

UTILIZATION OF FUNGAL TREATED WASTE FOUNDRY SAND IN CONCRETE

*A thesis
Submitted in partial fulfillment of the requirement
For the award of the degree of*

**DOCTOR OF PHILOSOPHY
IN
BIOTECHNOLOGY**

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CERTIFICATE

Certify that the thesis entitled "Utilization of Fungal Treated Waste Foundry Sand in Concrete" which is submitted by Ms. Gurdeep Kaur, in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy in Biotechnology, Department of Biotechnology of Thapar University, Patiala is a record of the candidate's own independent and original research work carried out by him under my supervision and guidance. The matter embodied in this thesis has not been submitted in part or full to any other university or institute for the award of any degree.

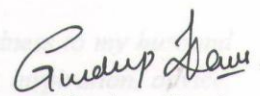

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DECLARATION

I hereby certify that the work which is being presented in this thesis entitled "Utilization of Fungal Treated Waste Foundry Sand in concrete" being in partial fulfillment of the requirements for the award of degree of Doctor of Philosophy in Biotechnology submitted in Department of Biotechnology, Thapar University, Patiala is an authentic record of my own work carried out under the supervision of Dr. Anita Rajor (School of Energy & Environment) and Dr. Rafat Siddique (Civil Engineering Department) and refers other research's work are duly listed in the reference section.

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


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LIST OF PUBLICATIONS FROM PRESENT WORK

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ABSTRACT

Solid waste management has become one of the global environmental issues, as there is continuous increase in industrial by-products and waste materials. . Due to lack of land filling space and its ever increasing cost, utilization of waste material and by-products has become an attractive alternative to disposal. The beneficial use of such by-products in construction materials results in reducing the cost of construction materials' ingredients and also helps in reducing disposal problem. Waste foundry sand (WFS) is one of such industrial by-product which could be used in construction materials. The leachate obtained from such materials may contain hazardous compounds, which may possibly effect the environment. Leaching characteristics are essential in understanding the environmental impact or toxicity, disposal and potential development of beneficial applications of WFS.

This study aimed to present the utilization of fungal treated WFS in concrete. This experimental investigation was performed to evaluate the properties of M20 (31 N/mm²) Grade of concrete mixes, in which sand was partially replaced with waste foundry sand (WFS). Sand was replaced with four percentages of untreated and fungal treated WFS (0%, 10%, 15% and 20%). Fungal (*Aspergillus niger* and *Eupenicillium crustaceum*) inoculums (2%) were used for the treatment of WFS. Fungal treated WFS were utilized for the preparation of concrete. Concrete mixes were prepared with partial replacement of untreated WFS and fungal treated WFS. Compressive strength, water absorption and porosity were carried out to evaluate the properties of concrete containing untreated WFS and concrete containing fungal treated WFS at the age of 28 days. Study also included leachate analysis obtained from the concrete mixes made with fungal treated

WFS, untreated WFS (0%, 10%, 15% and 20%). Results showed the metal concentration of Cd, Cr, Fe, Mo, Mn, Ni and Pb were reduced to significant levels as compared with World Health Organization (WHO) standard limits and ground water quality standards (GWQS).

Concrete containing fungal (*Aspergillus niger* and *Eupenicillium crustaceum*) treated WFS showed increase in compressive strength and decrease in water absorption and porosity in concrete made fungal treated WFS as compared to concrete made with untreated WFS. Results showed that the treatment of WFS by *Eupenicillium crustaceum* is more effective than *Aspergillus niger* in the case of metal leaching and improvement of concrete properties. X-ray diffraction (XRD) showed some extra peaks of calcium aluminum silicate hydrate (Gismondine) in concrete containing fungal treated WFS as compared to concrete containing untreated WFS. The strength improvement was due to microbially induced mineral formed by fungi often referred as ‘calcified filaments’ confirms by scanning electron microscopy (SEM) and energy dispersive spectrum (EDS). This ability of fungal culture to form the biomineral results in improvement of concrete properties. Results showed that there was better enhancement in strength properties at 20% replacement of fine aggregate with fungal (*Eupenicillium crustaceum*) treated WFS as compared to concrete made with untreated WFS.

Keyword: Fungi; Metal Leaching; Microbial concrete; Solid Waste Management; SEM; WFS; XRD

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Abbreviations

Abbreviations		Word (s)
ACI	-	American Concrete Institute
BLAST	-	Basic local Alignment Search Tool
ASTM	-	American Standards of testing Materials
CA	-	Coarse aggregate
CB	-	Crushed Brick
CC	-	Crushed Concrete
CLSM	-	Controlled Low Strength Material
COD	-	Chemical Oxygen Demand
CSH	-	Calcium Silicate Hydrate
DNA	-	Deoxyribo Nucleic Acid
EDS	-	Energy Dispersive Spectrum
EPA	-	Environmental Protection Agency
ES	-	Enforcement Standards
FA	-	Fine Aggregate
FHA	-	Federal Highway Administration
FS	-	Foundry Sand
GFN	-	Grain Fineness Number
GPa	-	Giga pascals
GWQS	-	Ground Water Quality Standards
HPLC	-	High Performance Liquid Chromatography

HRWRA	-	High Range Water Reducing Admixtures
IAWG	-	International Ash Working Group
ICPMS	-	Inductively Coupled Plasma Mass Spectrometry
IS	-	Indian Standard
ITS	-	Internal Transcribed Spacer
JCPDS	-	Joint Committee on Powder Diffraction Standards
LFL	-	Land- Fill Leachate
MK	-	Metakaolin
MMN	-	Melin Norkrans Medium
MNR	-	Ministry of Natural Resources
MOEE	-	Ministry of Energy and Environment
MPa	-	Mega Pascals
NCBI	-	National Centre for Biotechnology Information
PCR	-	Polymerase Chain reaction
PAHs	-	Polycyclic Aromatic Hydrocarbons
PAL	-	Preventive Action Limits
PDA	-	Potato Dextrose Agar
PDB	-	Potato Dextrose Broth
RAC	-	Recycled Aggregate Concrete
RCA	-	Recycled Coarse Aggregate
RCPT	-	Rapid Chloride Permeability Test
SCC	-	Self Consolidating Materials
SEM	-	Scanning Electron Microscopy
SPLP	-	Synthetic Precipitation Leaching Procedure
SSA	-	Sewage Sludge Ash

SSD	-	Saturated Surface Dry
TCLP	-	Toxicity Characteristic Leaching Procedure
TPH	-	Total Petroleum Hydrocarbon
USF	-	Used Found Sand
VSS	-	Virgin Silica Sand
WDNR	-	Wisconsin Department of Natural Resources
WFS	-	Waste Foundry Sand
WHO	-	World Health Organization
XRD	-	X- Ray Diffraction

CHAPTER-1

INTRODUCTION

This chapter introduces utilization of waste foundry sand (WFS) in concrete along with their types and its global production in brief. It also includes leachate and its generation, WFS leachate, role of fungi in bioleaching and the objectives of the present work.

1.1 WASTE FOUNDRY SAND (WFS)

Foundry sand consists primarily of, uniformly sized, high-quality silica sand that is bonded to form molds for ferrous (iron and steel) and non-ferrous (copper, aluminum, brass) metal castings. Foundries use high-quality size specific silica sands for use in their molding and casting operations. The raw sand is normally of a higher quality than the typical bank run or natural sands used in fill construction sites.

In the casting process, molding sands are recycled and reused multiple times. Eventually, however, the recycled sand degrades to the point that it can no longer be reused in the casting process. When it is not possible to further reuse in the foundry it is removed from the foundry and termed as waste foundry sand (WFS). The automotive industry and its parts suppliers are the major generators of foundry sand. The physical and chemical characteristics of foundry sand will depend in great part on the type of casting process and the industry sector from which it originates.

Waste foundry sand are by-products which appears to possess the potential to partially replace regular sand as a fine aggregate in concretes, providing a recycling opportunity for them. If such types of materials can be substituted partly/fully for natural sand (fine aggregates) in concrete mixtures without sacrificing or even improving strength and durability, there are clear economic and environmental gains. Currently, very limited literature is available on the use of these by-products in concrete. The typical physical and chemical property of WFS is dependent upon the

type of metal being poured, casting process, technology employed, type of furnaces (induction, electric arc and cupola) and type of finishing process (grinding, blast cleaning and coating).

1.2 TYPES OF WASTE FOUNDRY SAND

Classification of foundry sands depends upon the type of binder systems used in metal casting. On the basis of binder systems foundry sands are categorized into two parts: clay-bonded sand (green sand) and chemically bonded sand. In foundry industry, sand cast system is the common casting process. Virtually all sand cast molds for ferrous castings are of the green sand type. Clay-bonded (Green) sand is composed of naturally occurring materials, such as high-quality silica sand (85–95%), bentonite clay (4–10%) as a binder, a carbonaceous additive (2–10%) to improve the casting surface finish and water (2–5%). It is black in color due to carbon content.

1.2.1 Green (Clay Bonded) Sand

In foundries, the most commonly used molding media is green sand. Silica sand is the bulk medium that resists high temperatures while the coating of clay binds the sand together. The water adds plasticity. Addition of carbonaceous material leads to prevent the “burn-on” or fusing of sand onto the casting surface. Green sand also contains trace chemicals such as MgO, K₂O, and TiO₂.

1.2.2 Chemically Bonded Sand

Chemically-bonded sand is used both in mold making and core making where high strength is necessary to withstand the heat of molten metal. These systems involve the use of one or more organic binders along with catalysts and different hardening or setting procedures. Chemically bonded sand consists of 93–99% silica and 1–3% chemical binder. Chemically bonded sands are generally light in color than clay-bonded sands (Fig. 1.1).

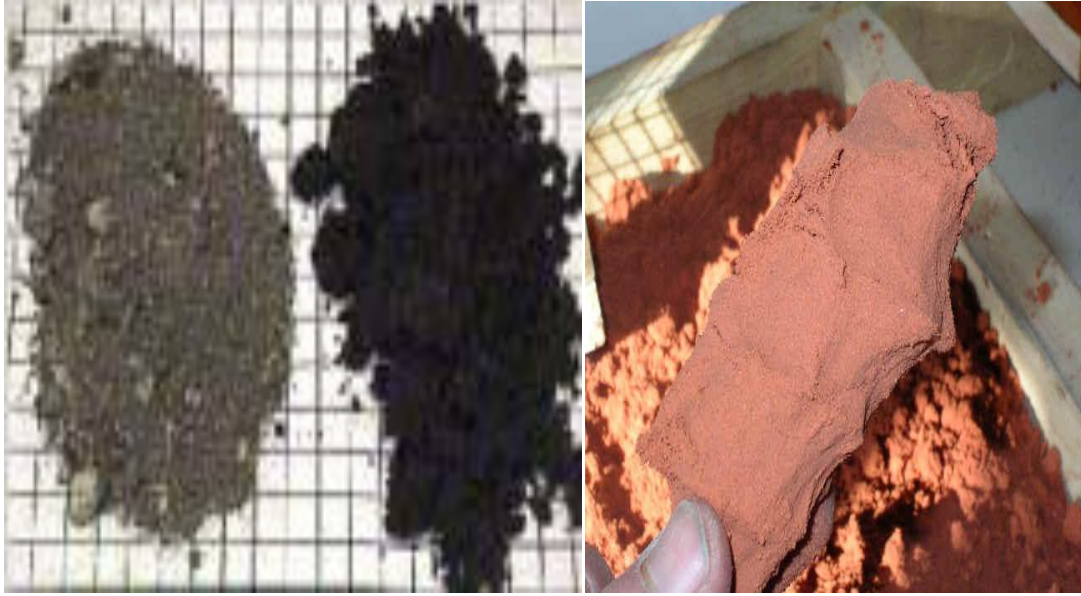


Fig 1.1: Clay Bonded Sand and Chemically Bonded Sand (Federal Highway Administration, 2004)

1.3 PROPERTIES OF WFS

1.3.1 Physical Properties

Waste foundry sand is generally sub-angular to round in shape. Chemically bonded sand is typically of medium tan or off-white color and Green sand is black or gray. Waste foundry sand has low absorption capacity. Javed and Lovell (1994), suggested that the grain size distribution of waste foundry sand is uniform, with 85–95% of the material between 0.6 mm and 0.15 mm; 5–12% of foundry sand can be smaller than 0.075 mm. The specific gravity of foundry sand varies between 2.39 and 2.55 (Table 1.1).

1.3.2 Chemical Properties

The chemical composition of the waste foundry sand depends upon three main factors: type of metal molded at the foundry, type of binder and combustible used. The performance of WFS depends on its chemical properties. WFS is mainly composed of silica sand, coated with a thin film of burnt carbon, residual binder (bentonite, sea coal, resins/chemicals) and dust. Table 1.2

Table 1.1: Typical Physical Properties of Waste Foundry Sand

Properties	Javed and Lovell (1994)	Naik et al. (2001)	Guney et al. (2010)	Siddique et al. (2011)
Specific gravity	2.39-2.55	2.79	2.45	2.61
Fineness modulus	-	2.32	-	1.78
Unit Weight (kg/m ³)	-	1784	-	1638
Absorption (%)	0.45	5.0	-	1.3
Moisture content (%)	0.1-10.1	-	3.25	-
Clay lumps and friable particles	1- 44	0.4	-	0.9
Materials finer than 75µm (%)	-	1.08	24	18

lists the chemical composition of a typical sample of waste foundry sand. Silica sand is hydrophilic and consequently attracts water to its surface. Johnson (1981) suggested the pH value of waste foundry sand can vary between 4 and 8. There is various chemical binder system used in foundry industry, such as furfuryl alcohol, phenolic urethane, phenolic no bake-acid, phenolic resole-ester, sodium silicate, phosphate, alkyd (oil) urethane, shell liquid/powdered and flake resins. When binder mixes with the silica sand, then catalyst start the reaction that cures the chemical resin and hardens the sand core or mould. Some of the most common chemically bonded sands are resins coated sand, hot box, cold box and CO₂ sand. Majority of binder used in the foundry are self setting chemical binder.

Ministry of Natural resources (1993) reported that some waste foundry sands can be corrosive to metals due to the presence of phenols in foundry sand. There is some concern that precipitation percolating through stockpiles could mobilize leachable fractions, resulting in phenol discharges into surface or ground water supplies. Foundry sand sources and stockpiles must be monitored to assess the need to establish controls for potential phenol discharges (Ministry of Energy and Environment MOEE, 1993; Johnson, 1981).The maximum phenol content in foundry sand should be less than 2ppm (Federal Highway Administration, 2004).

Table 1.2: Typical Chemical Composition of Waste Foundry Sand

Constituent	Value (%)			
	American Foundry men's Society (1991)	Guney et al. (2010)	Etxeberria et al. (2010)	Siddique et al. (2011)
SiO ₂	87.91	98	95.10	78.81
Al ₂ O ₃	4.70	0.8	1.47	6.32
Fe ₂ O ₃	0.94	0.25	0.49	4.83
CaO	0.14	0.035	0.19	1.88
MgO	0.30	0.023	0.19	1.95
SO ₃	0.09	0.01	0.03	0.05
Na ₂ O	0.19	0.04	0.26	0.10
K ₂ O	0.25	0.04	0.68	-
TiO ₂	0.15	-	0.04	-
Mn ₂ O ₃	0.02	-		-
SrO	0.03	-		-
LOI	5.15	-	1.32	2.15

1.4 BENEFICIAL REUSE OF WFS

Non-hazardous and non-dangerous waste foundry sand has traditionally been used as "clean fill. However, "clean fill" opportunities have declined in recent years, making exploration and implementation of other applications essential. Waste foundry sand has been successfully used in various applications. Below are some recycling options for waste foundry sand:

- **Asphalt Concrete:** Substitution of up to 15% spent sand for conventional asphalt concrete fine aggregate.
- **Compost Additive:** Bulking agent for composted yard waste, to produce topsoil or topsoil additive.
- **Concrete:** Substitution for regular sand in structural grade concrete, at low percentages.

