward and reverse sequences were then processed manually by aligning and a consensus sequences was formed for each isolate. The consensus sequence of isolate KG1 has the length of 1408 bp whereas isolate KG4, KG5 and KGMD1 has the length of 1466, 1381 and 1360 bp, respectively.

The consensus sequences were then analyzed for sequence similarity with the sequences submitted in the NCBI database using BLAST tool. The alignment and comparison of the 16S rRNA sequence of the bacterial isolates (KG1, KG4, KG5 and KGMD1) to the reference 16S rRNA sequences belonging to different strains of *Bacillus* and confirmed the 94.7%, 94.7%, 96.6% and 96.6% sequence similarity with *B. halodurans* strain DSM 497 (AJ302709), *B. sonorensis* NRRL B-23154 (AF302118), *B. aerius* strain :24K (AJ831843) and *B. halodurans* strain DSM 497 (AJ302709), respectively.

Consensus sequence of isolate KG1 (1408 bp)

ACATTACTGGAAAATGGCTCCTCTATATAATATACTTAAGGGGTGAGTAACACGTGGGCAACCTGCCTGTAGACTGGGATAACATCGAGAAATCGGTGCTAATACCGGATAATAAAAAGAACTGCAGGTTCTTTTTTGAAAGATGGTTTCGGCTATCACTTACAGATGGGCCCGCGGCGCACTAGCTAGTTGGTGGGGTAACGGCTCACCAAGGCGACATGCGTAGCCCACCTGAGAGGGTGATCGGCCACACTGGGACTGAGACACGGCCCAACTCCTACGGGAGGCAGCAGTAGGGAATCTTCCGCAATGGACGAAAGTCTGACGGAGCAACGCCTCGCGAGTGATGAAGGTTTTCGGATCGTAAAACTCTATTGTTAGGGAAGAACAAGTGCCGTTCGAAAGGGCGGCACCTTGACGGTGCCTAACGAGAAAGCCACGGCTAACTACATGCCAGCAGCCGCGGTAATACGTATGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGCGCGCGCAGGCGGTCTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGGTCATTGGAAACTGGGAGACTTGAGTACAGAAGAGGAGAGTGGAATTCCACGTGTAGCGGTGAAATGCGTAGAGATGTGGAGGAACACCAGTGGCGAACGCGACTCTCTGGTCTGTAACTGACGCTCAGGCGCGAAGCGTGCGGAGCAGACAGGATCAGATACC

CTGGTACTCCACGCCTTAACGATGAGTGCTATGTGTTAGGGGTTTCGATGCCCTCAG
TGCCGAAGTTGACACCTTAATGCACTACGACCGGGGCAGTACGACCGCAGGGTCGA
TCCTCATCAGAATTAGACAGAGCGGCCCGCACACGCTTGCAGCAAGATGATTTCATT
ACGAAGCACGCGACGTACCTTAACAGTCTGACATGCATTGACTACCCTAGAGATATG
TGCTTTCCCCTTCGGGGGACAAAGTGACAGGTGGTGCATGGTGGTCGTCAGCTCGTG
TCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGACCTTAGTTGCCAG
CATTCAGTTGGGCACTCTAAGGTGACGCCGGTGACAAACCGGAGGAAGGTGGGGAT
GACGTCAAATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGATGG
TACAAAGGGTTGCGAAGCCGCGAGGTGAAGCCAATCCCAGAAAGCCATTCTCAGTT
CGGATTGCAGGCTGCAACTCGCCTGCATGAAGCCGGAATTGCTGGTAATCGCGGATC
AGCATGCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACAA
GAGTTTGTATCTCCCGTAAGCCGTTGGGGTAGCTCATCTCAGTAGCCCCCAAA

Consensus sequence of isolate KG4 (1466 bp)

CNCNCCTTGGCGCCGGGCCTAAAACTGGCAGTCGAGCGGACAGATGGGAGCTTGCT
CCCTGATGTCAGCGGCGGACGGGTGAGTAACACGTGGGTAACCTGCCTGTAAGACT
GGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGATTGAACCGCAGGTTC
AATTATAAAAGGTGGCTTTTAGCTACCACTTACGAATGGACCCGCGGCGCATTACCT
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ATCGGCCACACTGGGACTGAAACACGGCCCAAACTCCTACGGGAGGCACCATTAGG
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TGGGGAACTTGAGTGCAAAAAAGGAAAGTGGAATTCCACGTGTAGCGTTTAAAATG
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CGCCGTCAACGATGAGTGCTAAGTGTTAGAGGGTTTCCGCCCTTTAGTGCTGCAGCA
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TTGACGGGGGCCCGCACCAGCGGTGGAGCATGTGGTTTAATTCGAAGCAACGCGAA
GAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTCCCCTTCG
GGGGCAGAGTGACAGGTGGTGCATGGTTGTCGTCAGCTCGTGTCGTGAGATGTTGG
GTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGTTGGGCA
CTTTAAGGTGACTGCCGGTGACAAACCGGAGGAAGGTGGGGATGACGTCAAATCAT
CATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGCAGAACAAAGGGCAG
CGAAGCCGCGAGGCTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATCGCAGTAT
GCAACTCGCCTGCGTGAAGCTGGAATCTCTGGTAATCGCGGATCAGCATGCCGCGGT
GAATACGTTCCCGGGCCTTGTACACACCGCCTGTCACACCACGAGAGTTTGTAACAC
CGGAGCTAGCTGAGGTAACCCCTGGAGACTCGCACGTGTAAGCCGGNCACCATGTC

Consensus sequence of isolate KG5 (1381 bp)

CGGGAGCTTGCTCCCTTAGGTCAGCGGCGGACGGGTGAGTAACACGTGGGTAACCT
GCCTGTAAGACTGGGATAACTCCGGGAAACCGGGGCTAATACCGGATGCTTGATTG
AACCGCATGGTTCCATCCTTAAAGGGGGCTTTTAACTACCCCTTTCCGATGGACCCG
CGGCGCATTAACTAATTGGGGAGGTAACGGCTCCCCCAGGGGACCAAGCCTAACCC
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TGAGTGATGAAGGTTTTCCGAACCTAAAACTCTGGTGGTAGGGAGACAGTACCGTTC
GAATAGGGCGGTACCTTGACGGTACCTAACCAGAAAGCACGGCTAACTACGTGCCA
GCAGCCGCGGTAATACGTAGGTGGCAAGCGTTGTCCGGAATTATTGGGCGTAAAGC
GCGCGCAGGCGGTTTCTTAAGTCTGATGTGAAAGCCCCCGGCTCAACCGGGGAGGG
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ACGCGAAGAACCTTACCAGGTCTTGACATCCTCTGACAACCCTAGAGATAGGGCTTC
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TGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTTGATCTTAGTTGCCAGCATTCAGT
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AATCATCATGCCCCTTATGACCTGGGCTACACACGTGCTACAATGGGCAGAACAAA
GGGCAGCGAAGCCGCGAGGCTAAGCCAATCCCACAAATCTGTTCTCAGTTCGGATC
GCAGTCTGCAACTCGACTGCGTGAAGCTGGAATCGCTAGTAATCGCGGATCAGCAT
GCCGCGGTGAATACGTTCCCGGGCCTTGTACACACCGCCCGTCACACCACGAGAGTT
TGTAACACCCGAAGTCGGTGAGGTAACCTT

Consensus sequence of isolate KGMD1 (1360 bp)

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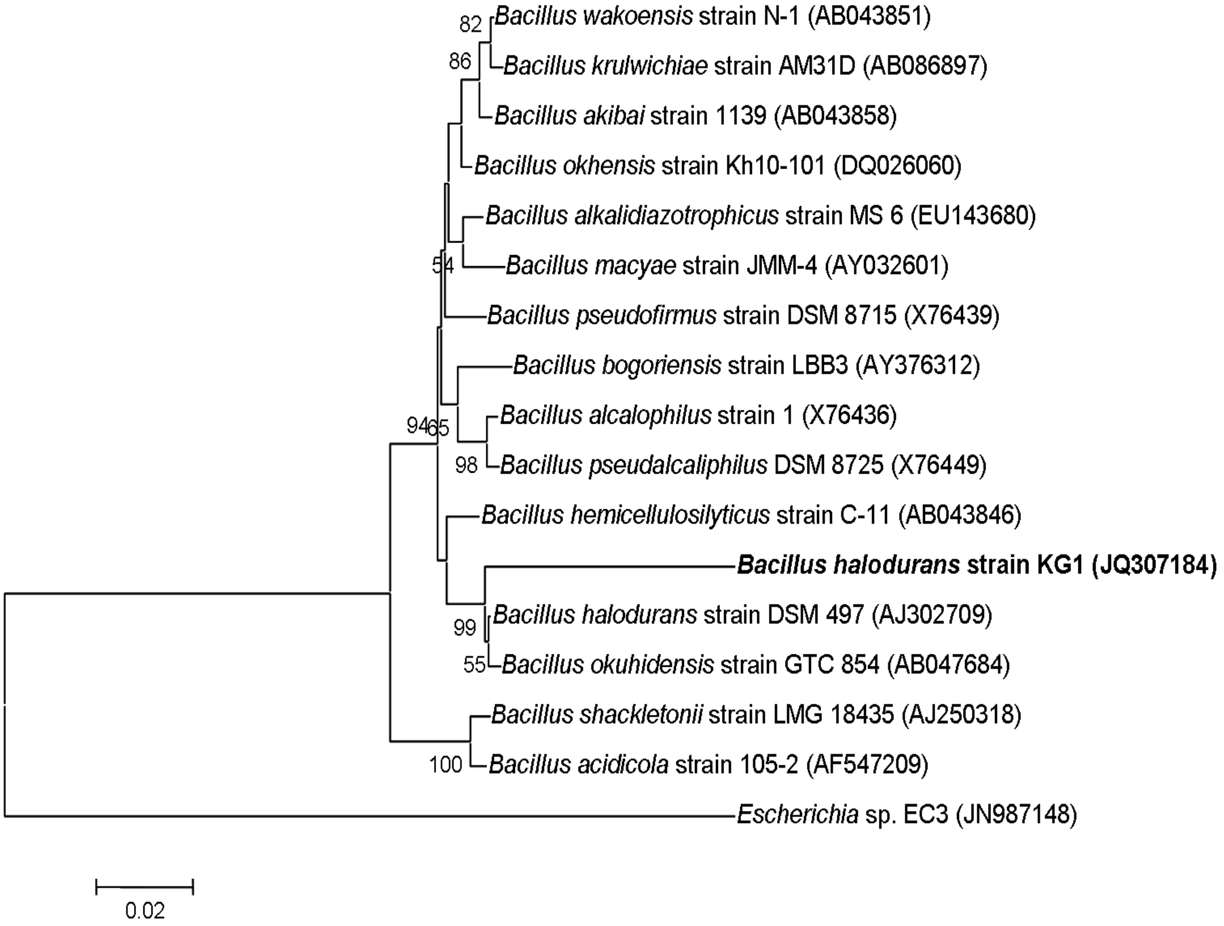


Fig. 4.20: Phylogenetic tree showing the relationships of the identified bacterial strain (KG1) and the type strains of closely related *Bacillus* species, constructed using the neighbour-joining method based on 16S rRNA gene sequences. GenBank accession numbers are given in parentheses. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at the branch points. Bar, 0.02 nucleotide substitutions per site

Phylogenetic tree of all the isolates was constructed using closely related taxa retrieved from the GenBank-NCBI database by neighbour joining method (Fig. 4.20-4.23). The results of phylogenetic analysis suggested that isolate KG1 formed a clade with *B. halodurans* strain

DSM497 (AJ302709) with a bootstrap value of 99%. Pair wise sequence analysis revealed that the highest sequence similarity was with *B. halodurans* strain DSM 497 (94.7%) followed by *B. okuhidensis* strain GTC 854 (93.9%) and the remaining species with validly published names showed less than 94% similarity (Fig. 4.20). Similarly, isolate KG4, KG5 and KGMD1 formed a clade with *B. sonorensis* NRRL B-23154 (AF302118), *B. aerius* :24K (AJ831843) and *B. halodurans* strain DSM 497 (AJ302709) with bootstrap value of 99, 94 and 52%, respectively (Fig 4.21-4.23).

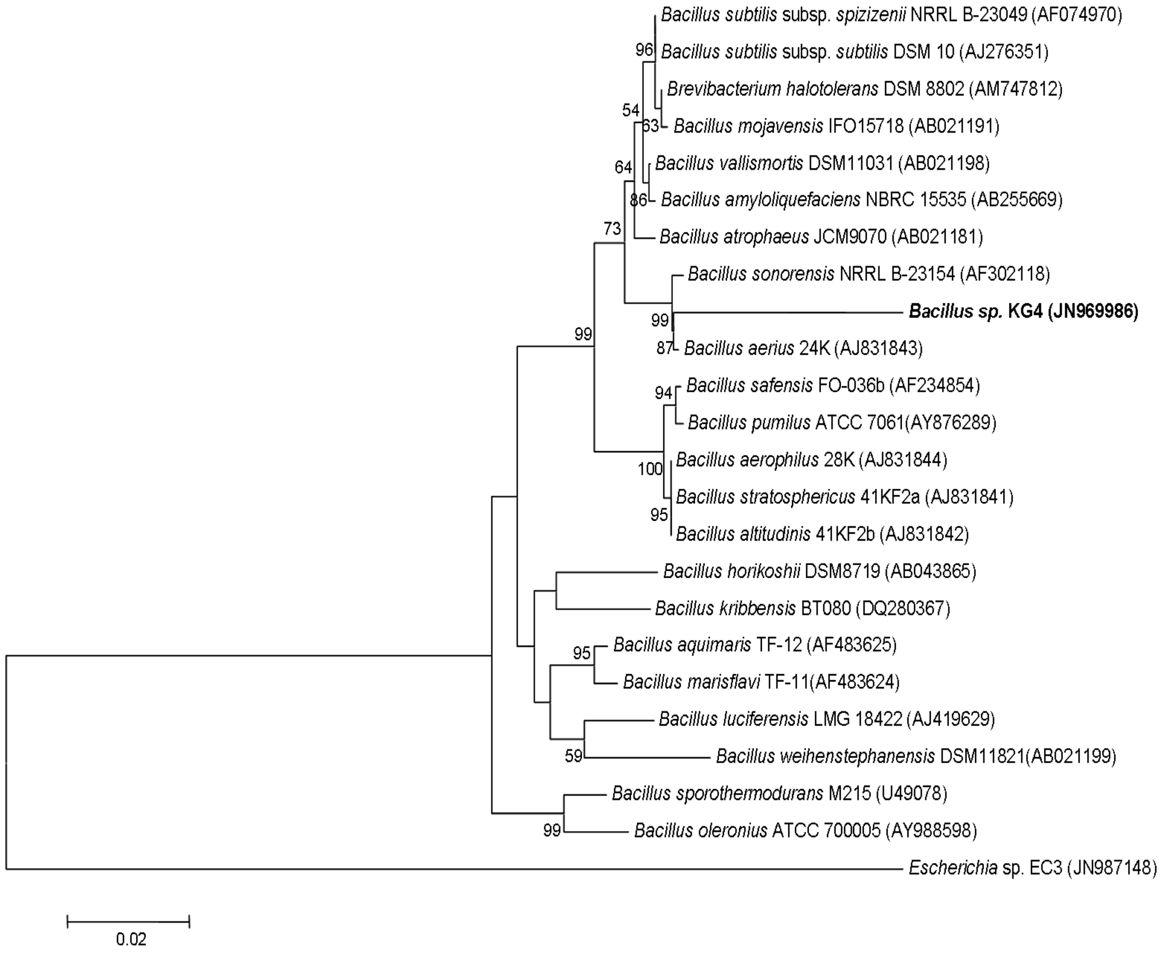


Fig. 4.21: Phylogenetic tree showing the relationships of the identified bacterial strain (KG4) and the type strains of closely related *Bacillus* species, constructed using the neighbour-joining method based on 16S rRNA gene sequences. GenBank accession numbers are given in parentheses. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at the branch points. Bar, 0.02 nucleotide substitutions per site

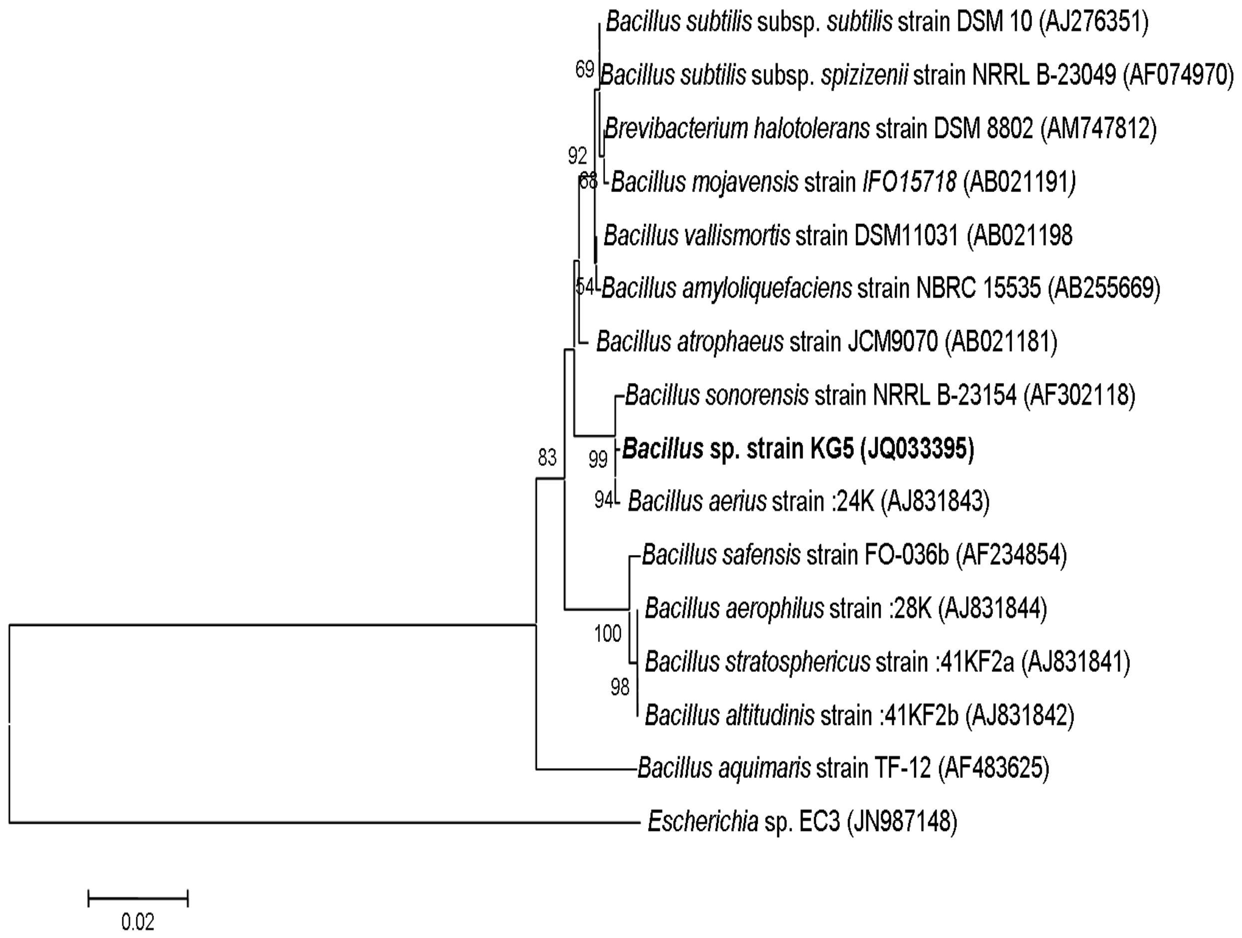


Fig. 4.22: Phylogenetic tree showing the relationships of the identified bacterial strain (KG5) and the type strains of closely related *Bacillus* species, constructed using the neighbour-joining method based on 16S rRNA gene sequences. GenBank accession numbers are given in parentheses. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at the branch points. Bar, 0.02 nucleotide substitutions per site

The generally recommended and accepted criteria for depicting bacterial species state that strains with a 16S rRNA gene sequence dissimilarity greater than 3% or with a DNA-DNA hybridization relatedness of less than 70% are considered to belong separate species (Nogi et al., 2005). It is recommended that bacterial strains with difference in 16S rRNA gene sequence of less than 3% cannot be allocated as new species without the support of DNA-DNA relatedness studies. In our study, all the bacterial strains showed difference in 16S rRNA gene sequence in the range of 4.4-5.3% with the closely related *Bacillus* species. Thus, on the basis of morphological, biochemical, physiological and phylogenetic results, all the strains be classified as the type strain of the novel species, *Bacillus* species.

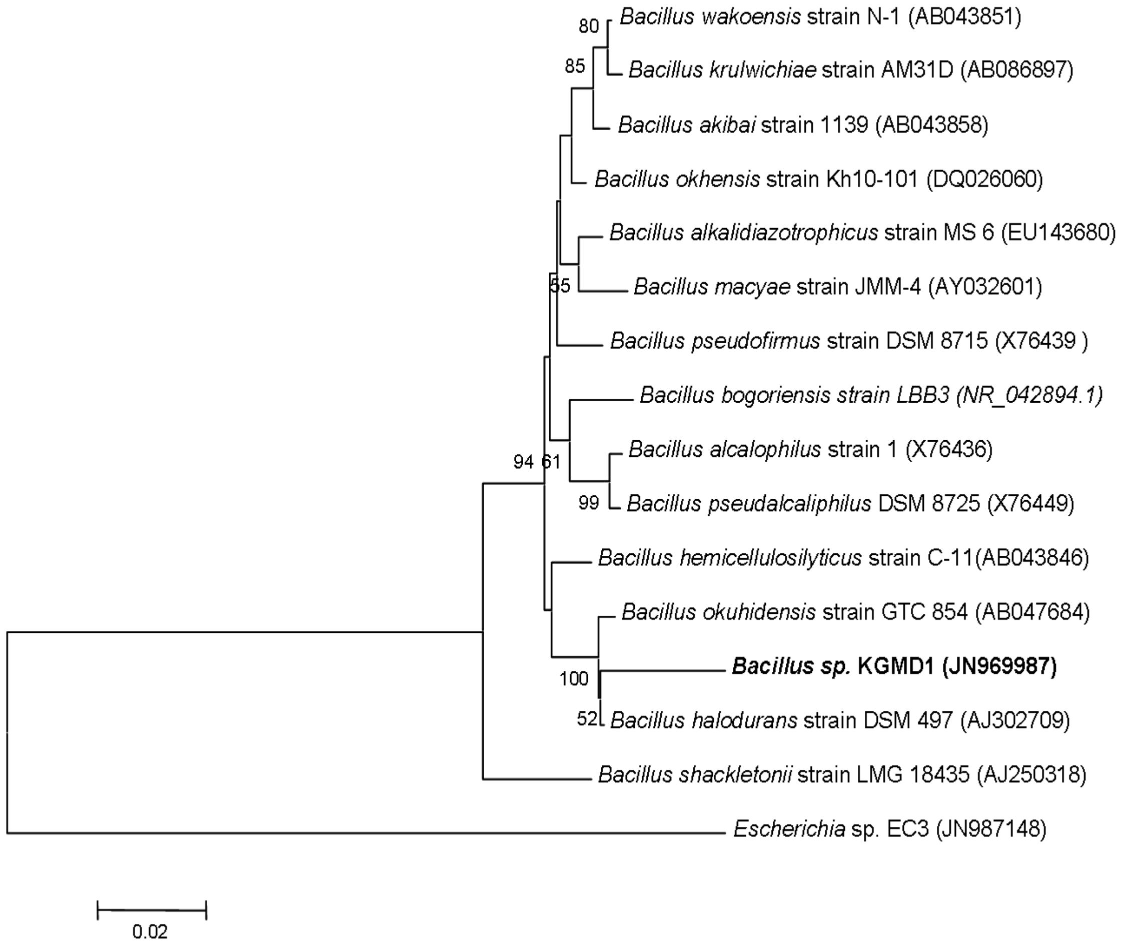


Fig. 4.23: Phylogenetic tree showing the relationships of the identified bacterial strain (KGMD1) and the type strains of closely related *Bacillus* species, constructed using the neighbour-joining method based on 16S rRNA gene sequences. GenBank accession numbers are given in parentheses. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at the branch points. Bar, 0.02 nucleotide substitutions per site

The 16S rRNA sequences of the isolates were submitted in GenBank-National Centre for Biotechnology Information (NCBI) and accession numbers were assigned as JQ307184 (*B. halodurans* strain KG1), JN969986 (*Bacillus* sp. KG4), JQ033395 (*Bacillus* sp. KG5) and JN969987 (*Bacillus* sp. KGMD1), respectively. The cultures were also deposited to National

Collection of Industrial Microorganisms (NCIM), National Chemical Laboratory, Pune (India) under deposition number NCIM 5439 (KG1), NCIM 5440 (KG4), NCIM 5441 (KGMD1) and NCIM 5442 (KG5). Table 4.12 shows the taxonomic name of all the isolates with closely related *Bacillus* species along with NCBI accession and NCIM deposition numbers.

Table 4.12: Tabular representation of taxonomic name of the isolates (KG1, KG4, KG5 and KGMD1) with closely related *Bacillus* species along with NCBI accession and NCIM deposition numbers

Isolate (s)	Sequence similarity with accession number	Taxonomic name of the isolates	NCBI accession number	NCIM deposition numbers
KG1	*B. halodurans* strain DSM 497 (AJ302709; 94.7%)	*Bacillus halodurans* strain KG1	JQ307184	NCIM 5439
KG4	*B. sonorensis* NRRL B-23154 (AF302118; 94.7%)	*Bacillus* sp. KG4	JN969986	NCIM 5440
KG5	*B. aerius* strain :24K (AJ831843; 96.6%)	*Bacillus* sp. KG5	JQ033395	NCIM 5442
KGMD1	*B. halodurans* strain DSM 497 (AJ302709; 96.6%)	*Bacillus* sp. KGMD1	JN969987	NCIM 5441

4.5 STUDY OF MORTAR AND CONCRETE PROPERTIES

4.5.1 Effect of bacterial treated CKD on cement-mortar properties

4.5.1.1 Water for normal consistency

Water for normal consistency cement paste containing different concentration of cement dust is shown in Table 4.13. It is seen that with increasing CKD content (5-30%) water demand increases to maintain a normal consistency. The increase in water demand is due to high alkali, sulfate, volatile salts and free lime content in CKD compared with Portland cement (El-Aleem et al., 2005; Marku et al., 2012). But, in this study the alkali (K_2O), sulfate (SO_3) and free lime content in untreated CKD is less than that of cement (Table 4.6), still the water demand increases. This is attributed to the coarseness, irregular particle size and lower density of the CKD than cement which increases the viscosity of the cement-CKD paste and thus, their water demand. These results are supported by the findings of Cyr et al. (2007) where addition of sewage sludge ash in cement reduced the workability and increased the water demand due to irregular shape of ash grains and higher surface area.

Table 4.13: Water for normal consistency of CKD blended cement paste

Treatment	Consistency (%)	
	Control	Treated
0% CKD	34.00	--
5% CKD	34.50	34.75
10% CKD	35.25	35.00
15% CKD	36.00	35.00
20% CKD	36.75	35.00
25% CKD	37.25	35.25
30% CKD	38.00	35.25

The reduction of alkali and sulfate content in bacterial treated CKD-cement paste and change in the chemical composition reduces the water demand compared with untreated CKD-cement paste, but in different percentages of bacterial treated CKD-cement pastes (5-30%) non-significant increase in water demand was observed (Table 4.13).

4.5.1.2 Setting time

Table 4.14 shows the setting time of Portland cement and CKD cement blends. A non-significant decrease in initial setting time of CKD-cement pastes was observed compared to Portland cement paste whereas at above 15% CKD addition increase in setting time was observed. Similarly, up to 15% CKD (control) addition decrease in the final setting time of the cement paste was observed but above this percentage the final setting time increases. In bacterial treated blended paste increased initial and final setting time was observed due to reduction in alkali and sulfate content (Table 4.14). Though there was variation in the setting time (initial and final) of untreated and bacterial treated CKD-cement paste but these findings were within allowable values of initial (45 min) and final (375 min) setting times specified by ASTM C-150 (Maslehuddin et al., 2008).

Table 4.14: Setting time of CKD blended cement paste

Treatment	Initial Setting (min)		Final Setting (min)	
	Control	Treated	Control	Treated
0% CKD	120.0	--	240.0	--
5% CKD	115.8	132.6	230.4	235.8
10% CKD	118.5	138.6	232.8	238.8
15% CKD	118.5	140.4	234.0	244.8
20% CKD	121.2	141.0	240.0	249.4
25% CKD	129.0	148.8	244.8	258.0
30% CKD	141.0	157.6	254.2	273.0

4.5.1.3 Hydration

The hydration process in cement and CKD blended cement pastes is shown in Fig. 4.24. In all hardened cement paste the degree of hydration increased with curing time due to continuous hydration and accumulation of hydration products such as calcium silicate and sulfoaluminate hydrate (El-Aleem et al., 2005). At curing ages of 28 and 91 days, the hydration increased significantly up to 10% CKD content which later on decreases with increase in CKD content (Fig. 4.24). During mixing of cement paste with water, the calcium and silica may leach out with water which upon hydration not available for the formation of hydration products where as in CKD blended cement pastes the loss of calcium and silica decreased due to less alkaline nature of blended pastes resulted in increased hydration. Wild and Khatib (1997) reported that partial replacement of cement with metakaolin increased the initial hydration of cement pastes which was evident from reduced content of portlandite (calcium hydroxide), That was utilized during the pozzolanic reaction, compared to control cement paste. Above 10% CKD additions the reduction in alkali content significantly affected the hydration process resulted in decreased hydration which was far decreased in bacterial treated CKD blended cement pastes.

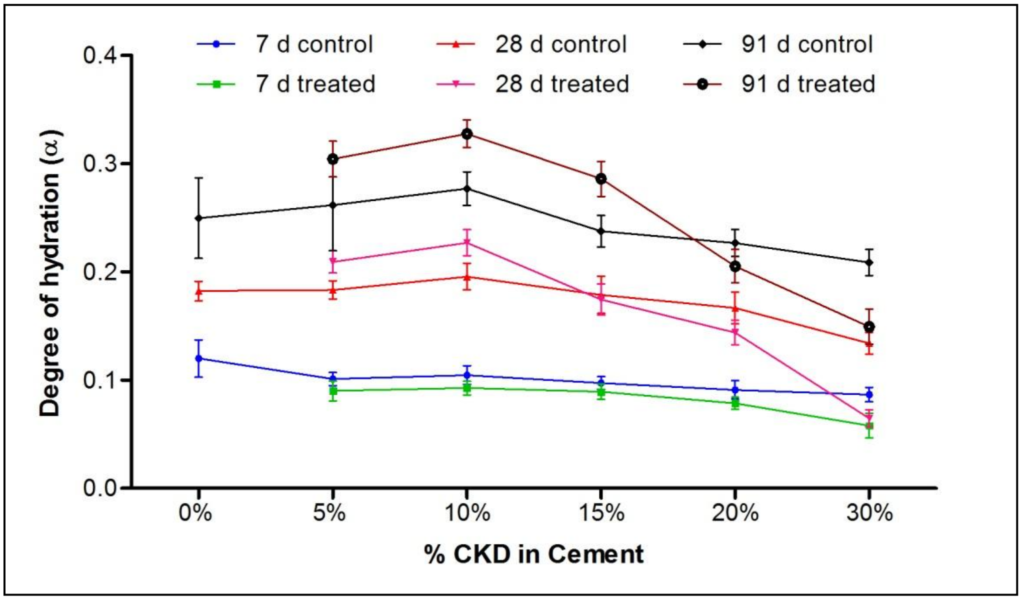


Fig. 4.24: Degree of hydration in cement pastes with different percentages of cement kiln dust at curing period of 7, 28 and 91 days

4.5.1.4 Compressive strength

The compressive strength increases with curing time for all hardened cement mortars. Figs. 4.25-4.27 show the compressive strength of hardened cement mortars containing control and bacterial treated CKD at 7, 28 and 91 days, respectively. High alkalinity in cement reduced the quality of cement and affected the strength and microstructure of the cement paste. In early curing age of 7 days, the strength of untreated CKD mortar increased up to 10% CKD additions whereas in bacterial treated CKD mortar, strength decreases with increase in CKD content. At the age of 28 days, 14.45% (25.82±1.97 N/mm^2) increase in compressive strength was observed in 10% CKD mortar compared to 0% CKD mortar (22.56±0.37 N/mm^2). This is due to the reduced alkalinity and proper packaging of materials in mortar resulted in increased strength where as above 10% CKD content decreased strength was due to decrease in cement content and hydration (Fig. 4.26). In bacterial treated CKD mortar at the age of 28 days, 26.37% and 10.42% increased compressive strength was observed in 10% CKD content (28.51±0.34 N/mm^2) compared to 0% (22.56±0.37 N/mm^2) and 10% (25.82±1.97 N/mm^2) untreated CKD mortar. Similar trend was also observed at 91 days of curing where 19.54% and 10.57% increased compressive strength was observed in 10% bacterial treated CKD mortar (36.40±0.26 N/mm^2) compared to 0% (30.45±1.98 N/mm^2) and 10% (32.92±1.48 N/mm^2) untreated CKD mortar (Fig. 4.27).