- **Bricks and Pavers:** Encapsulation in a proprietary, high pressure, pozzolanic process that can encapsulate and chemically bind various waste materials in C-grade fly ash (a fine particulate ash produced by coal-burning electrical power plants). The ambient-temperature process results in bricks that are cost effective and can be shaped to meet end-user requirements.
- **Portland Cement:** Cement kiln feed for Portland cement. A study by the American Foundrymen's Society indicates that portland cement manufactured with up to 13% of spent foundry sand exhibited slightly higher compressive strengths than conventionally produced portland cement, without any degradation of key characteristics such as set time.
- **Mineral Wool Products:** Potential silica source.
- **Flowable Fill:** Substitution for regular sand in flowable fill, a mixture of sand, flyash, and water that is mixed into a slurry and poured. Flowable fill is a self-leveling and self-compacting mix that hardens and develops strength over time, similar to concrete, and is commonly used as backfill for trenches (sewer, conduit, utility).

1.5 POSSIBLE HARMFUL COMPOUNDS IN WFS

1.5.1 Heavy Metals

Heavy metals are common environmental pollutants that are released in WFS as a result of casting in metal foundries. These include chromium, cadmium, mercury, lead, nickel, arsenic and silver (Ji et al., 2000). These heavy metals as well as their compounds are toxic to living organisms. The type and concentration of the heavy metal present in WFS depends on the type of metal foundry including steel, iron, aluminium and copper alloys and type of binder being used (Clean Washington Centre Report, 1996).

Mixed foundry by-products from non-ferrous foundries (which contain a combination of sand, dusts, and slag) have occasionally been found to have metal leachate concentrations above RCRA thresholds. For example, some foundry dusts have been found to have higher concentrations of metals relative to sand (Winkler and Bolshakov, 2000).

1.5.2 Organic Compounds

The binder system is the primary source of organic contaminants in sand. The primary organic contaminants from foundry sand cores are acetone and 1, 1, 1-trichloroethane, and a number of aromatic compounds (Table 1.3). Green sand casting, which generally does not involve the use of organic binders, has been shown to have lower potential for leaching organic compounds than chemically bonded systems. Depending on the curing and pouring process, some of the organic binder constituents may be altered from their original composition. The more reactive organic compounds commonly used in binders and resins are of special concern, because they can be transformed into new hazardous compounds under incomplete combustion conditions.

Harmful organic compounds that may be present in most commonly used WFS: Phenol, Formaldehyde, Isoforonide, isocyanate, Diphenylmetanide isocyanate Polymethyl-siloxane, heptanes, Benzaldehyde, Isopropyl alcohol, Dimethylisopropylamine, Triethylamine, Hexamine, Furfuryl alcohol, Sodium silicate and other polycyclic aromatic hydrocarbons (PAHs) (Ji et al., 2000)

Table 1.3: Primary Organic Compounds Found in Foundry Sand Cores (Winkler and Bolshakov, 2000)

Acetone	Formaldehyde
Benzene	Isopropylbenzene
2-chlorophenol	1- and 2-Methylnaphthalene
m/p-Cresol	Naphthalene
1,2-Dichlorobenzene	Phenol
1,4-Dichlorobenzene	Tetrachloroethane
Diethylbenzenes	Toluene
Dimethylnaphthalenes	1,1,1-Trichloroethane
2,4-Dimethylphenol	1,2,4-Trimethylbenzene
Ethylbenzene	1,3,5-Trimethylphenol
p-Ethyltoluene	Xylenes

1.6 Leachate

Leachate is the liquid that drains or 'leaches' from a landfill. Henry and Heinke (1996) suggested that its composition depends upon the type of waste that it contains and age of the landfill. It usually contains both dissolved and suspended material. Once leachate comes in contact with ground water, it gets contaminated and deteriorates the quality of ground water.

1.6.1 Generation of Leachate

Leachate generation is caused principally by precipitation percolating through waste deposited in a landfill. The percolating water becomes contaminated when it comes in contact with decomposing solid waste, and it flows out of the waste material and termed as leachate. A wide range of other materials including methane, carbon dioxide and a complex mixture of organic acids, aldehydes, alcohols and simple sugars are produced during the decomposition of carbonaceous material which adds to the additional volume of leachate. However despite much stricter statutory controls leachates from modern sites are found to contain a range of contaminants that may either be associated with some level of illegal activity or may reflect the ubiquitous use of a range of difficult materials in household and domestic products which enter the waste stream legally. However, most toxic materials are now specifically excluded from land filling

1.6.2 Composition of Leachate

Leachate may be characterized as a water-based solution of four groups of contaminants ; dissolved organic matter (alcohols, acids, aldehydes, short chain sugars etc.), inorganic macro components (common cations and anions including sulfate, chloride, Iron, aluminium, zinc and ammonia), heavy metals (Pb, Ni, Cu and Hg) and xenobiotic organic compounds such as halogenated organics. The physical appearance of leachate when it emerges from a typical landfill site is a strongly-odoured yellow colored cloudy liquid. The smell is acidic, offensive and may be very pervasive because of hydrogen, nitrogen and sulfur rich organic species.

Bacteria and fungi promote and assist the process of decomposition, when water percolates through the waste. In actively decomposing waste the temperature rises and the pH falls rapidly

and many metal ions which are relatively insoluble at neutral pH can become dissolved in the developing leachate. These processes in turn release by-products of decomposition and rapidly use up any available oxygen which creates an anoxic environment. The decomposition processes then release further water which adds to the volume of leachate. The reaction of leachate with the gypsum can generate large volumes of hydrogen sulfide which may be released in the leachate and may also form a large component of the landfill gas. It also reacts with materials that are not themselves prone to decomposition such as fire ash and cement based building materials.

Landfills have served for many decades as ultimate disposal sites for waste foundry sand but lack of land-filling space, its environmental hazards and due to its high cost, recycling and utilization in construction materials has become a possible alternative to disposal. The use of WFS as a substitute for fine aggregates in concrete mix is one option that can alleviate waste disposal problem and also provide a new resource for construction purposes. However some concerns were raised with the use of WFS as a fine aggregate regarding the potential leaching of hazardous metals. Ji et al. (2000) determined the concentration of As, Ba, Cd, Cr, Pb, Hg, Se, Ag, Cu and Zn in 11 different spent foundry sand with regard to leaching characteristics which were found to be quite low. Leachate analysis of used foundry sand was carried out by Naik et al. (2001) and showed the presence of Fe, Ba, Mn, Zn, As, Cr, Pb, Se, Cd and Hg. Dungan and Dees (2009) also reported the presence of heavy metals in waste molding sand.

1.7 Role of Fungi

Fungi are ubiquitous in natural environment and more than 70,000 species of fungi have been described. Some estimates suggest that 1.5 million species may exist (Hawksworth and Mueller, 2005). The filamentous fungi isolated from heavy metal contaminated soil such as *Aspergillus* and *Penicillium* has high level of resistance to a number of metals which makes them attractive potential candidates for further investigations regarding their ability to remove metals from contaminated waste water (Ezzouhri et al., 2009).

1.7.1 Bioleaching

Bioleaching may be defined as an interaction between microbes and metals that causes the solubilization of metal, and is based on the ability of microorganisms to transform solid compounds, thus resulting in soluble and extractable elements (Brandl et al., 1997). The bioleaching is based on three principles, namely (i) the transformation of organic or inorganic acids (protons); (ii) oxidation and reduction reactions; and (iii) the excretion of complexing agents. Metals can be leached either directly (i.e., physical contact between microorganisms and solid material) or indirectly (Fig. 1.2).

Schinner and Burgstaller (1989) suggested that bioleaching mainly includes three groups of microorganisms: autotrophic bacteria, heterotrophic bacteria, and fungi. The use of autotrophic *Acidithiobacilli* is advantageous, because no organic carbon source is needed for their growth. On the other hand heterotrophic bacteria and fungi can be used with higher pH (i.e., alkaline and acid-consuming materials). The most effective and common bacteria for metal solubilization belong to the genus *Acidithiobacillus*. In addition, the *Aspergillus* and *Penicillium* genera are the most well studied fungi used in bioleaching studies.

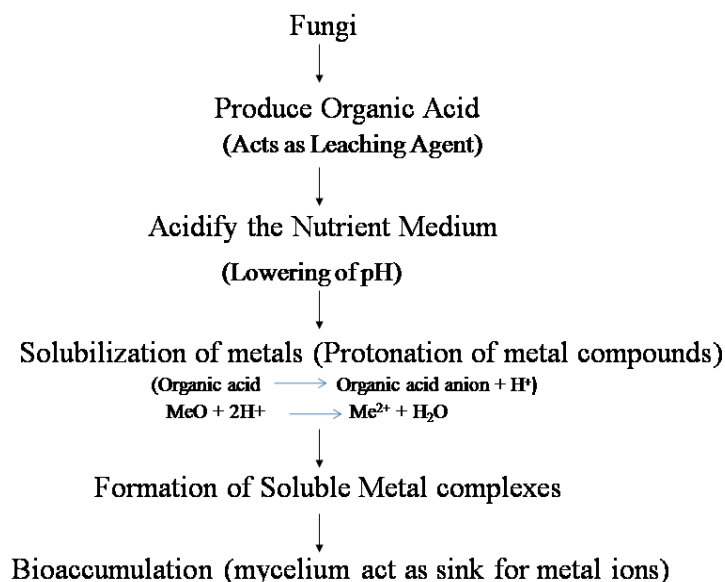


Fig 1.2: Flow Chart Showing Role of Fungi in Metal solubilization

1.7.2 Metal Immobilization

Fungal biomass provides a metal sink, either by metal biosorption to biomass (cell walls, pigments and extracellular polysaccharides), intracellular accumulation and sequestration, or precipitation of metal compounds onto and/or around hyphae. Fungi are highly effective biosorbents for a variety of metals including Ni, Zn, Ag, Cu, Cd and Pb. Fungi can precipitate a number of inorganic and organic compounds, e.g. oxalates, oxides and carbonates (Fig. 1.3).

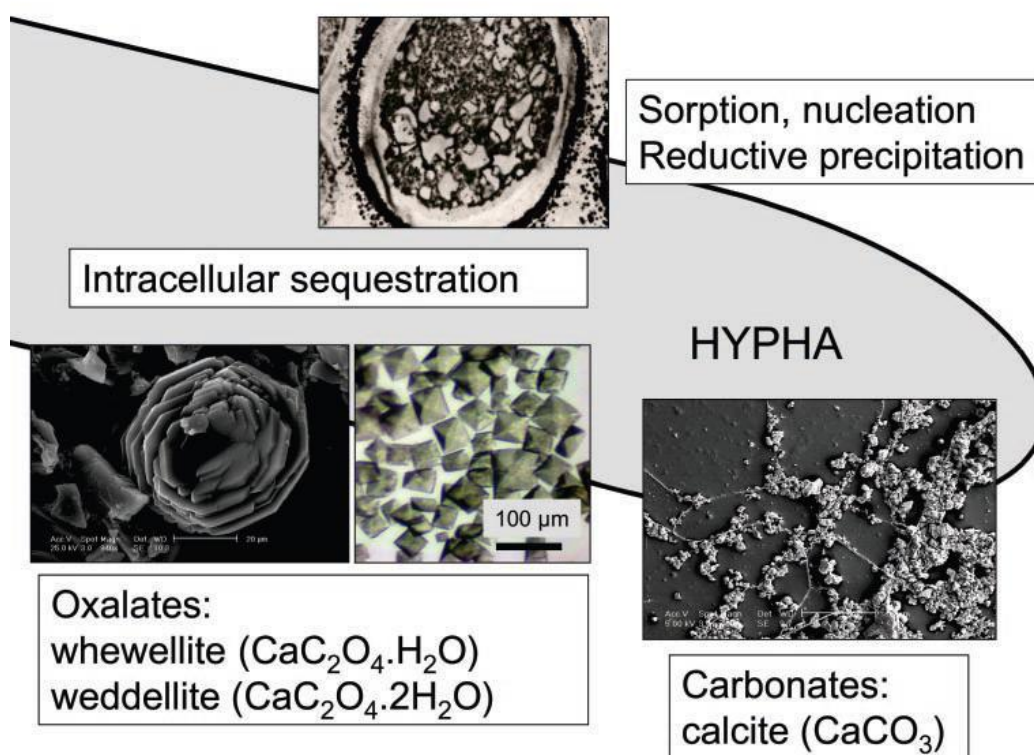


Fig 1.3: Metal Immobilization Mechanisms Exhibited by Fungi (Gadd, 2004)

1.7.3 Metal Mobilization

Fungi can solubilize metals by means of proton efflux, the production of Fe-binding siderophores, and as a result of other excreted metabolites with metal-complexing properties, e.g. amino acids, phenolic compounds, and organic acids may also be involved. The organic acids provide a source of protons for solubilization and metal-chelating anions to complex the metal

cations (Fig. 1.4). Many metal citrates are highly mobile and not readily degraded. Oxalic acid can act as a leaching agent for those metals that form soluble oxalate complexes, including Al and Fe.

Burgstaller and Schinner (1993) concluded the removal of metals from industrial wastes and by-products, low grade ores and metal-bearing minerals by fungal "heterotrophic leaching" was relevant to metal recovery and recycling and/or bioremediation of contaminated solid wastes. Sayer et al. (1995) suggested that fungal solubilization activities may also release potentially toxic metal cations into the soil solution.

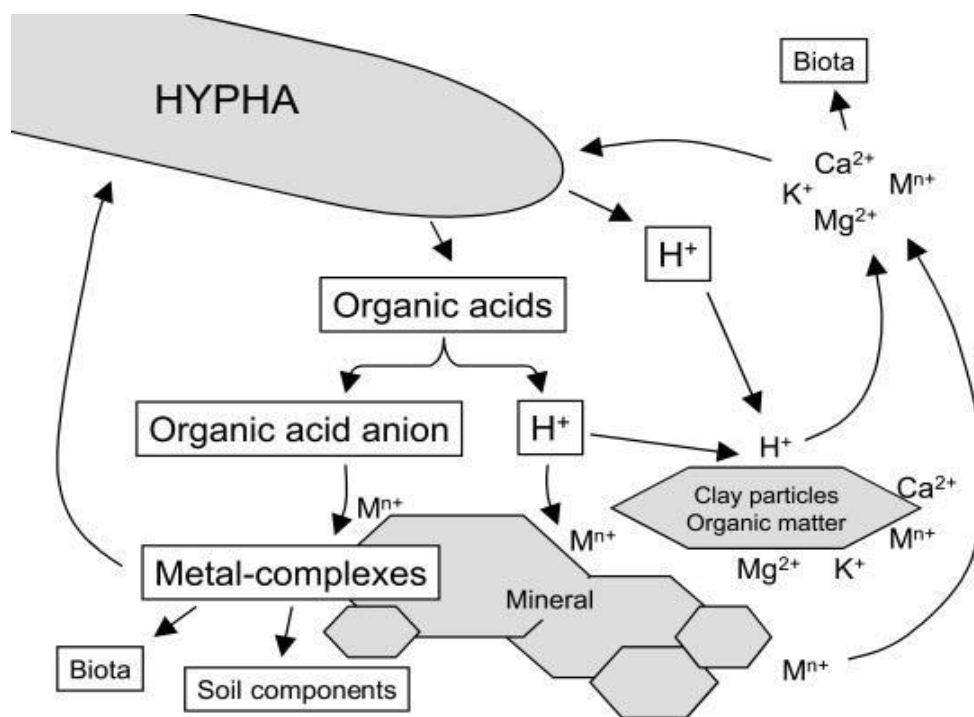


Fig 1.4: Proton and Organic Acid Ligand-Mediated Dissolution of Metals (Gadd, 2004).

1.7.4 Biomineralization

Biomineralization is the process by which living organisms produce minerals, often to harden or stiffen existing tissues. Such tissues are called mineralized tissues. It is an extremely widespread phenomenon; all six taxonomic kingdoms contain members that are able to form minerals, and over 60 different minerals have been identified in organisms. Examples include silicates in algae and diatoms in invertebrates, and calcium, phosphate and carbonate in vertebrates. Other

examples include copper, iron and gold deposits involving bacteria. Biologically-formed minerals often have special uses such as magnetic sensors in bacteria (Fe_3O_4), gravity sensing devices (CaCO_3 , CaSO_4 , BaSO_4) and iron storage and mobilization ($\text{Fe}_2\text{O}_3 \cdot \text{H}_2\text{O}$ in the protein ferritin).