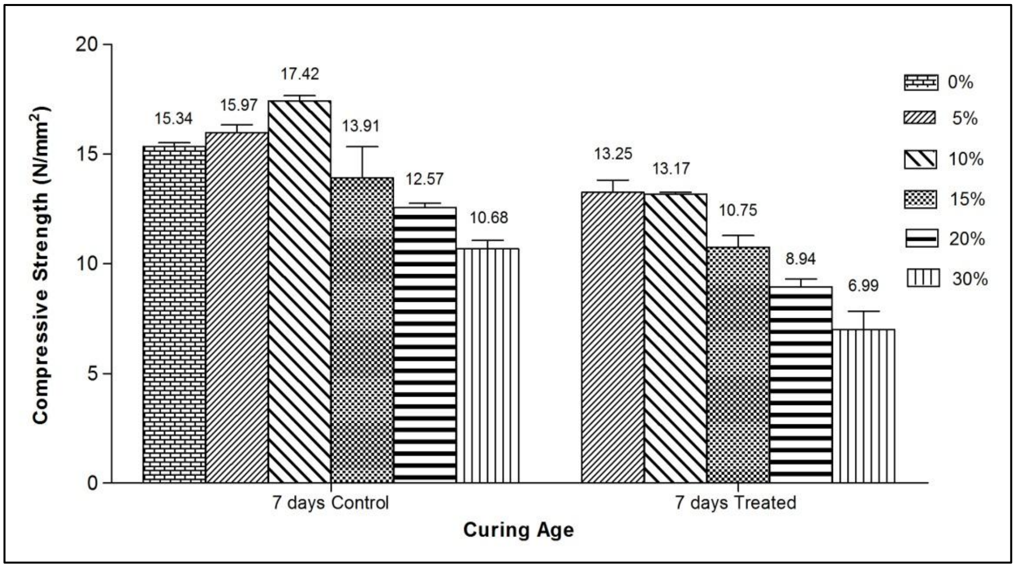


Fig. 4.25: Compressive strength of hardened mortar with different percentages of cement kiln dust (untreated and bacterial treated) at curing age of 7 days

The increased strength in 10% CKD concrete is due to an appropriate alkalinity that increases the dissolution of silicate and formation of calcium silicate hydrate (Maslehuddin et al., 2009). Makhloufi et al. (2012) observed that partial replacement of cement with slag and pozzolana improved in mechanical properties of mortar by consuming the portlandite to form hydration products during the pozzolanic reaction. Above 10% CKD content, the reduction in compressive strength is attributed to the reduction in cement content which weakened the hydration matrix and generates voids due to change in packing between the crystals. Al-Harthy et al. (2003) found that mortars with 5% CKD substitution produced close compressive strength where as above this substitution strength decreases. Similarly, Daous (2004) observed satisfactory mechanical strength in mortars containing 10% CKD and 4% fly ash content as blended waste material where as decrease in strength in mortars containing 15, 30 and 45% CKD was observed by Marku et al. (2012) due to high alkalinity and free lime content in CKD.

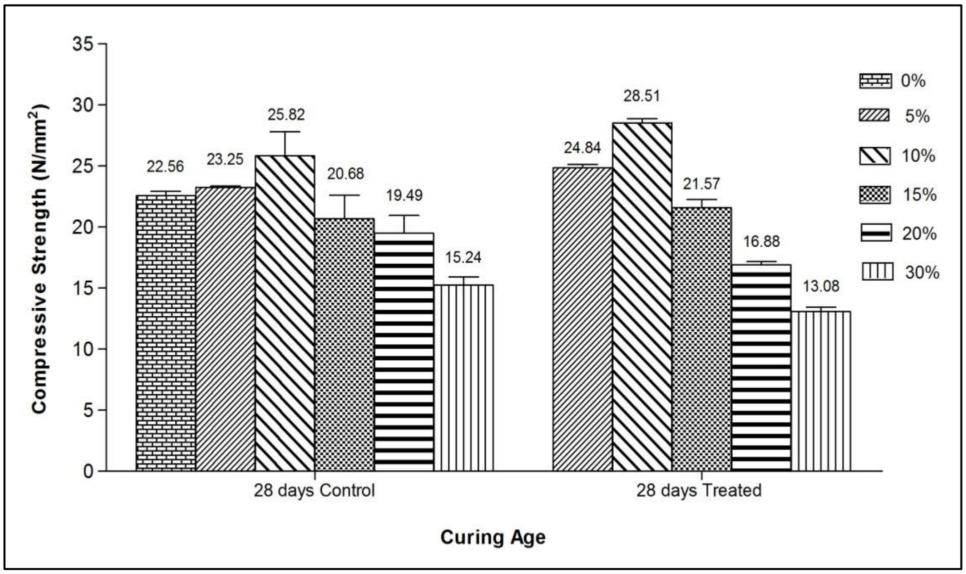


Fig. 4.26: Compressive strength of hardened mortar with different percentages of cement kiln dust (untreated and bacterial treated) at curing age of 28 days

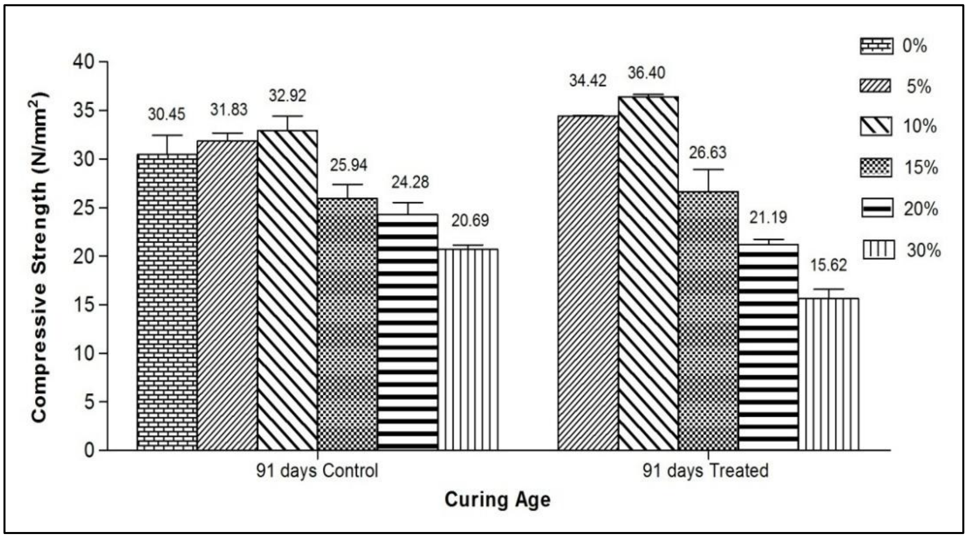


Fig. 4.27: Compressive strength of hardened mortar with different percentages of cement kiln dust (untreated and bacterial treated) at curing age of 91 days

4.5.2 Effect of Bacterial Treated CKD on Concrete Properties

4.5.2.1 Compressive strength

The compressive strength of the CKD control (untreated) concrete increased with increase in curing period. The compressive strength of the control CKD concrete (0% CKD) at the age of 7 days was 23.23±0.16 N/mm^2, whereas 5, 10, 15, 20 and 30% CKD (untreated) concrete showed compressive strength of 23.78±0.99, 24.31±1.39, 23.03±1.48, 22.28±0.93 and 19.87±0.14 N/mm^2, respectively (Fig. 4.28). After casting at the age of 28 days, the compressive strength of the control CKD concrete (0% CKD) was 34.82±1.03 N/mm^2 whereas of 5, 10, 15, 20 and 30% control CKD concrete was 35.78±1.19, 36.29±0.92, 34.53±1.95, 33.26±1.42 and 30.09±1.24 N/mm^2, respectively (Fig. 4.29). Similarly at 91 days of curing period, there were 40.44±4.03, 41.89±4.45, 44.12±1.36, 43.83±0.82, 41.87±1.50 and 39.78±1.37 N/mm^2 of compressive strength in 0, 5, 10, 15, 20 and 30% CKD control concrete (Fig. 4.30).

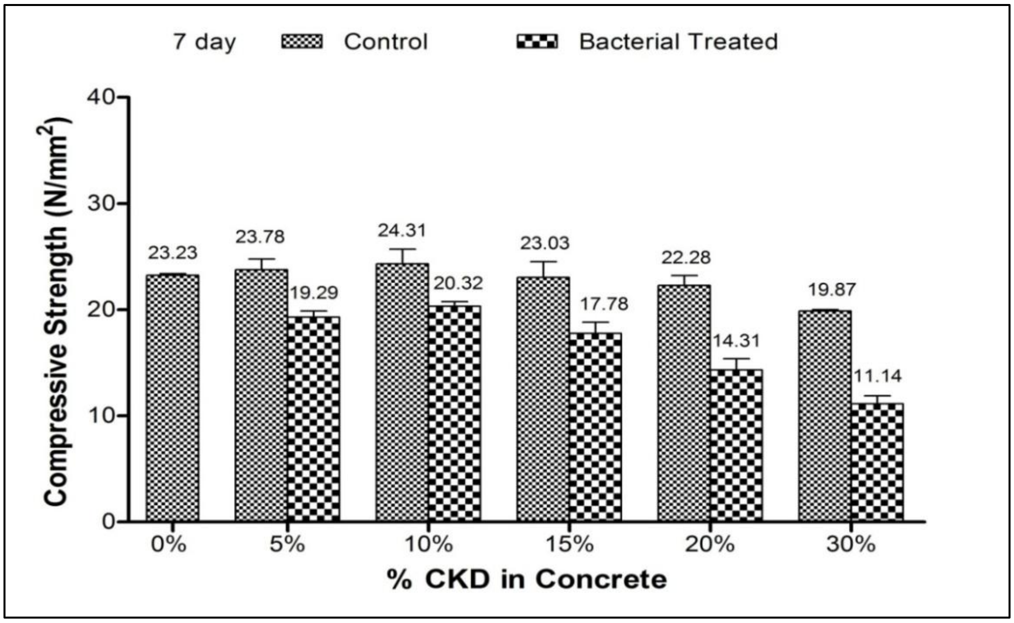


Fig. 4.28: Compressive strength of concrete with different percentages of cement kiln dust (untreated and bacterial treated) at curing age of 7 days

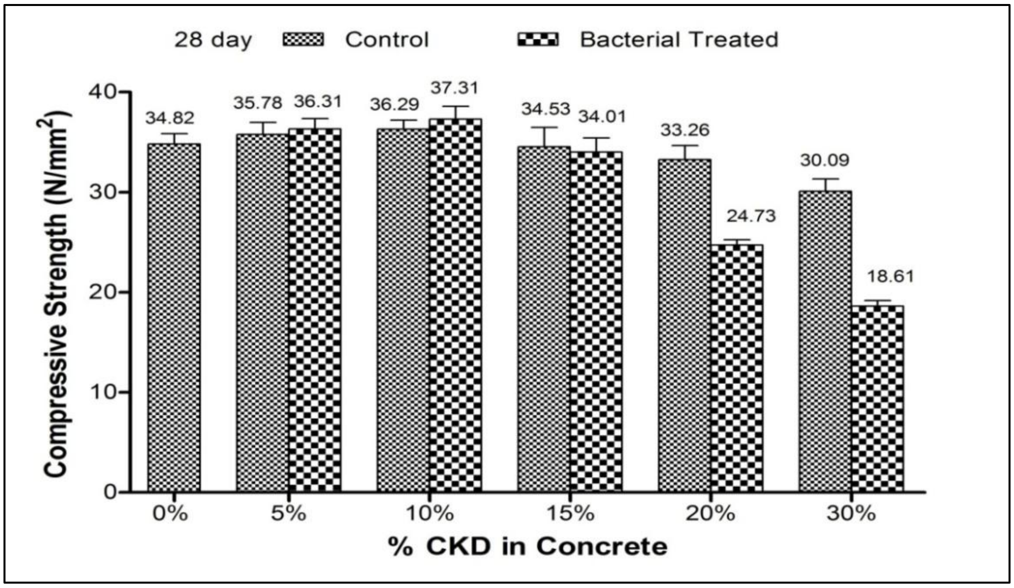


Fig. 4.29: Compressive strength of concrete with different percentages of cement kiln dust (untreated and bacterial treated) at curing age of 28 days

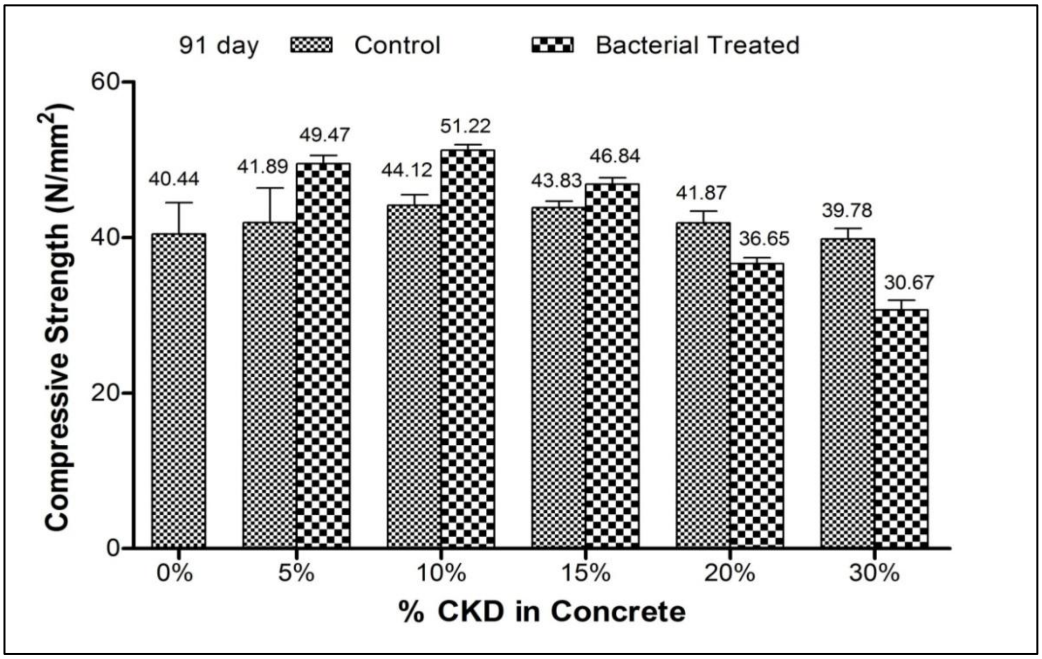


Fig. 4.30: Compressive strength of concrete with different percentages of cement kiln dust (untreated and bacterial treated) at curing age of 91 days

Maslehuddin et al. (2008) reported decrease in compressive strength (>5%) of concrete mixes (10 and 15% replacement) at all ages (3, 7, 14, 28, 56 and 91 days). The authors concluded that up to 5% CKD could be used without compromising the compressive strength of concrete. It was observed that up to 10% CKD replacement (control CKD concrete) strength increases whereas above 10% CKD addition compressive strength decreased at all ages with exception to 91 days curing where up to 20% CKD additions the increased strength was observed compared to 0% control concrete (Figs. 4.28-4.30).

The increased compressive strength in 10% CKD replacement levels is due to an appropriate alkalinity that increases the dissolution of silicate and formation of calcium silicate hydrate which is responsible for increased compressive strength whereas decreased compressive strength (above 10% CKD control concrete) is due to porosity and lower cement content at 91 days of curing (Wang et al., 2002). These results were in concomitant with the studies reported that high alkalinity in dust causes cracks, reduces the quality of cement and concrete, affects the hydration and microstructure of cement paste and ultimately reduced the strength of concrete (Davis and Hooks, 1975; Juenger and Jennings, 2001; Ghanem et al., 2010). Similar trend of increased compressive strength was also found in bacterial treated CKD concrete at all curing ages (Figs. 4.28-4.30).

The strength developed in bacterial treated CKD concrete at later ages (28 and 91 days) is due to the formation of additional hydrates obtained from the secondary reaction between silica and alumina in the cementitious materials (CKD in this case) with free calcium hydroxides present in the clinker (Pu, 1999). Cachim et al. (2014) reported that when pozzolans (calcined clay and volcanic ash) used as cement replacement up to 20% in concrete, strength of 60 MPa was achieved at 28 days. This was due to the decrease in pore size and pozzolanic action of natural pozzolans with lower heat of hydration and higher setting time resulted in increased long term strength.

In bacterial treated CKD concrete, at early ages (7 days) the decrease in strength values were observed compared to untreated CKD concrete cubes. In 10% bacterial treated CKD concrete (37.31±1.26 N/mm^2), 7.15 and 2.81% increase was observed in compressive strength compared to 0 (34.82±1.03 N/mm^2) and 10% (36.29±0.92 N/mm^2) CKD control concrete, respectively at

28 days of curing. At the age of 91 days, the increase in strength was 26.6 and 16.09%, respectively, in 10% bacterial treated CKD concrete (51.22±0.69 N/mm^2) compared with 0 (40.44±4.03 N/mm^2) and 10% (44.12±1.36 N/mm^2) CKD control concrete. Above 10% CKD addition (bacterial treated) the strength decreased at 28 days of curing and this may be attributed to decrease in cement content and hydration reaction. At 91 days, the strength was higher up to 15% CKD additions (bacterial treated) compared to control above which the strength decreases. Aggarwal et al. (2007) observed slower rate of strength gain at 7 days of curing by concrete containing bottom ash whereas beyond 28 days, the strength increased at faster rate due to pozzolanic nature of bottom ash. In early ages, bottom ash reacted slowly with calcium hydroxide generated during hydration reaction and less hydration products were formed resulted in decreased strength.

4.5.2.2 Split tensile strength

The effect of untreated and bacterial treated CKD (5, 10, 15, 20 and 30%) on splitting tensile strength of concrete mixtures at 7, 28 and 91 days is summarized in Figs. 4.31, 4.32 and 4.33, respectively. Test results showed 10.31% (2.15±0.12 N/mm^2), 5.36% (3.73±0.12 N/mm^2) and 11.9% (4.32±0.12 N/mm^2) increase in tensile strength at 7, 28 and 91 days of curing, respectively, in 10% untreated CKD concrete compared to 0% CKD concrete (1.95±0.11, 3.54±0.28 and 3.86±0.19 N/mm^2). Studies reported that addition of CKD decreases the split tensile strength of concrete. Udoeyo and Hyee (2002) reported 24% reduction in split tensile strength for concrete containing 20% CKD by weight replacing Portland cement. Similar reduction was also observed by Shoaib et al. (2000) with increase in CKD content due to poor bonding between aggregates and cement. In this study with addition of 10% untreated CKD in concrete, the strength increases as CKD acts as a filler material and improved the interfacial zone between cement and aggregates, thus increases the compaction of the structure (Khatib and Hibbert, 2005).

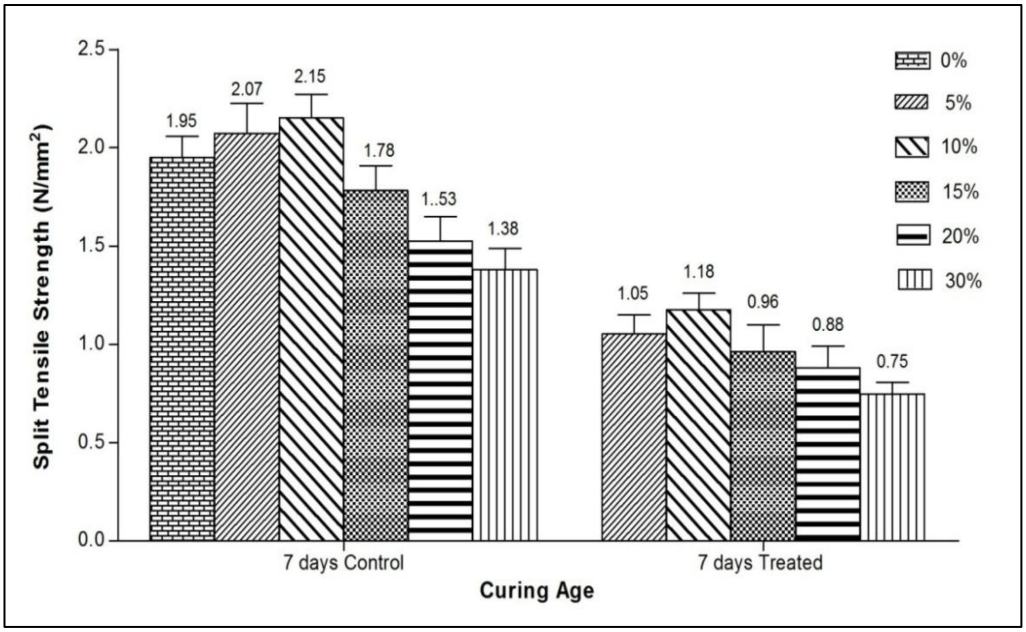


Fig. 4.31: Split tensile strength of concrete with different percentages of cement kiln dust (untreated and bacterial treated) at curing age of 7 days

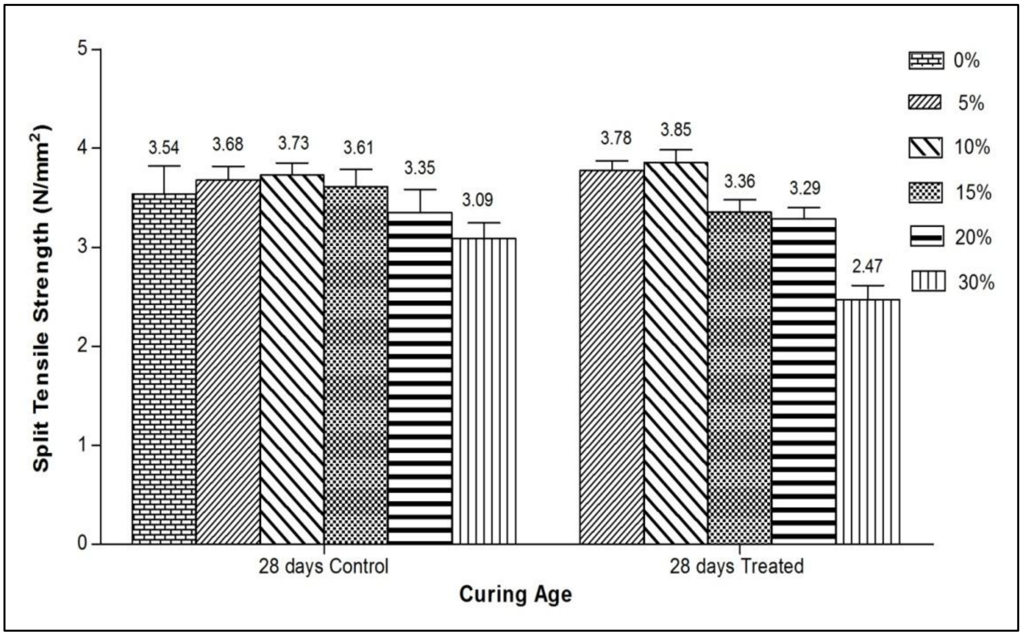


Fig. 4.32: Split tensile strength of concrete with different percentages of cement kiln dust (untreated and bacterial treated) at curing age of 28 days

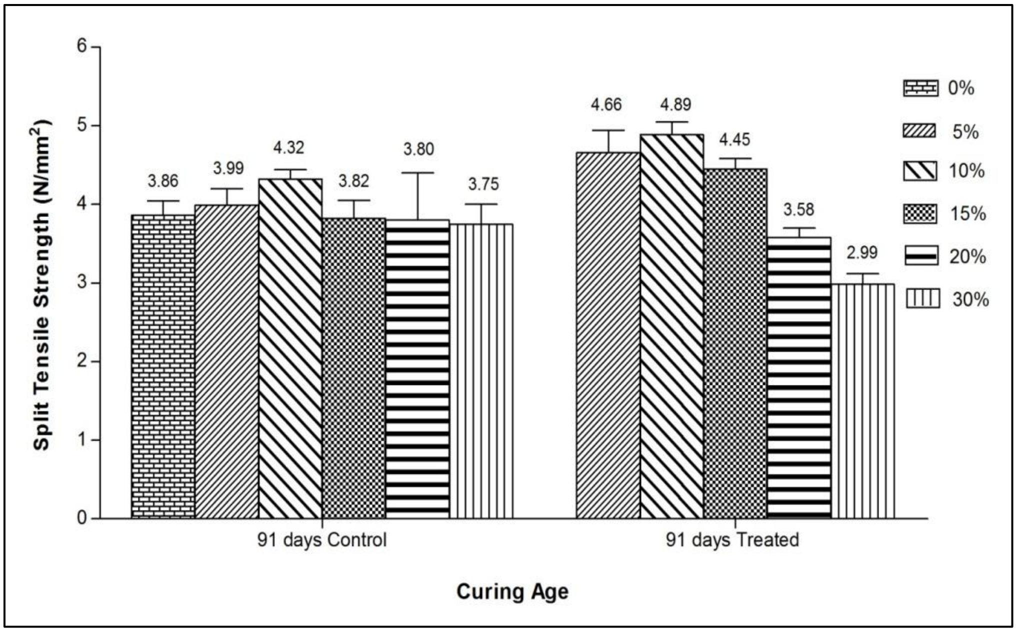


Fig. 4.33: Split tensile strength of concrete with different percentages of cement kiln dust (untreated and bacterial treated) at curing age of 91 days

Similar trend of splitting tensile strength was also observed in bacterial treated CKD concrete. Increase in split tensile strength in 10% bacterial treated CKD concrete from 3.85±0.13 (at 28 days) to 4.89±0.16 N/mm^2 (at 91 days) was observed (Figs. 4.32 and 4.33) which may be due to appropriate alkalinity and is responsible for late strength development (at 91 days) compared to control concrete. These results were supported by the findings of Wang et al. (2002) where increased strength in concrete with up to 15% CKD may be attributed to appropriate alkalinity and the reduced size of CKD significantly fills the pores and resulted in improved strength.

4.5.2.3 Ultrasonic pulse velocity

This test represents the quality of concrete using mechanical waves without damaging it. Table 4.15 shows the pulse velocity in different concrete specimens (cylinder size 100 x 200 mm) containing untreated and bacterial treated CKD. All the specimens at different curing periods (7, 28 and 91 days) showed pulse velocity in the range 3500-4500 m/s and termed the quality of concrete “good”.

Table 4.15: Ultrasonic Pulse Velocity Test of untreated and bacterial treated CKD concrete at 28 and 91 days of curing. (Values are ± S.D, n=3)

Treatment (% CKD Concrete)	Ultrasonic Pulse Velocity (UPV)					
	7 day curing		28 day curing		91 day curing	
	Control	Bacterial treated	Control	Bacterial treated	Control	Bacterial treated
0%	4053±8.82	--	4137±26.03	--	4160±32.15	--
5%	4087±8.82	3950±23.09	4163±52.39	4190±32.15	4197±43.333	4270±20.82
10%	4127±21.86	3990±32.15	4210±37.86	4237±29.06	4257±28.48	4380±28.87
15%	4047±24.04	3870±32.15	4080±28.87	4130±26.46	4140±32.15	4210±37.86
20%	4037±12.02	3660±32.15	4067±20.28	4050±20.82	4090±28.87	4120±32.15
30%	4020±41.63	3600±37.86	4033±26.03	3870±32.15	4073±55.48	4065±31.80

At the age of 7 days, bacterial treated CKD concrete showed decrease in pulse velocity compared to control. This decrease was due to reduced hydration reaction compared to control concrete specimens resulted in less formation of hydration products and porous nature of concrete matrix. In 10% CKD concrete (both untreated and treated), the pulse velocity is maximum at 28 (4210±37.86 and 4237±29.06 m/s, respectively) and 91 days of curing (4257±28.48 and 4380±28.87 m/s, respectively) compared to other treatments. This shows that concrete specimen containing 10% CKD is dense with fewer pores which help in the fast propagation of waves. This dense structure with fewer pores in 10% CKD concrete is responsible for higher pulse velocity that exhibited higher strength. Similarly, increased pulse velocity was also observed by Khatib (2008) in mixes containing 40% fly ash with 0.7% admixture compared with other mixes.

4.5.2.4 Rapid chloride permeability test

The chloride permeability of concrete specimens containing untreated and bacterial treated CKD showed decrease in chloride permeability with increasing curing period from 7 to 91 days (Table 4.16). The relationship between chloride penetrating rate and the charge passed in coulombs is already given in Table 3.23. At 7 days of curing, all the untreated CKD concrete specimens were within “low” range for chloride permeability whereas in bacterial treated CKD, all the specimens categorized under “moderate” chloride permeability. Specimens with 0, 5 and 10% CKD concrete after 28 days of curing were within “very low” range (1066±23, 1006±20 and 985±26.5 coulombs, respectively) for chloride permeability as per ASTM C 1202-07 classification while the specimens containing 15, 20 and 30% CKD were within the “low range” (1108±28.5, 1254±21.5 and 1677±25.5 coulombs, respectively). At 91 days of curing, all the treatments were within “very low” range. In 10% bacterial treated CKD concrete at 91 days, 22.45% (665±22 coulombs) decrease in chloride permeability was observed compared to control (857±31.5 coulombs). Rukzon and Chindaprasirt (2008) observed that the reduced chloride penetration is due to reduced average pore size and the improved interfacial zone. Studies reported that as more and more CKD is added, more free chloride ions are penetrated due to large pore size and thus increases the chloride permeability. Al Harthy et al. (2003) reported that that incorporation of CKD in the mortar mixes decreases the permeability due to very fine particle size of CKD which acts as fine filler material between the cement grains, therefore enhances durability. Maslehuddin et al. (2008) observed 6% increase in chloride permeability at 5% CKD replacement whereas at 15% CKD replacement the increase in permeability was 62%. The increase in chloride permeability was due to increased chloride content of the CKD that may lead to decrease in electrical resistivity of the concrete.

Table 4.16: Rapid chloride permeability test (RCPT) of untreated and bacterial treated CKD concrete at 7, 28 and 91 days of curing. (Values are ± S.D, n=3)

Treatment (% CKD Concrete)	Rapid Chloride Permeability Test (RCPT; coulombs)					
	7 day curing		28 day curing		91 day curing	
	Control	Treated	Control	Treated	Control	Treated
0%	1771±21.0	--	1066±23.0	--	858±31.5	--
5%	1757±23.5	2551±11.5	1006±20.0	983±23.5	835±24.0	761±23.0
10%	1720±30.0	2545±13.0	985±26.5	857±20.0	814±36.0	665±22.0
15%	1783±15.5	2730±8.0	1108±28.5	1203±22.0	924±21.0	794±15.5
20%	1842±10.0	2944±7.0	1254±21.5	2042±22.0	981±26.0	1155±26.0
30%	2228±12.5	4172±24.0	1677±25.5	2515±24.5	1242±15.0	1637±18.0

According to ASTM C 1202 classification, when the charge passed through concrete during a 6 h period is below 1000 coulombs it is characterized as “very low” chloride permeability. At 91 days of curing, all the treatments containing bacterial treated CKD showed “very low” permeability except 20 and 30% CKD concrete which falls in low chloride permeability range compared to control CKD concrete treatment. This is attributed to the reduced hydration reaction, increased CSH gel formation and tight packaging of materials which eventually reduced the pore size and thus, permeability.

4.5.2.5 Water absorption and porosity

Water absorption and porosity of concrete specimens containing untreated and bacterial treated CKD was determined by using ASTM C 642-97 method at the ages of 7, 28 and 91 days. Water absorption and porosity has the direct relation with the compressive strength of concrete. Increased water absorption and pore size decreased the compressive strength of concrete whereas decreased water absorption and pore size increased the compressive strength. The water absorption of the control CKD concrete (0% CKD) at the age of 7 days was 3.14±0.05%, whereas 5, 10, 15, 20 and 30% CKD (untreated) concrete showed water absorption of 3.07±0.13, 2.93±0.08, 3.19±0.30, 3.33±0.05 and 4.50±0.36%, respectively (Fig. 4.34). In bacterial treated CKD concrete minimum water absorption (1.84±0.13%) was observed in 10% CKD concrete.

At 28 days of curing, water absorption of 2.28±0.14, 1.24±0.29, 2.39±0.14, 3.17±0.06 and 3.39±0.29% in untreated CKD concrete whereas 1.72±0.01, 1.42±0.05, 2.17±0.12, 2.76±0.02 and 3.70±0.32% in bacterial treated CKD concrete (5, 10, 15, 20 and 30%, respectively) was observed. The water absorption of 0% control concrete was observed as 2.79±0.34% (Fig. 4.35). Fig. 4.36 shows the water absorption of concrete containing untreated and bacterial treated CKD at the age of 91 days.

Porosity of concrete decreased with increase in curing age. Figs. 4.37, 4.38 and 4.39 shows the porosity of concrete containing untreated and bacterial treated CKD at the age of 7, 28 and 91 days, respectively. The maximum reduction in porosity was observed in 10% bacterial treated concrete at the age of 7 (4.55±0.21%), 28 (3.56±0.11%) and 91 (2.71±0.03%) days compared to 0% CKD control concrete (7.52±0.44, 6.88±0.53 and 5.74±0.62%, respectively).