Skinner and Jahren (2003) suggested that biomineralization is a widespread complex phenomenon by which organisms form heterogeneous materials composed of biologic (or organic) and inorganic compounds like carbonate, phosphate, oxalate, silica, iron, or sulfur-containing minerals. Biologically induced mineralization is also an important geological process that helps in the formation of microfossil, hot spring deposition and transfer of chemical elements (Konhauser and Ferris, 1996; Jones et al., 1997).

The unique properties and functions of biomineralization have inspired innovative high-performance composites for construction applications, as well as other new materials. Use of microorganisms within mortar/concrete leading to the process of biomineralization is now a potential field of research in concrete technology (Ramachandran et al., 2001). Thus we need to develop a strategy to reduce the environmental hazards and risks associated with the use of WFS in concrete technology. Microbial research and the need for new methods for the removal of heavy metals and other contaminants led to the great deal of expansion in the field of biological methods of industrial waste cleanup.

1.8 SIGNIFICANCE AND OBJECTIVE OF THE RESEARCH

1.8.1 Significance in Research Area

Solid waste management has become the principal environmental concerns due to increasing quantities of industrial byproducts and waste materials. Due to scarcity space and increasing cost of land filling, utilization/recycling of byproducts/waste has become an attractive alternative to disposal. Several types of byproducts and waste materials are generated. The utilization of such materials in concrete not only makes it economical, but also do help in reducing disposal problems. Reuse of bulk wastes is considered the best environmental alternative for solving the problem of disposal. One of such industrial byproducts is waste foundry sand (WFS). Waste foundry sand is a by-product of ferrous and nonferrous metal casting industries. Foundries

successfully recycle and reuse the sand many times in a foundry. When the sand can no longer be reused in the foundry, it is removed from the foundry and is termed as waste foundry sand. No work has been reported on the quality of leachate obtained from concrete made with untreated and fungal treated WFS. Therefore, it is essential to study the leachate quality and further improve it by using biological tools for its proper utilization in concrete.

1.8.2 Gap in the Research Area

Indian foundry industry is the 4th largest casting producer in the world (Metal World, 2007). There are more than 5,000 foundry units in India, having an installed capacity of approximately 7.5 million tons per annum. The majority (nearly 95%) of the foundry units in India falls under the category of small-scale industry (Metal World, 2007). These foundry units generate approximately 1710000t (1.71 million MT) waste foundry sand per year (The Institute of Indian Foundry men). In Punjab region, there are approximately 750 foundries. The main clusters of foundries are Batala (450), Ludhiana (150) and Jalandhar (150). Punjab region generate approx. 270000t (0.27 million MT) waste foundry sand per year.

Siddique et al. (2007), Naik et al. (2004), Khatib and Ellis (2001) suggested the waste foundry sand as a potential material for use in concrete. However when waste materials are used in concrete, the leachate obtained from it may contain hazardous compounds, which may possibly affect the environment. As not much work has been reported on the quality of leachate from concrete made with waste foundry sand, influence of fungi on leachate obtained from concrete using waste foundry sand and its utilization in concrete was investigated.

1.8.3 Objective of the Research Work

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

- Isolation of fungal cultures from foundry soil and characterization of waste foundry sand
- Development of appropriate fungal treatment of WFS for use in concrete
- Assessment of impacts of using fungal treated WFS on concrete properties and its leachate quality.

1.9 ORGANIZATION OF THE THESIS

The thesis consists in five chapters:

Chapter 1 introduces utilization of waste foundry sand in concrete along with their types and its global production in brief. It also includes leachate and its generation, WFS leachate, role of fungi in bioleaching and the objectives of the present work.

Chapter 2 reviews the existing literature on the use of foundry sand in concrete, strength properties of concrete using waste foundry sand, application of WFS, leachate test methods, leachate analysis of WFS, bioleaching by fungi, mechanism involved in bioleaching, metal uptake ability and biomineralization of fungi.

Chapter 3 details the materials used with their properties, isolation and screening procedure along with molecular characterization for fungal cultures, fungal treatment of WFS, initial mix design, casting of specimens for studying various properties and methodology adopted for testing of different properties.

Chapter 4 presents the results, and their analysis for isolation, screening and molecular characterization of fungal cultures, growth and pH profile of fungal cultures in WFS and without WFS, organic acid production by fungal cultures along with HPLC. Leachate analysis of control, untreated WFS containing concrete mix and fungal treated WFS containing concrete mix by ICP-MS and compared with standard limits. It also presents the concrete properties including compressive strength, water absorption and porosity of control concrete, untreated WFS containing concrete and fungal treated WFS containing concrete along with the XRD, SEM and EDS studies to identify the presence of various phases of compounds and the microstructure of the concrete made with waste foundry sand (untreated and fungal treated) in varying percentages replacement of fine aggregate.

Chapter 5 summarizes and concludes the findings of the study. Few recommendations for further studies are also discussed.

References are placed at the end.

CHAPTER 2

LITERATURE REVIEW

This chapter reviews the existing literature on the use of foundry sand in concrete, strength properties of concrete using waste foundry sand, application of WFS, leachate test methods, leachate analysis of WFS, bioleaching by fungi, mechanism involved in bioleaching, metal uptake ability and biomineralization of fungi.

2.1 By-Products

Due to large scale construction natural sand is getting depleted. So it is necessary to find out an alternative of natural sand, which can be used as partial replacement of natural sand (fine aggregate). The beneficial use of such by-products in construction materials results in reducing the cost of construction materials' ingredients and also helps in reducing disposal problem. The leachate obtained from such materials may contain hazardous compounds, which may possibly effect the environment. So, it is important to know the characteristics of leachate obtained from waste foundry sand. Understanding the leachate characteristics of WFS is essential in its disposal, environmental impact, and potential development for beneficial utilization towards solid waste management.

Several researchers have investigated the utilization of waste materials and industrial by-products in concrete and construction materials such as fly ash (Shi and Qian, 2003); sewage sludge ash (Cyr et al., 2007); crushed stone dust (Sahu et al., 2003); silica fume (Scott and Singh, 2011) and WFS (Santurde et al., 2011). Shi C and Qian J (2003) studied chemical activation of reactivity of fly ash is an effective method to increase the use of fly ash in concrete. Cyr et al. (2007) showed that sewage sludge ash (SSA) is composed of irregular grains with high specific surface area and thus lead to a significantly high water demand. SSA induced short delays of cement hydration, probably due to both minor elements of the ash and dilution effect. Compressive strengths of mortars containing 25% and 50% of SSA were always lower than those of reference mortars but it was shown that SSA has a long-term positive effect which might

be related to a slight pozzolanic activity. The amount of elements leached from SSA mortars was slightly higher than from the reference mortar.

Raoa et al. (2007) reviewed the international scenario of construction and demolition waste generated. They summarized the effect of use of recycled aggregate on the properties of fresh and hardened concrete and concluded some of the major barriers in more widespread use of RA in recycled aggregate concrete (RAC), including lack of awareness, lack of government support, nonexistence of specifications/codes for reusing these aggregates in new concrete.

Velosa and Cachim (2009) tested compositions with different percentages of hydraulic lime and a pozzolanic material. Concrete specimens were tested for mechanical strength at various ages and a pozzolanic index was determined in order to evaluate the influence of the pozzolanic material on attained mechanical strength. Schmidt et al. (2011) studied the influence of environmental temperatures on the performance of polymeric stabilizing agent in fresh cementitious materials.

Cwirzen A (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 0.23wt% 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.

Rakshvir and Barai (2006) studied various physical along with mechanical properties of recycled concrete aggregates. Recycled concrete aggregates are different from natural aggregates and concrete made from them had specific properties. The percentages of recycled concrete aggregates were varied and it was observed that properties such as compressive strength showed a decrease of up to 10% as the percentage of recycled concrete aggregates increased. Water absorption of recycled aggregates was found to be greater than natural aggregates.

Aggarwal et al. (2008) presented an experimental procedure for the design of self-compacting concrete mixes. The test results for acceptance characteristics of self-compacting concrete such

as slump flow; J-ring, V-funnel and L-Box are presented. Further, compressive strength at the ages of 7, 28, and 90 days was also determined.

Charkradhara et al. (2011) investigated the influence of different amounts of recycled coarse aggregates (RCC) obtained from a demolished RCC culvert 15 years old on the properties of recycled aggregate concrete (RAC). To analyze the behavior of concrete in both the fresh and hardened state, a coarse aggregate replacement ratio of 0, 0.25, 0.50 and 1.0 was adopted in the concrete mixes. The properties namely compressive and indirect tensile strengths, modulus of elasticity, water absorption, volume of voids, and density of hardened concrete and depth of chloride penetration were studied. From the experimental results it is observed that the concrete cured in air after 7 days of wet curing shows better strength than concrete cured completely under water for 28 days for all coarse aggregate replacement ratios. The volume of voids and water absorption of recycled aggregate concrete were 2.61 and 1.82% higher than those of normal concrete due to the high absorption capacity of old mortar adhered to recycled aggregates.

Sahu et al. (2003) studied the suitability of crushed stone dust waste as fine aggregate for concrete. Two basic mixes were chosen for natural sand to achieve M20 and M30 grade concrete. Results indicated that concrete made with this replacement attained the same compressive strength, comparable tensile strength, modulus of rupture and lower degree of shrinkage as compared to control concrete.

Cachim (2009) investigated the workability and the density of fresh concrete, and the compressive strength, tensile splitting strength, modulus of elasticity and stress-strain behaviour of hardened concrete. Replacement ratios of natural aggregates by 15% and 30% were investigated as well as water/cement ratios of 0.45 and 0.5. Results indicated that ceramic residuals could be used as partial replacement of natural aggregates in concrete without reduction of concrete properties for 15% replacement and with reductions up to 20% for 30% replacement.

Khatib (2005) investigated the properties of concrete containing fine recycled aggregate. Recycled aggregate consisted of crushed concrete (CC) or crushed brick (CB) with particles less than 5 mm in diameter. The fine aggregate in concrete was replaced with 0%, 25%, 50% and 100% CC or CB. Results showed that concrete incorporating up to 50% CB exhibited similar

long-term strength to that of the control. Even at 100% replacement of fine aggregate with CB, the reduction in strength was only 10%. Beyond 28 days of curing, the rate of strength development in concrete containing either CC or CB was higher than that of the control indicates further cementing action in the presence of fine recycled aggregate. There are several types of waste material/byproducts, which have been explored for possible use in concrete as a partial replacement of fine aggregate. Such types of materials are coal bottom ash, recycled fine aggregate, sewage sludge ash, stone dust and glass cullet, and waste foundry sand, etc.

2.2 WASTE FOUNDRY SAND (WFS)

In foundry industry, millions tones of spent materials are disposed in the world. No significant work has been reported on the use of waste foundry sand in concrete. Use of waste foundry sand in concrete and concrete related products like bricks, blocks and paving stones has been reported by Khatib and Ellis (2001), Naik et al. (2004), and Siddique et al. (2007). Bakis et al. (2006) reported the use of waste foundry sand (WFS) in asphalt concrete.

Siddique et al. (2007) investigated the effect of spent foundry sand as partial replacement of fine aggregate on the compressive strength, splitting tensile strength and modulus of elasticity of concrete. The proportions of fine aggregate replaced were 10, 20, and 30%. They concluded that compressive strength, splitting strength and modulus of elasticity increase marginally with the increase in foundry sand content.

Bakis et al. (2006) investigated the use of waste foundry sand (WFS) in asphalt concrete. Asphalt concrete mixtures were prepared with 0, 4, 7, 10, 14, 17 and 20% replacement of fine aggregate with WFS. The strength of the asphalt concrete mixtures decreases as the percentage of WFS was increased.

Naik et al. (1994) investigated the performance of fresh and hardened concrete containing discarded foundry sands in place of fine aggregate. Concrete mixes were proportioned to replace 25% and 35% by weight of regular concrete sand with clean/new foundry sand and used foundry sand. Concrete performance was evaluated with respect to compressive strength, tensile strength, and modulus of elasticity. At 28-day age, concrete containing used foundry sand showed about 20-30% lower values than concrete without used foundry sand. But concrete containing 25% and

35% clean/new foundry sand gave almost the same compressive strength as that of the control mix.

Guney et al. (2006) conducted a laboratory testing program on soil-foundry sand mixtures amended with cement and lime to assess their applicability as highway sub- base materials. The mixtures were compacted in the laboratory at a variety of moisture contents and compactive efforts and subjected to unconfined compression, California bearing ratio, and hydraulic conductivity tests. The environmental suitability of the prepared mixtures was evaluated by analyzing the effluent collected during hydraulic conductivity tests. Study showed that the strength of a mixture was highly dependent on the curing period, compactive energy, lime or cement presence, and water content at compaction. Laboratory leaching tests indicated that if these mixtures later come in contact with water that has been discharged directly to the environment (e.g., drainage through asphalt pavement), the quality of water will not be affected.

Carey and Sturtz (1995) suggested that physical properties (such as particle gradation, grain shape, fine contents, density, absorption and specific gravity, bleeding, setting time, hydraulic conductivity and leaching characteristics) of WFS help to recognize its workability and suitability in flowable fill. They suggested that particle gradation, grain fineness number (GFN) and grain shape is important determinants of flowability, compacted density and strength of a WFS mixture. The greater the GFN, the finer the average grains. Absorption indicates the process by which a liquid is drawn into the WFS matrix and fills pore space.

Siddique et al. (2009) evaluated the mechanical properties of concrete mixtures in which fine aggregate (regular sand) was partially replaced with used-foundry sand (UFS). Fine aggregate was replaced with three percentages (10%, 20%, and 30%) of UFS by weight. Compressive strength, splitting- tensile strength, flexural strength, and modulus of elasticity were determined at 28, 56, 91, and 365 days. Test results indicated a marginal increase in the strength properties of plain concrete by the inclusion of UFS as partial replacement of fine aggregate (sand) and that can be effectively used in making good quality concrete and construction materials.

Naik et al. (2001) determined the physical properties of regular concrete sand, clean foundry sand and used foundry sand. A modified ASTM C 88 was used to measure soundness of the foundry sands. In accordance with the ASTM C 88 test standard, the test samples shall be such

that it contains 100 grams of all materials retained on each sieve of the 4.75mm, 2.36 mm, 1.18 mm, 600 μ m and 300 μ m. Since foundry sand was finer than 600 μ m, only about 0.2- 2.1% of clean and used foundry sands was retained on the 4.75 μ m to 600 μ m sieve. Therefore, the ASTM sample requirement was modified to evaluate the soundness of the foundry sands for this investigation.

The material finer than the 75 μ m sieve were slightly higher for the used foundry sand relative to the clean foundry sand. This difference in the result was probably due to the presence of binders in the used foundry sand. The results for the used sand showed low values than the allowable limit. However, the used foundry sand had the highest value of all the sands tested. This is primarily due to the presence of bentonite clay binders in the used foundry sand during the soaking in the water for 24 hrs and was washed away when sieved in accordance with ASTM C 142.

Kraus et al. (2009) conducted experimental work on the use of foundry silica-dust in self-consolidating concrete (SCC). A control SCC mixture was made, in which ASTM C 618 Class C fly ash constituted 40 mass% of the total cementitious materials. Silica-dust was used to replace 10%, 20%, and 30% of fly ash at a 1:2 (fly ash to foundry dust) ratio by mass. Use of foundry dust in SCC resulted in high air content (7–10%), possibly due to reaction between foundry dust and the particular chemical admixtures used. With the increase in foundry dust content containing iron, the color of concrete changed to dark gray or black. At 30% replacement of fly ash with foundry silica-dust, the demand for high-range water-reducing admixture (HRWRA) increased. It was concluded that foundry silica-dust material could be used in producing economical SCC.

Reddia et al. (1996) conducted a series of experiments to stabilize the phenolics in foundry sands by using four different types of binders (Portland cement, fly ash, kaolinite, and bentonite). Results suggested that compressive strength was acquired relatively faster in fly ash than in cement. Lesser amounts of phenolic compounds leached from fly ash-stabilized mixes than from cement-stabilized mixes. The leachate analysis for total phenolics and 2, 4, 6-trichlorophenol indicated that stabilization increases with increase in percent replacement of foundry sand.