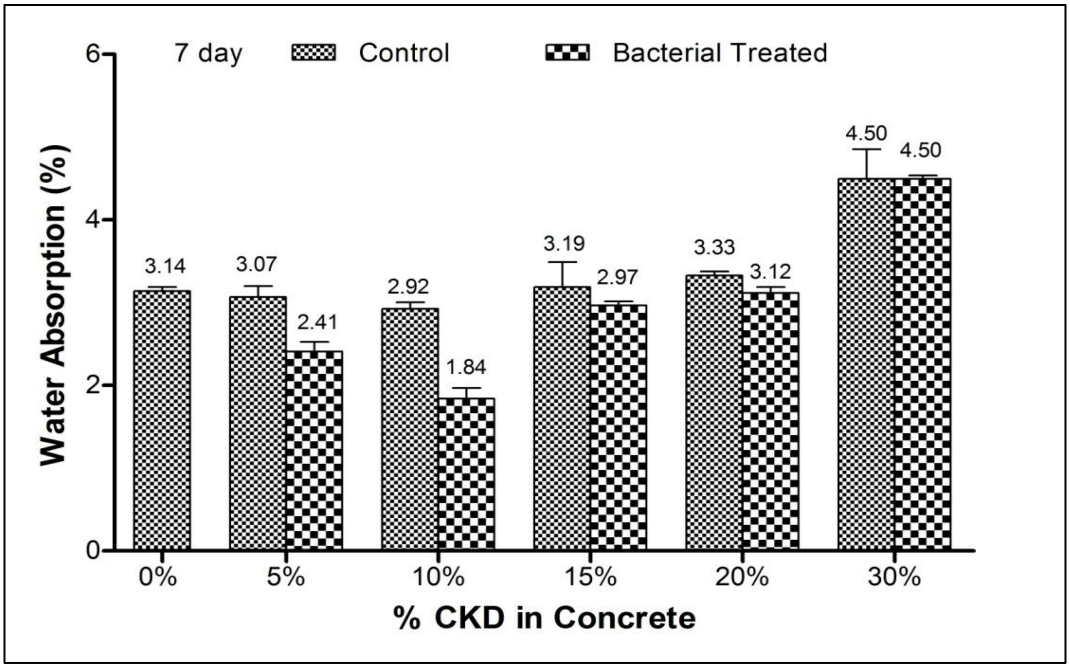


Fig. 4.34: Water absorption of concrete with different percentages of cement kiln dust (untreated and bacterial treated) at curing age of 7 days

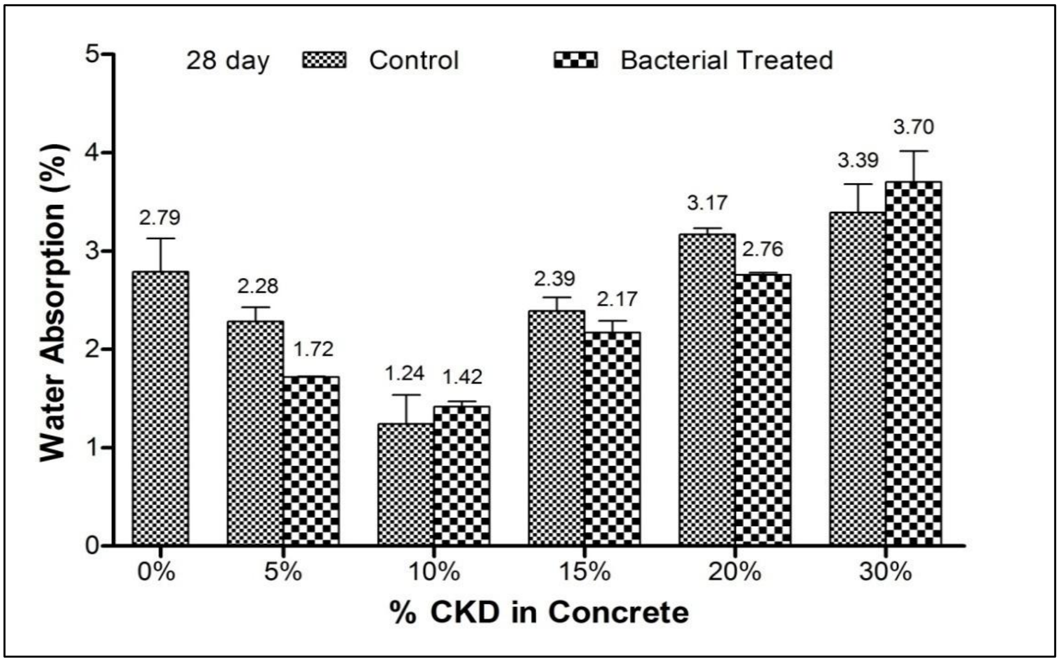


Fig. 4.35: Water absorption of concrete with different percentages of cement kiln dust (untreated and bacterial treated) at curing age of 28 days

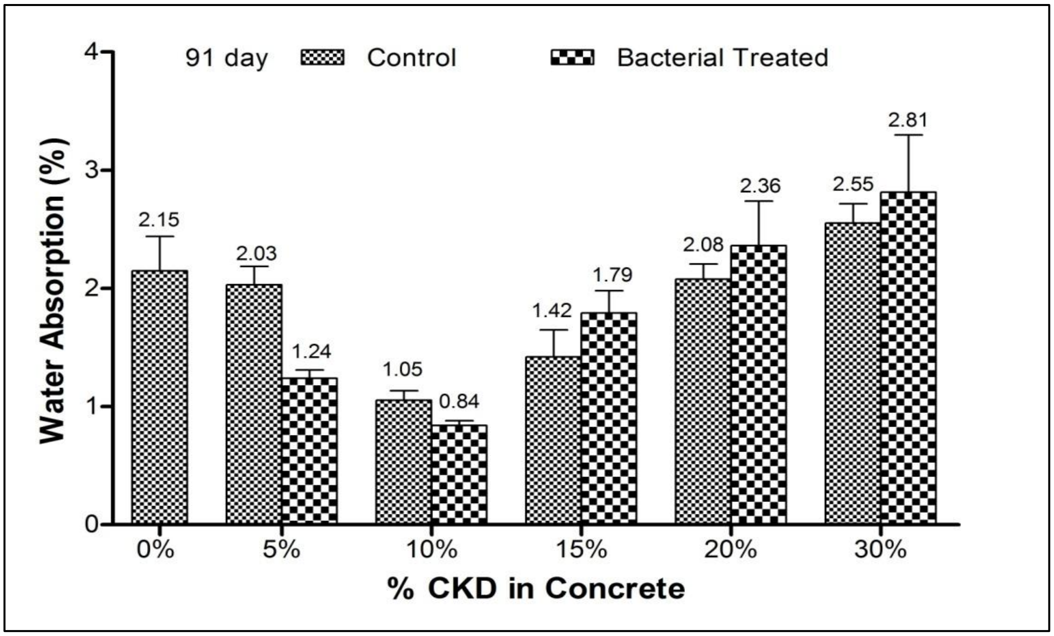


Fig. 4.36: Water absorption of concrete with different percentages of cement kiln dust (untreated and bacterial treated) at curing age of 91 days

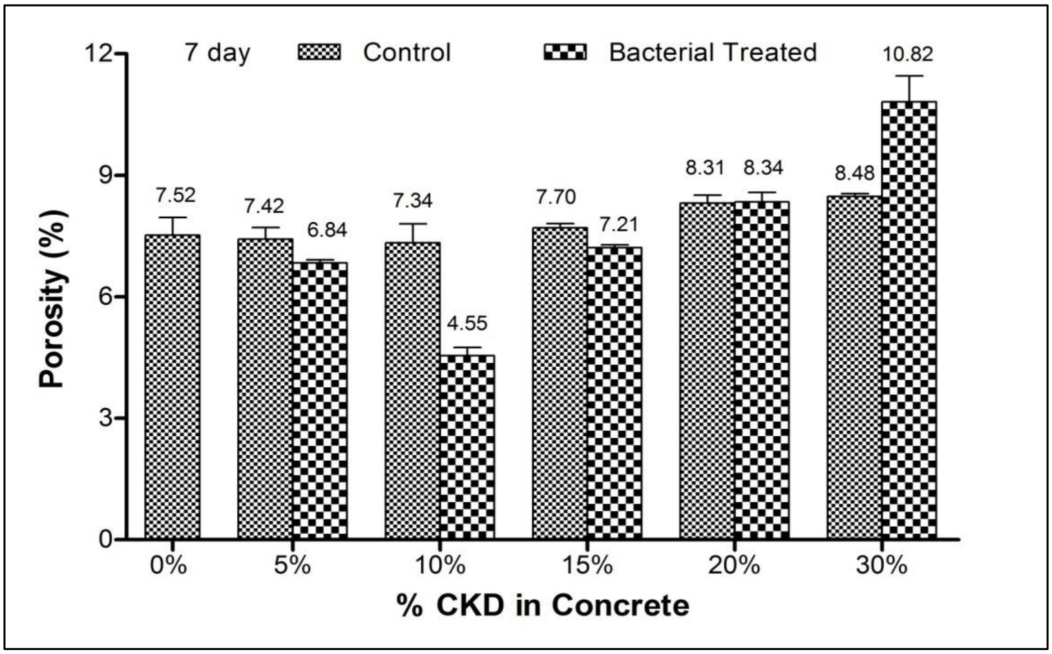


Fig. 4.37: Porosity of concrete with different percentages of cement kiln dust (untreated and bacterial treated) at curing age of 7 days

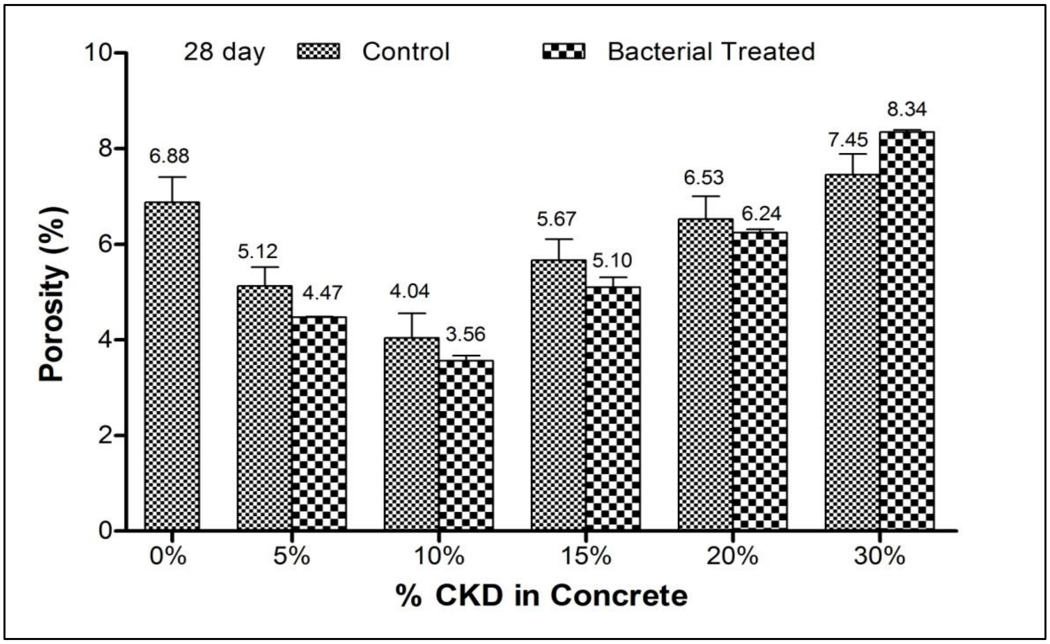


Fig. 4.38: **Porosity of concrete with different percentages of cement kiln dust (untreated and bacterial treated) at curing age of 28 days**

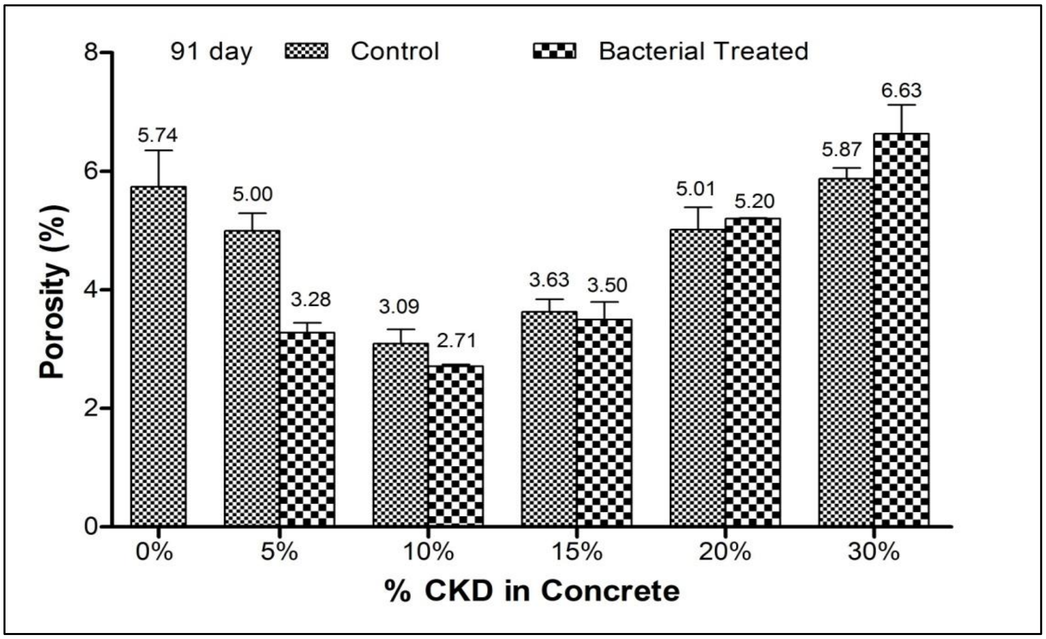


Fig. 4.39: **Porosity of concrete with different percentages of cement kiln dust (untreated and bacterial treated) at curing age of 91 days**

Results of water absorption and porosity showed decrease in 20% (0.84±0.04%) and 12.35% (2.71±0.03%), respectively, in 10% bacterial treated CKD concrete compared to untreated 10% CKD control concrete (1.05±0.08 and 3.09±0.24%) at 91 days of curing. Water absorption and porosity decreases with increase in CKD concentration but above 10% the water absorption and porosity increases in both control and bacterial treated CKD concrete at the age of 7, 28 and 91 days. This is due to decrease in cement content and increase in CKD concentration which decreases the binding of CKD-cement-aggregate in concrete and develops pores.

4.6 CHARACTERIZATION OF LEACHATE FROM CKD AND CONCRETE MIXTURE

4.6.1 Alkalinity and Chloride Analysis of Leachate

During fresh concrete mixing, the samples were collected from different places and the leachate generated was analyzed for alkalinity and chloride (APHA, 2005) of the fresh concrete mix leachate. The alkalinity of the cement and 0% CKD concrete (control) was 1893.33 and 1666.67±13.33 mg/L, respectively. When the cement was replaced with untreated CKD in different percentages (5, 10, 15, 20 and 30%) decrease in alkalinity (1553.33±17.64, 1526.67±35.28, 1440±34.64, 1253.33±17.64 and 1153.33±17.64 mg/L) was observed compared to control. After addition of bacterial treated CKD in concrete, 36 (1066.67±29.06 mg/L), 37.2 (1046.67±29.06 mg/L), 40 (1000±23.09 mg/L), 47.2 (880±11.55 mg/L) and 54.8% (753.33±40.55 mg/L) reduction in alkalinity was observed in 5, 10 15, 20 and 30% treated CKD concrete compared to control concrete mix (Fig. 4.40).

Similarly, the chloride content in 0% CKD concrete (control) was 800±23.09 mg/L whereas in 5, 10, 15, 20 and 30% untreated CKD concrete it was observed as 760±34.64, 740±34.64, 740±41.63, 720±41.63 and 720±46.19 mg/L. After addition of bacterial treated CKD in concrete (5, 10 15, 20 and 30%) the chloride content was observed as 740±23.09, 733.33±29.06, 673.33±40.55, 573.33±17.64, 480±34.64 mg/L, respectively. There was no significant decrease in chloride content up to 10% bacterial treated CKD addition whereas above 10% CKD additions decrease in chloride content was observed (Fig. 4.41). This reduction was due to decrease in cement content and addition of bacterial treated CKD with reduced chloride content.

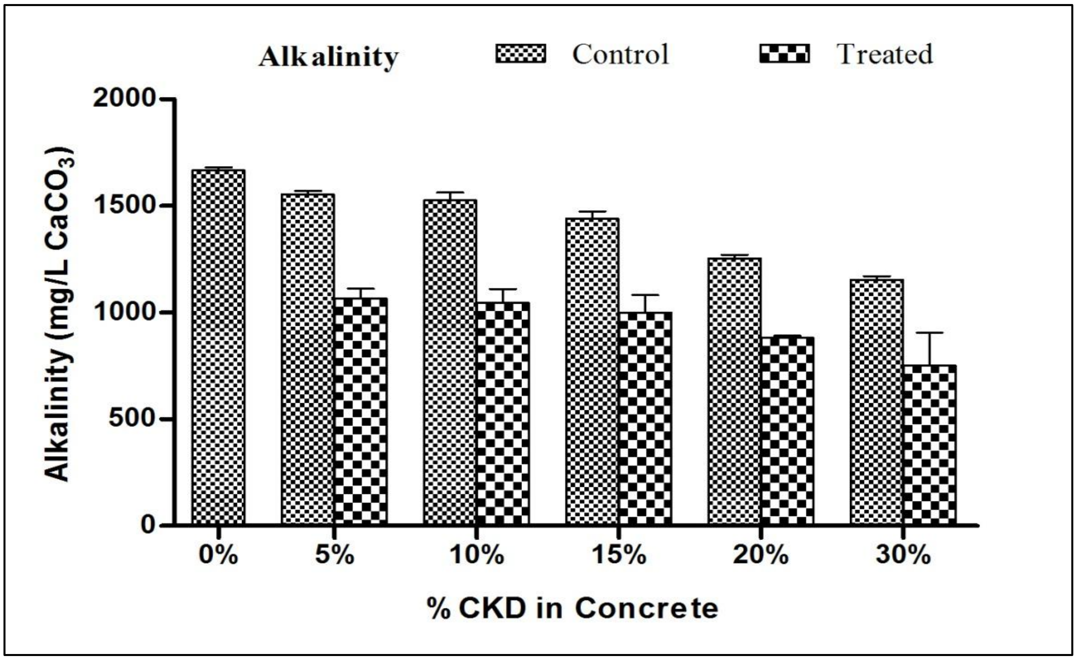


Fig. 4.40: Alkalinity of fresh concrete mix leachate containing different percentages of cement kiln dust (untreated and bacterial treated)

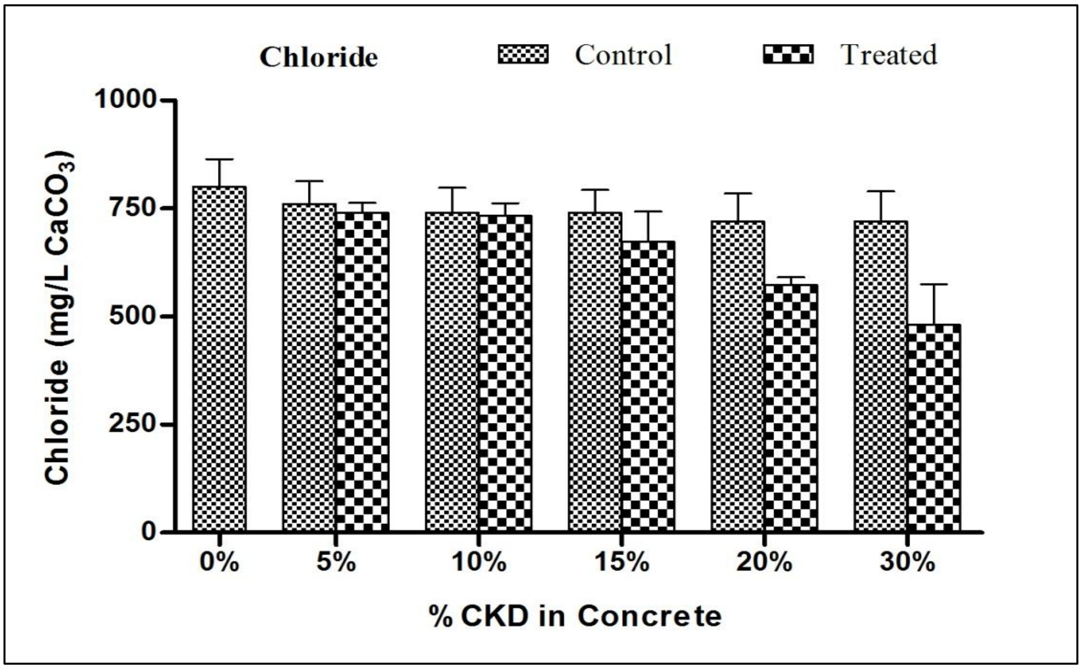


Fig. 4.41: Chloride of fresh concrete mix leachate containing different percentages of cement kiln dust (untreated and bacterial treated)

4.6.2 Metal Analysis

Metal analysis of leachate samples from control mix, untreated and bacterial (KG1, KG4, KG5 and KGMD1) treated CKD, and fresh concrete mixture of different treatments (0, 5, 10, 15, 20 and 30% CKD) were done by inductive coupled plasma mass spectrometer (ICP-MS). The leachate was analyzed for 10 metals including Chromium (Cr), Cobalt (Co), Iron (Fe), Lead (Pb), Silver (Ag), Cadmium (Cd), Nickel (Ni), Zinc (Zn), Copper (Cu) and Molybdenum (Mo).

4.6.2.1 CKD leachate

Bacterial treatment of CKD by isolate KG1, KG4, KG5 and KGMD1 showed reduction in metal content in leachate (Table 4.17). The content of silver (Ag) in control CKD was 0.338±0.008 mg/L which then reduced to 0.029±0.010, 0.031±0.009, 0.026±0.007 and 0.024±0.005 mg/L in KG1, KG4, KG5 and KGMD1, respectively, treated CKD. Similarly, chromium (Cr) in control CKD was 2.066±0.011 mg/L, and after KG1, KG4, KG5 and KGMD1 treatment the Cr content was reduced to 0.519±0.008, 0.492±0.008, 0.522±0.008 and 0.568±0.007 mg/L, respectively. Lead (Pb) was also reduced by 68.99 (0.040±0.004 mg/L), 68.11 (0.041±0.003 mg/L), 67.42 (0.042±0.004 mg/L) and 65.45% (0.045±0.004 mg/L), respectively, in KG1, KG4, KG5 and KGMD1 treated CKD compare to untreated (control) CKD (0.130±0.004 mg/L). The reduction in metal content was varied from 34-92% in different metals by different isolates (Table 4.17). Leaching of silver (Ag) is maximally inhibited by bacterial treatment followed by Cr, Cu, Ni and Zn.

Bacterial strains KG1, KG4, KG5 and KGMD1 are capable of producing organic acid (acetic and formic acid) which can solubilize and complex metal cations as the low pH favours acidolysis (solubilization of metals through organic acid produced by microorganisms) which enhanced the mobility of free cations by protonation. The pH of the untreated CKD is around 12 which upon treated with bacterial strains reduced to 8. This increased the mobility and complexation of the metal ions compared to untreated CKD but lesser than the mobility at low pH (1-2). At pH 8, absorption of the metal ions by bacterial cultures were also affected, therefore, the efficiency of the isolates to absorb the metal ions was also reduced (range varies for 34-92% depending upon the metal).

Table 4.17: Metal analysis of leachate obtained from untreated (control) and bacterial treated cement kiln dust. (Values are ± S.D, n=3)

Metals	Control	CKD+KG1	CKD+KG4	CKD+KG5	CKD+KGMD1
Cr	2.066±0.011	0.519±0.008±	0.492±0.008	0.522±0.008	0.568±0.007
Co	0.014±0.003	0.005±0.001	0.005±0.001	0.005±0.001	0.006±0.001
Fe	0.850±0.007	0.362±0.008	0.564±0.010	0.487±0.012	0.536±0.012
Pb	0.129±0.130	0.040±0.001	0.041±0.003	0.042±0.004	0.045±0.004
Ag	0.338±0.008	0.029±0.010	0.031±0.009	0.026±0.007	0.024±0.005
Cd	0.024±0.003	0.008±0.002	0.008±0.001	0.008±0.002	0.009± 0.002
Ni	0.122±0.005	0.036±0.003	0.036±0.004	0.039±0.007	0.042±0.001
Zn	5.096±0.022	1.519±0.012	1.462±0.010	1.529±0.002	1.682±0.022
Mo	0.127±0.006	0.039±0.005	0.035±0.005	0.038±0.006	0.041±0.004
Cu	0.285±0.008	0.081±0.005	0.083±0.007	0.088±0.012	0.098±0.008

All values are in mg/L

4.6.2.2 Fresh concrete leachate

Utilization of CKD in concrete leads to increase in metal content in leachate obtained from untreated CKD compared to control (0%) CKD concrete, whereas decrease in metal content in bacterial treated CKD was observed. The decrease in metal content is not significant as the 0% CKD control concrete already contains metals (Table 4.18) due to presence of fly ash in cement (Pozzolanic Portland Cement). Thus, on replacing the cement with CKD very less reduction in metal content was observed. The metal concentration (Ag, Cd, Cr, Cu, Fe, Mo, Ni, Pb and Zn) in 5% bacterial treated leachate were reduced from 0.101 mg/L to 0.084 mg/L, 0.009 mg/L to 0.007 mg/L, 0.700 mg/L to 0.624 mg/L, 0.343 mg/L to 0.323 mg/L, 0.713 mg/L to 0.680 mg/L, 0.122 mg/L to 0.117 mg/L, 0.310 mg/L to 0.300 mg/L, 0.056 mg/L to 0.050 mg/L and 0.931 mg/L to 0.741 mg/L, respectively. No such change in Co concentration was observed. Similar reduction in metal concentration was also observed in 10, 15, 20 and 30% bacterial treated CKD concrete (Table 4.18).

Table 4.18: Metal analysis of leachate obtained from untreated and bacterial treated (KG1) CKD (5 and 10%) fresh mix concrete. (Values are ± S.D, n=3)

Metal(s)	CKD control	CKD treated	0% CKD control	5% CKD concrete		10% CKD concrete	
				Control	Treated	Control	Treated
Cr	2.066± 0.011	0.519± 0.008±	0.630 ± 0.047	0.700 ± 0.053	0.624 ± 0.009	0.774 ± 0.006	0.619 ± 0.008
Co	0.014± 0.003	0.005± 0.001	0.037 ± 0.005	0.036 ± 0.007	0.035 ± 0.005	0.034 ± 0.005	0.034 ± 0.004
Fe	0.850± 0.007	0.362± 0.008	0.757 ± 0.064	0.713 ± 0.006	0.680 ± 0.081	0.642 ± 0.009	0.620 ± 0.072
Pb	0.129± 0.130	0.040± 0.001	0.053 ± 0.008	0.056 ± 0.005	0.050 ± 0.006	0.061 ± 0.007	0.042 ± 0.005
Ag	0.338± 0.008	0.029± 0.010	0.087 ± 0.006	0.101 ± 0.005	0.084 ± 0.005	0.122 ± 0.004	0.081 ± 0.008
Cd	0.024± 0.003	0.008± 0.002	0.008 ± 0.002	0.009 ± 0.002	0.007± 0.001	0.009 ± 0.001	0.007 ± 0.001
Ni	0.122± 0.005	0.036± 0.003	0.313 ± 0.061	0.310 ± 0.044	0.300 ± 0.067	0.330 ± 0.047	0.280 ± 0.081
Zn	5.096± 0.022	1.519± 0.012	0.710 ± 0.055	0.931 ± 0.007	0.741 ± 0.006	1.123 ± 0.006	0.791 ± 0.087
Mo	0.127± 0.006	0.039± 0.005	0.121 ± 0.004	0.122 ± 0.007	0.117 ± 0.005	0.122 ± 0.005	0.112 ± 0.007
Cu	0.285± 0.008	0.081± 0.005	0.346 ± 0.004	0.343± 0.006	0.323 ± 0.007	0.338 ± 0.006	0.318 ± 0.008

All values are in mg/L

Horikoshi (1999, 2011) reported that alkaliphilic microorganisms are suitable candidates for the bio-remediation process under alkaline conditions as they have optimum growth at high pH values (pH 9 to 11) and tolerate high salt concentration. *Bacillus halodurans* strain KG1 (used in this study) is capable to grow at high pH values 8-9 and tolerate upto 8% of salinity in minimal growth medium. This represents the alkaliphilic and halophilic nature of bacterial strain KG1. Ventosa (1998) reported that for halophilic or halotolerant microorganisms high concentration of anions and cations are needed for their growth which may prove toxic to other microorganisms. The reason for the requirement of cations and anions for growth is that the microbial isolates utilize the metal elements in their metabolic processes by reducing them from toxic to non- toxic elements. The toxicity of copper (Cu) is based on the production of hydro-peroxide radicals that damages cell membrane (Nies, 1999), but it also acts as a main functioning agent in cytochrome C-oxidase and related enzymes in respiratory transport chain of microbial metabolism (Madigan et al., 2009; Nies, 1999). Iron (Fe) has low solubility in neutral or alkaline medium and is not toxic to aerobic bacteria, thus its uptake is favoured by microbial formation of ligands such as siderophores which helps in the solubilization of ferric iron and absorbed by microorganisms (Madigan et al., 2009). Studies reported that heavy metal cations in aerobic bacteria may react to oxygen to form metal oxyanions which interfere with the metabolism of structurally related non metals such as chromate with sulfate etc., which on reduction leads to the production of radicals that may prove toxic to cell (Kachur et al., 1998). Sodium and potassium are essential elements for enzymatic activity and ionic pumps in alkaliphiles and halophiles which bind to these radicals and detoxify them, thereby enhances the bacterial tolerance to toxic metals.

Table 4.19: Metal Analysis of leachate obtained from untreated and bacterial treated (KG1) CKD (15, 20 and 30%) fresh mix concrete. (Values are ± S.D, n=3)

Metal(s) mg/L	15% CKD concrete		20% CKD concrete		30% CKD concrete	
	Control	Treated	Control	Treated	Control	Treated
Cr	0.846 ± 0.005	0.602 ± 0.008	0.917 ± 0.007	0.59 ± 0.008	1.063 ± 0.009	0.561 ± 0.010
Co	0.032 ± 0.007	0.031 ± 0.006	0.028 ± 0.004	0.028 ± 0.007	0.025 ± 0.008	0.024 ± 0.005
Fe	0.595 ± 0.007	0.580 ± 0.038	0.539 ± 0.008	0.513 ± 0.010	0.411 ± 0.006	0.402 ± 0.014
Pb	0.067 ± 0.006	0.040 ± 0.004	0.073 ± 0.007	0.038 ± 0.007	0.078 ± 0.007	0.033 ± 0.009
Ag	0.135 ± 0.006	0.075 ± 0.009	0.148 ± 0.009	0.071 ± 0.008	0.172 ± 0.006	0.059 ± 0.009
Cd	0.100 ± 0.006	0.006 ± 0.001	0.011 ± 0.003	0.005 ± 0.001	0.012 ± 0.002	0.004 ± 0.001
Ni	0.340 ± 0.069	0.270 ± 0.064	0.363 ± 0.052	0.27 ± 0.099	0.39 ± 0.069	0.24 ± 0.058
Zn	1.321 ± 0.009	0.835 ± 0.011	1.432 ± 0.008	0.887 ± 0.010	1.659 ± 0.007	0.992 ± 0.010
Mo	0.123 ± 0.005	0.105 ± 0.008	0.124 ± 0.006	0.102 ± 0.007	0.125 ± 0.008	0.080 ± 0.015
Cu	0.327 ± 0.008	0.308 ± 0.007	0.319 ± 0.006	0.302 ± 0.007	0.307 ± 0.006	0.282 ± 0.066

Bacterial strain KG1 is capable of producing organic acid (acetic and formic acid) which can solubilize and complex metal cations. Studies reported that low pH favours acidolysis (solubilization of metals through organic acid produced by microorganisms) which enhanced the mobility of free cations by protonation (Sayer et al., 1997). The pH of the untreated CKD is around 12 which upon treated with bacterial strain KG1 reduced to 8. This increased the mobility and complexation of the metal ions compared to untreated CKD but lesser than the mobility at low pH (1-2). At pH 8, absorption of the metal ions by bacterial culture is also affected, therefore, the efficiency of the isolate to absorb the metal ions is also reduced (range varies for 58-92% depending upon the metal).

Aravindhan et al. (2004) studied that different functional groups such as carboxyl, phosphate or hydroxyl group of the biomass were involved in several chemical reactions like ionic interactions, hydrogen bonding, for complexation with metal (chromate) ions which was found to be favoured by acidic pH due to organic acid production by *Bacillus* sp., and provides resistance to bacterial cell and reduces the metal ions. The metal ions that do not absorb by the bacterial isolate might be precipitated or chelated by bacteria outside the cell membrane. This can be supported by the findings of Beveridge and Murray (1976) and Doyle et al. (1980) that in many Gram positive bacteria (*Bacillus* sp.) the high density of electronegative sites (due to presence of repeated phosphodiester residues) with in the wall is found to be responsible for the affinity of metals and its ability to form metal precipitates. Thus, the results revealed that isolate KG1 was able to reduce the metal content in CKD under alkaline conditions without any amendment of nutrient (except sucrose once at 5 days of incubation) which suggests its potential application in bioremediation of other solid alkaline industrial wastes.

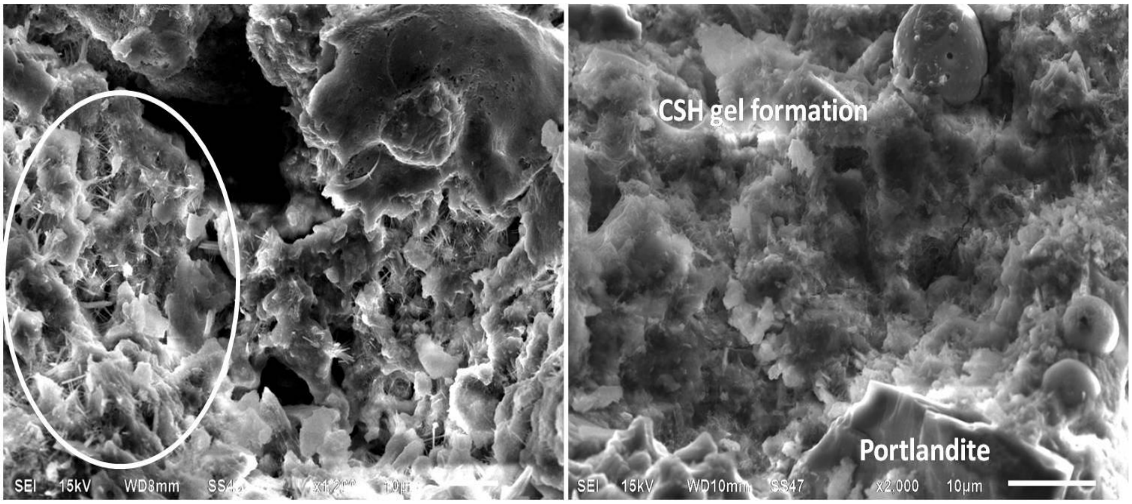
4.7 MICROSTRUCTURE ANALYSIS

4.7.1 SEM Analysis

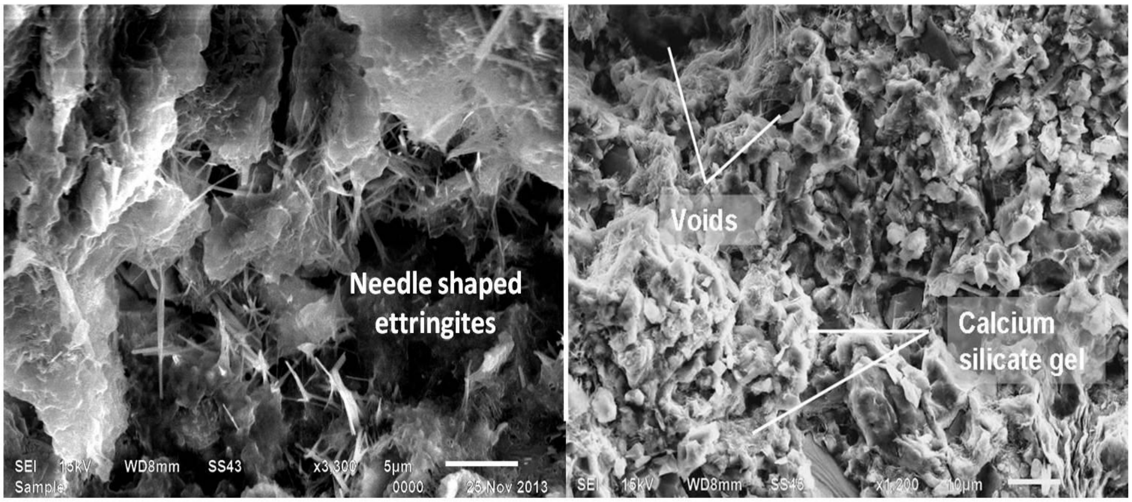
4.7.1.1 Mortar

Figs. 4.42-4.47 shows the SEM analysis of control (0% CKD) and, untreated and bacterial treated CKD (5, 10, 15, 20 and 30%) blended cement mortars. In all the treatments with curing age change in microstructure of the specimens was observed. In 0% CKD mortar, the SEM image shows the entangled mass of calcium silicate hydrate (CSH) gel, main hydration product responsible for increased compressive strength along with voids (Fig. 4.42). At 7 days of curing, initiation of pozzolanic reaction and formation of CSH gel was observed. Due to presence of alkalis and sulfates in cement needle shaped ettingites (calcium aluminium sulfate) were formed on the surface of the mortar matrix. As curing time increases the increased hydration product tends to densify and harden the specimen. Unreacted portlandite (calcium hydroxide) was also observed at days of curing in the crystal form.

Similar type structures was also observed in 5 and 10% CKD mortars but are less porous than 0% control at 91 days of curing period. In bacterial treated CKD mortar (5 and 10%) ettringites in pores were observed (Figs. 4.43 and 4.44) that fills the voids and reduces the size of pores. At 91 days of curing compact packaging of matrix was observed due to increased formation of CSH gel. In 15, 20 and 30% CKD control mortar (Figs. 4.45-4.47) voids were observed which made the structure porous and resulted in decreased strength. In 10% bacterial treated CKD mortar less porous and highly dense structure was formed at 91 days of curing (Fig. 4.44) due to continuous hydration, reduced alkalinity and compactness of the materials due to increased CSH gel. The reduction in strength in 20 and 30% bacterial treated CKD mortar is due to expansive nature of ettringites formed on the outer surface of calcium silicate hydrate at the interfaces of cement paste which exerts pressure by forming gaps at the interfaces resulting in increased porosity and reduced strength (Famy et al., 2000).

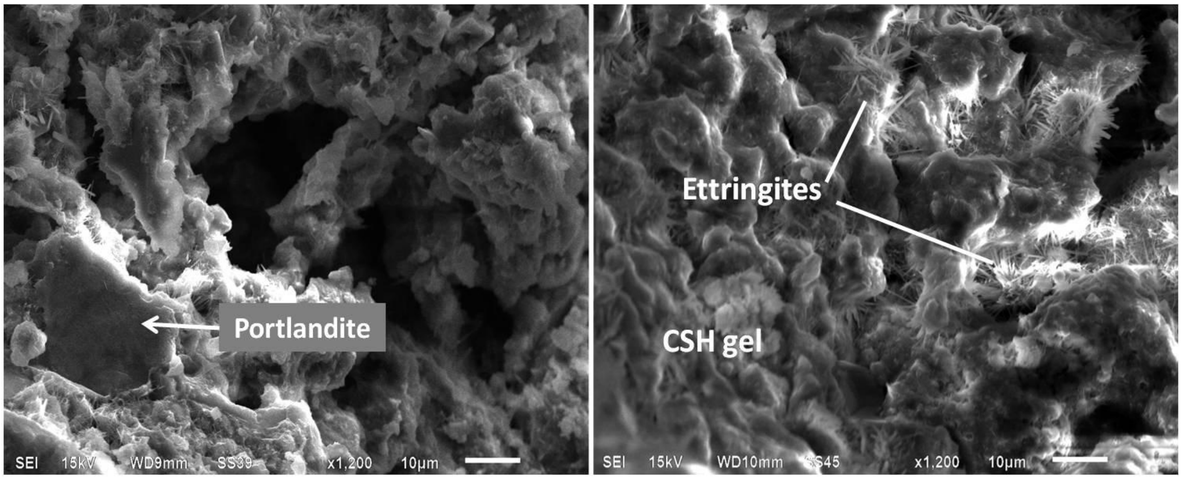


7 days **28 days**

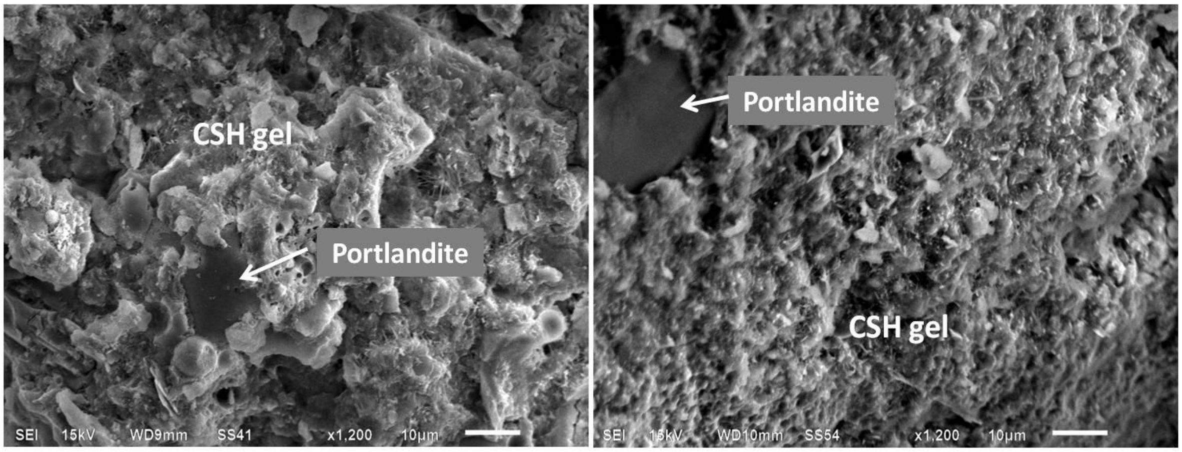


Enlarged portion **91 days**

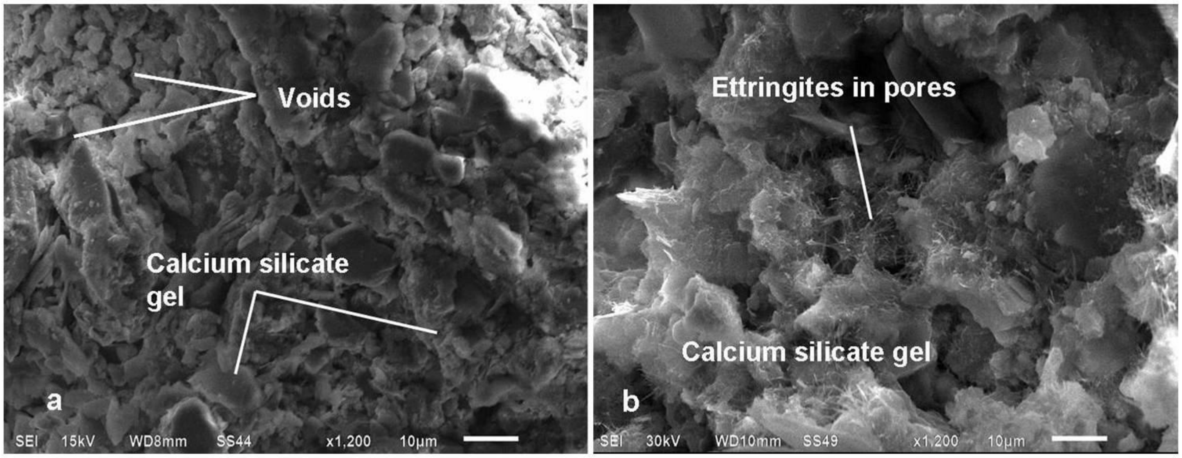
Fig. 4.42: SEM image shows control mortar (0% CKD) at 7, 28 and 91 days of curing. At 7 days of curing needle shaped ettringites are seen (enlarged portion)



7 days **Control** **Treated**



28 days **Control** **Treated**



91 days **Control** **Treated**

Fig. 4.43: SEM image shows 5% CKD mortar (control and treated) at 7, 28 and 91 days of curing

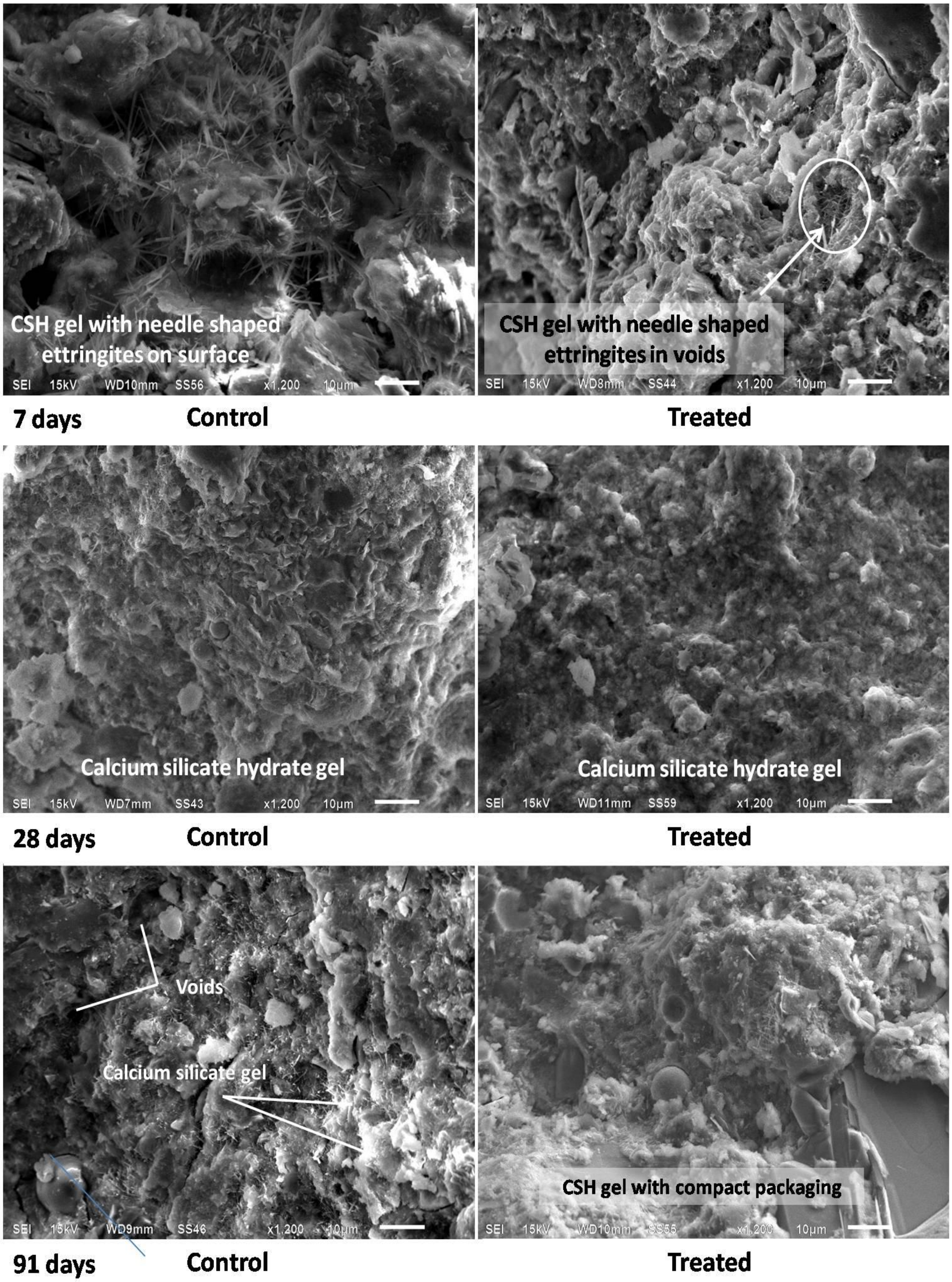
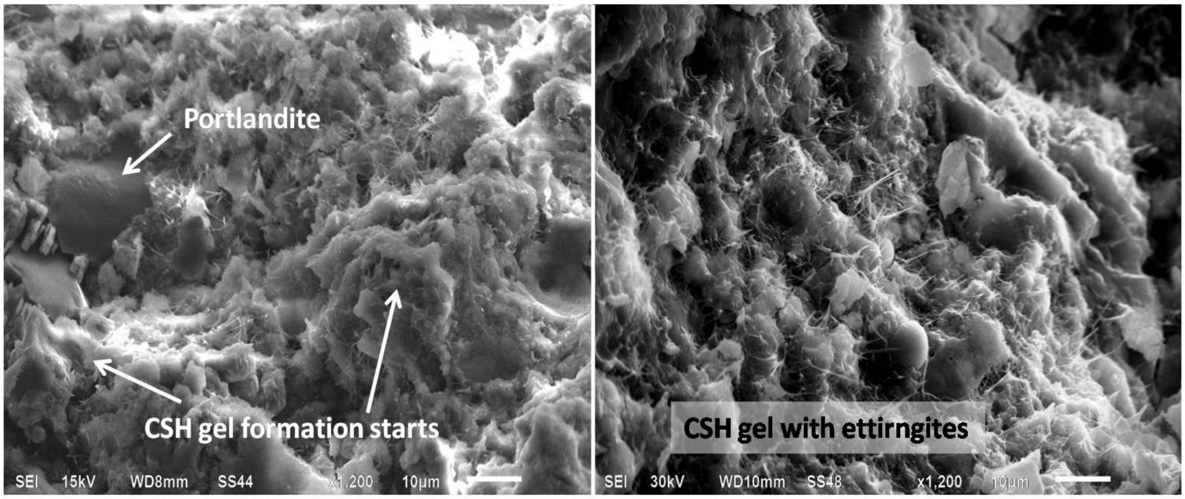
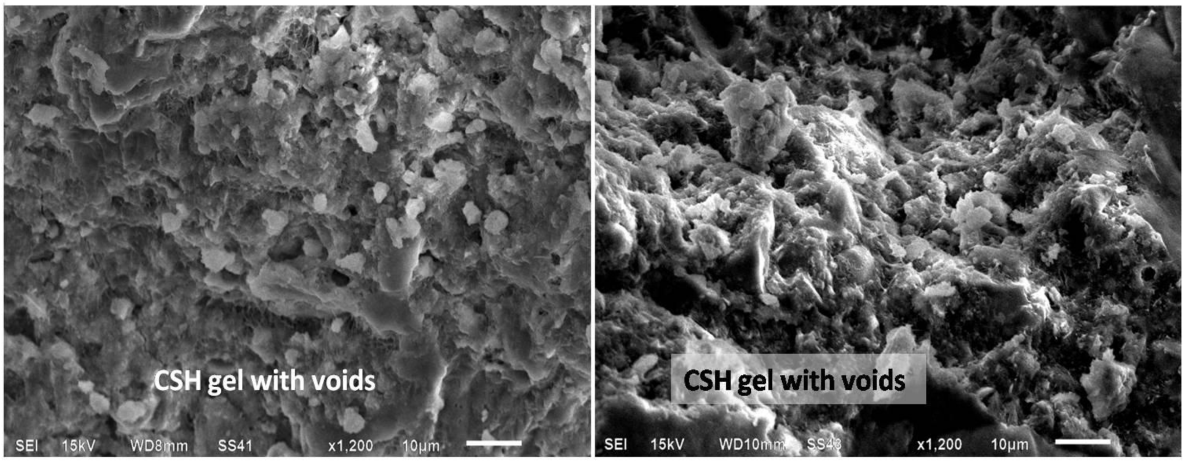


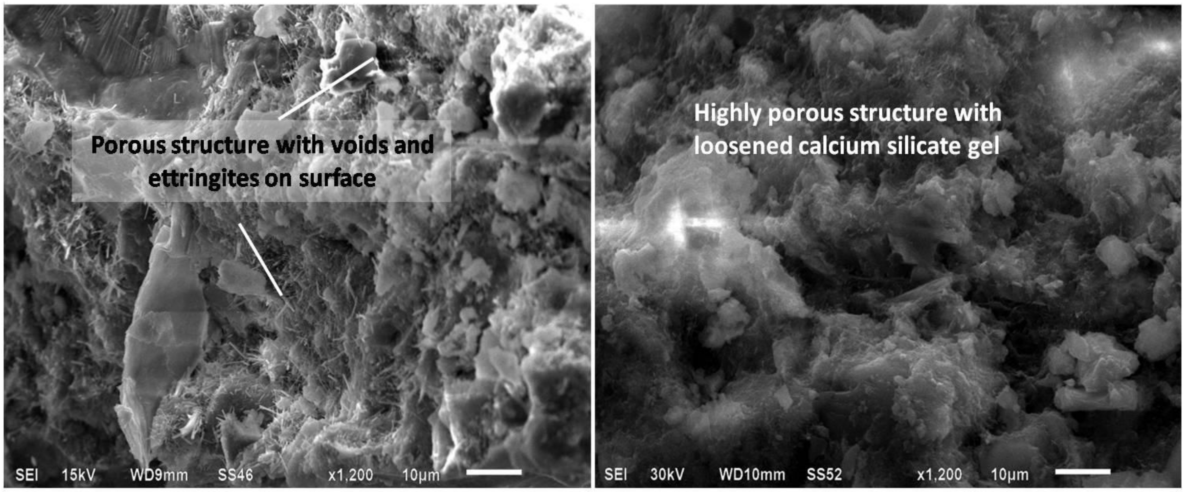
Fig. 4.44: SEM image shows 10% CKD mortar (control and treated) at 7, 28 and 91 days of curing



7 days **Control** **Treated**



28 days **Control** **Treated**



91 days **Control** **Treated**

Fig. 4.45: SEM image shows 15% CKD mortar (control and treated) at 7, 28 and 91 days of curing

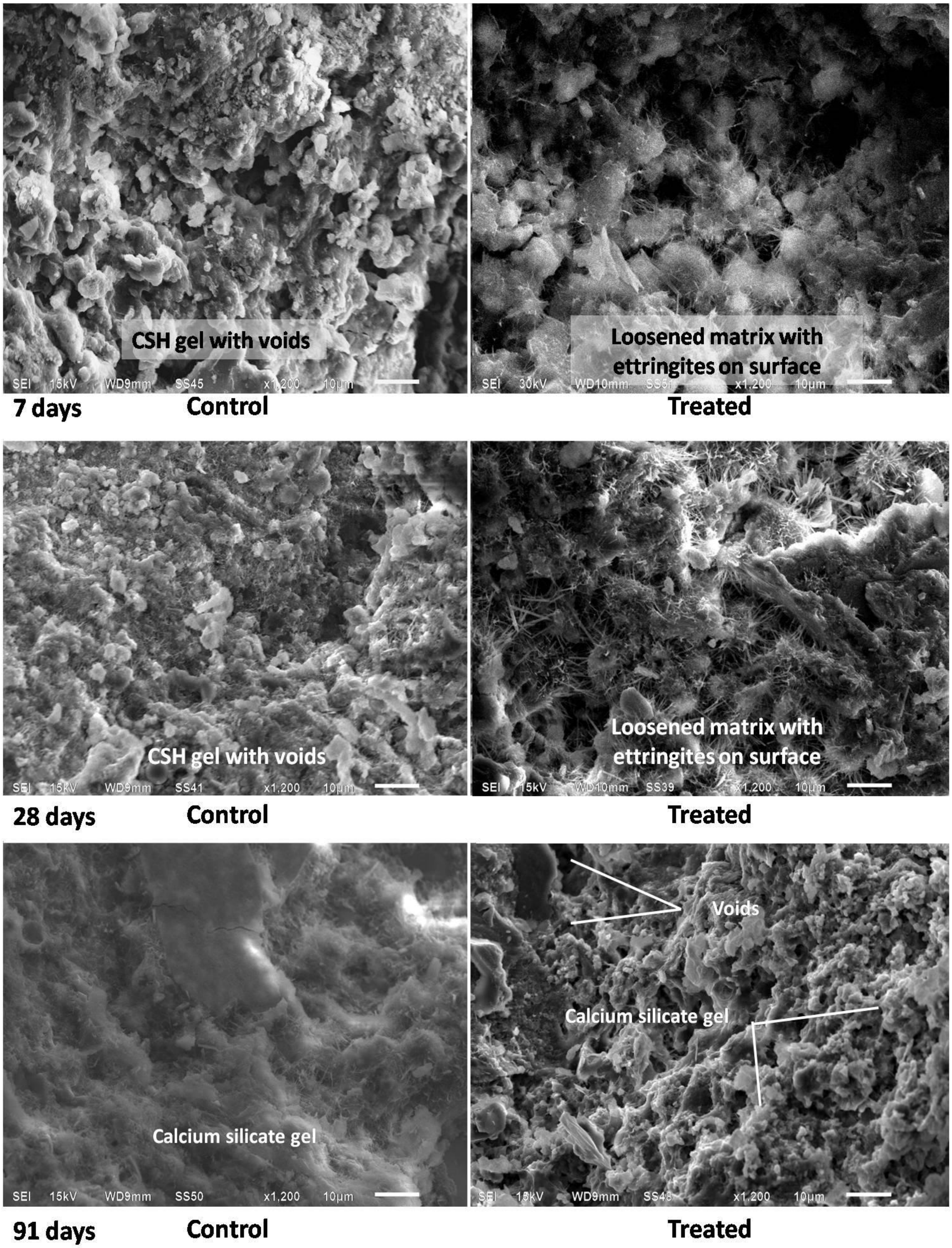
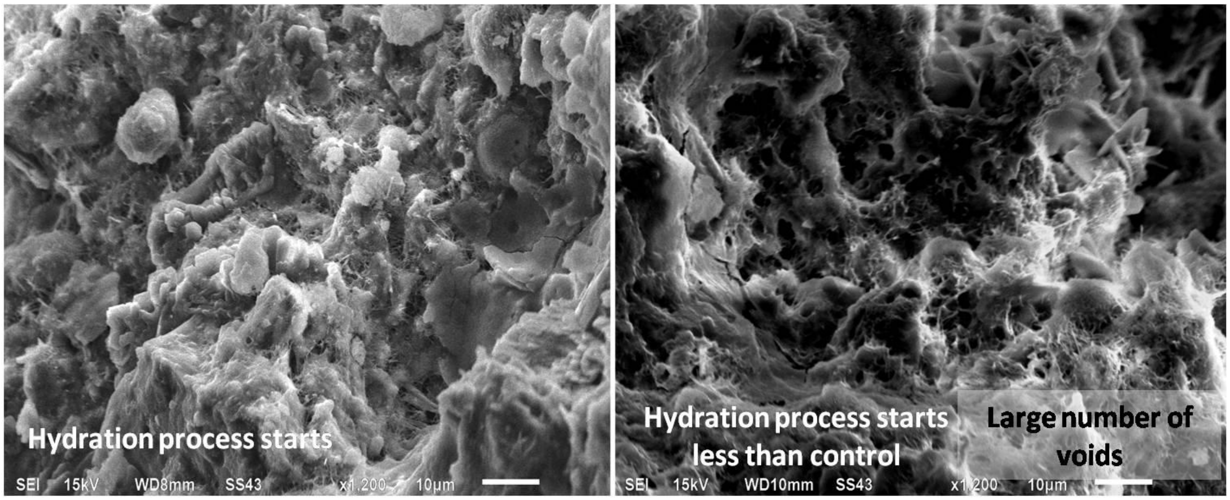
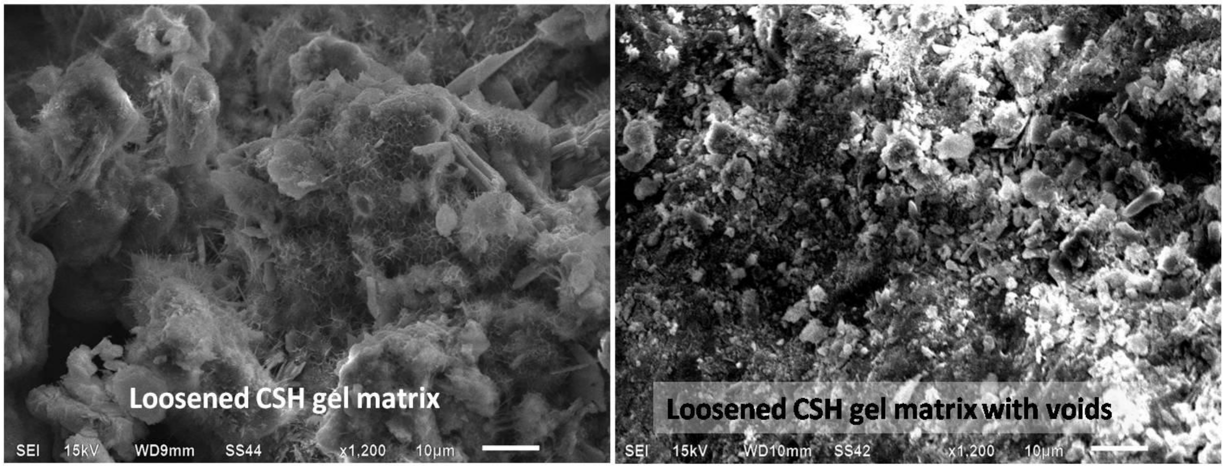


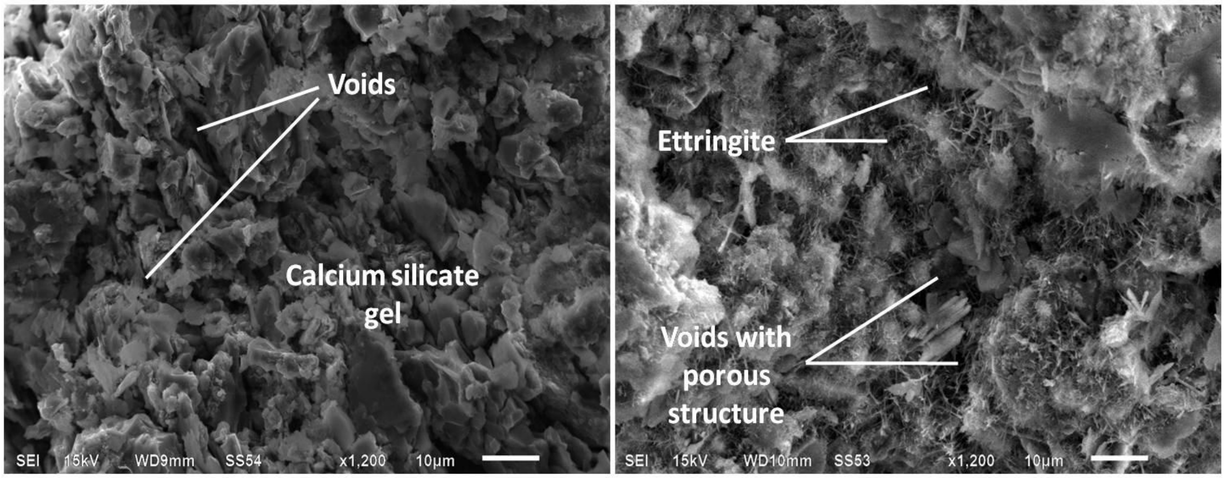
Fig. 4.46: SEM image shows 20% CKD mortar (control and treated) at 7, 28 and 91 days of curing



7 days **Control** **Treated**



28 days **Control** **Treated**



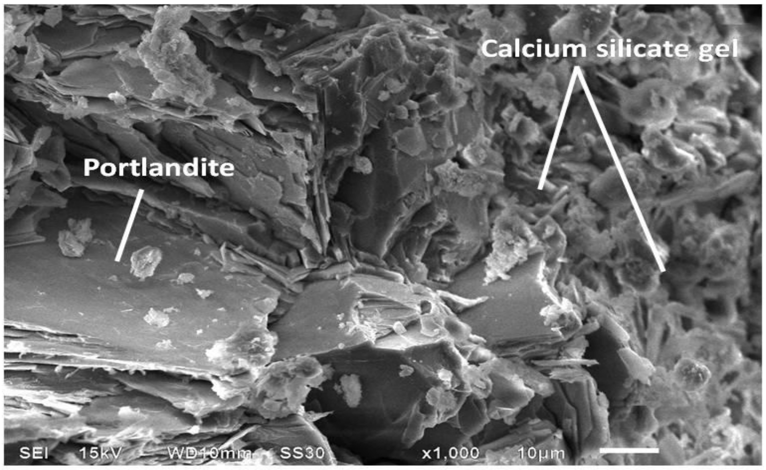
91 days **Control** **Treated**

Fig. 4.47: SEM image shows 30% CKD mortar (control and treated) at 7, 28 and 91 days of curing

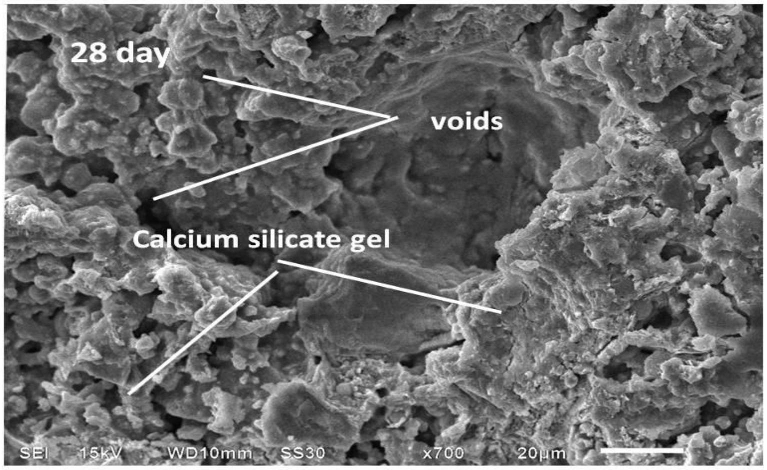
4.7.1.2 Concrete

Figs. 4.48-4.53 show the SEM analysis of control (0% CKD) and, untreated and bacterial treated CKD (5, 10, 15, 20 and 30%) containing concrete. SEM images shows the formation of calcium silicate hydrate (CSH) and the hydration reaction formed dense structure resulted in increased compressive strength in the concrete specimens. In 0% CKD concrete at 7 days of curing, formation of CSH gel was observed along with unreacted portlandite. With curing age formation of CSH gel increased and at 91 days, a compact matrix with reduced voids was observed (Fig. 4.48). Similar type of compact matrix was also observed in 5 and 10% CKD concrete at 91 days of curing period but is less porous than 0% control (Figs. 4.49 and 4.50). With increasing CKD content porosity of the concrete increases. In 15, 20 and 30% CKD control concrete (Figs. 4.51-4.53) voids and ettringites on surfaces were shown resulted in highly porous structure, and exhibited decrease in strength compared to other control treatments.