Siddique and Kadri (2011) studied the effect of foundry sand (FS) and metakaolin (MK) on the near surface characteristics of concrete. Results indicated that with the increase in MK content, there was a decrease in the surface absorption and sorptivity till 10% metakaolin replacement. All mixtures showed low water and compressive strength showed an inverse relation with sorptivity. Inclusion of foundry sand resulted in reduction in compressive strength.

Siddique et al. (2010) concluded that industrial by-products can produce concrete with sufficient strength and durability to replace normal concrete. Compressive strength, and split-tensile strength, was determined at 28, 90 and 365 days along with carbonation and rapid chloride penetration resistance at 90 and 365 days. The Rapid Chloride Permeability Test (RCPT) values were less than 750 coulombs at 90 days and 500 coulombs at 365 days which comes under very low category. The micro-structural investigations also suggest the nature of variation in strength at the different replacements of fine aggregates with foundry sand in concrete.

Guney et al. (2010) investigated the potential re-use of waste foundry sand in high-strength concrete production in which natural fine sand was replaced with waste foundry sand (0%, 5%, 10%, and 15%). Results showed the reduction in compressive and tensile strengths, and the elasticity modulus which is directly related to waste foundry inclusion in concrete. Concrete with 10% waste foundry sand showed similar results to that of the control one. The slump and the workability of the fresh concrete decreased with the increase of the waste foundry sand ratio. The obtained results satisfy the acceptable limits set by the American Concrete Institute (ACI).

Etxeberria et al. (2010) studied the effect of two types of foundry sands on the 28-day compressive strength and modulus of elasticity of concrete. They used chemical foundry sand and green foundry sand. Results indicated that concrete with chemical foundry sand achieved 28-day strength of 28.4 MPa, where as it was 25 MPa for green foundry sand concrete. Modulus of elasticity values at 28 days were 27.92 GPa and 27.39 GPa for concrete incorporating chemical foundry sand and green foundry sand, respectively.

Singh and Siddique (2012a) investigated the abrasion resistance and strength properties of concrete containing waste foundry sand (WFS). They replaced sand (fine aggregate) with 0%, 5%, 10%, 15% and 20% of WFS by mass. The water-to-cement ratio was maintained at 0.040 and the workability of mixtures was maintained at 85 ± 5 mm. Results showed that replacement

of sand with WFS enhanced the 28-day compressive strength by 8.3–17%, splitting tensile strength by 3.6–10.4% and modulus of elasticity by 1.7–6.4% depending upon the WFS content, and showed continuous improvement in mechanical properties up to the ages of 365 days. Inclusion of WFS as sand replacement led to improve the abrasion resistance of concrete at all ages.

Singh and Siddique (2012b) evaluated the strength and durability properties of concrete mixtures, in which natural sand was partial replaced with (WFS). They replaced natural sand five percentage (0%, 5%, 10%, 15%, and 20%) of WFS by weight. A total of five concrete mix proportions (M-1, M-2, M-3, M-4 and M-5) with and without WFS were developed. Results showed marginal increase in strength and durability properties of plain concrete by inclusion of WFS as a partial replacement of fine aggregate.

2.2.1 Applications of WFS

In 2000, approximately 9 million metric tons of waste foundry sand was disposed off in landfills by metal casting foundries in the US (Winkler and Bolshakov, 2000). Annual cost of WFS disposal was around US\$ 135–675 million including national average landfill tipping fee of foundry byproducts of US \$15–75 per ton inclusive of storage, transportation and labor costs (Winkler et al., 1999). WFS land filling not only imposes the financial burden to the foundries; also lead to cause remediation problems. This issue is increasingly addressed by alternate scenarios of beneficially reusing WFS.

Beneficial reuses of WFS span a variety of applications related to infrastructure engineering and rehabilitation works, e.g., highway embankment construction (Ham et al., 1990; Javed and Lovell, 1994; Mast and Fox, 1998; Kleven et al., 2000; Abichou et al., 2004), ground improvement (Vipulanandan et al., 2000), hydraulic barrier or liner (Abichou et al., 2004) and clay bricks (Santurde et al., 2011). These alternate applications provide cost savings for both foundries and user industries, and an environmental benefit at the local and national level.

A report “User Guidelines for Waste and By-Product Materials in Pavement Construction,” was issued by Federal Highway Administration (FHWA), which covers in detail the use of foundry sand in Controlled Low Strength Materials (CLSM) and provides guidelines for its proper usage (Federal Highway Administration, 1998). Foundry sand along with fly ash was also recognized

as suitable materials for CLSM by the U.S. Environmental Protection Agency (EPA). Utilization of foundry sand in CLSM showed negative impacts on environment due to leaching of heavy metals present in the foundry sand. Ferrous foundry sands are more commonly used in CLSM because there are concerns about the heavy metals content of non-ferrous foundry sands. The EPA does not recommend the use of non-ferrous foundry sand in CLSM because of concerns over the potential leaching of phenols and heavy metals, such as cadmium, lead, copper, nickel, and zinc (Environmental Protection Agency, 1998).

In recent years, foundry sand has been studied and used successfully in CLSM (Bhat and Lovell, 1996). Due to its lower cost, increasing availability, and satisfactory performance; it is becoming a more viable candidate for use in CLSM. Green sand is the most commonly used waste foundry sand in CLSM. After molding is completed, the sand is discarded, often in landfills, and typically at a cost of \$20/ton to \$40/ton (Bhat and Lovell, 1996).

Use of spent foundry sand in concrete related products like bricks, blocks and paving stones has been reported by Khatib and Ellis (2001), Naik et al. (2004), Fiore and Zanetti (2007), and Siddique et al. (2007). Bakis et al. (2006) investigated the use of waste foundry sand in asphalt concrete. Results showed that the strength of asphalt concrete mixtures indicated a linear decrease as the percentage of WFS was decreased, yielding values from 1.39 MPa with 0% WFS to 0.94 MPa with 20% WFS. Siddique et al. (2007) investigated the effects of spent foundry sand as partial replacement of fine aggregate on the compressive strength of concrete. Based on the test results, it was concluded that compressive strength of concrete mixes with 10%, 20% and 30% fine aggregate replacement with foundry sand was higher than the control mix in all ages which indicated that foundry sand could be successfully used in making concrete as partial replacement of fine aggregate.

Braganca et al. (2006) evaluated the production feasibility of triaxial white ware using sand from cast iron moulds as a raw material instead of silica and also concluded that (i) it is possible to produce white ware bodies with formulations containing white firing clay, glass waste and foundry sand; (ii) There was no significant variation in the density of the formulations with the foundry sand contents, which can be as high as 45% in the triaxial ceramic mass; (iii) The higher densification after firing was achieved for firing temperatures between 1200 and 1250°C, and the amount of added flux was more important than the variation in the foundry sand content. It

should be noted that it was possible to add up to 45% of foundry sand to the triaxial ceramic mass without compromising the densification of the final product. The maximum density (2.19 g cm^{-3}) was achieved in the temperature of 1200 to 1250°C.

Waste foundry sand often demonstrate soil like qualities making them potentially attractive components in manufactured soils and useful for enhancing soil blend, physical and chemical properties. It contains plant nutrients, organic carbon and clay. Dayton et al. (2010) collected waste foundry sand from 11 foundries and evaluated WFS as plant growth media for lettuce seeds. Results showed a range in germination relative to the control of from 56.3% to 125% with a medium of 96.9%. Eighty five percent of WFS germination was between 80% to 120% relative to control blends. So, WFS at varying rated with other materials will allow tailoring of manufactured soil chemical and physical properties to meet the specific growing needs. Dungan and Dees (2009) studied that total metal concentrations in the molding sands were similar to those of found in agricultural soils in which the leaching of metals were assessed by TCLP, SPLP and ASTM water leach test. It was found that the total metal concentration of majority of waste sands was at the low end of the range measured in agricultural soils.

2.3 LEACHATE TEST METHODS

Leaching methods are often categorized by static extraction tests (is a single addition) and dynamic tests (leaching fluid is renewed). Methods can also be classified as batch leaching in which the sample is placed in a given volume of leachant solution, as column or flow through systems, and as bulk or flow around systems for monolithic samples. 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). In many methods, the liquid to solid ratio (L/S) is used to quantify the volume of leachant with respect to the amount of solid sample, usually as mL/g or L/kg. It includes three types of methods:

- (i) Batch methods
- (ii) Column methods
- (iii) Combined methods

2.3.1 Batch Methods

In these methods, a sample is placed in a given volume of leachant solution for a set period of time. Most of these methods require some type of agitation to insure the constant contact between the sample and the leachant. At the end of the leaching period, the liquid is removed and analyzed. The most commonly used batch leaching methods are the Toxicity Characteristic Leaching Procedure (TCLP), Synthetic Precipitation Leaching Procedure (SPLP), and Standard Test Method for Shake Extraction of Solid Waste with Water. Further Batch methods includes: (i) Toxicity characteristic leaching procedure; (ii) Synthetic precipitation leaching procedure (SPLP); (iii) Synthetic ground-water leaching procedure; (iv) Shake extraction of solid waste with water (ASTM D 3987-1985).

2.3.2 Column Methods

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 leachant solution. Precipitation or sorption within the column may affect the results. Column leaching tests are designed to simulate the flow of percolating groundwater through a porous bed of granular material. The flow of the leaching solution may be in either down-flow or up-flow direction, and continuous or intermittent. The flow rate is generally accelerated when compared to natural flow conditions. However, it should be slow enough to allow leaching reactions to occur.

The Standard Test Method for Leaching Solid Material in a Column Apparatus (ASTM D 4874-1995) is intended to maximize the leaching of metallic species from a solid. The aqueous fluid passes through particles of known mass in a saturated up-flow mode. The Dutch Standard Column Test is also an up-flow application, and the Nord test Column Method (NORD TEST 1995) is similar to the Dutch Column test, except that column dimensions are optional. The up-flow column procedures are designed to insure that the leachant solution is equally distributed throughout the column.

2.3.3 Monolithic and Bulk Methods

Monolithic leaching methods frequently used to characterize the release of pollutants from stabilized waste materials and also used to evaluate the release of elements from a material that

normally exists as massive solid. The release of an element is a function of the exposed surface area as opposed to the mass. Flow around systems relates solubility to the surface area of a particular volume. Flow-through systems also consider the internal pore surface. And some systems take into account the rate of diffusion of the leachant solution into the pores.

2.3.4 Combined Methods

A combined leaching protocol has designed by the International Ash Working Group (IAWG) which quickly determine the total leachable elements in a material and to estimate metal release in a normal environmental setting (van der Sloot, 1998). It combines the sequential batch availability test (NEN 7341- 1993) with a serial batch extraction using water. A two-step availability test of fine-grained material, at an L/S of 50 and controlled pH 4 and 8, is used to determine leachability at the upper and lower pH limits found in natural environments. From the total acid consumption, the acid neutralization capacity of the material is estimated. Total elemental release as a function of time is estimated by leaching at several L/S values between 1 and 100 in a serial batch test with water. The release of contaminants is usually expressed in mg/kg leached against the L/S ratio

2.4 LEACHATE ANALYSIS OF WASTE FOUNDRY SAND

Presently there are a number of alternative leachate analyses available, each designed specifically to deal with various waste types and stockpiling applications. Leachate analysis is conducted in an effort to characterize the mobile phase of water or stockpiled material. Thus data obtained from detailed leachate analysis are then used to classify the waste or to estimate ground water or surface water contamination.

Earlier, the concentration of organic compounds in ground water leached from an iron foundry landfill was determined by Fero et al. (1986). Test samples derived from all monitoring wells showed all measured organics were below their respective detection limits. Later on, Engroff et al. (1989) evaluated the leachate characteristics of foundry sand derived from nine common core binder systems using the toxicity characteristic leaching procedure (TCLP) test method. The test data showed the presence of a wide range of organic compounds, but their concentrations were low.

Naik et al. (2001) conducted tests on various samples to determine the leachability of CLSM mixtures. For this purpose, shake extraction of solid waste with water (ASTM D 3987- 1985), leaching method was selected. They concluded that: (i) these materials contained very little organics; leachate derived from each material was analyzed for inorganic constituents in accordance with WDNR requirements; (ii) clean foundry sand met both the WDNR preventive action limits (PAL) and the enforcement standards (ES) of Ground Water Quality Standards (GWQS); (iii) spent foundry sand met all parameters of ES, but it exceeds the PAL for lead and chromium. However, spent foundry sand met all requirements, except for Fe, for public welfare-related GWQS; (iv) both foundry sands met all the requirements for the Drinking Water Standard (DWS).

Douglas (2003) examined the TCLP extractions on waste sand obtained directly from the foundry and on mixtures of waste sand with various additives. Results showed that TCLP extracts of used foundry sand without any additives contain high concentrations of copper, lead and zinc well over the regulatory limits for hazardous waste of 5 mg/L. The difference between copper and lead results for each of the treated sands and the untreated sands were statistically significant ($p < 0.01$). Adding iron to the TCLP extraction of foundry sand decreases the copper and lead concentrations in the extract because of reduction by iron.

On the contrary, results shown by Deng and Tikalsky (2008) indicated that sand is not hazardous with high confidence per metallic elements, and a leaching program is not mandatory in assessing whether sand is hazardous or not regarding metallic elements. They collected and assessed one thousand one hundred eighty two data sets addressing bulk and leaching characterization of up to 24 metallic elements in sand, dust, and slag using statistical methodologies.

Only a fraction of metallic elements are extracted from as-received waste streams. Through comparisons of statistical parameters including estimated means, 95th percentiles and data distributions, sand is found less contaminated by most metallic elements than dust and slag, but metallic elements in sand leachate are in the similar levels as those in dust and slag leachates. Fe, Zn, and Al are highly concentrated in dust and slag on average, which suggests their potential reclamations. In this study, three leaching protocols are involved across collected data sets

including TCLP, synthetic precipitation leaching procedure SPLP, and standard test method for shake extraction of solid waste with water (ASTM D 3987-1985).

They concluded that dust and slag are more contaminated with most reported metallic elements than sand. Metallic elements including B, Cd, Cu, Fe, Pb, and Zn in dust have factors over 10 versus those in sand. Metallic elements including Ba, Be, Mn, and Ti in slag have factors over 10 versus those in sand. Fe in dust at average mass ratio of 16.7%, and Fe, Al, and Mg in slag at average mass ratios of 9.99, 1.18, and 1.04%, respectively, are relatively highly concentrated and thus to be considered reclaimable.

Although dust and slag are more metallically contaminated than sand, these three waste streams have similar levels of most metallic elements in their leachates by TCLP, SPLP, and ASTM D 3987- 1985, respectively. It is found, regarding mean concentrations of metallic elements, that leachates of sand, dust, and slag by three leaching protocols contain generally less than 1% of the concentrations measured in corresponding bulk waste streams, which indicates that only a fraction of metallic elements can be extracted from as-received waste streams.

Ji et al. (2000) presented the chemical analysis and leaching characteristics of waste foundry sand of four sand types (Green sands, Furan/acid sand, Phenolic sands and silicate sands). It was found that all spent/waste foundry sands contain poly aromatic hydrocarbon (PAH) compounds. The PAHs in green sands are much higher than those in chemical binder spent sands, even though phenolic/ester sands have higher PAHs than furan/acid and silicate sands. The leached metals were very low in all waste foundry sands. The leached Cr increases with increasing pH of the eluted solution, which can be used in practice of shortening the leaching time of the waste foundry sands.

Deng (2009) analyzed the leaching characteristics of WFS by three leaching protocols, namely, TCLP, SPLP and ASTM D3987. TCLP and SPLP leachates are on average moderate acid, with final pH values between 5 and 7. According to the 95th percentiles, upper boundary indicators, all leachate streams had regulated metallic chemicals (Ag, As, Ba, Cd, Cr, Hg, Pb and Se) well below TCLP toxicity thresholds. According to the mean and median, middle levels or most metallic chemicals in TCLP leachates are higher than those in SPLP or ASTM D3987 leachates

by varying factors up to 50, which was thought associated with TCLP was more aggressive than other two protocols.

Dungan and Dees (2009) studied total metal concentrations in molding sands and the leached metals were assessed via TCLP, SPLP and ASTM water leach test. They used TCLP to assess the leachability of other metals including Be, Cu, Ni, Sb and Zn. The vast majority of molding sands were found to leach these metals at the levels below the metal detection limits if 0.01, 0.10, 0.14, 0.02 and 0.41 mg/L respectively. In this study it was concluded that total metal concentrations in the majority of the waste molding sands were at the low end of range measured in the agricultural soils. With the exceptions of few sands, only Co, Cu and Ni were above the range found in the soils.