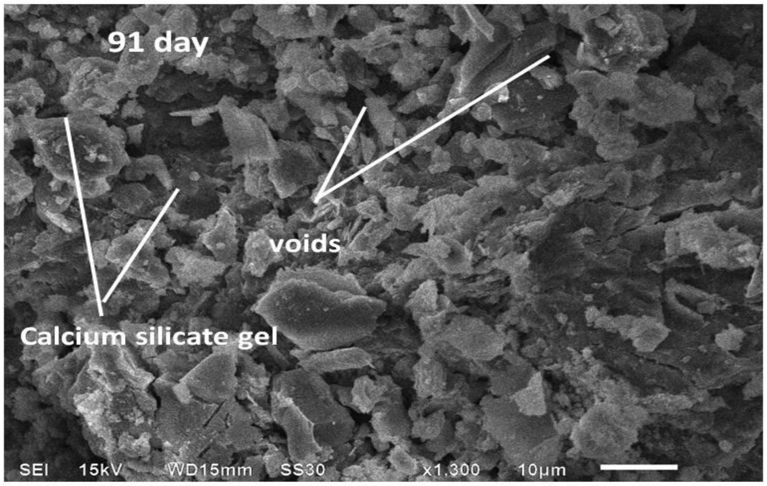
Samoui et al. (2005) observed more reticular and porous structure in high alkali cement paste compared to low alkali paste. In 10% bacterial treated CKD concrete less porous and highly dense structure was formed at 91 days of curing (Fig. 4.50) due to reduced hydration and alkalinity, and compactness of the materials in concrete whereas in 15, 20 and 30% bacterial treated CKD concrete highly porous nature and voids due to less cement content and CSH formation that do not bind actively the materials, thus resulted in reduced strength in concrete (Figs. 4.51-4.53). Lee et al. (2005) observed the microcracks in concrete by using high magnification SEM and reported that ettringites does not occur in microcracks that extend from ettringite filled voids instead they are associated with rim ettringite that developed outside the voids i.e. on the surface. The presence of ettringites on the surface of untreated CKD concrete was more compared to bacterial treated CKD concrete. Famy et al. (2000) indicating the expansive nature of ettringites on the outer surface of calcium silicate hydrate surface at the interfaces of cement paste-aggregate and non-expansive nature of ettringite in the voids of the concrete i.e. microporous zones in the cement paste, pores or bubbles. The expansive ettringite exerts pressure to the aggregates forming gaps at the interfaces resulting in increased porosity and reduced strength.



7 day

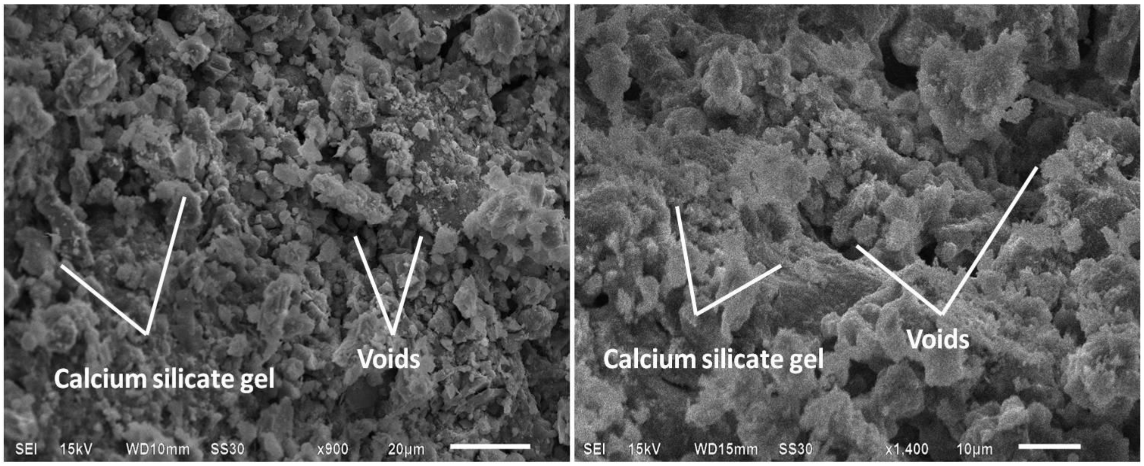


28 day

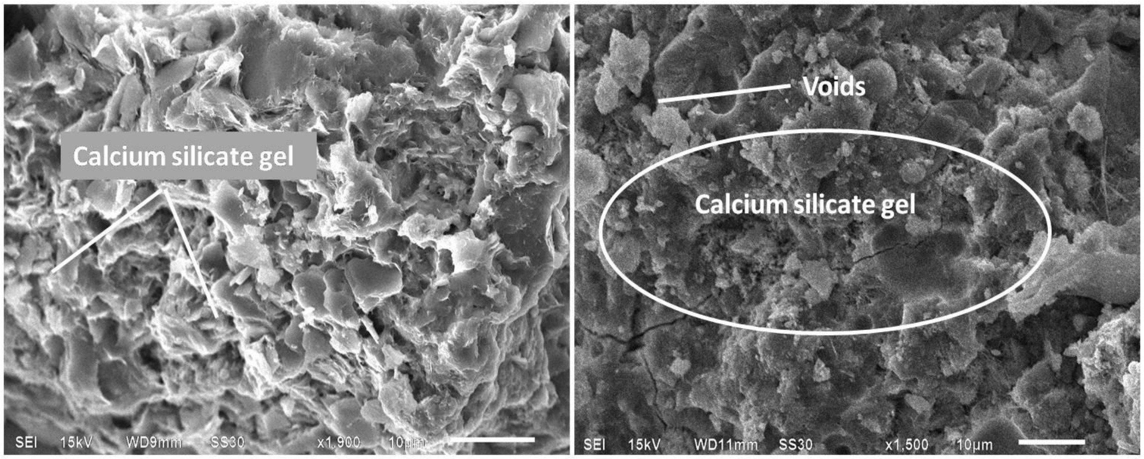


91 day

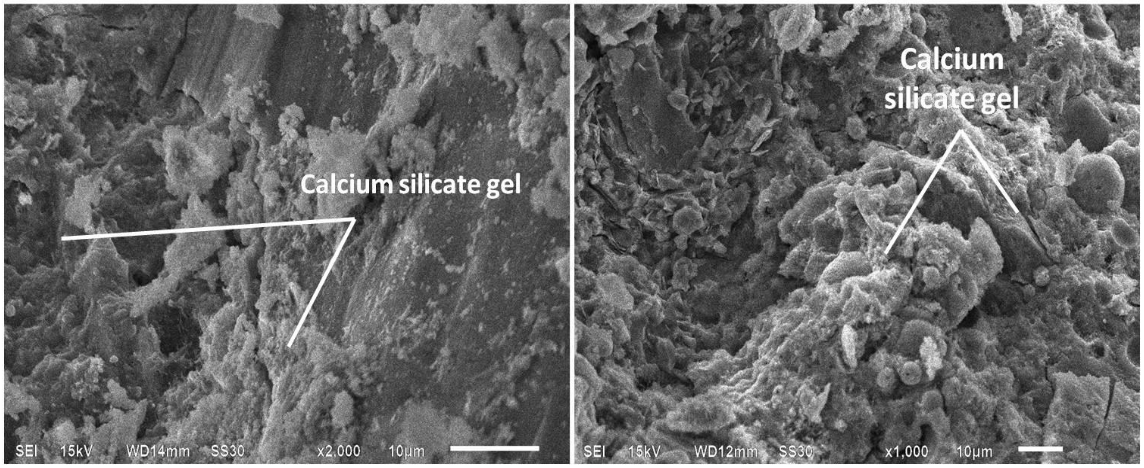
Fig. 4.48: SEM image shows control concrete (0% CKD) at 7, 28 and 91 days of curing



7 days **Control** **Treated**



28 days **Control** **Treated**



91 days **Control** **Treated**

Fig. 4.49: SEM image shows 5% CKD concrete (control and treated) at 7, 28 and 91 days of curing

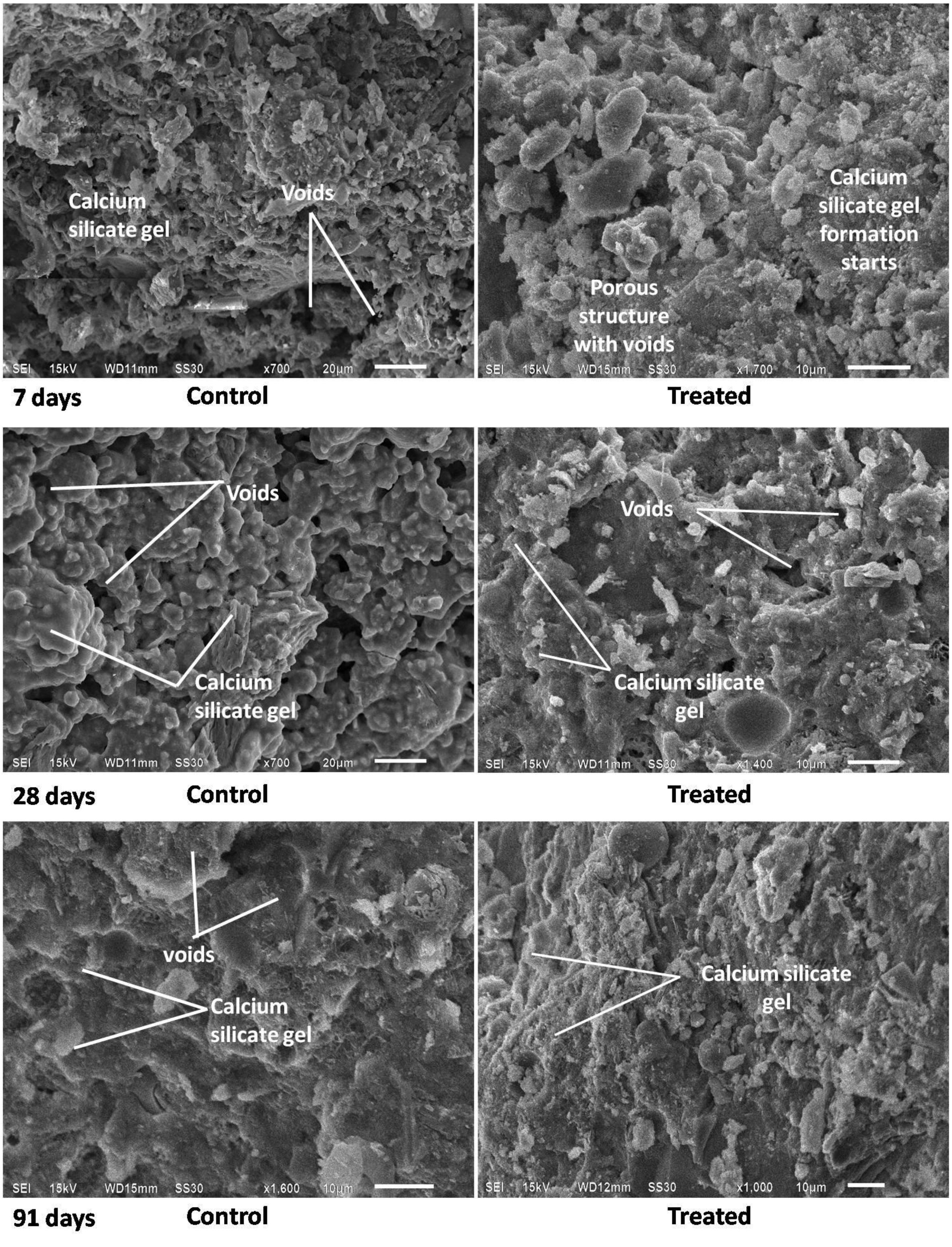
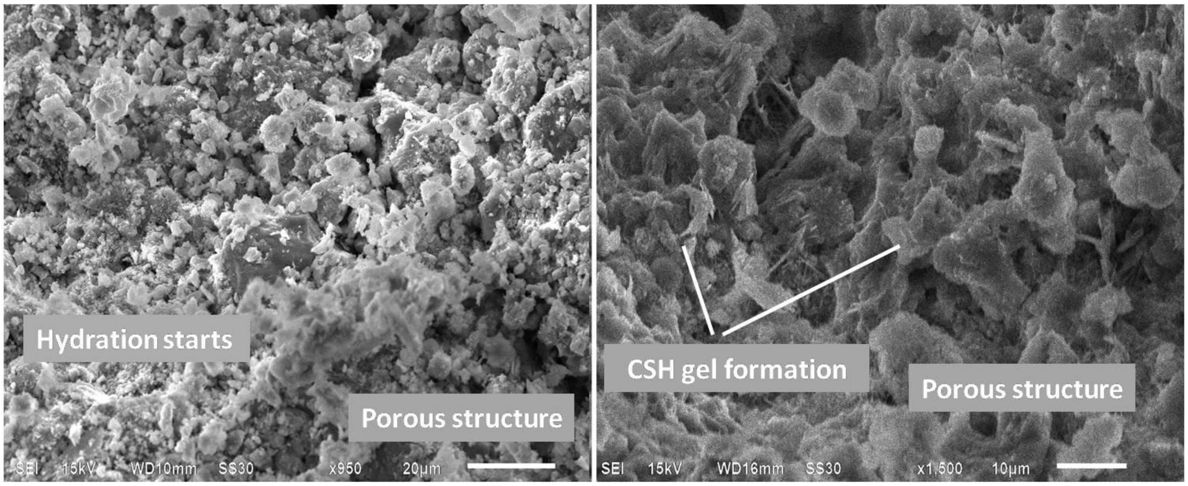
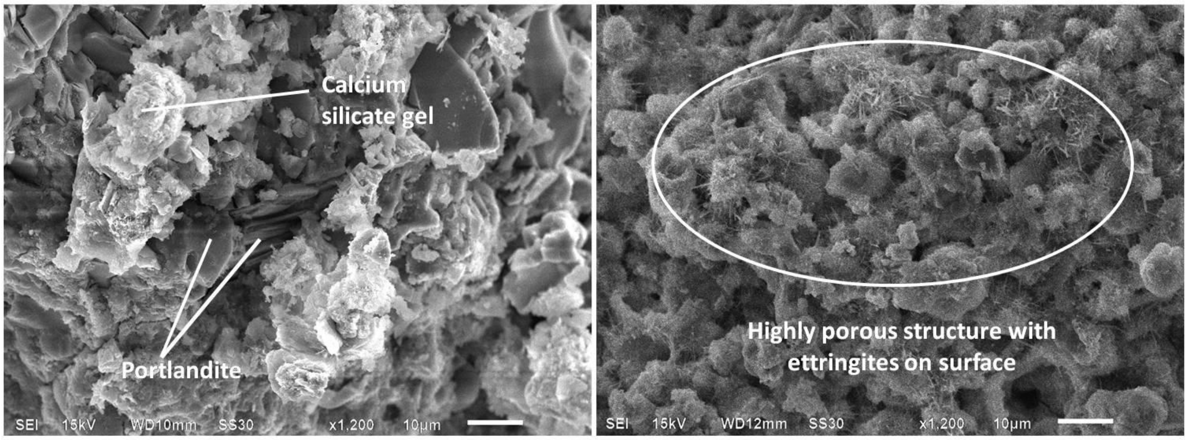


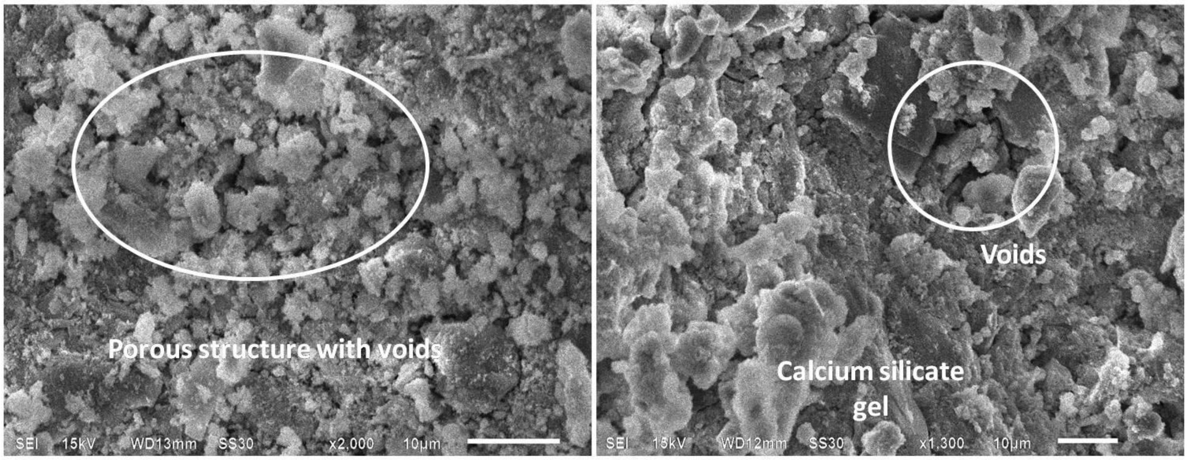
Fig. 4.50: SEM image shows 10% CKD concrete (control and treated) at 7, 28 and 91 days of curing



7 days Control Treated



28 days Control Treated



91 days Control Treated

Fig. 4.51: SEM image shows 15% CKD concrete (control and treated) at 7, 28 and 91 days of curing

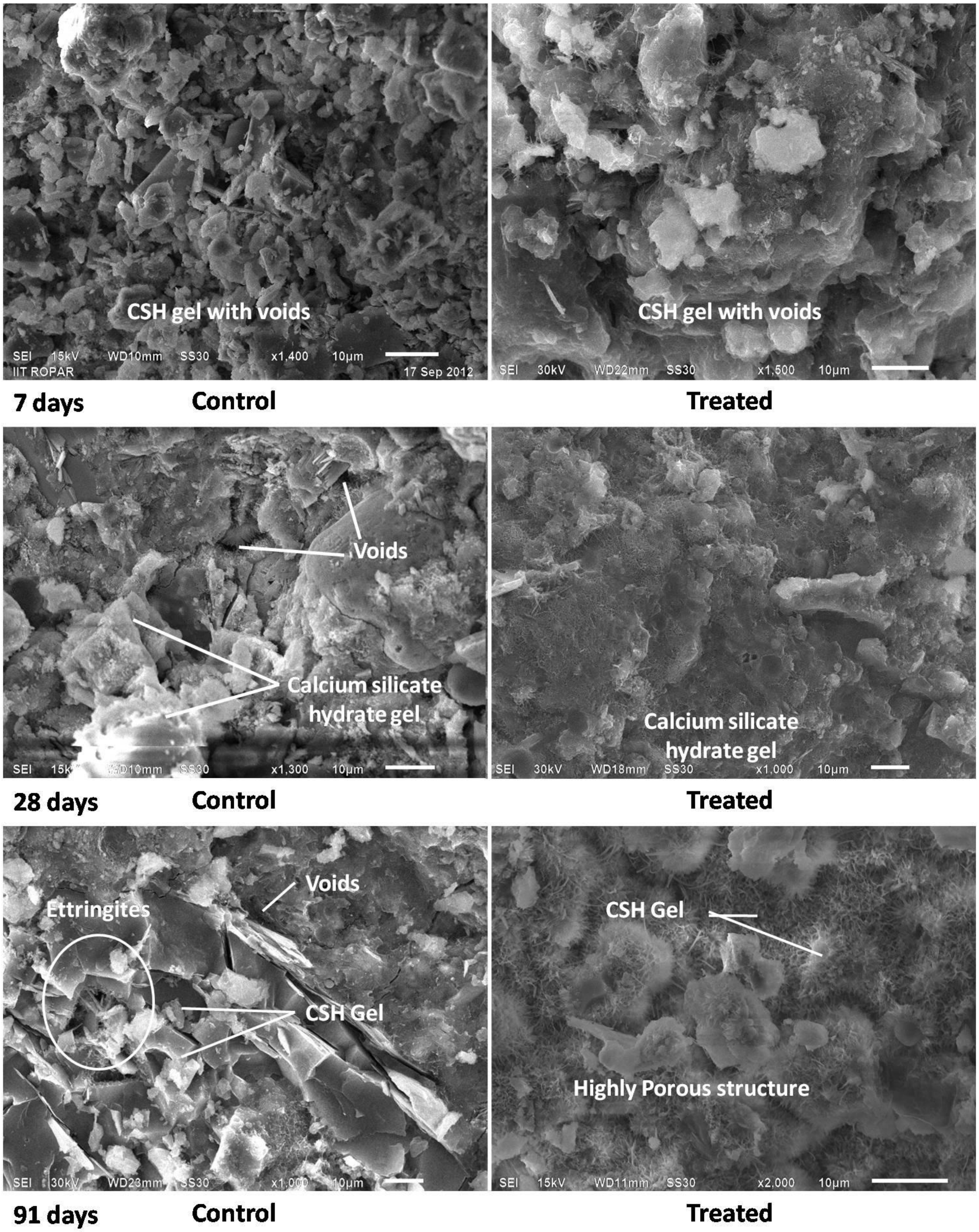


Fig. 4.52: SEM image shows 20% CKD concrete (control and treated) at 7, 28 and 91 days of curing

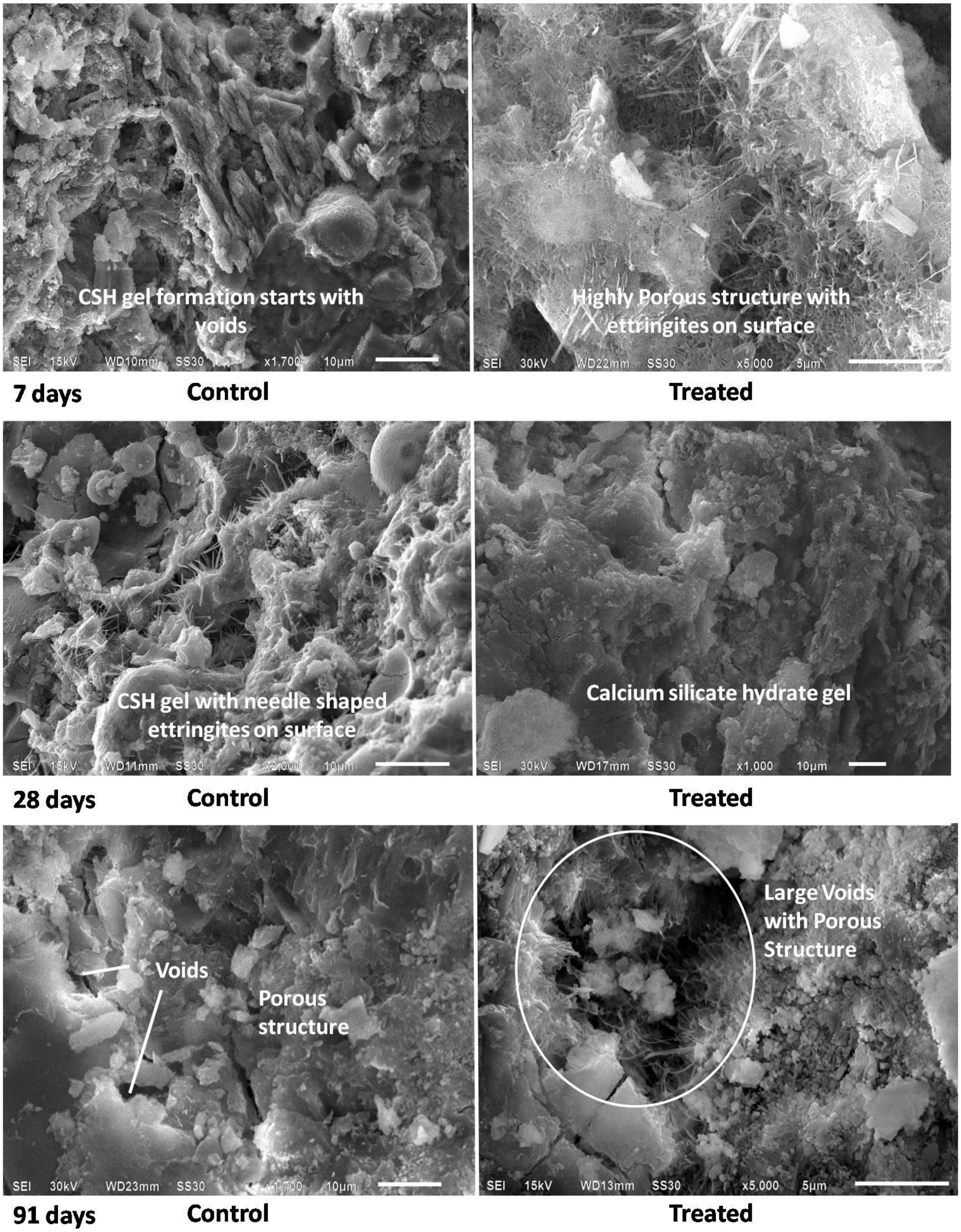


Fig. 4.53: SEM image shows 30% CKD concrete (control and treated) at 7, 28 and 91 days of curing

4.7.2 XRD Analysis

4.7.2.1 Mortar

X-ray diffraction (XRD) analysis of mortar samples with and without bacterial treated cement kiln dust is shown in Figs. 4.54-4.64. It is shown that peaks of quartz (Q), calcium silicate hydrate (CSH), calcite (C), larnite (L) and ettringite (E) phases are present on comparing the values of 2θ/d/I/I of the peaks by JCPDS data file. The peaks of different phases in treatments show the intensity corresponding to the strength of concrete. The peak of larnite (Ca_2SiO_4) or C_2S or Belite was observed at 37 degree 2θ. Larnite reacts with water to form calcium silicate hydrate and responsible for the late strength development in the specimens. The increase in peak for CSH was observed with curing age in all treatments.

In 10% bacterial treated CKD mortar (Fig. 4.58) the increased formation of CSH resulted in increased strength compared to 10% CKD control mortar (Fig. 4.57). Ettringite formation in 10% bacterial treated CKD mortar, due to less alkali content, was non expansive and filled the pore structure resulted in dense structure and increased the compressive strength. With increasing CKD concentration beyond 10%, reduced formation of CSH was observed which is due to reduced cement content and degree of hydration thus reduced the strength of the specimens. This reduction in CSH formation was more in bacterial treated CKD mortar due to less alkalinity required for hydration process. Min and Mingshu (1994) stated that the high concentration of hydroxyl ions (i.e. high pH values) due to higher alkali content of the solution results in the expansive type of ettringite. According to Heinz and Ludwig (1987), several factors affect the formation of ettringite such as sulfate content, pH of the solution and availability of the calcium hydroxide. Higher alkali content increased the solubility of sulfate ions in solution which being absorbed by CSH resulted in formation of expansive type of ettringite. The XRD results (Figs. 4.56 and 4.58) shows increased intensity of CSH (21, 26, 29 degree 2θ) and non expansive ettringite (7, 17 and 34 degree 2θ) in bacterial treated CKD mortar (5 and 10%) responsible for the strength development in mortar where as in 15, 20 and 30% bacterial treated CKD mortar, reduction in cement content reduced the required alkalinity (needed for hydration reaction) which in turn decreased the CSH and thus reduced the strength compared to control mortar (Figs. 4.59-4.64).

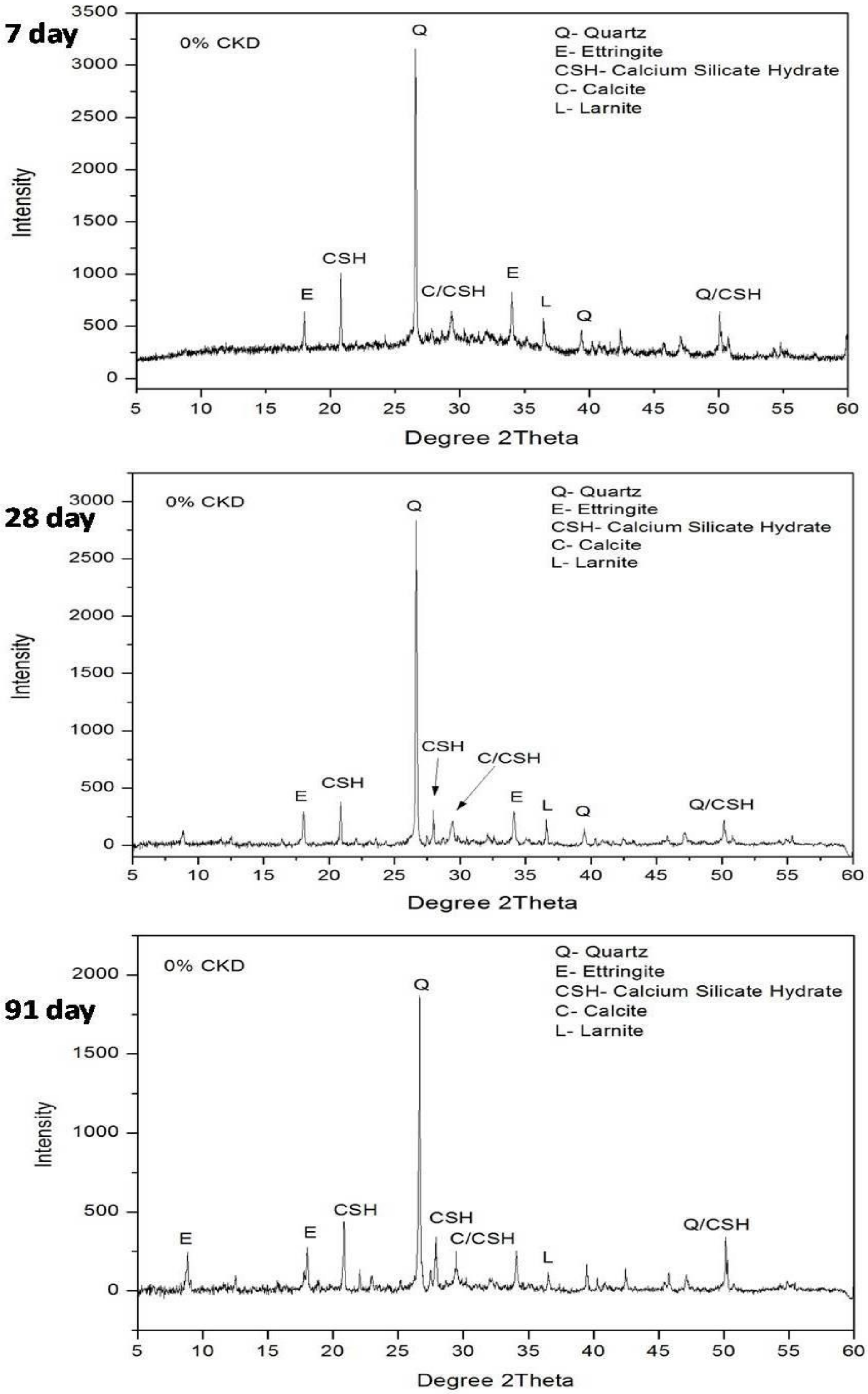


Fig. 4.54: X-ray diffraction shows control mortar (0% CKD) at 7, 28 and 91 days of curing

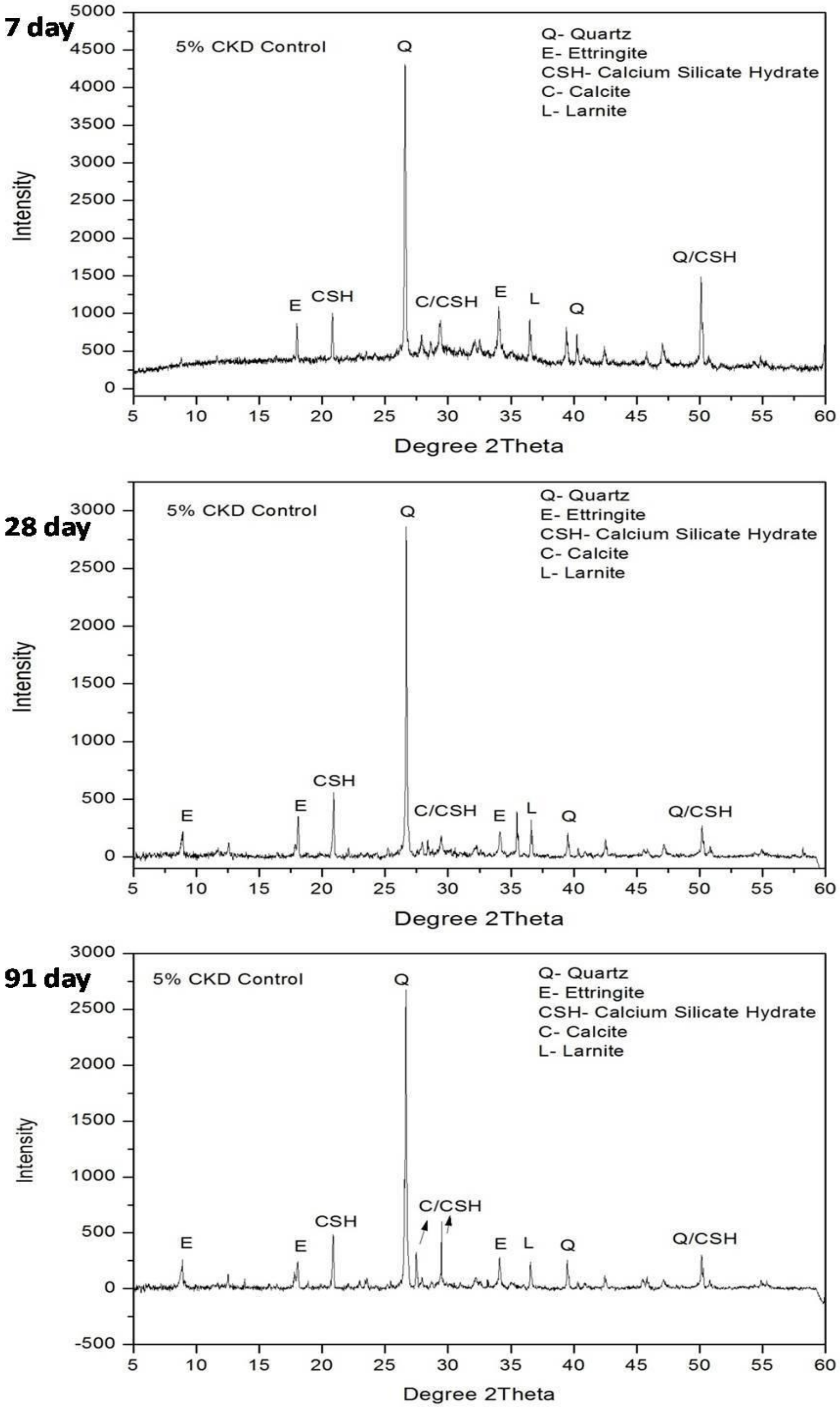


Fig. 4.55: X-ray diffraction shows 5% CKD mortar (control) at 7, 28 and 91 days of curing

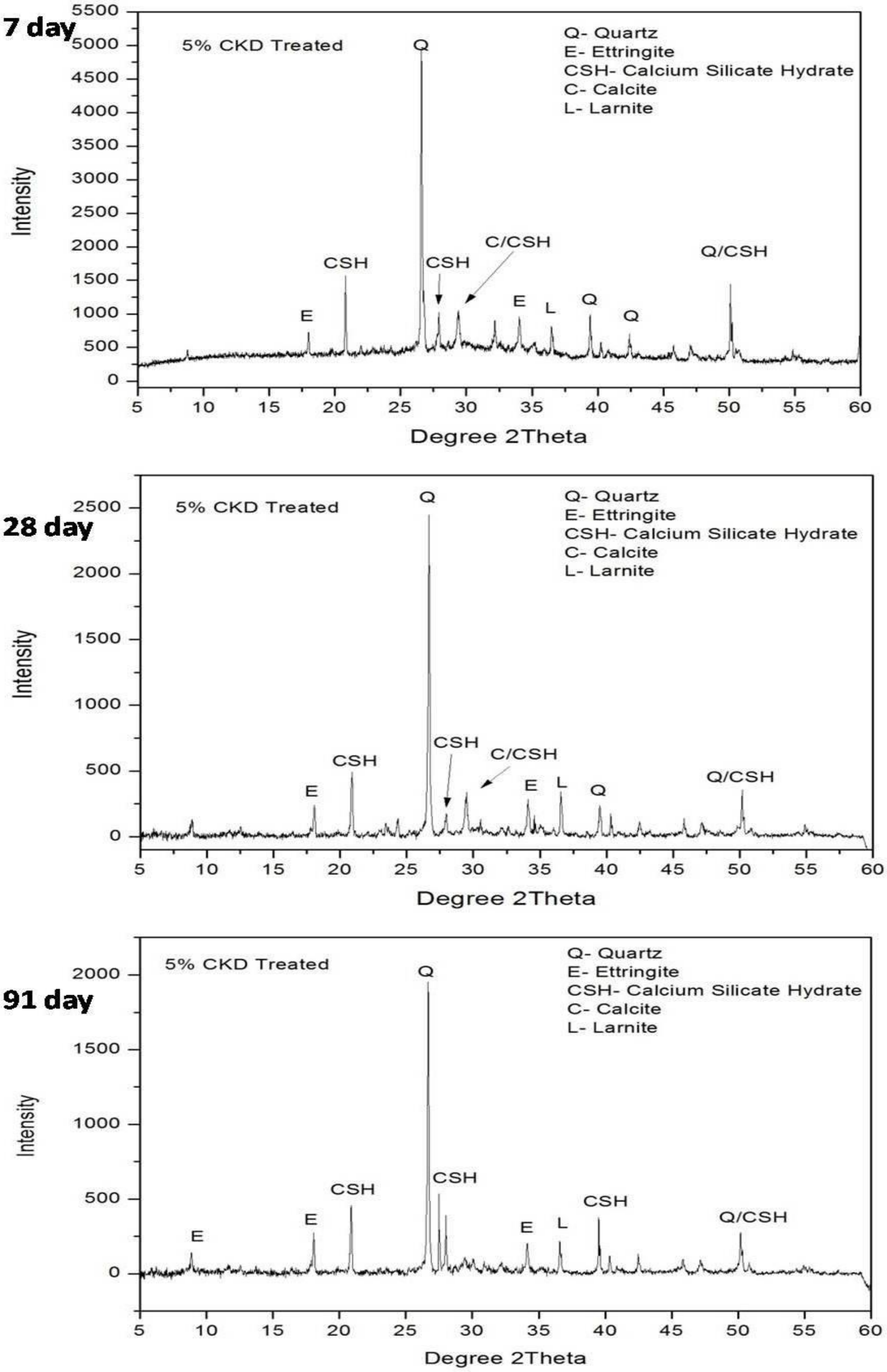


Fig. 4.56: X-ray diffraction shows 5% CKD mortar (treated) at 7, 28 and 91 days of curing

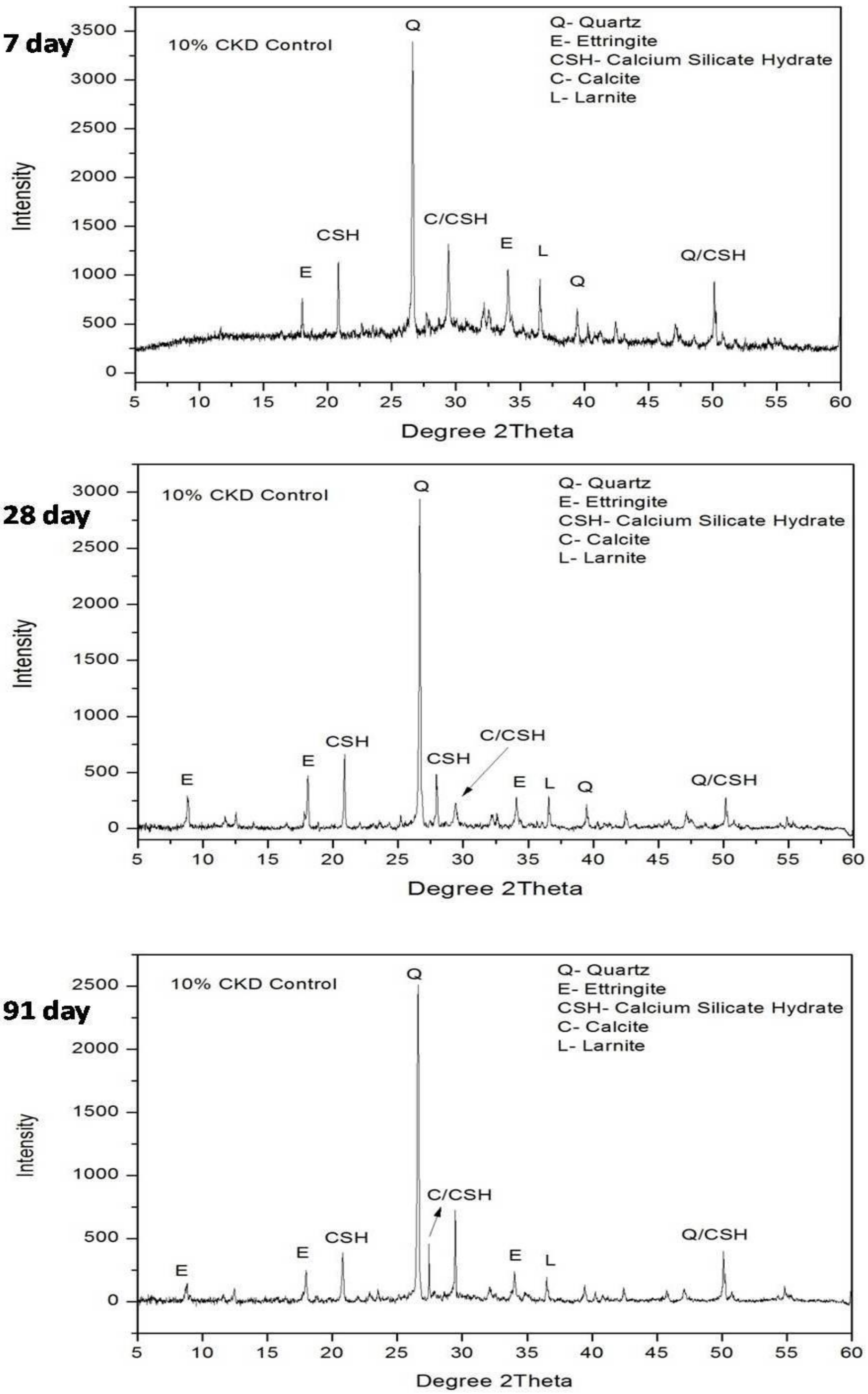


Fig. 4.57: X-ray diffraction shows 10% CKD mortar (control) at 7, 28 and 91 days of curing

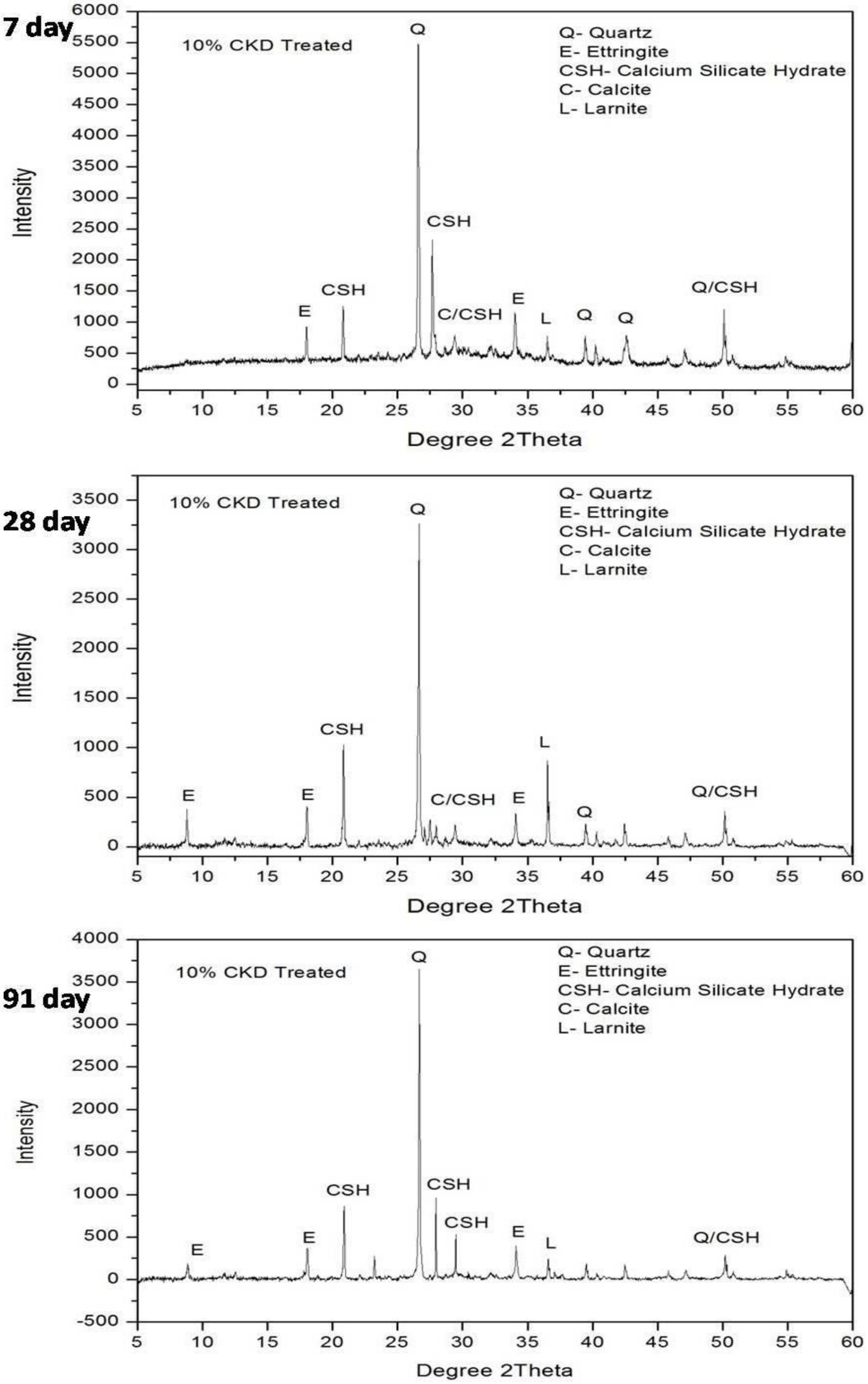


Fig. 4.58: X-ray diffraction shows 10% CKD mortar (treated) at 7, 28 and 91 days of curing

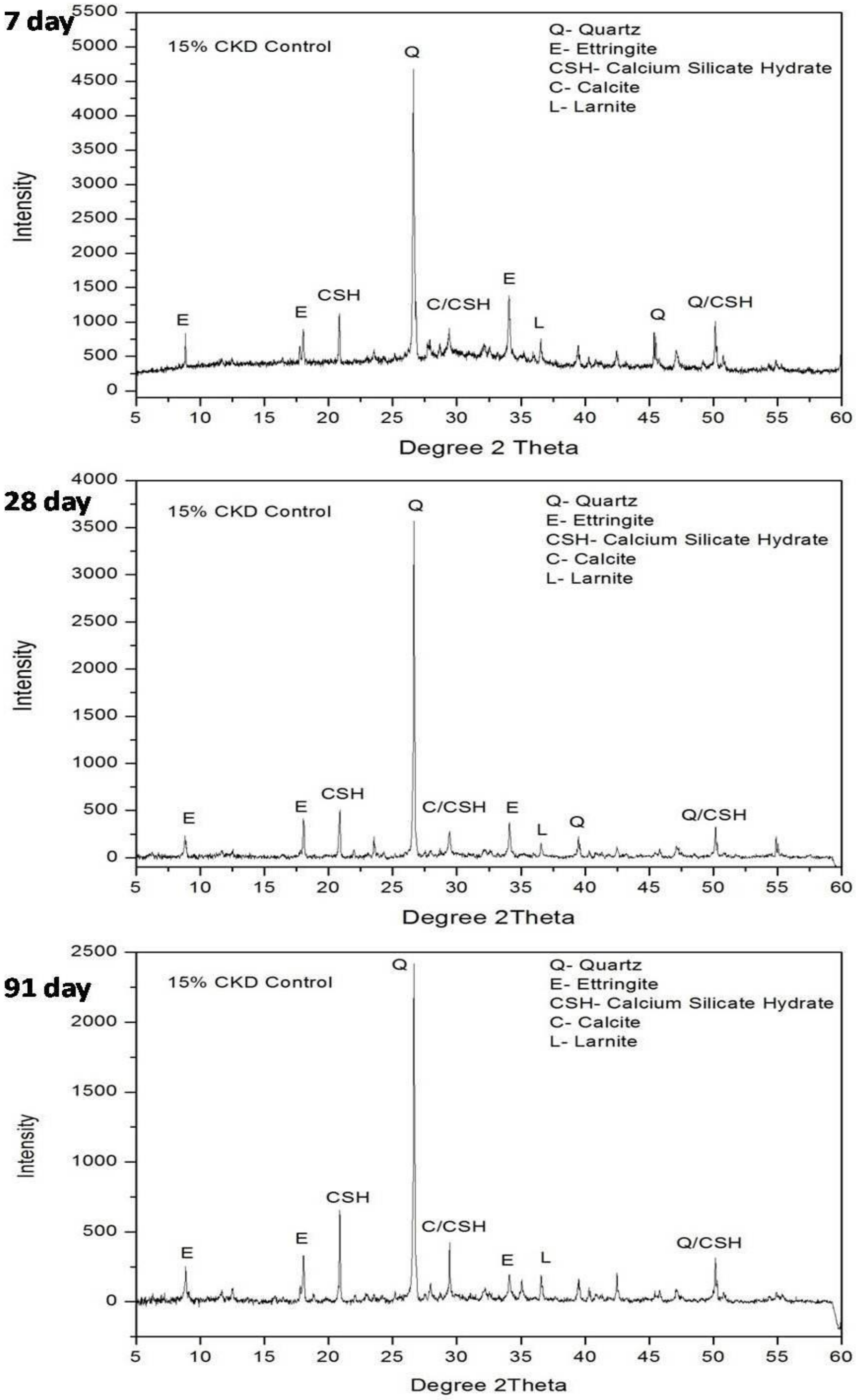


Fig. 4.59: X-ray diffraction shows 15% CKD mortar (control) at 7, 28 and 91 days of curing

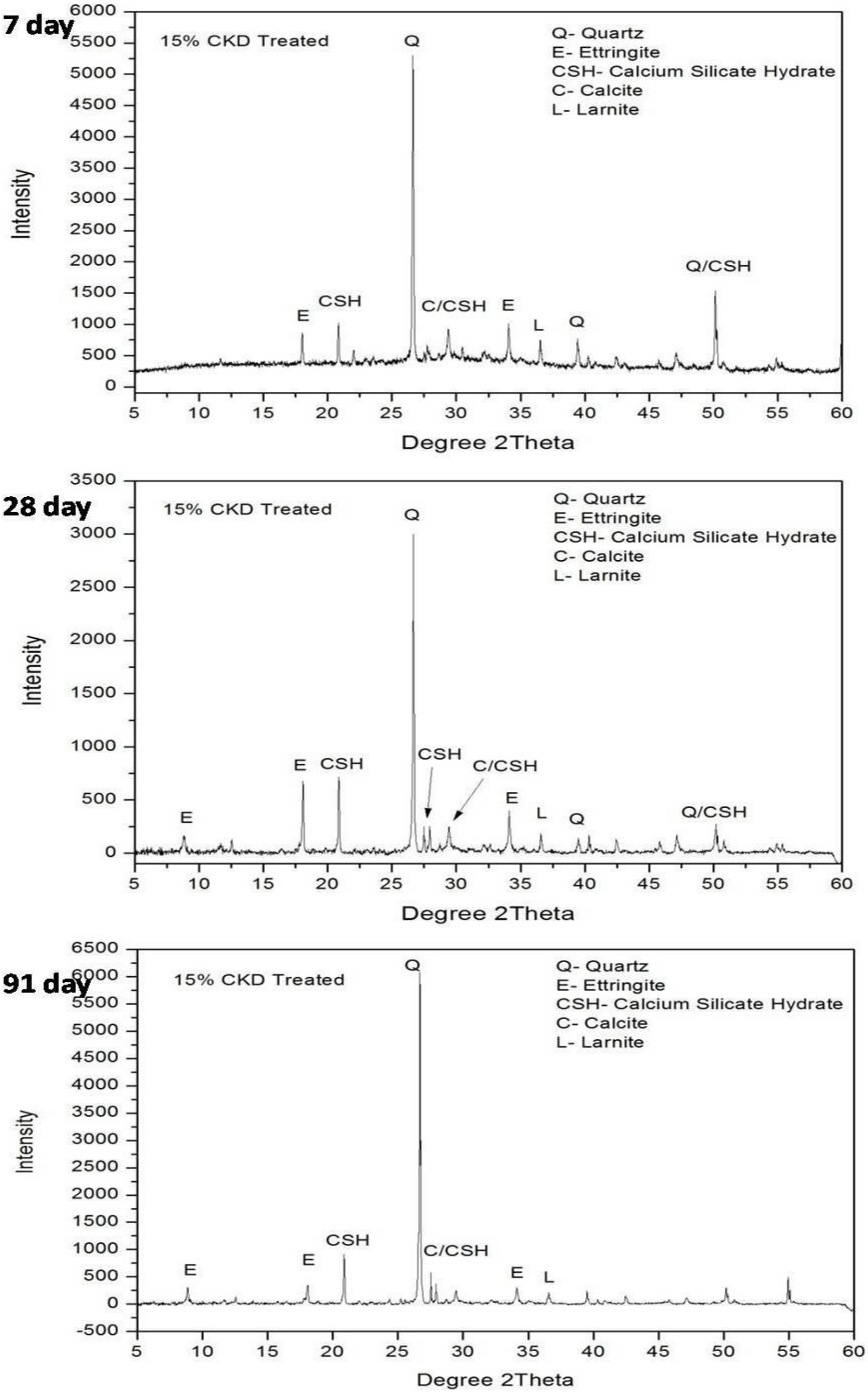


Fig. 4.60: X-ray diffraction shows 15% CKD mortar (treated) at 7, 28 and 91 days of curing

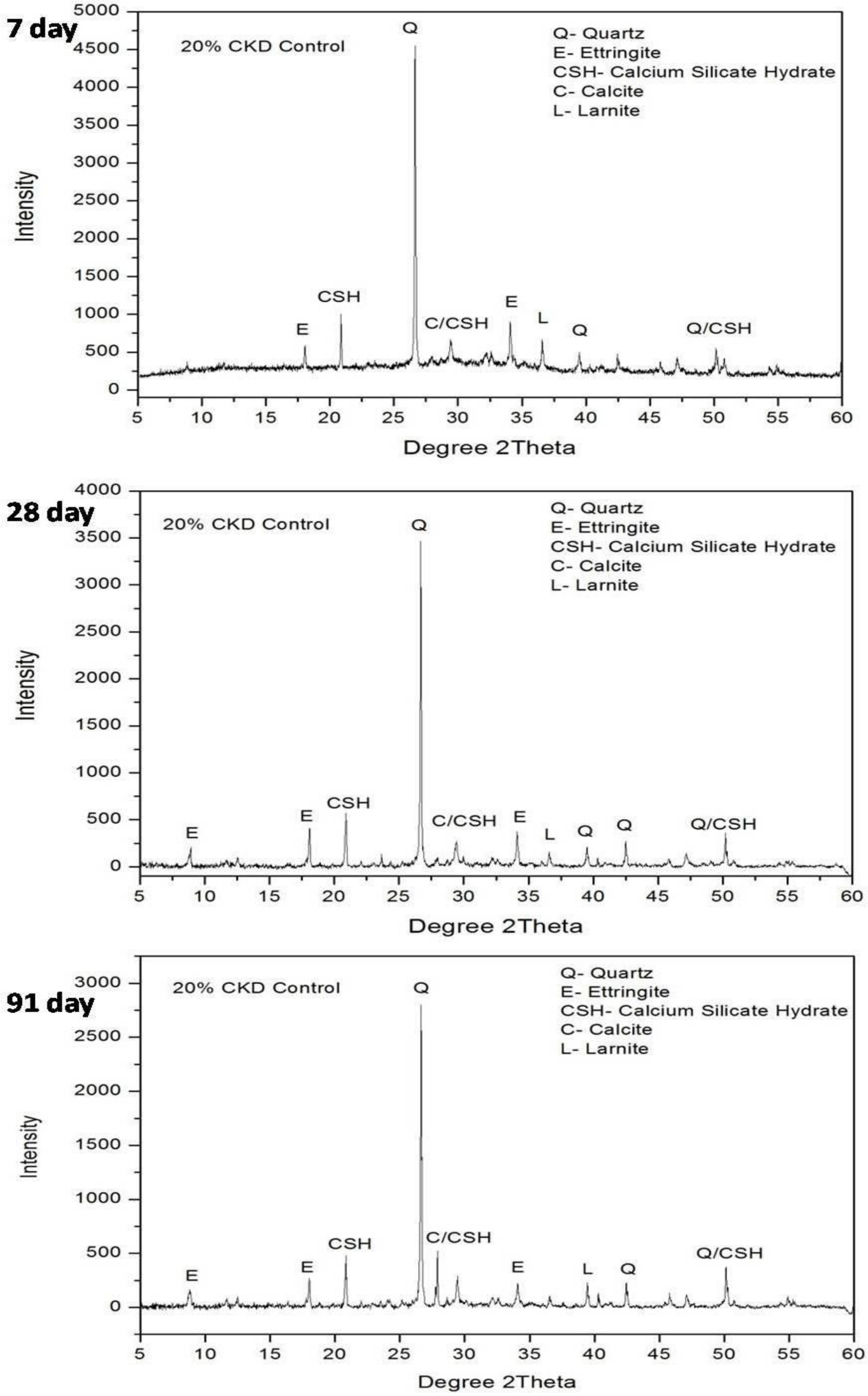


Fig. 4.61: X-ray diffraction shows 20% CKD mortar (control) at 7, 28 and 91 days of curing

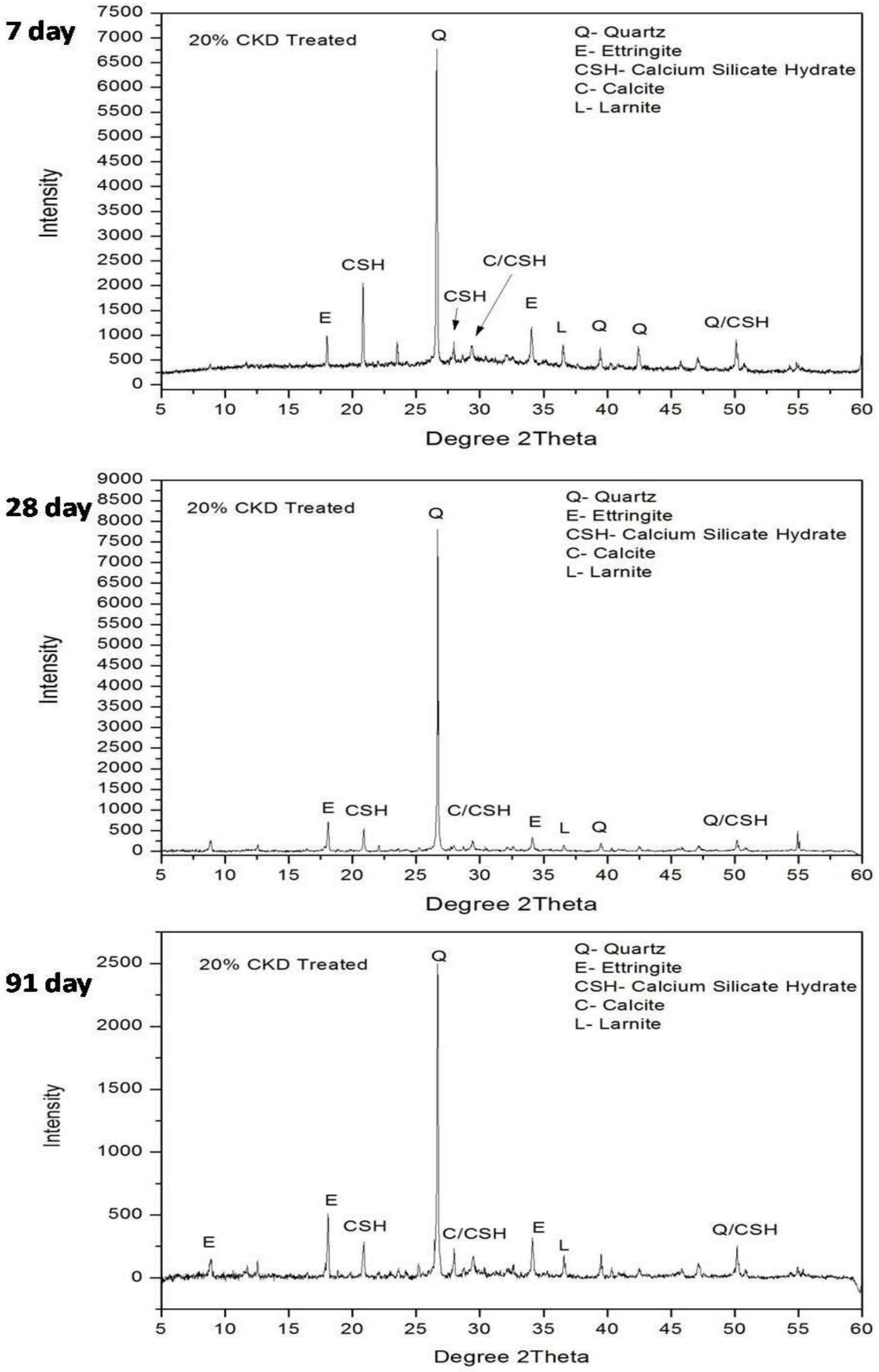


Fig. 4.62: X-ray diffraction shows 20% CKD mortar (treated) at 7, 28 and 91 days of curing

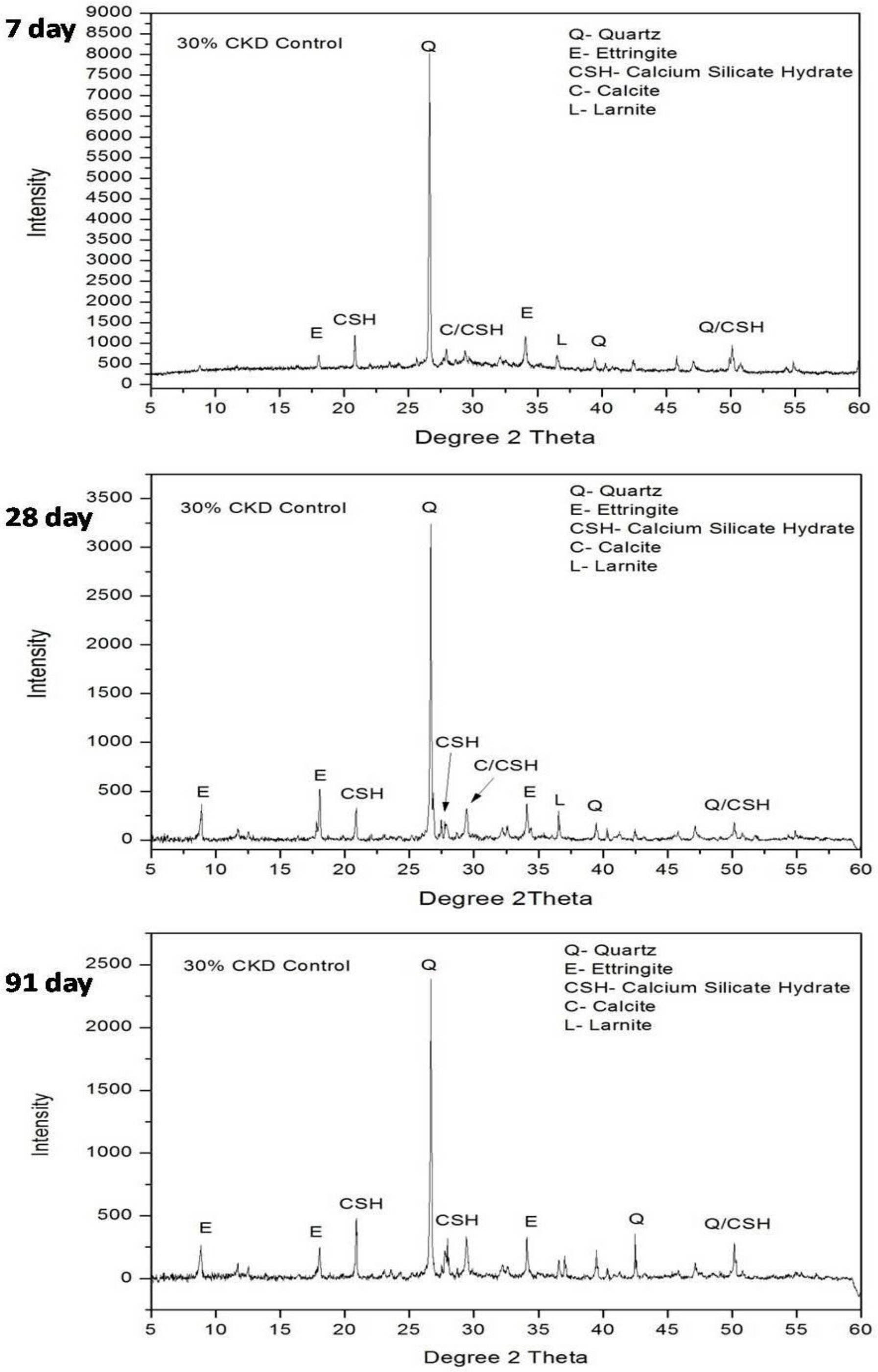


Fig. 4.63: X-ray diffraction shows 30% CKD mortar (control) at 7, 28 and 91 days of curing

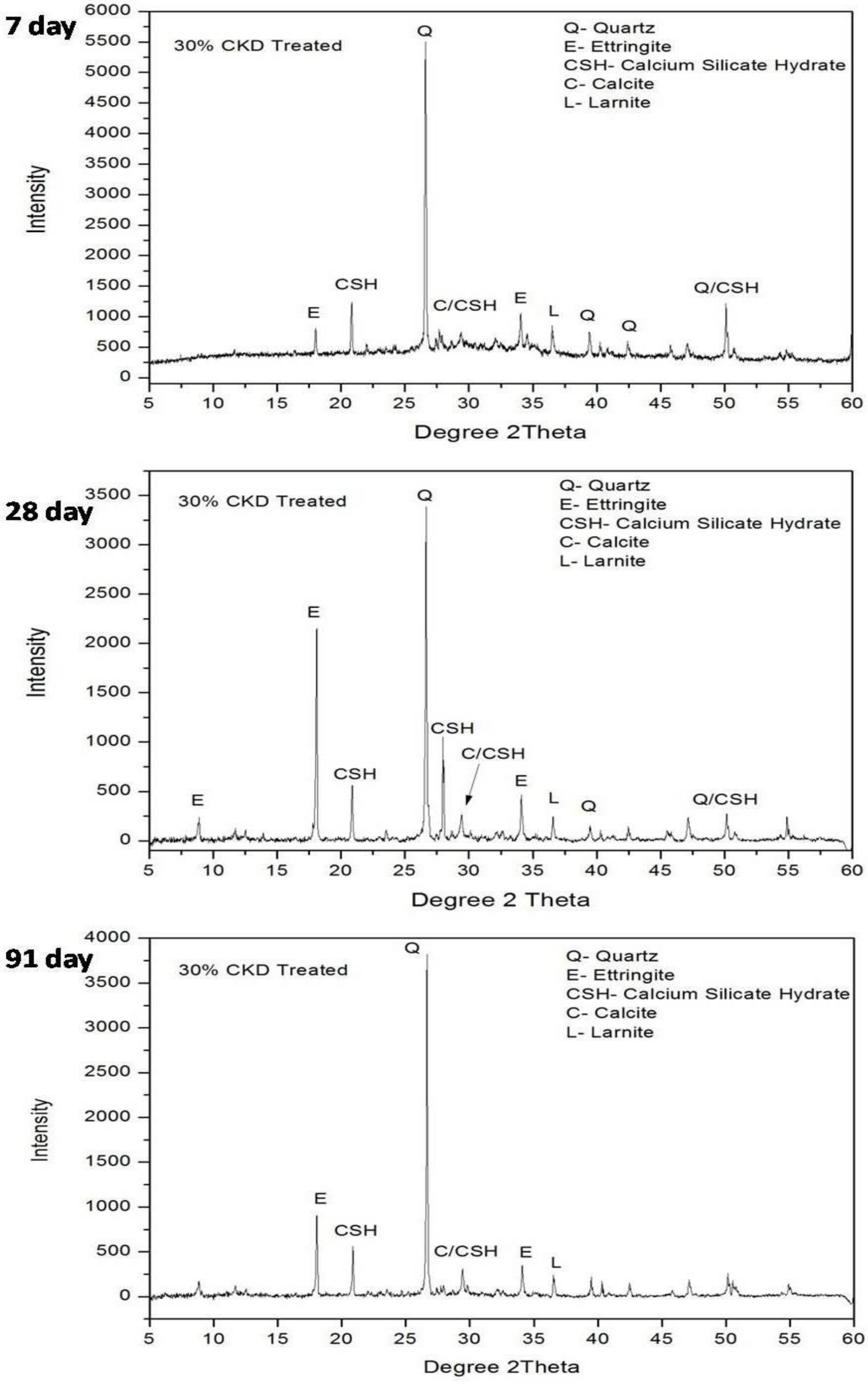


Fig. 4.64: X-ray diffraction shows 30% CKD mortar (treated) at 7, 28 and 91 days of curing

4.7.2.2 Concrete

X-ray diffraction (XRD) analysis of concrete samples with or without bacterial treated cement kiln dust shows peaks of quartz (Q), calcium silicate hydrate (CSH), calcite (C), larnite (L) and ettringite (E) phases on comparing the values of 2θ/d/I/I of the peaks by JCPDS data file (Figs. 4.65-4.75). Peaks of different phases in treatments shows the intensity corresponding to the strength of concrete. Alite (C_3S) is the major mineral component (>50%) found in cement and upon hydration forms calcium silicate hydrate or calcium silicate which hardens the cement slurry and is responsible for initial (1-3 days) and final strengths (Jumate and Manea, 2011).