Miguel et al. (2012) investigated metal concentrations in WFSs and compared to those in virgin silica sands (VSSs), surface soils and soil guidance levels. A total analysis for Ag, Al, Ba, Be, Cd, Co, Cr, Cu, Fe, Mg, Mn, Mo, Ni, Pb, Sb, Te, Tl, V, and Zn was conducted on 96 WFSs and 14 VSSs collected from 17 small and medium-sized foundries. The majority of WFSs analyzed, regardless of metal cast and binder type, contained metal concentrations similar to those found in VSSs and native soils. In several cases where alkyd urethane binder was used, Co and Pb concentrations were elevated in the waste sands. Elevated Cr, Mo, Ni, and Tl concentrations associated with VSSs should not be an issue since these metals are bound within the silica sand matrix. Because of the naturally low metal concentrations found in most WFSs examined in this study, they should not be considered hazardous waste, thus making them available for encapsulated and unencapsulated beneficial use applications.

The waste sands are then often disposed of in uncontrolled landfills, sometimes at the foundries, or sent to controlled landfills. Due to high tipping fees, the disposal of sands in controlled landfills is an economic burden to foundries, especially as landfills reach the end of their life expectancy. While there is economic advantage to dispose of waste foundry sands (WFSs) in uncontrolled landfills, this practice could potentially contribute to groundwater pollution (Ham et al., 1987; Riediker et al., 2000). Although disposal regulations for WFSs do vary among countries, the majority of molding sands are not considered to be hazardous in nature (Deng, 2009; Dungan and Dees, 2009; Siddique et al., 2010). As a result, there are efforts underway in

countries like Argentina, Brazil, South Africa, and the United States to beneficially use WFSs in geotechnical (e.g. concrete, asphalt road-sub base) and agricultural (e.g. manufactured soils) applications (Lindsay and Logan, 2005; Siddique et al., 2009). Beneficial use of WFSs preserves natural resources by decreasing the demand for virgin materials, conserves energy and reduces greenhouse gas emissions through reduced mining activities, and decreases the economic and environmental burdens of disposal. Despite these potential benefits, many regulators are reluctant to issue beneficial use permits due to limited information about constituent concentrations in WFSs.

Dungan (2008) characterize trace metals and EPA-priority polycyclic aromatic hydrocarbons (PAHs) and phenolics in ferrous and non-ferrous SFSs over a one-year period. Overall, the total metal concentrations in the SFSs were similar to those found in native soils, while the PAHs and phenolic concentrations were relatively low. Metal leaching tests were also performed, which revealed that the SFSs have a low metal leaching potential under the specific test conditions. The data from this study suggests that the majority of SFSs are not hazardous in nature, except those that use olivine sands or are from brass foundries, due to the presence of elevated concentrations of Ni or Cu, Pb, and Zn, respectively. This information will be useful to environmental regulators who are considering including SFSs in their beneficial use regulations.

Kendall (2003) examined the limitations of the TCLP and the way it is used by studying a particular example. Waste casting sand from brass foundries to which iron metal has been added passes the TCLP test but when placed in a landfill for several years may start to leach lead, copper, and zinc. Results of TCLP tests of waste sand alone and with the additives iron metal, zinc metal, hydrous ferric oxide, and hematite are reported. Three processes were studied: reduction by metallic iron, sorption by hydrous ferric oxide, and precipitation of hydroxides. Lead, copper, and zinc behave differently with respect to these three processes, and their measurement allows some deductions as to what is occurring in a TCLP test or a landfill. Iron addition does not result in long-term stabilization of a waste placed in the ground. The chemistry of a laboratory extraction can be very different from the chemistry of a waste placed in the environment. Wastes that are treated to pass the TCLP test, but are not permanently stabilized, are a threat to the environment.

Dungan et al. (2009) characterize the structure and composition of bacterial communities in sandy loam and silty clay soils amended with 30% spent sand from iron, aluminum, and steel foundries. All spent foundry sand (SFS) blends were grown with or without perennial ryegrass and samples were collected at 4 weeks and 6 months. Phylogenetic analysis of bacterial isolates (total of 309) from the SFS blends showed that they were dominated by Actinobacteria (46%), Proteobacteria (29%), and Bacilli (20%), with fewer numbers belonging to Bacteroidetes (5%). While the addition of SFS to soil does bring about bacterial community level changes, these changes are similar to that of blending soil with clean silica sand.

2.5 BIOLEACHING

In general, bioleaching is a process described as “the dissolution of metals from their mineral source by certain naturally occurring microorganisms” or “the use of microorganisms to transform elements so that the elements can be extracted from a material when water is filtered through it”. Additionally, Brandl (2001) used the term “biooxidation”. The following are the various definitions of bioleaching have been given:

- Boscawen (1987) defined as “The winning of metals with the aid of bacteria, based on the capacity of certain bacteria of the genus *Thiobacillus* to convert sparingly soluble metal compounds by biochemical reaction mechanisms into water-soluble metal sulfates.”
- Krebs et al. (1997) defined as “Bioleaching processes are based on the ability of microorganisms (i.e., bacteria and fungi) to transform solid compounds, resulting in soluble and extractable elements which can be recovered.”
- Lundgren et al. (1986) defined as “Biochemical oxidation process involving microorganisms as the catalyst. An insoluble inorganic substrate is oxidized to a soluble form.”

The function of the microbes in the bioleaching process was more clearly defined only from the 1950s, however Brierley (1990) and Brombacher et al. (1997) demonstrated the bacterial catalysis of iron oxidation and sulfuric acid formation in mine waters. The biotechnology of microbial mining was started where bacterial oxidation processes were used for copper extraction from sulphidic ores (Lundgren et al., 1986). Since then, these microbiological techniques have gained more attention, and are currently used on an industrial scale for the

recovery of copper, gold, uranium and zinc from low-grade ores or from low-grade mineral resources (Brombacher et al., 1997).

2.5.1 Bioleaching of Solid Waste

Recently, there have been some interests in the application of bioleaching from mining ores to industrial wastes, as increasingly vast quantities of hazardous industrial waste (e.g., fly ash, waste foundry sand, slag and filter dust etc.) are produced worldwide. Initially, the main focus of bioleaching was the recovery of metals from insoluble metal sulphide minerals in mining ores. It was based on the ability of microorganisms to oxidize reduced iron and sulphur compounds. The examples of industrial waste materials that are microbially treated for metal recovery are presented in Table 3. Due to their high metal concentrations, these waste materials can be considered as ‘artificial ores’. They can serve as secondary raw materials and may reduce the demand for primary mineral resources (Brandl, 2001). Brombacher et al. (1997) suggested the removal of metals from these industrial wastes brings about a detoxification of the residues and thus improves environmental quality.

2.5.2 Microorganisms in Bioleaching

Chemolithoautotrophic bacteria, heterotrophic bacteria, and heterotrophic fungi are three groups of microorganisms used for the leaching process. The most effective and common bacteria for metal solubilization belong to the genus *Bacillus*, but *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* are of industrial importance. In case of fungi, *Aspergillus* and *Penicillium* are the most significant genera used in bioleaching studies. Table 2.1 gives an overview on some selected microorganisms that are well known to mediate metal bioleaching reactions (Krebs et al., 1997).

All fungi are chemoheterotrophs, requiring organic compounds for energy and carbon. Fungi solubilize metal compounds by excreted acidity, mainly in the form of organic acids. This is greatly advantageous, because organic acids increase the solubility of metal ions at non-acidic pH values by complexation.

Additionally, complexation between metal ions and organic acid anions may reduce the toxicity of heavy metal ions. Among these, the strains *Aspergillus niger* and *Penicillium simplicissimum*

are probably the most widely used. *Aspergillus niger* and *Penicillium simplicissimum* have been extensively used to leach and detoxify metals from a variety of materials, such as silicates (Castro et al., 2000), low grade laterite ores (Valix et al., 2001), copper mine residues (Mulligan et al., 1999), municipal solid waste fly ash (Bosshard et al., 1996), filter dust (Burgstaller et al., 1992; Muller et al., 1995) and electronic scrap (Brandl et al., 1997).

Table 2.1: Industrial Waste Materials that are Microbially Treated for Metal Recovery

Waste Material	Metals Leached	Organisms Applied	References
Catalysts	V	<i>Thiobacillus thiooxidans</i>	Briand et al. (1999)
		<i>Bacillus</i> spp.	
	Cu	filamentous fungus	Hahn et al. (1993)
Electronic scrap	Al, Cu, Ni, Pb, Sn, Zn	<i>Thiobacillus thiooxidans</i> , <i>Thiobacillus ferrooxidans</i> , <i>Aspergillus,niger</i> , <i>Penicillium simplicissimum</i>	Brandl et al. (1997)
Fly ash	Al, Cd, Cr, Cu, Fe, Mn, Ni, Pb, Zn	<i>Aspergillus niger</i>	Bosshard et al. (1996)
Fly ash	Cd, Cr, Cu, Ni, Pb, Zn	<i>Pseudomonas putida</i> , <i>Thiobacillus thiooxidans</i>	Krebs et al. (1997)
Filter residue	Filter residue	<i>Penicillium</i> spp.	Schinner and Burgstaller (1989)
Belt filter press Solids	Cu	<i>Thiobacillus ferrooxidans</i>	Clark and Ehrlich (1992)

2.5.3 Role of Fungi in Bioleaching

Bioleaching of waste products by chemolithoautotrophs and heterotrophs was reported in several different ways such as; spent catalyst (Bredberg et al., 2004), coal fly ash (Seidel et al., 2001) by chemolithoautotrophs and the leaching of copper mine residues (Mulligan et al., 1999), municipal solid waste fly ash (Bosshard et al., 1996), electronic scrap (Brandl et al., 1997) by heterotrophic fungi. For the commercially important bacterial and fungal species, the temperature for optimal growth and activity is ~ 30° C. The pH range for the growth and leaching activities of chemolithoautotrophs lies between 1.0 and 5.5, with the optimum at around

pH of 2.4. For the growth and production of leaching agents heterotrophic fungi are dependent on an organic carbon, whereas chemolithoautotrophic bacteria (*Acidithiobacillus*) only need carbon dioxide and a reduced iron or sulfur compound for growth. Therefore, a significant amount of organic carbon has to be supplied externally for the fungal leaching.

Burgstaller and Schinner (1993a) suggested that bioleaching of alkaline solid waste by chemolitho autotrophs require a pH adjustment using commercial acids. In contrast, fungi can withstand a much wider pH range, typically from 2 to 7. This is an important characteristic property of fungi that enables them to be used with many types of solid waste, including those of alkaline, acid-reducing materials. However, metabolites excreted by fungi are strongly influenced by the medium composition and the leaching environment. The organic acids excreted by *Aspergillus niger* are different when the pH varies from acidic to alkaline. Variations in the quantity or quality of metabolites excreted due to the leaching environment leads to extra cautions during the control and optimization of fungal bioleaching.

The organic acids produced by the fungi form complexes with the metal ions and enhance metal solubilization. The complexes reduce the toxicity of heavy metal ions to the fungi and thus the fungi are fairly resistant to heavy metals. Burgstaller and Schinner (1993a) reported that fungi are generally more tolerant of heavy metals than heterotrophic bacteria and therefore, the inhibition of fungal leaching processes by heavy metal toxicity seems to be less than the latter. Burgstaller and Schinner (1993b) concluded the utilization of heterotrophic fungi to leach solid wastes generally resulted in a faster leaching process and with a shorter lag phase. It has been reported that the growth of autotrophic bacteria is slow compared to heterotrophic microorganisms due to the lower energy yield at comparable substrate consumption

2.5.4 Mechanisms of Bioleaching

Brandl (2001) suggested the ability of a variety of microorganisms (i.e., bacteria and fungi) to mobilize and leach metals from solid materials was based mainly on three principles: redoxolysis (oxidation and reduction reactions), acidolysis (the formation of organic or inorganic acids), and complexolysis (the excretion of complexing agents).

2.5.4 (a) Redoxolysis

Redoxolysis includes direct and indirect mechanism. In the direct mechanism, bacterial/ fungal metal leaching via redox reaction, metals are solubilized by enzymatic reactions through a physical contact between the microorganisms and the leaching materials, which lead to the destruction of the mineral. In the indirect redox mechanism, the oxidation of ions, such as Fe^{2+} to Fe^{3+} by bacteria and subsequently the Fe^{3+} (as the oxidizing agent) in turn dissolves the metal chemically from the solid material.

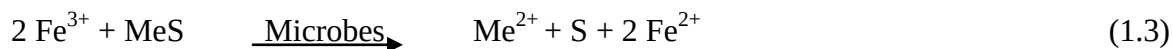
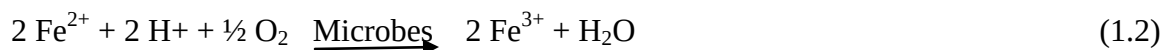
(i) *Direct Mechanism*

In direct leaching of metals, oxidation or reduction may occur, which involves the transfer of electron either from the solid structure (i.e., oxidation) to an electron acceptor (normally oxygen); or the injection of electrons into the solid structure from an electron donor, such as H_2 (i.e., reduction) (Ehrlich, 1992). Direct leaching requires physical contact between the microbial cell and the mineral sulphide surface, and the oxidation to sulphate takes place via several enzymatically catalyzed steps. Direct microbial leaching can be described according to the following reaction: In the following equation, MeS is the metal sulphide (Bosecker, 1997).



(ii) *Indirect Mechanism*

The oxidation of reduced metals through the “indirect” mechanism is mediated by a ferric iron (Fe^{3+}), originating from the microbial oxidation of ferrous iron (Fe^{2+}) compounds present in the minerals or added externally as an energy source. Ferric iron is an oxidizing agent and can oxidize reduced compounds (e.g., metal sulphides), resulting in it being reduced to a ferrous iron, which in turn can be microbially oxidized again (Brandl, 2001). Indirect leaching of metal sulphides can be described according to the following reaction (Ehrlich, 1997):





In the above MeS is the metal sulphide. The above reactions occur best in a pH range of 1.5 to 2.5. An example of redoxolysis in fungal bioleaching is the reduction of ferric iron and manganese, mediated by an oxalic acid in an acidic environment (Burgstaller and Schinner, 1993a).

2.5.4 (b) Acidolysis

The second mechanism of metal solubilization is through the formation of organic or inorganic acids. Examples include the production of citric acid by *Aspergillus niger* and *Penicillium simplicissimum*, and sulphuric acid by *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. In this process, solubilization occurs via the protonation of the metal compound, making it less available to the metal cation and promoting the dissolution of the solid (Hughes and Poole, 1991). For instance, protons protonate the anion of the insoluble metal compounds, such as the oxygen atoms covering the surface of the metal compound. The protons and the oxygen combine with water and the metal is therefore detached from the surface (Burgstaller and Schinner, 1993a). Equation 1.5 shows an acidolysis reaction. Protons are obtained from the acids produced, and its maximum amount available determines the amount of metal oxides solubilized (Burgstaller et al., 1992). This process is usually fast and it is the most important mechanism for fungal bioleaching.



In the above, MeO is the metal oxide.

2.5.4 (c) Complexolysis

This mechanism involves the metal extraction occurred by complexing agents. It is a relatively slower mechanism compared to acidolysis. Organic acids can leach metals through complexation to form soluble metal complexes. The solubilization of metal ions is based on the complexing capacity of a molecule. If the bonds between metal ions and ligands are stronger than the lattice bonds between metal ions with solid particles, the metal will be successfully leached out from the solid particles (Ehrlich, 1992). Apart from organic acids, other metabolites, such as

siderophores can also complex and solubilize metals. Siderophores are low-molecular weight chelating agents which could solubilize ferric iron, magnesium, manganese and chromium (Gadd and Sayer, 2000). Additionally, the complexation of heavy metals can reduce the metal toxicity to the fungi when high concentrations of metals are present.