The second major component found in cement is C_2S or Belite or Larnite (Ca_2SiO_4) which reacts with water to form hydration products such as calcium silicate hydrate or calcium silicate and portlandite, and responsible for the development of late strength. Neville (2003), Molnar et al. (2010) and Jumate and Manea (2011) studied that hydration and hydrolysis reaction of C_3S and C_2S mineral components produce calcium silicate hydrate (also known as Tobermorite) gels and later the solid phase develops crystals during curing period leading to strengthening of the cement-concrete mixes.

In 5 and 10% bacterial treated CKD concrete (Figs. 4.67 and 4.69) the increased formation of CSH resulted in increased strength compared to 5 and 10% CKD control concrete (Figs. 4.66 and 4.68). Ettringite formation in 10% bacterial treated CKD concrete was non expansive due to less alkali content and filled the pore structure in concrete resulted in dense structure and increased compressive strength. Min and Mingshu (1994) and Heinz and Ludwig (1987) stated that the high concentration of hydroxyl ions (i.e. high pH values) due to higher alkali content of the solution results in the expansive type of ettringite. The formation of ettringite depends upon several factors such as sulfate content, pH of the solution and availability of the calcium hydroxide. Higher alkali content increased the solubility of sulfate ions in solution which being absorbed by CSH resulted in formation of expansive type of ettringite. The XRD results shows increased intensity of CSH (21, 26, 29 degree 2θ) and non expansive ettringite (7, 17 and 34 degree 2θ) in bacterial treated CKD concrete (5 and 10%) responsible for the strength development in concrete (Figs. 4.67 and 4.69) where as above 10% bacterial treated CKD content, reduction in cement content reduced the required alkalinity (needed for hydration reaction) which in turn decreased the CSH and thus reduced the strength compared to control concrete (Figs. 4.70 – 4.75).

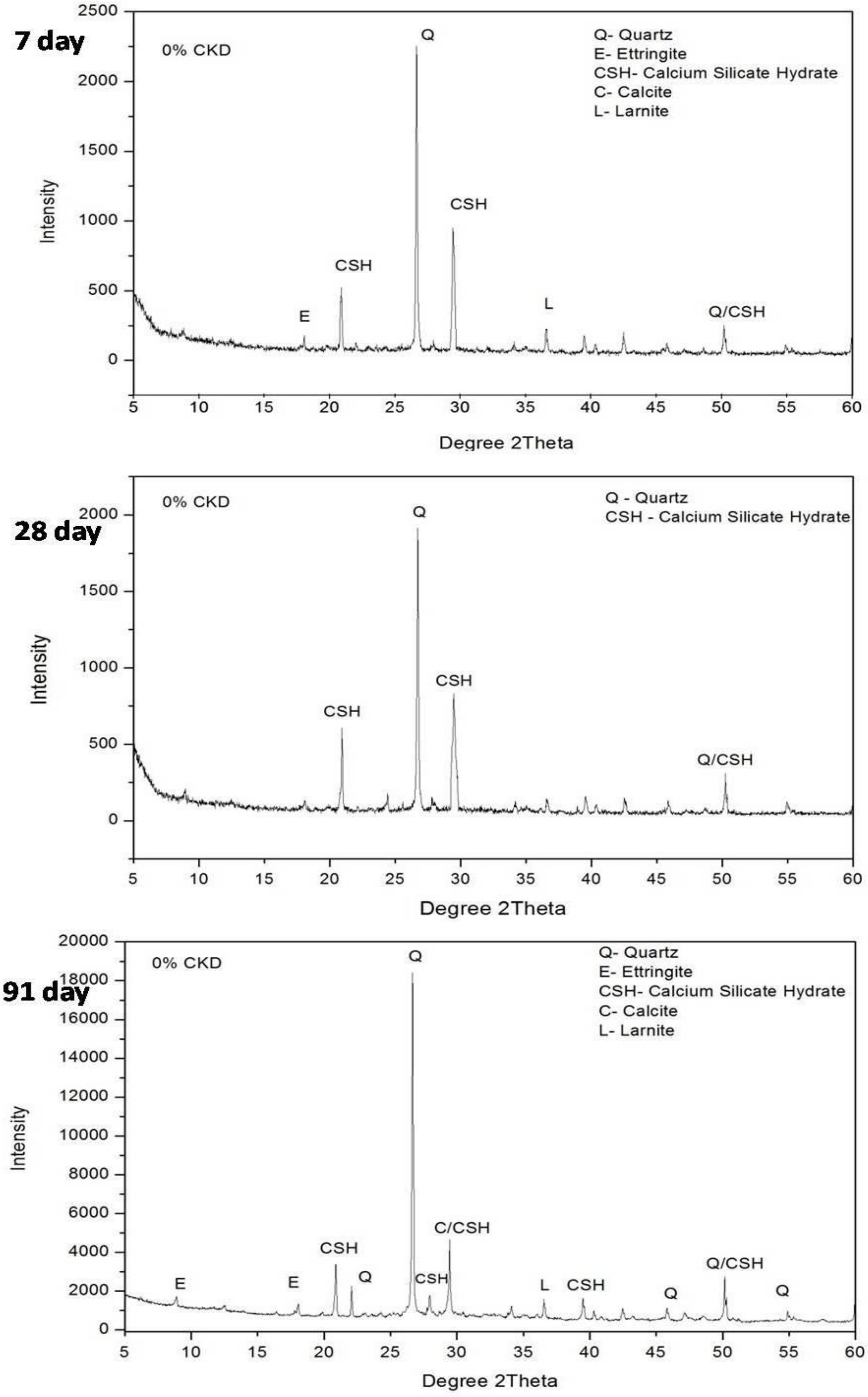


Fig. 4.65: X-ray diffraction shows control concrete (0% CKD) at 7, 28 and 91 days of curing

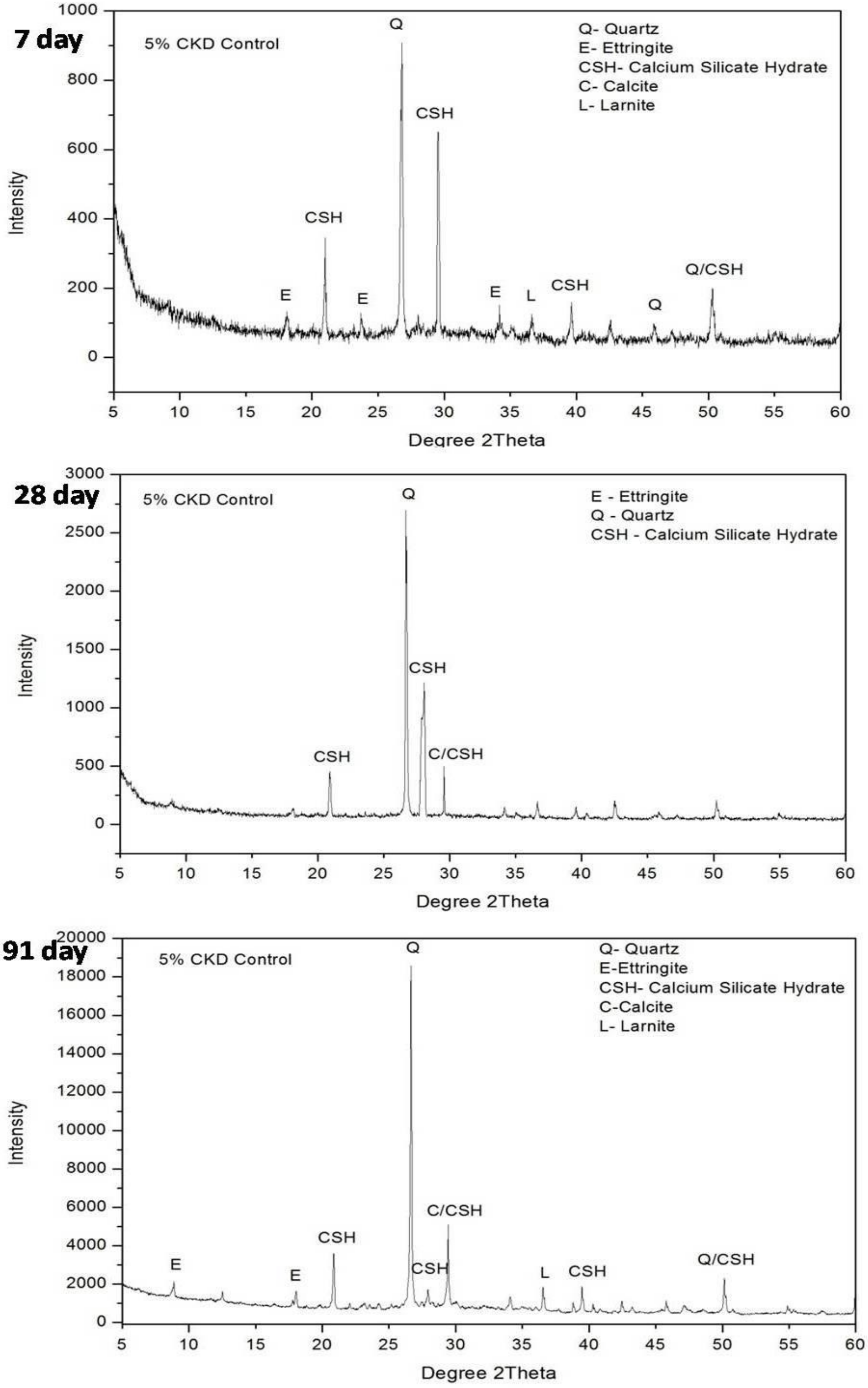


Fig. 4.66: X-ray diffraction shows 5% CKD concrete (control) at 7, 28 and 91 days of curing

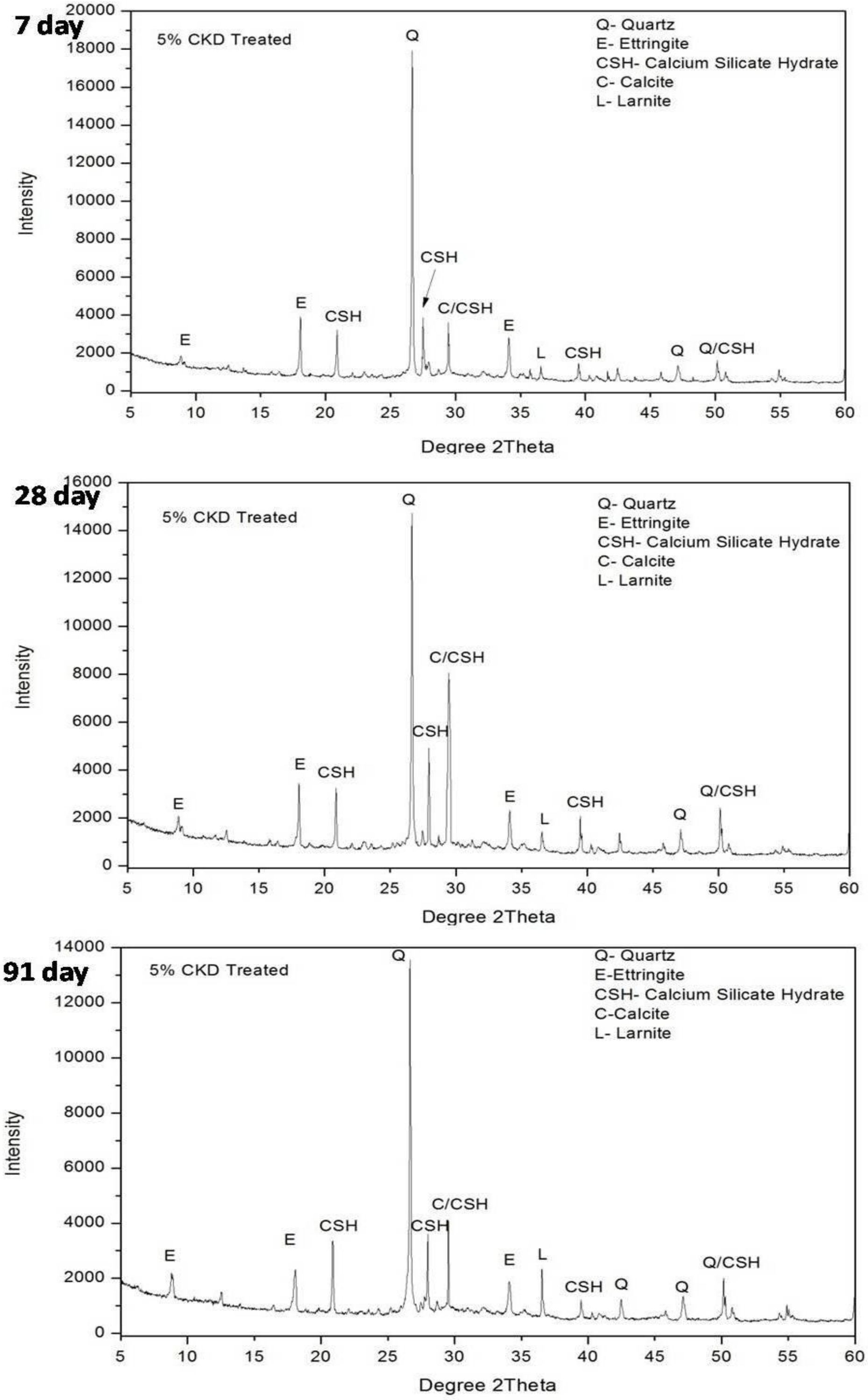


Fig. 4.67: X-ray diffraction shows 5% CKD concrete (treated) at 7, 28 and 91 days of curing

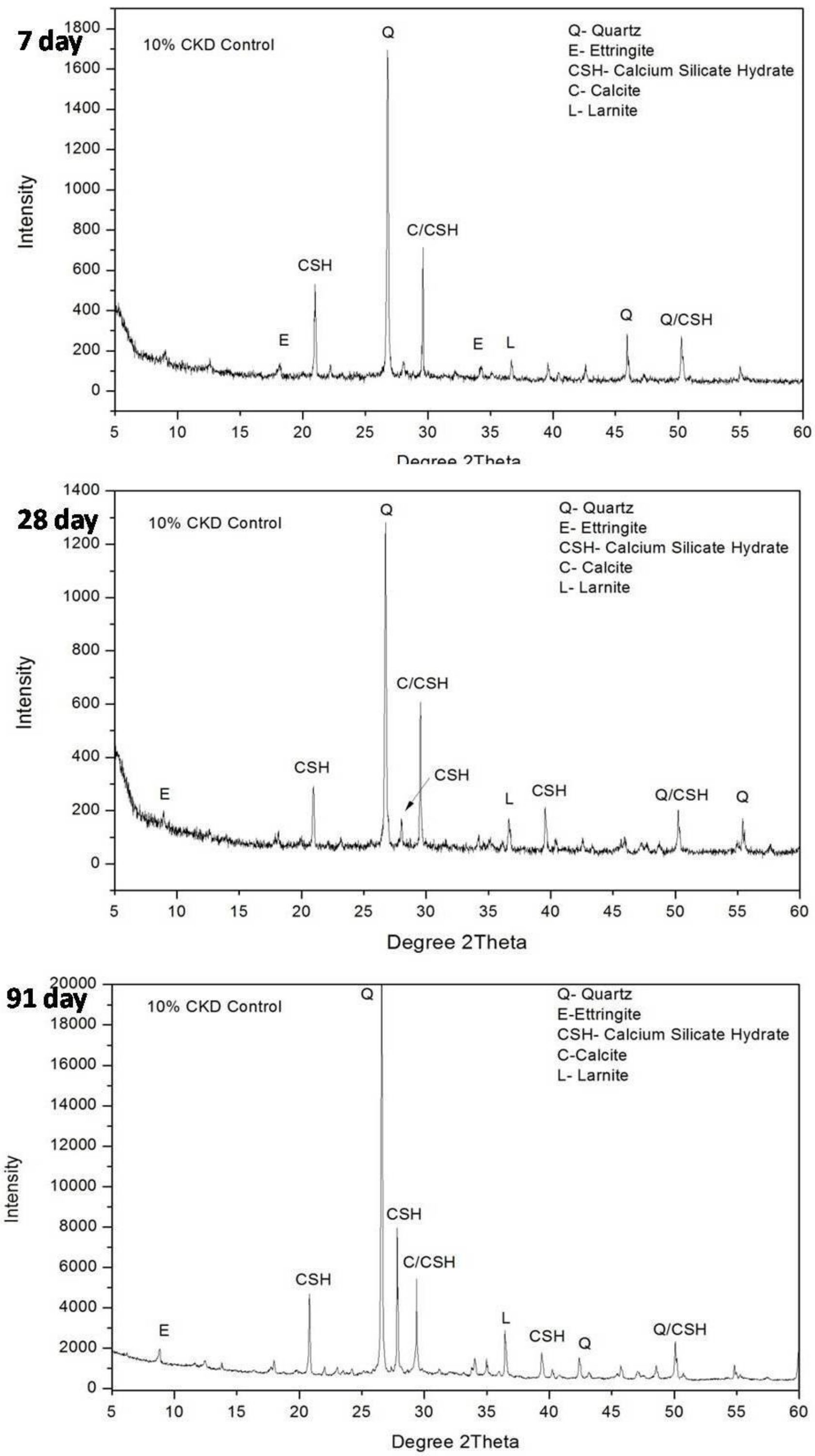


Fig. 4.68: X-ray diffraction shows 10% CKD concrete (control) at 7, 28 and 91 days of curing

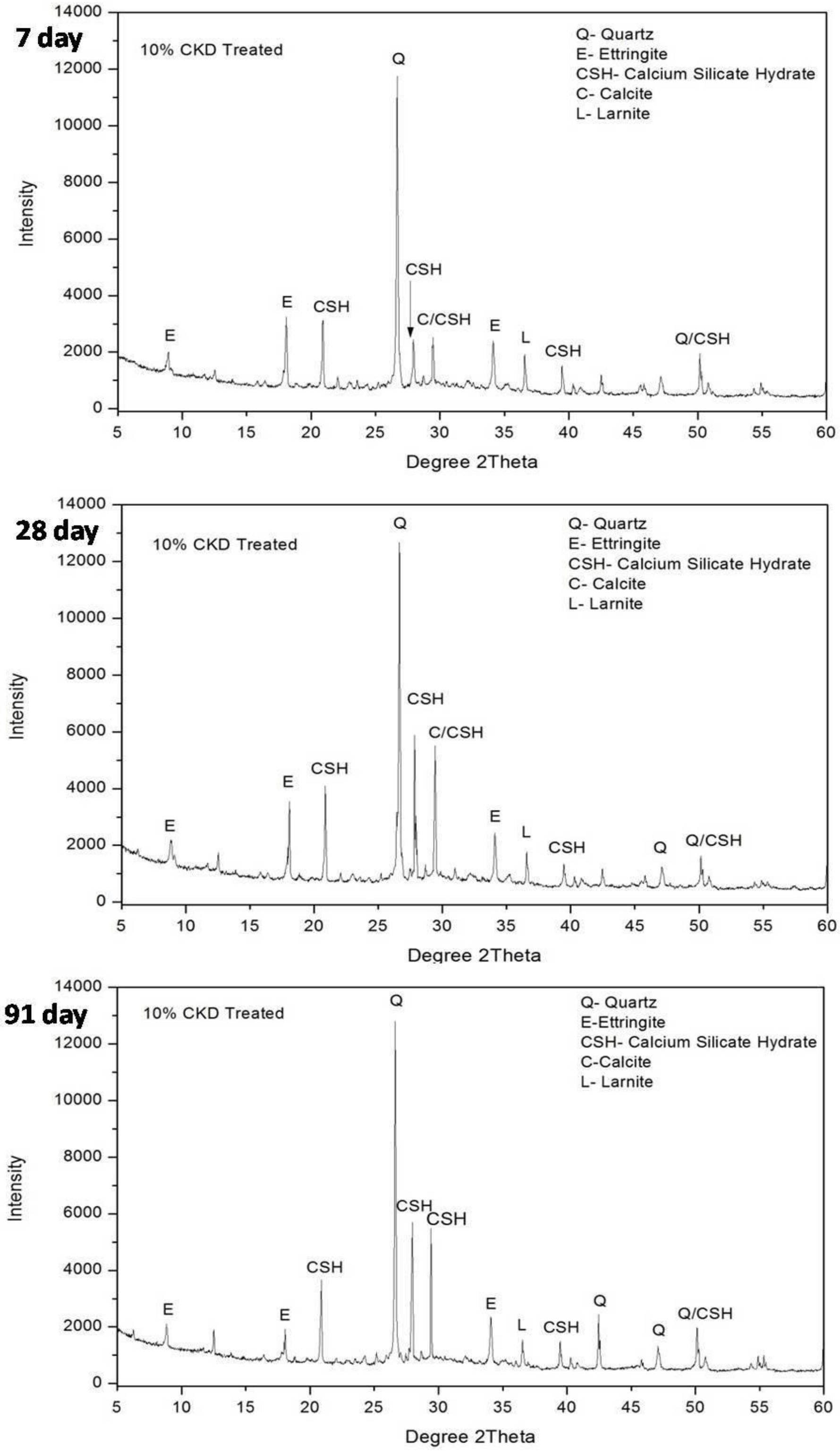


Fig. 4.69: X-ray diffraction shows 10% CKD concrete (treated) at 7, 28 and 91 days of curing

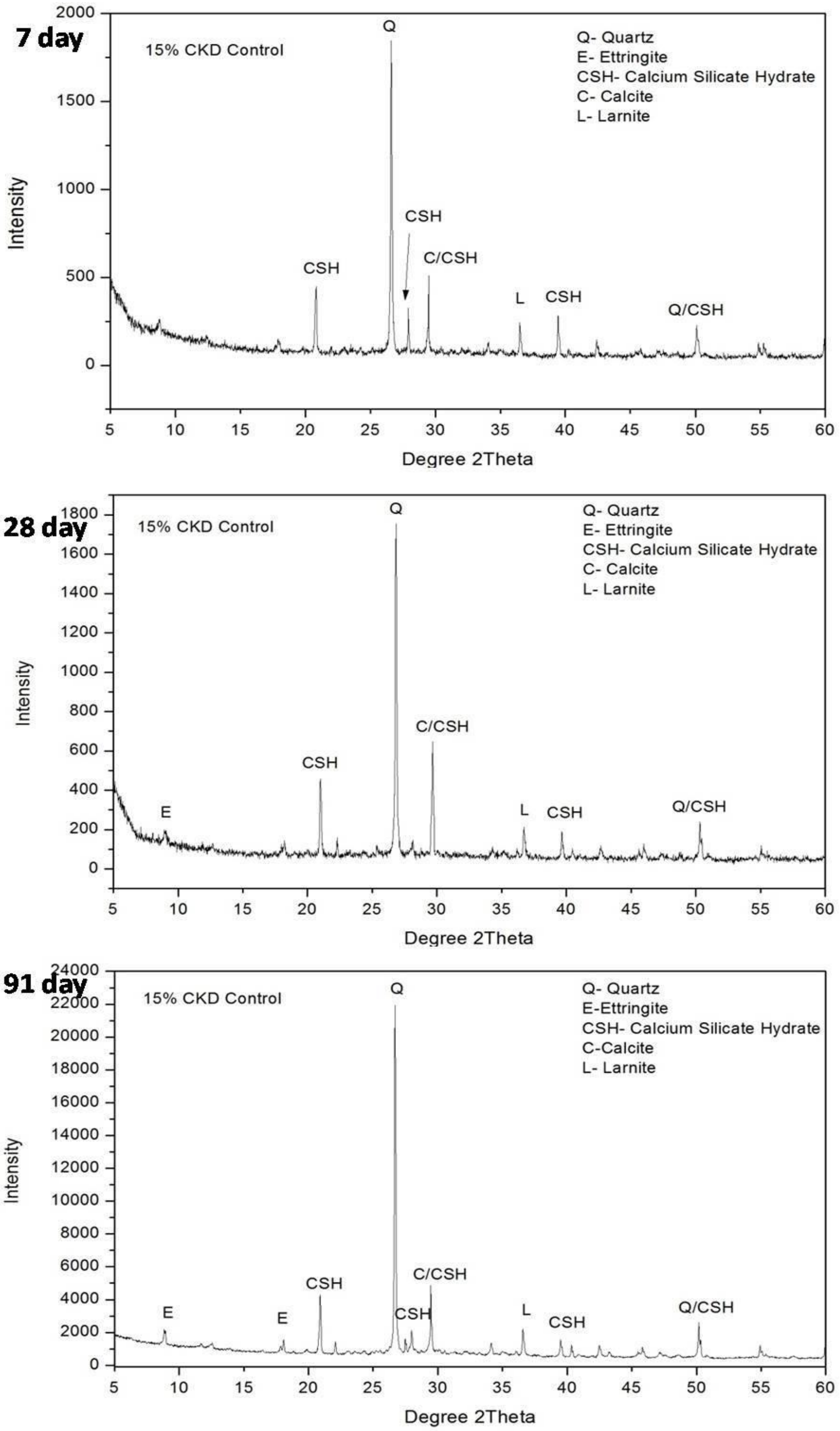


Fig. 4.70: X-ray diffraction shows 15% CKD concrete (control) at 7, 28 and 91 days of curing

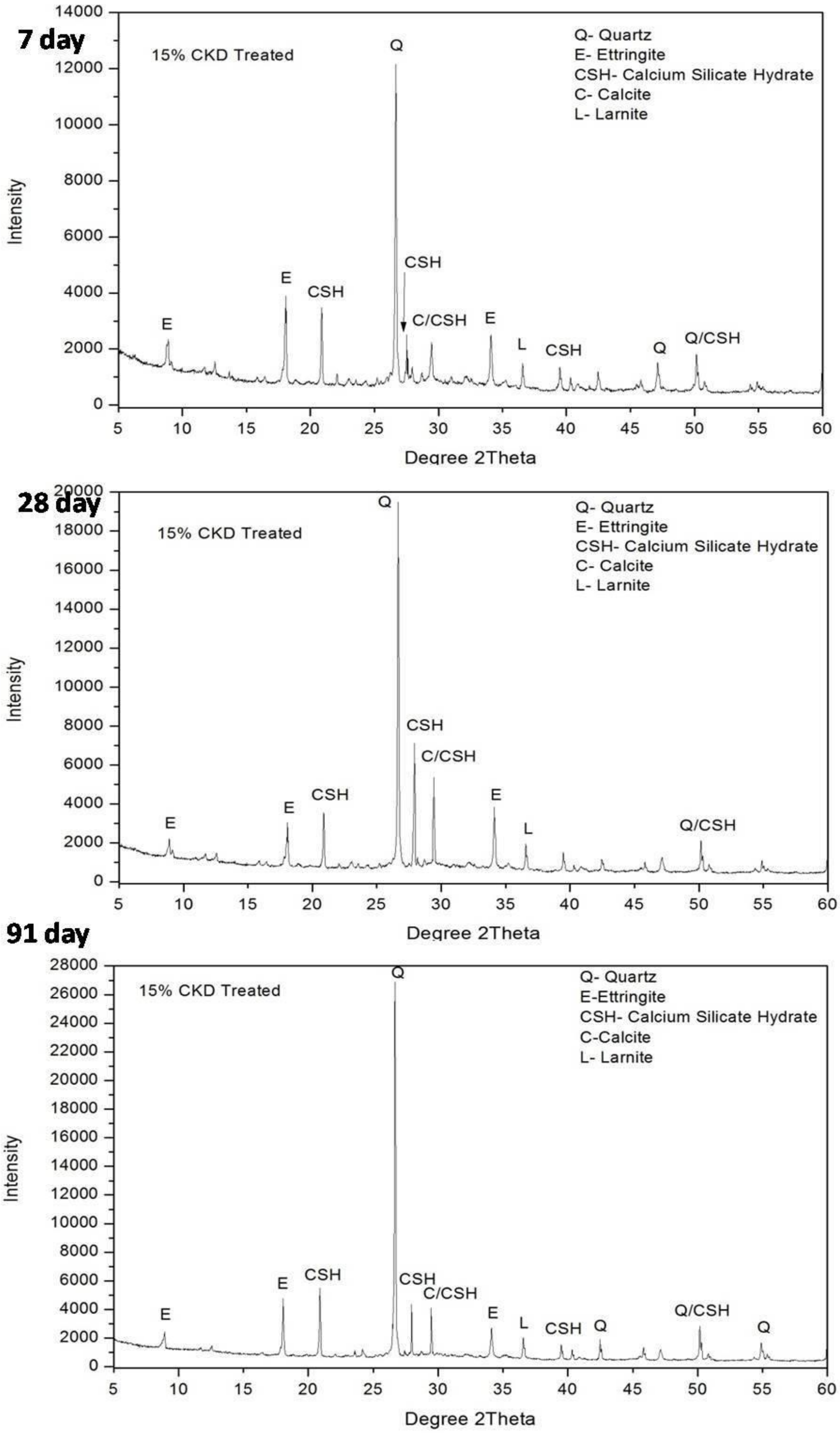


Fig. 4.71: X-ray diffraction shows 15% CKD concrete (treated) at 7, 28 and 91 days of curing

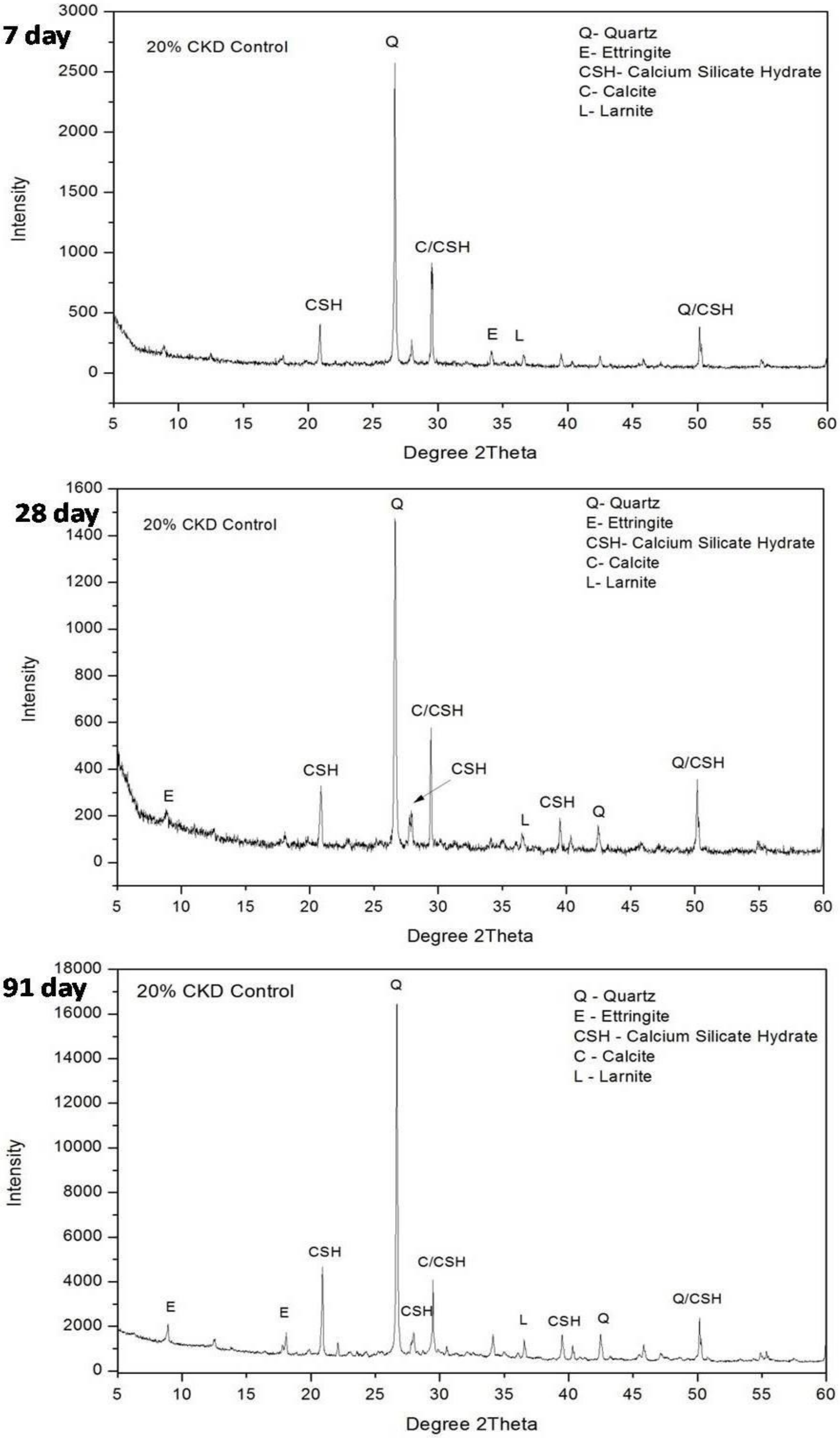


Fig. 4.72: X-ray diffraction shows 20% CKD concrete (control) at 7, 28 and 91 days of curing

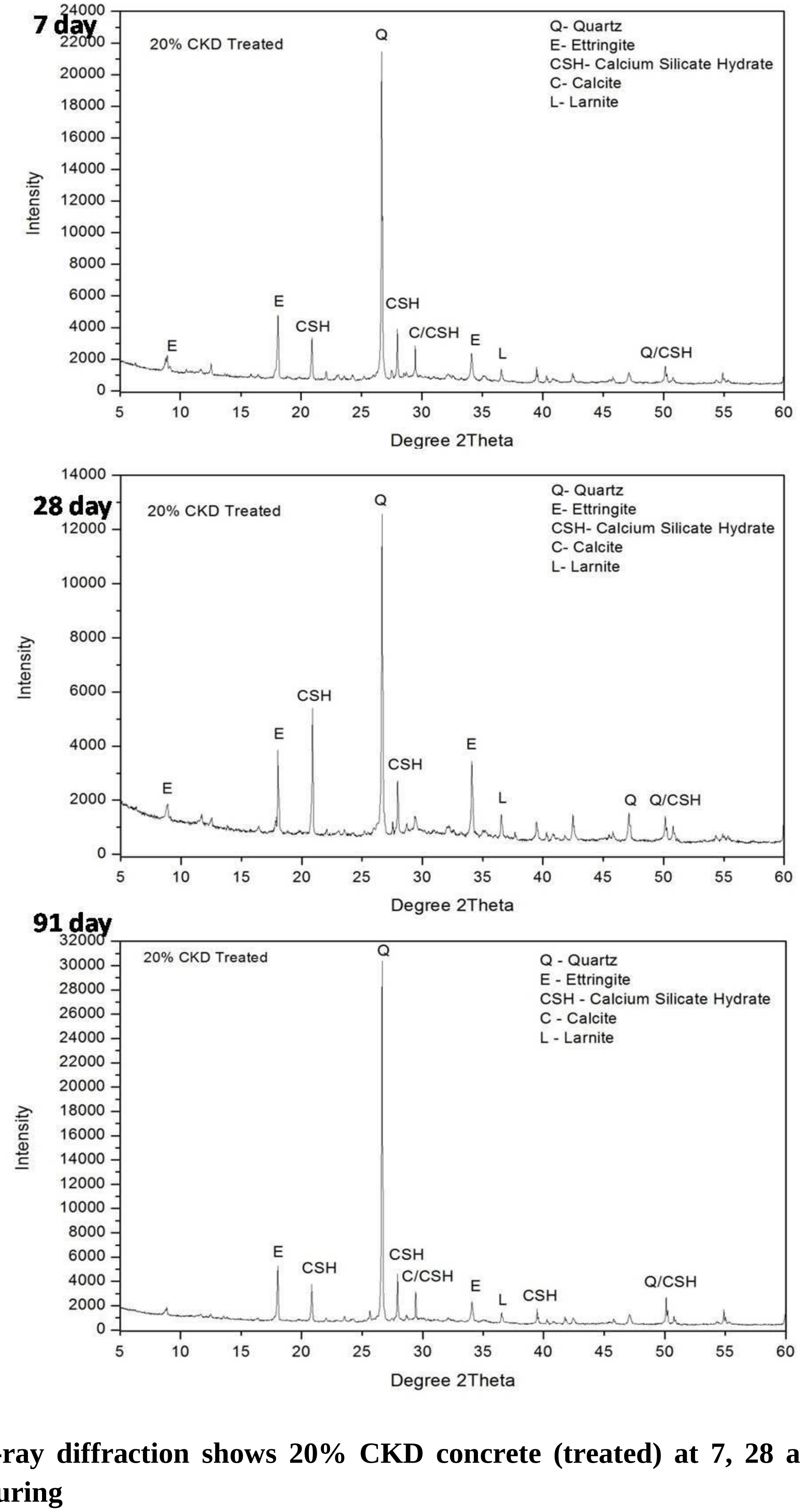


Fig. 4.73: X-ray diffraction shows 20% CKD concrete (treated) at 7, 28 and 91 days of curing

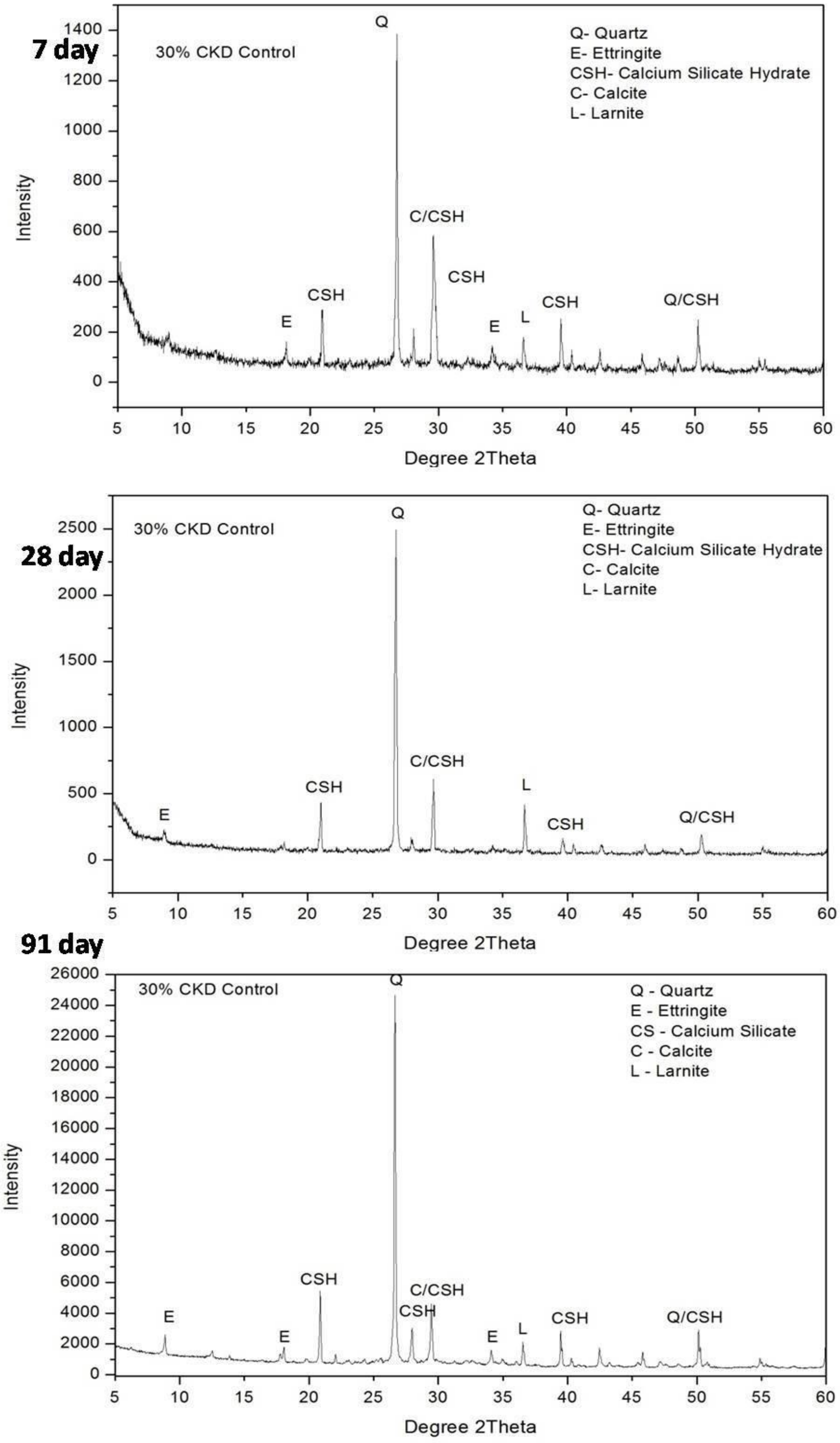


Fig. 4.74: X-ray diffraction shows 30% CKD concrete (control) at 7, 28 and 91 days of curing

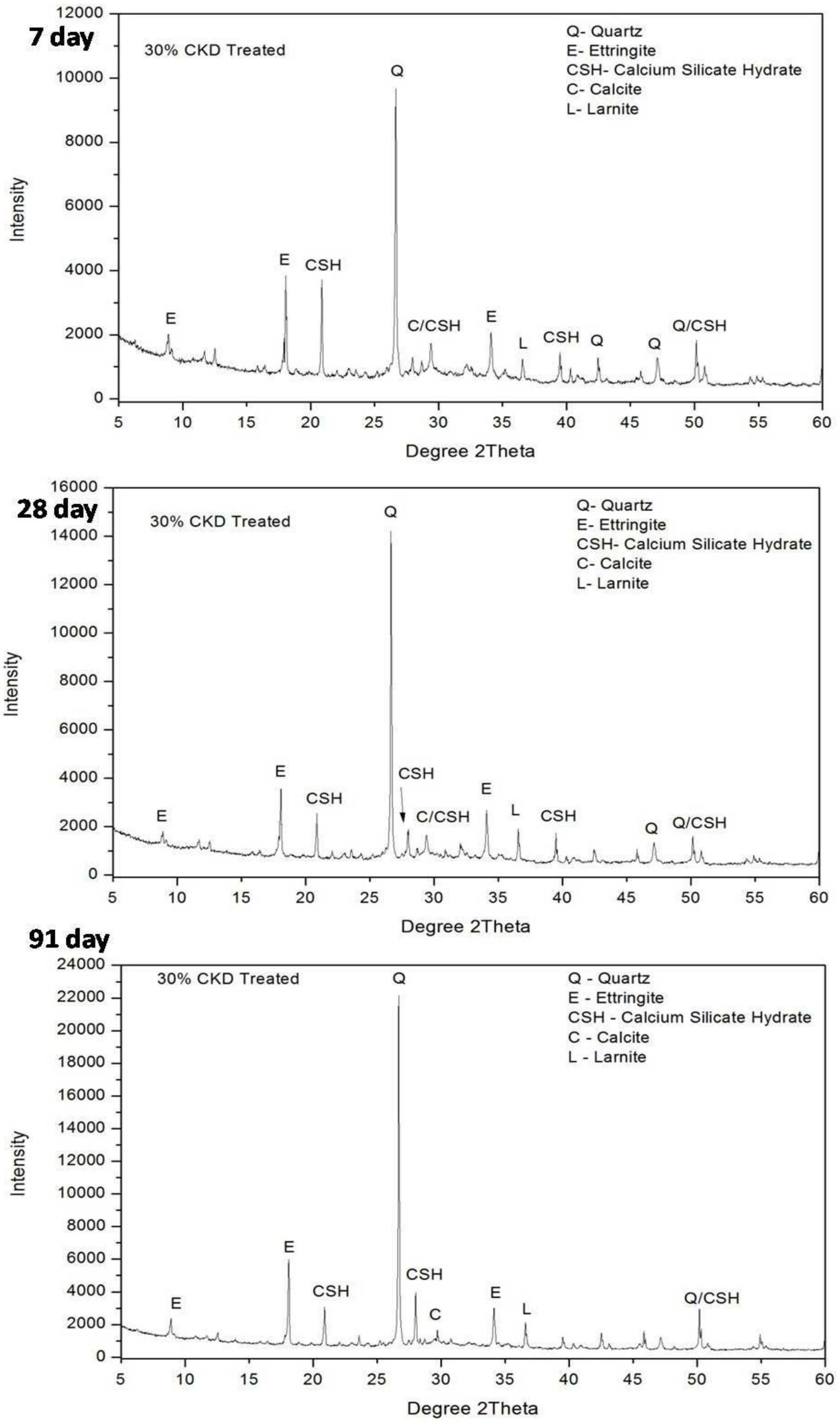


Fig. 4.75: X-ray diffraction shows 30% CKD concrete (treated) at 7, 28 and 91 days of curing

CHAPTER – 5

CONCLUSIONS

This study investigated the results for the isolation and screening of alkali-tolerant bacteria producing acid, physical and chemical characterization of CKD, optimization of bacterial treatment and identification and characterization of bacterial isolates. Water consistency, setting time, hydration of cement blended pastes, compressive strength of mortar and concrete, split tensile strength, ultrasonic pulse velocity, RCPT, water absorption and porosity of concrete containing (untreated and bacterial treated) CKD (0, 5, 10, 15, 20 and 30%) were analyzed. Leachate analysis of untreated and bacterial treated CKD (alkalinity, chloride, metal analysis) and metal leachate analysis of fresh concrete mix were also analyzed. On the basis of the results from the present study, following conclusions are drawn.

5.1 ISOLATION OF ALKALI-TOLERANT BACTERIAL ISOLATES

i. Four alkalitolerant bacterial isolates designated as KG1, KG4, KG5 and KGMD1, isolated from soil and textile industry waste water were able to tolerate and grew at higher pH values (11 and 12).

ii. Isolates were able to reduce the pH of the alkaline minimal medium by 3.49-3.89 units whereas in enrichment medium 2.56-3.19 pH units were reduced within 3 days of culture inoculation. Similarly, fading of the colored medium from pink to colorless was observed within 30-42 h of incubation. This showed their ability to neutralize alkaline medium.

5.2 CHARACTERIZATION OF CEMENT KILN DUST

i. Cement kiln dust (CKD), a by-product generated during the manufacturing of Portland cement, is fine powdery material of grey-black in color and relatively uniform in size.

ii. Fineness of CKD was observed as 22.5% (percent mass retained on 90 µm sieve). Specific gravity of CKD was 2.39 and pH was 12.15. The average particle size of CKD was below 90 µm and moisture content was 0.02%.

iii. The chemical composition of CKD was similar to that of the Portland cement and mainly composed of CaO (55.78%), SiO_2 (13.17%), Al_2O_3 (2.38%) and Fe_2O_3 (2.62%) followed by K_2O (1.12%) and SO_3 (1.13%).

5.3 BACTERIAL TREATMENT OF CEMENT KILN DUST

i. The removal of the alkalinity of CKD by using alkali-tolerant bacterial strain is an eco-friendly method. The alkalinity of control CKD was 1467±26.58 mg/L $CaCO_3$ which after bacterial treatment (KG1, KG4, KG5 and KGMD1) reduced by 72.7 (400 mg/L), 70.7 (430 mg/L), 72.0 (410 mg/L) and 70.0% (440 mg/L), respectively, after 20 days of incubation.

ii. Inoculum size of 0.8 O.D. at 20 days of incubation showed maximum reduction in alkalinity (91.82%) of CKD after treatment with isolate KG5 followed by KG1 (83.64%), KG4 (79.52%) and KGMD1 (71.37%), respectively.

iii. The chloride content of CKD leachate upon treatment with isolate KG1 (0.8 O.D. at 20 days) showed 86.96% (60 mg/L $CaCO_3$) reduction followed by KG4, KG5 and KGMD1 (82.61%) compared to control (460 mg/L $CaCO_3$).

iv. EDX and XRD analysis supported the reduction in alkalinity in terms of absence of sulfate and crystalline alkaline sulfate phases (arcanite) in treated powdered CKD. In KG4 treated cement kiln dust SO_3 content was not detected.

5.4 IDENTIFICATION OF ALKALI-TOLERANT BACTERIAL CULTURES

i. Morphological, biochemical and physiological characteristics of isolate KG1, KG4, KG5 and KGMD1 showed that these strains are the member of group six (alkaliphile) of the genus *Bacillus* and designated as *Bacillus* sp. KG1, KG4, KG5 and KGMD1.

ii. Molecular characterization of 16S rRNA sequences of KG1, KG4, KG5 and KGMD1 isolates confirmed the 94.7%, 94.7%, 96.6% and 96.6% sequence similarity with *B. halodurans* strain DSM 497 (AJ302709), *B. sonorensis* NRRL B-23154 (AF302118), *B. aerius* strain :24K (AJ831843) and *B. halodurans* strain DSM 497 (AJ302709), respectively.

iii. The 16S rRNA sequences of the isolates (KG1, KG4, KG5 and KGMD1) received NCBI accession numbers JQ307184, JN969986, JQ033395 and JN969987, respectively and deposited to NCIM Pune (India) under deposition number NCIM 5439, 5440, 5442 and 5441.

5.5 STUDY OF MORTAR AND CONCRETE PROPERTIES

5.5.1 Mortar Properties

i. *Water for normal consistency*- Water demand for normal consistency increases with increase in CKD content, whereas in bacterial treated CKD mortar the increase was non-significant.

ii. *Setting time* - Up to 15% untreated CKD addition decrease in the final setting time of the cement paste was observed but above this percentage the final setting time increases. In bacterial treated blended paste increased initial and final setting time was observed due to reduction in alkali and sulfate content.

iii. *Hydration* - The hydration increased significantly up to 10% CKD content which later on decreases with increase in CKD content at curing ages of 28 and 91 days. Above 10% CKD additions the reduction in alkali content resulted in decreased hydration which was far decreased in bacterial treated CKD blended cement pastes.

iv. *Compressive strength* - Increase of 14.45 and 8.11% compressive strength was observed in 10% untreated CKD mortar compared to 0% CKD mortar at the age of 28 and 91 days, respectively. Similarly, in bacterial treated CKD mortar (10%), this increase was 26.37 and 19.54%, respectively.

5.5.2 Concrete Properties

5.5.2.1 Compressive strength

i. The compressive strength of the control CKD concrete (0% CKD) at the age of 7 days was 23.23 N/mm^2, whereas 5, 10, 15, 20 and 30% CKD (untreated) concrete showed compressive strength of 23.78, 24.31, 23.03, 22.28 and 19.87 N/mm^2, respectively.

ii. At the age of 28 days, the compressive strength of the 0% CKD concrete was 34.82 N/mm^2 whereas of 5, 10, 15, 20 and 30% control CKD concrete was 35.78, 36.29, 34.53, 33.26 and 30.09 N/mm^2, respectively. Similar trend of increase in compressive strength up to 10% CKD control concrete was observed at 91 days of curing period, above which reduction in strength was observed.

iii. Up to 10% CKD replacement (control CKD concrete) strength increases whereas above 10% CKD addition compressive strength decreased at all ages with exception to 91 days curing where up to 20% CKD additions the increased strength was observed compared to control concrete (0%).

iv. In 10% bacterial treated CKD concrete at the age of 28 days, 7.15 and 2.81% increased compressive strength was observed compared to 0% (34.82 N/mm^2) and 10% (36.29 N/mm^2) CKD control concrete, respectively.

v. Increase in strength of 26.6 and 16.09%, respectively, was observed in 10% bacterial treated CKD concrete (51.22 N/mm^2) compared with 0% (40.44 N/mm^2) and 10% (44.12 N/mm^2) CKD control concrete at the age of 91 days.

5.5.2.2 Split tensile strength

i. There was 10.31% (2.15 N/mm^2), 5.36% (3.73 N/mm^2) and 11.9% (4.32 N/mm^2) increase in tensile strength at 7, 28 and 91 days of curing, respectively, in 10% untreated CKD concrete was observed compared to 0% CKD concrete (1.95, 3.54 and 3.86 N/mm^2).

ii. Increase in split tensile strength in 10% bacterial treated CKD concrete from 3.85 (at 28 days) to 4.89 N/mm^2 (at 91 days) was observed which may be due to appropriate alkalinity which is responsible for late strength development (at 91 days) compared to control concrete.

5.5.2.3 Ultrasonic pulse velocity

i. All the concrete specimens at different curing periods (7, 28 and 91 days) showed pulse velocity in the range 3500-4500 m/s and termed the quality of concrete "good".

ii. In 10% CKD concrete (both untreated and treated), the pulse velocity is maximum at 28 (4210 and 4237 m/s, respectively) and 91 days of curing (4257 and 4380 m/s, respectively) compared to other treatments.

5.5.2.4 Rapid chloride permeability test

i. The chloride permeability of concrete specimens containing untreated and bacterial treated CKD showed reduction in chloride permeability with increasing curing period from 7 to 91 days.

ii. At 7 days of curing, all the untreated CKD concrete specimens were within "low" range whereas in bacterial treated CKD, all the specimens categorized under "moderate" chloride permeability.

iii. In 10% bacterial treated CKD concrete at 91 days, 22.45% decrease in chloride permeability was observed compared to control (857 coulombs). At 91 days of curing, all the treatments containing bacterial treated CKD showed "very low" permeability except 20 and 30% CKD concrete which falls in low chloride permeability range compared to control CKD concrete treatment.

5.5.2.5 Water absorption and porosity

i. Increased water absorption and pore size decreased the compressive strength of concrete and vice-versa. The water absorption of the 0% CKD concrete at the age of 7 days was 3.14%, whereas 5, 10, 15, 20 and 30% CKD (untreated) concrete showed water absorption of 3.07, 2.93, 3.19, 3.33and 4.50%, respectively. In bacterial treated CKD concrete minimum water absorption (1.84%) was observed in 10% CKD concrete.

ii. At 28 days of curing, minimum water absorption of 1.24 and 1.42% in 10% untreated and bacterial treated CKD concrete was observed compared to 0% control concrete (2.79%).

iii. The maximum reduction in porosity at the age of 7 (4.55%), 28 (3.56%) and 91 (2.71%) days was observed in 10% bacterial treated concrete compared to 0% CKD control concrete.

iv. Results of water absorption and porosity showed decrease of 20% and 12.35%, respectively, in 10% bacterial treated CKD concrete compared to 10% untreated CKD concrete at 91 days of curing.

5.6 LEACHATE ANALYSIS OF CKD AND CONCRETE MIXTURE

5.6.1 Alkalinity and chloride analysis of leachate

i. The alkalinity of the cement and 0% CKD concrete (control) was 1893.33 and 1666.67 mg/L, respectively. Decrease in alkalinity (1553.33, 1526.67, 1440, 1253.33 and 1153.33 mg/L) was observed in 5, 10, 15, 20 and 30% untreated CKD concrete compared to 0% control whereas in bacterial treated CKD concrete, 36.0, 37.2, 40, 47.2 and 54.8% reduction in alkalinity was observed in leachate.

ii. Chloride content in 0, 5, 10, 15, 20 and 30% untreated CKD concrete was observed as 800, 760, 740, 740, 720 and 720 mg/L, respectively, whereas in bacterial treated CKD concrete chloride content was observed as 740, 733.33, 673.33, 573.33, 480 mg/L, respectively.

5.6.2 Metal analysis of CKD and Fresh Concrete Mix Leachate

i. The content of silver (Ag) in control CKD leachate was 0.338 mg/L which then reduced to 0.029, 0.031, 0.026 and 0.024 mg/L in KG1, KG4, KG5 and KGMD1, respectively, in treated CKD. Similarly, chromium (Cr) and lead (Pb) was also reduced in the range of

0.492-0.568 and 0.040-0.045 mg/L, respectively, compared to untreated (control) CKD leachate (2.066 and 0.130 mg/L).

ii. The reduction in metal content was varied from 34-92% in different metals by different isolates. Leaching of silver (Ag) is maximally inhibited by bacterial treatment followed by Cr, Cu, Ni and Zn.

iii. Increase in metal content was observed with increasing untreated CKD in concrete whereas addition of bacterial treated CKD in concrete, non- significant reduction in metal content was observed.

iv. The metal concentration (Ag, Cd, Cr, Cu, Fe, Mo, Ni, Pb and Zn) in 5% bacterial treated leachate were reduced from 0.101 mg/L to 0.084 mg/L, 0.009 mg/L to 0.007 mg/L, 0.700 mg/L to 0.624 mg/L, 0.343 mg/L to 0.323 mg/L, 0.713 mg/L to 0.680 mg/L, 0.122 mg/L to 0.117 mg/L, 0.310 mg/L to 0.300 mg/L, 0.056 mg/L to 0.050 mg/L and 0.931 mg/L to 0.741 mg/L, respectively. No such change in Co concentration was observed. Similar reduction in metal concentration was also observed in 10, 15, 20 and 30% bacterial treated CKD concrete.

5.7 MICROSTRUCTURE ANALYSIS

i. SEM analysis revealed the increased formation of CSH gel and non expansive ettringite formation in the matrix of treated mortar and concrete which supports the increased compressive strength of the material.

ii. X-ray diffraction (XRD) analysis of mortar and concrete samples showed the peaks of quartz (Q), calcium silicate hydrate (CSH), calcite (C), larnite (L) and ettringite (E) phases. The peak of larnite (Ca_2SiO_4) or C_2S or Belite was observed at 37 degree 2θ responsible for late strength development in mortar and concrete.

iii. XRD analysis confirmed the formation of CSH (21, 26, 29 degree 2θ) and non expansive ettringite (7, 17 and 34 degree 2θ) within the matrix of treated mortar and concrete specimens, which causes an improvement in the strength of the material.

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APPENDIX – I

16S rRNA Sequences of Bacterial Strains – GenBank NCBI Report

Bacillus halodurans strain KG1 16S ribosomal RNA gene, partial sequence

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LOCUS       JQ307184              1408 bp    DNA     linear   BCT 12-FEB-2012
DEFINITION  Bacillus halodurans strain KG1 16S ribosomal RNA gene, partial
            sequence.
ACCESSION   JQ307184
VERSION     JQ307184.1  GI:374722916
KEYWORDS    .
SOURCE      Bacillus halodurans
  ORGANISM  Bacillus halodurans
            Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1408)
  AUTHORS   Garg,K., Rajor,A. and Aminedi,R.
  TITLE     Isolation and characterization of alkalophilic bacteria for
            treatment of cement kiln dust
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1408)
  AUTHORS   Garg,K., Rajor,A. and Aminedi,R.
  TITLE     Direct Submission
  JOURNAL   Submitted (19-DEC-2011) Department of Biotechnology & Environmental
             Sciences, Thapar University, Bhadson Road, Patiala, Punjab 147004,India
COMMENT     Sequences were screened for chimeras by the submitter using Bellerophon v.3.
FEATURES             Location/Qualifiers
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      181 actagctagt tggtggggta acggctcacc aaggcgacat gcgtagccca cctgagaggg
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      301 tcttccgcaa tggacgaaag tctgacggag caacgcctcg cgagtgatga aggttttcgg
      361 atcgtaaaac tctattgtta gggaagaaca agtgccgttc gaaagggcgg caccttgacg
      421 gtgcctaacg agaaagccac ggctaactac atgccagcag ccgcggtaat acgtatgtgg
      481 caagcgttgt ccggaattat tgggcgtaaa gcgcgcgcag gcggtctctt aagtctgatg
      541 tgaaagcccc cggctcaacc ggggagggtc attggaaact gggagacttg agtacagaag
      601 aggagagtgg aattccacgt gtagcggtga aatgcgtaga gatgtggagg aacaccagtg
      661 gcgaacgcga ctctctggtc tgtaactgac gctcaggcgc gaagcgtgcg gagcagacag
      721 gatcagatac cctggtactc cacgccttaa cgatgagtgc tatgtgttag ggtttcgat
      781 gccctcagtg ccgaagttga caccttaatg cactacgacc ggggcagtac gaccgcaggg
      841 tcgatcctca tcagaattag acagagcggc ccgcacacgc ttgcagcaag atgatttcat
      901 tacgaagcac gcgacgtacc ttaacagtct gacatgcatt gactacccta gagatatgtg
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***Bacillus* sp. KG4 16S ribosomal RNA gene, partial sequence**

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DEFINITION  Bacillus sp. KG4 16S ribosomal RNA gene, partial sequence.
ACCESSION   JN969986
VERSION     JN969986.1  GI:363804975
KEYWORDS    .
SOURCE      Bacillus sp. KG4
  ORGANISM  Bacillus sp. KG4
            Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1466)
  AUTHORS   Kunal and Rajor,A.
  TITLE     Isolation and characterization of microbial isolates capable of
            growing under highly alkaline conditions
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1466)
  AUTHORS   Kunal and Rajor,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (31-OCT-2011) Department of Biotechnology and Environmental
             Sciences, Thapar University, Patiala, Punjab 147004,India
FEATURES             Location/Qualifiers
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                     /isolation_source="soil"
                     /db_xref="taxon:1126829"
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      121 tccgggaaac cggggctaat accggatgct tgattgaacc gcaggttcaa ttataaaagg
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      421 gggaaaaaca agtaccgttc gaataggggg gtaccttgac ggtacctaac caaaaggcca
      481 cggctaatta cttgccacca cccgcggtat tacttaggtg gcaagcgttg tccggaatta
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      601 cggggagggt cattgaaaac tggggaactt gagtgcaaaa aaggaaagtg gaattccacg
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      961 ccttaccagg tcttgacatc ctctgacaac cctagagata gggcttcccc ttcgggggca
     1021 gagtgacagg tggtgcatgg ttgtcgtcag ctcgtgtcgt gagatgttgg gttaagtccc
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     1321 atctctggta atcgcggatc agcatgccgc ggtgaatacg ttcccgggcc ttgtacacac
     1381 cgcctgtcac accacgagag tttgtaacac cggagctagc tgaggtaacc cctggagact
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Bacillus sp. KG5 16S ribosomal RNA gene, partial sequence

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DEFINITION  Bacillus sp. KG5 16S ribosomal RNA gene, partial sequence.
ACCESSION   JQ033395
VERSION     JQ033395.1  GI:372863688
KEYWORDS    .
SOURCE      Bacillus sp. KG5
  ORGANISM  Bacillus sp. KG5
            Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1381)
  AUTHORS   Kunal and Rajor,A.
  TITLE     Isolation and characterization of microbial isolates capable of
            growing under highly alkaline conditions
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1381)
  AUTHORS   Kunal and Rajor,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (10-NOV-2011) Department of Biotechnology & Environmental
            Sciences, Thapar University, Bhadson Road, Patiala, Punjab 147004,
            India
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      121 ggttccatcc ttaaaggggg cttttaacta cccctttccg atggacccgc ggcgcattaa
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Bacillus sp. KGMD1 16S ribosomal RNA gene, partial sequence

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LOCUS       JN969987                1360 bp    DNA     linear   BCT 21-DEC-2011
DEFINITION  Bacillus sp. KGMD1 16S ribosomal RNA gene, partial sequence.
ACCESSION   JN969987
VERSION     JN969987.1  GI:363804976
KEYWORDS    .
SOURCE      Bacillus sp. KGMD1
  ORGANISM  Bacillus sp. KGMD1
            Bacteria; Firmicutes; Bacilli; Bacillales; Bacillaceae; Bacillus.
REFERENCE   1  (bases 1 to 1360)
  AUTHORS   Kunal and Rajor,A.
  TITLE     Isolation and characterization of microbial isolates capable of
            growing under highly alkaline conditions
  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 1360)
  AUTHORS   Kunal and Rajor,A.
  TITLE     Direct Submission
  JOURNAL   Submitted (31-OCT-2011) Department of Biotechnology and
            Environmental Sciences, Thapar University, Patiala, Punjab 147004,
            India
FEATURES             Location/Qualifiers
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