2.5.5 Bioaccumulation

Bioaccumulation can be another important mechanism in fungal bioleaching (Burgstaller and Schinner, 1993a). In the accumulation process, the mycelium functions as a “sink” for the metal ions. The accumulation of metal ions from the leaching solution through active metabolic reactions and passive adsorption upsets the equilibrium between the solid and dissolved metal, thus causing the continuous solubilization of the metal. The fungal cell wall contains many different functional groups (e.g. hydroxyl, amine, carboxyl, phosphate and sulphhydryl groups), which are able to bind metal ions to a greater or lesser extent (Kapoor and Viraraghavan, 1995). Among the filamentous fungi, the genera of *Aspergillus* and *Penicillium* have been reported to have a high ability to accumulate heavy metals from their external environment (Volesky, 1991).

Burgstaller and Schinner (1993a) isolated and used the most active leaching fungi which belong to the genera *Penicillium* or *Aspergillus*. They reported *Aspergillus niger* and *Penicillium simplicissimum* to be the most important species because of their ability to excrete abundant amounts of organic acids for solubilization of metal compounds such as oxides, carbonates, silicates and hydroxides.

Dutton et al. (1993); Makela et al. (2002) investigated the presence of unspecific metal-chelating compounds such as organic acids in the fungal cultures, as the low pH of the culture filtrates suggests the production of these acids. Oxalic, citric and succinic acids were detected in all fungal cultures, and the addition of iron to the liquid MMN (Melin-Norkrans Medium) medium decreased their production by different isolates.

Sayer et al. (1995) demonstrated that *Aspergillus niger* (*A. niger*) and *Penicillium simplicissimum* (*P. simplicissimum*), as well as several natural isolates, can solubilize a wide range of insoluble metal compounds which, for *A. niger* and *P. simplicissimum*, must primarily result from organic acid production.

Bosshard et al. (1996) examined the biological leaching of fly ash from municipal waste incineration by *Aspergillus niger* in batch cultures and compared it with chemical leaching. *Aspergillus niger* grew in the presence of up to 10% (w/v) of fly ash in the medium and produced gluconate, whereas in its absence citrate was produced (in one step process). Variation of the fly ash concentration in the growth medium resulted in different amounts of solubilized metals. A total of 3% (w/v) fly ash generally gave maximum extraction yields (in percent of the amount applied). In a two-step process *Aspergillus niger* first was cultivated in the growth medium, and subsequently the microbiologically produced citric acid was used as the leaching agent. At 6% (w/v) fly ash, the amounts of leached metals (leaching for 1 day) were 81% of Cd, 66% of Zn, 57% of Cu, 52% of Pb, 32% of Mn, 27% of Al, and less than 10% of Cr, Fe, and Ni, respectively. Chemical leaching with commercial citric acid of equal molarity was only slightly higher than microbial leaching.

Modak et al. (1996) used non-living waste biomass from *Aspergillus niger* along with wheat bran as a biosorbent for the removal of Zn and Cu from aqueous solution. The binding capacity of the biomass for Cu was observed to be higher than that of Zn. the metal uptake was found to be a function of the initial metal concentration, the biomass loading and pH. The metal uptake of Cu by the biomass decreased in the presence 16 of Co ion. The uptake of Cu by the biomass decreased in the presence of the Zn and vice versa. The decreased in the metal uptake was depends on the concentration of metal ions in two compounds in aqueous solution.

Wasay et al. (1998) developed a bioremediation process using the fungus *Aspergillus niger* to produce weak organic acids for the leaching of heavy metals from contaminated soils. The fungus was cultivated on the surface of three contaminated soils (a clay loam, a loam, and a sandy clay loam) for 15 days at 30°C and at a pH <4 to favor the production of citric acid rather than oxalic acid which hinders Pb leaching. For the clay loam, Cr, Mn, Pb, and Hg were leached to levels of 37, 41, 85, and 91%, respectively. For the loam, the leaching of Cd and Pb was found to reach levels of 99.7 and 83%, respectively. For the sandy clay loam, Cd, Cu, Pb, and Zn were leached to levels of 99, 94, 58, and 99%, respectively.

Yestis et al. (1998) carried out biosorption studies with the white-rot fungi *Polyporous versicolor* and *Phanaerochaete chrysosporium* for Cu (II), Cr (III), Cd (II), Ni (II) and Pb (II) under same

operating conditions. Result showed that both were effective in removing Pb (II) from aqueous solutions with maximum biosorption capacity of 57.5 and 110 mg Pb (II)/g dry biomass.

Kovacevic et al. (2000) used fungal mycelium pellets of *Aspergillus niger* 405 for biosorption of metal ions including Cu^{2+} , Zn^{2+} , Ni^{2+} and CrO_4^{2-} . It was found that the fungal biomass good affinity for binding of Cu^{2+} , Ni^{2+} and Zn^{2+} at pH ranging from 4 and 6. The uptake of copper and zinc was reported to be 46 %, nickel 30 % and chromium 20 %.

Mulligan and Cloutier (2003) evaluated the use of the fungus *A. niger* for bioleaching and then to determined the effect of process steps, the tailings concentration and type of substrate. An oxidized mining tailing containing mainly copper (7240 mg kg^{-1} tailings) was studied. A sucrose and mineral salts medium was initially used to produce citric and gluconic acids by *A. niger* at various concentrations of tailings (1, 5, 7, 10 and 15% w/v). Maximal removal of up to 60% of the copper was obtained for the 5% tailings when the organic acid supernatant was added to the tailings. In a single step process, *A. niger* was then grown in the presence of mining tailings at various concentrations. Maximum copper solubilization (63%) occurred with 10% mining tailings using sucrose as the substrate. Other substrates were then evaluated including molasses, corn cobs and brewery waste (10% mining tailings). Sucrose gave the best results for copper removal, followed by molasses, corn cobs and brewery waste.

Bishnoi and Garima, (2004) reported that common filamentous fungi can absorb heavy metals (Zn, Cd, Pb, Fe, Ni, Ag, Ra, U and Th) from aqueous solutions to varying extents. Fungal biosorption largely depends on parameters such as pH, metal ion, biomass concentration and presence of various legends in the solutions. When compared with commercial ion exchange resins, carbon and metal oxides, fungal derivatives generally perform well.

Sanyal et al. (2005) showed that reaction of the fungus, *Fusarium oxysporum*, with the aqueous heavy-metal ions Pb^{2+} and Cd^{2+} results in the one-step formation of the corresponding metal carbonates. The metal carbonates are formed by reaction of the heavy-metal ions with CO_2 produced by the fungus during metabolism and thus provide a completely biological method for production of crystals of metal carbonates. The PbCO_3 and CdCO_3 crystals thus produced have interesting morphologies that are shown to arise because of interaction of the growing crystals with specific proteins secreted by the fungus during reaction. An additional advantage of this

approach is that the reaction leads to detoxification of the aqueous solution and could have immense potential for bioremediation of heavy metals. Under conditions of this study, the metal ions are not toxic to the fungus, which readily grows after exposure to the metal ions.

Ahmad et al. (2005) evaluated the biosorption potential of two isolates belonging to the predominant genera *Aspergillus* and *Rhizopus* which were isolated from agricultural field treated with sewage/ industrial effluents for Cr and Cd. Pretreated, dead biomass of above fungi was used for bioadsorption experiment at pH value 4.5 with the biomass, 1-5 mg in a 100 ml metal solution of different concentration (2, 4, 6 and 8 mM) with a contact time of 18 hrs and agitation, 120 rpm. Bioadsorption of Cr ranged from 6.20- 9.5 mg/g of dry mass at one or other initial metal concentrations by *Aspergillus* and *Rhizopus* sp. The bioadsorption of Cd was ranged from 2.3-8.21 mg/g. On the comparative basis *Rhizopus* sp. could bioadsorbed higher concentration of both metals as compared to *Aspergillus* sp. The findings revealed that fungi of metal polluted sites showed higher metal tolerance and bioadsorption capacity of chromium and cadmium.

Mishra et al. (2001) conducted a field-scale study in a 4000 m² plot of land contaminated with an oily sludge by use of a carrier-based hydrocarbon-degrading bacterial consortium for bioremediation. The plot was divided into four blocks, based on the extent of contamination. Blocks A, B, and C were treated with the bacterial consortium, whereas Block D was maintained as an untreated control. In Block A, at time zero, i.e., at the beginning of the experiment, the soil contained as much as 99.2 g/kg of total petroleum hydrocarbon (TPH). The application of a bacterial consortium (1 kg carrier-based bacterial consortium/10 m² area) and nutrients degraded 90.2% of the TPH in 120 days, whereas in block D only 16.8% of the TPH was degraded. This study validated the large-scale use of a carrier-based bacterial consortium and nutrients for the treatment of land contaminated with oily sludge, a hazardous hydrocarbon waste generated by petroleum industry.

Ellouze et al. (2007) studied the efficiency of five different fungal strains in reducing the toxicity characteristics of landfill leachate. Young landfill leachates (LFL) collected from Djebel Chekir (Tunisia) was treated using selected strains of *Trametes trogii*, *Phanerochaete chrysosporium*, *Lentinus tigrinus* and *Aspergillus niger*. Experiments were undertaken at different concentrations of the effluent up to 100%. Chemical oxygen demand (COD) removal efficiencies for *P. chrysosporium*, *T. trogii* and *L. tigrinus* were of 68, 79 and 90%, respectively. Above 50% of

LFL, the effluent was toxic to these strains and caused growth inhibition indicating the sensitivity of these strains to concentrated LFL. Comparatively to the other tested strains, *A. niger* showed to tolerate raw LFL since it grew at 100% of LFL.

Zafar et al. (2007) isolated metal-resistant fungi from wastewater-treated soil belonging to genera *Aspergillus*, *Penicillium*, *Alternaria*, *Geotrichum*, *Fusarium*, *Rhizopus*, *Monilia* and *Trichoderma*. Minimum inhibitory concentrations (MIC) for Cd, Ni, Cr, Cu, and Co were determined. The MIC ranged from 0.2 to 5 mg ml⁻¹ for Cd, followed by Ni (0.1–4 mg ml⁻¹), Cr (0.3–7 mg ml⁻¹), Cu (0.6–9 mg ml⁻¹) and for Co (0.1–5 mg ml⁻¹) depending on the isolate.. Biosorption experiments were conducted with initial metal concentrations of 2, 4, 6 and 8 mM with a contact time of 4 h and wet fungal biomass (1–5 g) at 25 °C. Maximum biosorption of Cr and Cd ions was found at 6 mM initial metal concentration. *Aspergillus* sp.1 accumulated 1.20 mg of Cr and 2.72 mg of Cd per gram of biomass. Accumulation of these two metals by very tolerant *Aspergillus* sp.2 isolate was at par with relatively less tolerant *Aspergillus* sp.1 isolate. *Rhizopus* sp. accumulated 4.33 mg of Cr and 2.72 mg of Cd per g of biomass.

Diwaniyan et al. (2010) investigated decolorization of six synthetic dyes and two raw textile effluents (A and B) by eight basidiomycetous fungi. Among eight basidiomycetous fungi, fungal isolate RCK-1 decolorized textile effluent A maximally (42%), while fungal isolate RCK-3 was found to decolorize more of Congo Red (69%), Xylidine Ponceau 2R (100%), Poly R-478 (96%), Indigo Carmine (99%), Lissamine Green B (90%), Toluidine Blue (57%) and textile effluent B (54%), than the rest of fungi. Percentage decolorization of all synthetic dyes and textile effluents by the new fungal isolates RCK-1 and RCK-3 was higher compared to the most widely studied simultaneous lignin degrader, *Phanerochaete chrysosporium* and selective lignin degrader, *Pycnoporus cinnabarinus*, when tested in liquid cultures. A statistically significant positive correlation between laccase production and decolorization of dyes and effluents was obtained as compared to other ligninolytic enzymes (lignin peroxidase and manganese peroxidase) production.

Arwidsson et al. (2009) investigated fungi in a bioremediation process of three metal (lead) contaminated soils. *Aspergillus niger*, *Penicillium bilaiae*, and a *Penicillium* sp. were exposed to poor and rich nutrient conditions (no carbon availability or 0.11M D-glucose, respectively) and metal stress (25 µM lead or contaminated soils) for 5 days. Main organic acids identified were

oxalic acid (*A. niger*) and citric acid (*P. bilaiae*). Exudation rates of oxalate decreased in response to lead exposure, while exudation rates of citrate were less affected. There was a significant mobilization of metals from the soils in the carbon rich treatments and maximum release of Pb was 12% from the soils after 5 days. Maximum release of Ni, Zn, and Cu was 28%, 35%, and 90%, respectively. The release of metals was related to the production of chelating acids, but also to the pH-decrease.

Ezzouhri et al. (2009) isolated thirty-six micro-organisms, represented by fungi and yeasts strains, from heavy metal contaminated sites in Tangier, Morocco and studied their resistance to heavy metals. The results revealed that the majority of the isolates were resistant to Pb, Cr, Cu and Zn whereas to Cd, only the fungus *Penicillium* sp. was able to grow. Minimum inhibitory concentrations for Pb^{2+} , Cr^{6+} , Cu^{2+} and Zn^{2+} were also determined. *Aspergillus* and *Penicillium* isolates were the most tolerant to the heavy metals and exhibited strong growth. Their minimum inhibitory concentrations ranged from 20 - 25 mM for Pb, followed by 15 - 20 mM both for Cu and Zn and 10 - 15 mM for Cr.

Gutarowska and Czyzowska (2009) studied the acid production capacity of filamentous fungi. Sixty two filamentous fungi isolated from paint coatings, wallpaper, carton-gypsum board, and indoor air in buildings were screened for acid activity. Research indicated that filamentous fungi considerably decrease the pH of the medium when that medium containing building material. The greatest acid production and pH decrease of the medium was observed during the growth of filamentous fungi in a medium with mortar, while the production of acids was less in a medium with carton gypsum board, gypsum, and wallpaper. Filamentous fungi produced succinic, oxalic, malic and fumaric acids in the medium with indoor building materials.

Iram et al. (2009) conducted experiments to study the tolerance of isolated fungal strains towards the heavy metals. The most common fungal strains viz., *Aspergillus niger*, *Aspergillus* sp., *Fusarium* sp., and *Penicillium* sp., were tested for tolerance against the heavy metals Zn, Pb, Ni and Cd. Among the isolated fungal strains, *Aspergillus niger* was the most tolerant against all the tested heavy metals. It exhibited strong radial growth from 0-40 ppm followed by *Aspergillus* sp., *Pencillium* sp. and *Fusarium* sp. against all the tested heavy metals.

Ren et al. (2009) investigated the bioleaching of heavy metals from a contaminated soil in an industrial area using metabolites, mainly weak organic acids, produced by *Aspergillus niger*. Results showed that after the one-step process, maximum removals of 56%, 100%, 30% and 19% were achieved for copper, cadmium, lead and zinc, respectively. After the two-step process, highest removals of 97.5% Cu, 88.2% Cd, 26% Pb, and 14.5% Zn were obtained. Results of sequential extraction showed that organic acids produced by *Aspergillus niger* were effective in removing the exchangeable, carbonate, and Fe/Mn oxide fractions of Cu, Cd, Pb and Zn; and after both processes the metals remaining in the soil were mainly bound in stable fractions. Such a treatment procedure indicated that leaching of heavy metals from contaminated soil using *Aspergillus niger* has the potential for use in remediation of contaminated soils

2.6 BIOMINERALIZATION

Biomineralization is a widespread complex phenomenon by which living organisms produce minerals. Skinner and Jahren (2003) suggested that the heterogeneous materials was composed of organic and inorganic compounds like carbonate, phosphate, oxalate, silica, iron, or sulfur-containing minerals. Konhauser and Ferris (1996) concluded that biologically induced mineralization is important geological process that helps in the formation of microfossil, hot spring deposition and transfer of chemical elements. The unique properties and functions of biomineralization have inspired innovative high-performance composites for construction applications, as well as other new materials (Bright, 1994; Newnham, 1997; Travis, 1997).

Fungi interact with natural rock systems, soil and buildings under a broad variety of environmental conditions (Landeweert et al., 2001; Hoffland et al., 2004) results in the formation of secondary biomineral on the substrate (Chen et al., 2000; Burford et al., 2003b; Hoffland et al., 2004) and eventually in the diagenesis of the mineral substrates. Diagenesis induced by fungi can be attributed to biochemical and biomechanical alteration of natural rock systems.

Diagenesis involves the dissolution of the mineral substrates by organic acids exuded in the fungal growth environment. These strong chelators and metal-binding acids (Gadd, 1999) form complexes with various metals in the mineral substrates such as calcium (Ca), magnesium (Mg), manganese (Mn), Zinc (Zn), copper (Cu), aluminum (Al) and Iron (Fe) in a process of metal mobilization and immobilization that causes mineral dissolution and precipitation of new

minerals (Sayer and Gadd, 1997; Gadd, 2004; Gadd et al., 2002). The minerals formed by this process are mainly Ca- Mg-oxalates (weddellite: $\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$, whewellite: $\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$, and glushinskite: $\text{MgC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$), and carbonates (calcite) (Burford et al., 2003a, 2003b; Kolo and Claeys, 2005). Use of microorganisms within mortar/concrete leading to the process of biomineralization is now a potential field of research in concrete technology (Ramachandran et al., 2001).

Ramakrishnan et al. (1999) concluded that the inherent cement based biomaterial has been developed to remediate the cracks and fissures in concrete structures. Previous studies have shown that the addition of specific microorganisms to cement–sand mortar or concrete deposit inorganic substances inside the pores of the matrices, which can be used as a filling material to remediate cracks within the structures. It was also reported that the addition of an anaerobic hot spring bacterium (closely related to *Shewanella* species) to the mortar/concrete could increase the compressive strength (25–30%) of the material with respect to control (Ghosh et al., 2005; Ghosh et al., 2006). The biologically induced cement based material thus also exhibited better durability and crack repairing performance compared to normal concrete materials (Ghosh et al., 2008).

Verrecchia et al. (1993) suggested that biomineralized fungal filaments (often referred to as "calcified filaments") are of interest because of their role in the calcium cycle in calcretes. Energy dispersive spectrometry (EDS) microprobe coupled with a scanning electron microscope (SEM) permitted the measurement of the light elements C and O, as well as Ca. Comparison of crystal spectra to those of known standards demonstrated that the great majority of needles associated with the fungal filaments are calcium oxalate. Based on these results, a model of fungal filament mineralization is proposed that describes the formation of weddellite (polyhydrate calcium oxalate) into whewellite (monohydrate calcium oxalate) in a sequence that includes all of the crystal morphologies observed by SEM.

Tuason and Arocena (2009) examined the formation of calcium oxalate crystals by *Piloderma fallax* in response to calcium (0.0, 0.1, 0.5, 1, and 5 mM) and phosphorus (0.1 and 6 mM) additions in modified Melin-Norkrans agar medium. Both calcium and phosphorus supplementation significantly affected the amount of calcium oxalate formed. More calcium

oxalate was formed at high P levels. Concentrations of soluble oxalate in the fungus and medium were higher at low P levels. Calcium oxalate crystals were identified as the monohydrate form (calcium oxalate monohydrate or whewellite) by X-ray diffraction analysis. *P. fallax* may be capable of dissolving hyphal calcium oxalate under conditions of limited Ca.

Burford et al. (2006) investigated the formation of biogenic fabrics in limestone by two fungi, *Serpula himantoides* and a polymorphic fungal isolate from limestone identified as a *Cephalotrichum* (syn. *Doratomyces*) sp. Biomineralization of fungal filaments were observed in laboratory microcosms consisting of carboniferous limestone and after 21 days incubation at 25°C. Environmental electron scanning microscopy and X-ray micro-analysis of crystalline precipitates on the hyphae of *S. himantoides* demonstrated that the secondary crystals exhibited different crystalline forms but were similar in elemental composition to the original limestone. Powder X-ray diffraction (XRD) of crystalline precipitates showed they were composed of a mixture of calcite (CaCO_3) and calcium oxalate monohydrate ($\text{CaC}_2\text{O}_4 \cdot \text{H}_2\text{O}$). XRD showed that they were composed solely of calcite (CaCO_3) or of calcite with some calcium oxalate dehydrate ($\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$). These results provide direct experimental evidence for the precipitation of calcite (CaCO_3) and also secondary mycogenic minerals, on fungal hyphae in low nutrient calcareous environments.

CHAPTER 3

EXPERIMENTAL PROGRAM

This chapter describes the details of experimental programs for the physical & chemical analysis of waste foundry sand, isolation, screening and characterization of organic acid producing fungal cultures. It also describes the methods for fungal treatment used for WFS and organic acids production by the fungal cultures. It also presents the metal leachate analysis obtained from concrete mixes containing untreated and fungal treated WFS (0%, 10%, 15% and 20%). It includes the details for the measurements of compressive strength, water absorption and porosity of concrete containing (untreated and fungal treated) WFS with varying percentages. It also include the XRD technique to study the various phases formed in hardened concrete made with WFS (untreated and fungal treated), SEM to study the surface morphology and EDS for chemical composition of hardened concrete.

3.1 MATERIALS USED

3.1.1 Cement

Cement is a fine, grey powder. The cement contains two basic ingredients namely argillaceous and calcareous. IS mark 43 grade cement (Brand- Ambuja cement) was used for all mixes. The cement used was fresh and without any lumps. Testing of cement was done as per BIS-8112 (1989). The various tests results conducted on the cement are reported in Table 3.1.

3.1.2 Coarse and Fine Aggregates

Locally available coarse aggregates having the size of 12.5 mm was used in this work. Testing of fine and coarse aggregates was done as per BIS-383 (1970). The sand used for the experimental programme was locally procured and conformed to Indian Standard Specifications BIS-383(1970). The sand was first sieved through 4.75 mm sieve to remove any particles greater than 4.75 mm and remove the dust. Properties of the coarse and fine aggregate used in the experimental work are tabulated in Table 3.2.

Table 3.1: Physical Properties of Portland Pozzolana Cement

Properties	Values	Standard Values
Initial setting time (minutes)	90 min	Not less than 30
Final setting time (minutes)	255 min	Not greater than 600
Fineness (%)	3.5%	<10
Specific gravity	3.125	-

Table 3.2: Physical Properties of Coarse and Fine Aggregate

Characteristics	Coarse Aggregate	Fine Aggregate
Specific gravity	2.62	2.57
Water absorption (%)	0.80	1.02
Fineness modulus	2.0	2.28

3.1.3 Water

Water used for casting specimens conformed to the requirements of BIS- 456 (2000). Test results are given in Table 3.3.

Table 3.3: Properties of Water

Properties	Observed value
PH	8.0
Dissolved Solids (mg/l)	290
Suspended Solids	Nil
Chlorides (mg/l)	20
Sulphates (mg/l)	74
MPN Value/100 ml.	Nil

3.1.4 Waste Foundry Sand

Sample of waste foundry sand (WFS) was collected in bags from the dumped site of Delux Metal Foundry located at Mandi Gobindgarh (Punjab, India) (Fig. 3.1)

3.1.5 Soil Sample

Soil samples for isolation of fungi were collected from the areas surrounding the same foundry (Fig. 3.2).



Fig. 3.1: Sample Collection of WFS



Fig. 3.2: Soil Sample Collection

3.2 PHYSICAL AND CHEMICAL ANALYSIS OF WFS

3.2.1 Physical Analysis of WFS

Physical analysis including specific gravity, density, fineness and absorption were determined by ASTM C128 (2001).

3.2.1 (a) Apparatus

Pycnometer: (A flask or other suitable container into which the WFS can be readily introduced and in which the volume content can be reproduced within 0.1 cm^3), Drying Oven and Sample (Waste Foundry Sand).

3.2.1 (b) Preparation of Test Specimen

The specimen was dried at 110°C . It was then allowed to cool and covered with water by immersion. Then it was allowed to stand for 24 hours. Excess water was decanted off. Sample was spreaded on non-absorbent surface.

3.2.1 (c) Procedure

The pycnometer was partially filled with water. Immediately 500 g of saturated-surface-dry (SSD) specimen (WFS) was introduced. The weight of this SSD sample was placed in the pycnometer was recorded. The pycnometer was filled to 90% of its capacity. The pycnometer was rolled, inverted, and agitated to eliminate all air bubbles (this can take 15 to 20 min). The pycnometer was calibrated. The total weight of the pycnometer, specimen, and water was determined. Then the specimen was removed from the pycnometer and dried to constant weight in an oven at 110°C for 1 hour, then cooled to room temperature, and weighed. The weight of the pycnometer filled to its calibration capacity with water was also determined. Calculations were made on the basis of the following equations:

$$\text{Specific Gravity} = A / (B + S - C)$$

$$\text{Bulk Specific Gravity} = S / (B + S - C)$$

$$\text{Absorption (\%)} = 100 [(S - A) / A]$$

Where, A = weight of oven dried test sample in air, (in gms)

B = weight of pycnometer filled with water to calibration mark, (in gms)

S = weight of saturated surface dried (SSD) sample in air, (in gms) (prior to placement in pycnometer)

C = weight of pycnometer with test sample and water to calibration mark, (in gms)

3.2.2 Chemical Analysis of WFS

Chemical analysis of WFS was carried out by using Energy Dispersive spectroscopy (EDS). For this WFS sample was grinded and percent oxide forms of metals present in WFS samples were obtained (Table 3.4).

Table 3.4: Chemical Composition of WFS

S. No	CONSTITUENTS
1.	Silica (SiO ₂)
2.	Iron Oxide (Fe ₂ O ₃)
3.	Alumina (Al ₂ O ₃)
4.	Calcium Oxide (CaO)
5.	Magnesium oxide (MgO)
6.	Titanium Dioxide (TiO ₂)
7.	Sodium Oxide (Na ₂ O)
8.	Potassium Oxide (K ₂ O)
9.	Sulphur Trioxide (SO ₃)
10.	Manganese Oxide (Mn ₃ O ₄)
11.	Strontium Oxide (SrO)

3.3 ISOLATION OF FUNGAL CULTURES

3.3.1 Sterilization of Apparatus

Petri plates, flasks, test tubes, micro tips and distilled water were dipped in chromic acid for 24 hours. These were then washed and dried in oven at 95°C. After drying these were sterilized in autoclave for 20 minutes at 121°C, 15 psi.

3.3.2 Preparation of Growth Medium

Potato dextrose agar (PDA) medium was used for the growth of fungal cultures.

Composition of PDA (Himedia) g/L:

Potato Extract: 4

Dextrose: 20

Agar: 15

pH: 5.6 ± 0.2

It was sterilized at 121 °C, 15 psi.

3.3.3 Preparation of Petriplates

Poured the medium in sterilized Petri plates and allowed to solidify for 24 hour. Once the agar was solidified plates were kept in an inverted position for 24 hours at room temperature for sterility test.

3.3.4 Isolation of Fungi

Potato dextrose agar (PDA) was used for the isolation of fungi. The soil sample was processed with isolation procedure using the soil dilution plate method. Petri plates were incubated at $28 \pm 2^{\circ}\text{C}$ for 4 days. After incubation distinct colonies were counted and re-cultured for purification. The cultures were maintained on PDA slants at 4 °C.

3.4 QUALITATIVE ESTIMATION OF ACID ACTIVITY

The screening method given by Abin et al. (2002) was used for the estimation of acid activity. The acidity was estimated on a medium containing Bromocresol purple. Isolated fungal cultures were placed on the medium having following composition in g/L:

Glucose – 20

Peptone – 1

Yeast Extract – 5

Agar – 20

CaCO_3 - 5g

Bromocresol purple - 0.030

pH - 6.0

Petri plates were then incubated at 28⁰C for 5-6 days in triplicates. After 6 days the plates were observed for appearance of yellow zone around colonies as a result of acid production. The acidity of medium diameters (acidity = yellow zone around colonies) and the diameters of the colonies were measured. The acid activity coefficient (Q) was estimated:

$$Q = \frac{\text{Diameter of acidity of medium (mm)}}{\text{Diameter of colony (mm)}}$$

For the purpose of the experimental criteria, the acid activity was established using the following scale: very high activity $Q > 1.3$; high $Q = 1-1.3$; medium $Q = 0.5-1$; low $Q = 0.1-0.5$; no activity $Q = 0$.

3.5 CHARACTERIZATION OF FUNGAL CULTURE

3.5.1 Macroscopic Characteristics: Fungal cultures were identified on the basis of colony morphology, color, texture, elevation, appearance, and growth pattern of the fungal colonies obtained on PDA petriplates.

3.5.2 Microscopic Characteristics: Fungal cultured were observed for structure of hyphae, shape and structure of conidiophores, color, and pattern of conidia under compound microscope. For this purpose, drop of lactophenone blue stain was placed in the center of a clean slide. A fragment of the fungal colony was removed from the colony edge using an inoculating or teasing needle. The fragment was placed in the drop of stain and teased gently. After applying a cover slip the preparation was examined under compound microscope for the presence of characteristic mycelia and fruiting structures.

3.5.3 Molecular Identification of Fungal Cultures

The cultures used for the molecular studies were grown on PDB (potato dextrose agar). The cultures were incubated at 28⁰C for 7 days. DNA was isolated from the culture and its quality was evaluated on 1.2% Agarose Gel. DNA sequencing reaction of PCR amplicon was carried out by using the universal primers ITS1 (5' TCC GTA GGT GAA CCT GCG G 3') and ITS 4 (5' TCC TCC GCT TAT TGA TAT GC 3') using Cycle sequencing kit on ABI 3730xl Genetic Analyzer. Consensus sequence of was generated from forward and reverse sequence data using

aligner software. The 28S sequences of SA4 and SA2 isolate were used for a BLAST search in the Gen Bank database. The 28S sequence of the isolates were further aligned and compared to published 28S rRNA sequences searched with the taxonomy browser of the National Center for Biotechnology Information (NCBI). Based on maximum identity score first fifteen sequences were selected and the phylogenetic tree was constructed using MEGA 5.

The evolutionary history was inferred using the Neighbor-Joining method (Saitou and Nei, 1987). The bootstrap consensus tree inferred from 500 replicates were taken to represent the evolutionary history of the taxa analyzed (Felsenstein, 1985). Branches corresponding to partitions reproduced in less than 50% bootstrap replicates were collapsed. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches (Felsenstein, 1985). The evolutionary distances were computed using the Kimura 2-parameter method and are in the units of the number of base substitutions per site (Kimura, 1980). All positions containing gaps and missing data were eliminated from the dataset. Phylogenetic analyses were conducted in MEGA 5 (Tamura et al., 2007).

3.6 GROWTH AND PH PROFILE OF FUNGAL CULTURES

To study the growth and pH profile, both the fungal cultures were grown in PDB (pH- 5.6 ± 0.2) on shaker (150 rpm) at 28°C. After 7 days of incubation, bead formation and pH were studied for both the cultures. Series of experiments by using minimal medium were performed to study the effect of incubation days, glucose content, WFS percentages on reduction of pH due acid formation. Effect of glucose content on biomass production was also studied for fungal cultures. For fungal biomass production studies, *A. niger* and *E. crustaceum* were allowed to grow in minimal medium containing various percentages of glucose at 28 °C for 7 days. Biomass was filtered on filter paper and weighed to study the increase in biomass. To study the effect of incubation on acid production, both the cultures were grown in minimal medium for 30 days at 150 rpm at 28°C. Reduction pH was recorded after every five days by using pH meter. To study the effect of glucose content, minimal medium containing various percentages of glucose (1%, 2%, 3%, 4%, 5%, 6%, 7%, 8%, 9%, and 10%) were inoculated with both fungal cultures separately and incubated for 7days on shaker (150 rpm) at 28°C. Reduction in pH was recorded after 7 days of incubation. To study the effect of WFS percentage on acid production, fungal

cultures were inoculated in medium medium containing various percentages of WFS. Reduction in pH was in recorded in all flasks after 4th and 7th day of incubation.

3.7 FUNGAL TREATMENT OF WFS

For Fungal treatment, WFS was spread in plastic tray in specific manner (layers) for proper spread of fungal mycelium. For this, 1 cm thick layer of WFS was made as bottom layer and it was drenched with water. Middle layer was consisting of baggage which served as the source of medium for the growth of fungus.



Fig. 3.3: Untreated WFS



Fig. 3.4: Fungal Treated WFS

Five days grown fungal culture was used inoculate WFS and spore count was performed using Neubauer Haemocytometer (1.7×10^7 spores per ml). For optimization of percent fungal cultures, various percentages of fungal culture (0.5%, 1%, 1.5%, 2% and 2.5%) (w/v) were used

to inoculate the WFS. After 30th days samples were taken out for reduction in pH and metal analysis. The middle layer was covered by another layer of WFS. These plastic trays then placed at temperature of 28°C for 30 days. Fungal treated WFS were used for concrete mixes. Figs. 3.3 and 3.4 show WFS before and after treatment with fungal cultures.

The effect of incubation days, WFS percentages and glucose content on pH profile of fungal cultures was studied. Growth medium was prepared by using glucose (10%) in distilled water. Flasks containing growth medium were inoculated by fungal cultures separately for 21 days at 28°C. Samples were taken at regular intervals to check the reduction in pH. To study the effect of glucose content, flasks containing varying percentages of glucose were inoculated with fungal cultures and incubated at same conditions mentioned above. Samples were taken after 10 days to analyze the reduction in pH for various percentages of glucose content. To study the effect of WFS percentage on growth and pH, the flasks containing glucose and varying percentages of WFS were inoculated with fungal cultures. Samples were taken after 4th and 7th day of inoculation for pH reduction

3.8 CALCIUM OXALATE FORMATION BY *Eupenicillium crustaceum*

Fungal culture was inoculated in flask containing potato dextrose broth (PDB) along with urea- CaCl_2 and incubated for 20 days at 28°C. After 20 days the fungal mycelium was extracted from the broth and washed twice with sterilized distilled water. The fungal mycelium was crushed to form powder and was analyzed by using X- ray diffraction (XRD) technique for identification of phase of mineral formed.

3.9 ORGANIC ACID PRODUCTION BY FUNGAL CULTURES

Fungal cultures were analyzed for organic acid production by using High Performance Liquid Chromatography (HPLC) technique. For this, fungal cultures were allowed to grow in minimal medium for 15 days at 28°C. Fungal cultures were inoculated in both minimal medium containing WFS and without WFS. After 15 days of incubation the samples were allowed to filter through ordinary filter paper and then the extracts were filtered through 0.45 micron filter assembly in sterilized vials. For HPLC analysis, samples were analyzed by using model Agilent 1120 Compact LC. The mobile phase used for this was KH_2PO_4 at pH at 2.5 at a flow rate of

0.5ml/min. Gradient mobile phase containing 70% KH_2PO_4 and 30% methanol were used. The operation column for HPLC was C 18 column. Operation was carried out at temperature of 30°C at 226 nm. Standard solutions of citric acid and oxalic acid were prepared along with samples.

3.10 STUDY OF CONCRETE PROPERTIES

Concrete (M20) cubes having size 150 mm x150 mm x 150 mm were cast in triplicates using waste foundry sand (WFS) and fungal treated waste foundry sand as partial replacement of fine aggregate.

3.10.1 Mixture Preparation for Cube Casting

Ingredients of M20 concrete mix are given in Table 3.5. Mixture proportions of concrete M20 is shown in Table 3.6.

Table 3.5: Mix Proportion (M20)

Unit of Batch	Water (Liters)	Cement (Kg)	F.A (Kg)	C.A (Kg)
Cubic meter content	195	390	569	1165
Ratio of ingredients	0.5	1	1.45	2.98

Table 3.6: Mix Proportions of Concrete

WFS Type	Untreated WFS				Fungal Treated WFS		
WFS (%)	0	10	15	20	10	15	20
Cement (kg/m^3)	390	390	390	390	390	390	390
Fine Aggregate (kg/m^3)	568.87	511.98	483.54	455	511.98	483.54	455
Coarse Aggregate (kg/m^3)	1164.12	1164.12	1164.12	1164.12	1164.12	1164.12	1164.12
Water (kg/m^3)	185	185	185	185	185	185	185
WFS (kg/m^3)	-	56.88	85.33	113.77	56.88	85.33	113.77

3.10.2 Casting of Cubes

Cubes were made (Fig. 3.5) using concrete mixture without WFS and concrete mixture containing WFS as well as fungal treated WFS as partial replacement of fine aggregate (10%, 15%, and 20%).

3.10.3 Sample Collection for Leachate Characterization

10g of concrete mixture (control, with WFS and fungal treated WFS) samples were taken at the time of mixture preparation for leachate characterization (ASTM D 3987).



Fig 3.5: Casting of Concrete Cubes (M20)

3.10.4 Curing of Concrete Cubes

After casting, all the test specimens were stored at room temperature in the casting room. They were demolded after 24 hours, and were put into a water-curing tank for 28 days at room temperature.

3.10.5 Testing of Compressive Strength

Compressive Strength of concrete cubes prepared with WFS, without WFS and fungal treated WFS were determined at the age of 28 days by BIS: 516 (1959). Specimens were placed on the bearing surface of Digital Compression Testing Machine (Fig. 3.6). The maximum load was noted and the compressive strength was calculated by the equation given below. It was then compared with the concrete made without WFS.

Compressive strength = (Load in N/ Area in mm²) = ...N/mm²



Fig. 3.6: Universal Testing Machine (UTM)

3.10.6 Water Absorption and Porosity

After 28 days of curing, cubes were used to determine for the water absorption and porosity by using the ASTM C 642 (1997).

Procedure:

a) Oven-Dry Mass: Determine the mass of the portion, and dry in an oven at a temperature of 100 to 110°C for not less than 24h. After removing each specimen from the oven, allow it to cool in dry air to a temperature of 20 to 25°C and determine the mass. If the specimen was

comparatively dry when its mass was first determined, and the second mass closely agrees with the first, consider it dry. If the specimen was wet when its mass was first determined, place it in the oven for a second drying treatment of 24h and again determine the mass. If the third value checks the second, consider the specimen dry. If the difference between the values obtained from two successive values of mass exceeds 0.5% of the lesser value, return the specimens to the oven for an additional 24 h drying period, and repeat the process until the difference between two successive values is less than 0.5% of the lowest value obtained. Designate this last value as A.

b) Saturated Mass After Immersion: Immerse the specimen, after final drying, cooling and determination of mass, in water at approximately 21°C for not less than 48h and until two successive values of mass of the surface- dried samples at intervals of 24h show an increase in mass of less than 0.5% of the larger value. Surface-dry the specimen by removing surface moisture with a towel, determine the mass. Designate the final surface- dry mass after immersion B.

c) Saturated Mass After Boiling: Place the specimen in a suitable receptacle, covered with tap water and boil for 5h. Allow it to cool by natural loss of heat for not less than 14h to a final temperature of 20 to 25 °C. Remove the surface moisture with a towel and determine the mass of the specimen. Designate the soaked, boiled, surface- dried mass C.

d) Immersed Apparent Mass: Suspend the specimen, after immersion and boiling, by a wire and determine the apparent mass in water. Designate this apparent mass D.

Calculation:

Absorption after immersion, % = $[(B-A)/A] \times 100$

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

Bulk density, dry = $[A/(C-D)] \cdot \rho = g_1$

Bulk density after immersion = $[B/(C-D)] \cdot \rho$

Bulk density after immersion and boiling = $[C/(C-D)] \cdot \rho$

Apparent density = $[A/(A-D)] \cdot \rho = g_2$

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

Where:

A = mass of oven-dried sample in air, g

B = mass of surface- dry sample in air after immersion, g

C = mass of surface- dry sample in air after immersion and boiling, g

D = apparent mass of sample 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 = $1 \text{ Mg/m}^3 = 1 \text{ g/cm}^3$

3.11 CHARACTERIZATION OF LEACHATE FROM CONCRETE MIXTURE

Samples were taken from each concrete mix and leachate was obtained by using shake extraction of solid waste method (ASTM D 3987- 1985). Leachate obtained from concrete (Grade M20) made with WFS and fungal treated WFS as partial replacement of fine aggregate (10%, 15% and 20%) was characterized for metal concentration. The leachate was analyzed for 15 metals including Barium (Ba), Beryllium (Be), Cadmium (Cd), Chromium (Cr), Cobalt (Co), Copper (Cu), Iron (Fe), Mercury (Hg), Lithium (Li), Molybdenum (Mo), Magnesium (Mg), Manganese (Mn), Nickel (Ni) and Zinc (Zn).

3.11.1 Acid Digestion of Samples for Metal Analysis

The digestion vessels and volumetric ware were acid washed and rinsed with distilled water. Digestion vessels were labeled properly after drying. 2 ml of sample was added to digestion vessel. 5 ± 0.1 ml of 70% nitric acid and 1 ± 0.1 ml of concentrated hydrochloric acid to the vessel in a fume hood (or fume exhausted enclosure). The digestion vessels in the digestion block (fume hood) were maintained at 110°C throughout the digestion. Brown nitrogen dioxide fumes were observed within 5 minutes. 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. 2 ml of 72% perchloric acid was added to the vessel. The digestion was continued at 110°C for 16 hr. 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. The acid digested samples were then analyzed for metals content (Ba, Be, Cd, Co, Cu, Cr, Fe, Hg, Li, Mn, Mo, Mg, Ni, Pb and Zn) by using ICP-MS (Inductively Coupled Plasma-Mass Spectrometry) at IIT Roorkee.

3.11.2 Method for Leachate Characterization (ASTM D 3987-1985)

Each test sample obtained from concrete mixture weighing about 10 g was prepared and added to a 250 ml conical flask having water tight closure. A volume of deionized water equal to a 20 times the weight of the each test sample was added to the container. The container was continuously agitated for 18 hr at about 20 °C. The sample was then allowed to settle for 5 min and the bulk of aqueous phase was separated from the solid by filtration through a coarse filter paper. The liquid was then filtered through 0.45 µm membrane filter. The resulting extract was analyzed for various metals after acid digestion of the samples by ICP-MS (Fig. 3.7).



Fig. 3.7: Inductively Coupled Plasma Mass Spectrometry (ICP-MS) Instrument

3.12 X- RAY DIFFRACTION (XRD) ANALYSIS

X-ray powder diffraction technique is the most prominent technique used for unraveling the structure of the materials in bulk and thin film forms. X-ray diffraction is a non-destructive technique used to determine the elements present in any particular substance. 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. The samples for X-Ray diffraction

analysis were prepared in powdered form. The concrete sample was taken from the inner core of the matrix.

Concrete samples of each mix (after 28 days curing) were taken and crushed into fine powder by pestle-mortar. The powder samples were analyzed in a powder X-ray diffractometer (PANalytical X' Pro) (Fig. 3.8). The XRD spectrum were taken from $2\theta = 10^\circ$ to $2\theta = 80^\circ$. The peaks in the new positions of the spectrum were marked, compared and identified from the JCPDS data file.



Fig. 3.8 X-Ray Diffraction (XRD) Instrument

Procedure for Deduction of Minerals

- (i) For a given sample, XRD graphs are obtained with intensities on Y-axis and 2θ on X-axis.
- (ii) Match the d values and intensities of peaks of respective minerals with the fundamental peaks of X-ray powder diffraction files given in the software.
- (iii) For any mineral to be present, all the strong peaks should be present in the XRD graph, else the mineral is not present.

3.13 SCANNING ELECTRON MICROSCOPY (SEM) & ENERGY DISPERSIVE SPECTRUM (EDS) ANALYSIS

The broken concrete samples (with/without fungal treated WFS) collected after compressive strength testing was dried for 24 h at 80°C. The samples were kept in desiccator for over- night for removal of moisture. The samples were coated with gold and analyzed by using JEOL- JSM 6510 LV scanning electron microscope (SEM) at 20 kV; the instrument was equipped with an energy dispersive x-ray spectroscopy (EDS) facility (Fig. 3.9).



Fig. 3.9: Scanning Electron Microscope (SEM)

CHAPTER 4

RESULT AND DISCUSSION

In this chapter, the findings of an experimental investigation are presented. It includes the results for the physical & chemical analysis of waste foundry sand, isolation, screening and characterization of organic acid producing fungal cultures. It represents the results regarding fungal growth, pH profile on various conditions, organic acids production and treatment of WFS by the fungal cultures. It includes metal leachate analysis obtained from concrete mixes containing untreated and fungal treated WFS (0%, 10%, 15% and 20%). Results regarding compressive strength, water absorption and porosity of concrete containing (untreated and fungal treated) WFS with varying percentages are presented in this chapter. Results also include XRD, SEM and EDS of hardened concrete cubes containing untreated and fungal treated WFS with varying percentages.

4.1 PHYSICAL PROPERTIES OF WFS

Waste foundry sand is sub- angular to round in shape and black in color. Table 4.1 presents the physical properties of waste foundry sand. Specific gravity was observed as 2.16 and bulk relative density was 2255.9 kg/m³. Percent absorption was found to be observed as 6.4 and fineness modulus was obtained as 2.07. The pH of waste foundry sand was 6.8.

The typical specific gravity of foundry sand varies between 2.39 and 2.55 and bulk relative density was 2589 kg/m³ (Javed and Lovell, 1994). Dayton et al. (2010) mentioned the specific gravity of WFS varies between 2.39 and 2.55. Obtained value (2.16) of specific gravity was ranged between the values as mentioned by Javed and Lovell (1994); Dayton et al. (2010). Percent absorption of WFS was found to be higher (6.4) in present studies. The main reason for high water absorption is based on the fact that the clay based system sands requires approximately 10% water content to activate bentonite binding; nevertheless, 2-3% water is needed as solvent catalyst to activate organic binders in organic based system sands (Winkler

and Bolshakov, 2000). Moreover, its high absorption can be correlated with the fineness of the sand. It can be interpreted by the law that a fine particle leads to high surface area, which favours the absorption of water (Deng and Tikalsky, 2008).

Table 4.1: Physical Properties of Waste Foundry Sand

Physical Properties	Values
Specific gravity	2.16
Bulk relative density (kg/m^3)	2255.9
Absorption (%)	6.4
Fineness modulus	2.07
Ph	6.8

4.2 CHEMICAL PROPERTIES OF WFS

Chemical composition of the waste foundry sand depends on the type of metal molded at the foundry, type of binder and combustible used. Waste foundry sand was analyzed for percent metal oxides present in it. The chemical composition of the foundry sand may influence its performance. Table 4.2 presents data regarding the chemical composition of WFS. Silica shows the maximum value (83.3%). Iron oxide was observed as 5.39% and strontium oxide was found to be negligible in waste foundry sand. Alumina (Al_2O_3), Titanium Dioxide (TiO_2), Magnesium Oxide (MgO), Sodium Oxide (Na_2O), Potassium Oxide (K_2O), Sulphur Trioxide (SO_4) and Manganese (Mn_3O_4) were found in little quantities.

Earlier, Guney et al. (2010), Etxeberria et al. (2010), Siddique et al. (2011) and American Foundrymen Society (1991) reported that percent silica was found to be higher than the rest of metal oxides. So, waste foundry sand is rich in silica content. Results show iron oxide (5.39%) is present in WFS, as the foundry sand obtained from the ferrous based foundry.

Table 4.2: Chemical Composition of Waste Foundry Sand

Constituents	Values (%)
Silica (SiO ₂)	83.8
Alumina (Al ₂ O ₃)	0.81
Titanium Dioxide (TiO ₂)	0.21
Calcium Oxide (CaO)	1.42
Magnesium Oxide (MgO)	0.86
Iron Oxide (Fe ₂ O ₃)	5.39
Sodium Oxide (Na ₂ O)	0.87
Potassium Oxide (K ₂ O)	1.14
Sulphur Trioxide (SO ₄)	0.21
Manganese (Mn ₃ O ₄)	0.047
Strontium Oxide (SrO)	Nil

4.3 ISOLATION OF FUNGAL CULTURES

Fungal cultures were isolated on potato dextrose agar (PDA) at 28°C. Eight different fungal cultures were isolated from both soil and waste foundry sand by serial dilution method. Fungal cultures were designated as SO1, SO2, SO3 and SO4 isolated from soil and SA1, SA2, SA3 and SA4 isolated from waste foundry sand (Figs. 4.1 and 4.2). Fungal cultures were transferred to PDA slants for preservation at 4°C.

4.4 SCREENING OF FUNGAL CULTURES

Screening of fungal cultures was done to screen out the acid producing fungal isolates. For this, fungal cultures were stab inoculated on Bromocresol purple medium. This medium is basically used as pH indicator in which reduction in pH is indicated by change of purple color to yellow color. Eight isolated fungal cultures were stab inoculated on Bromocresol purple medium at 28 °C for 2-3 days for the appearance of zone around the fungal colony.

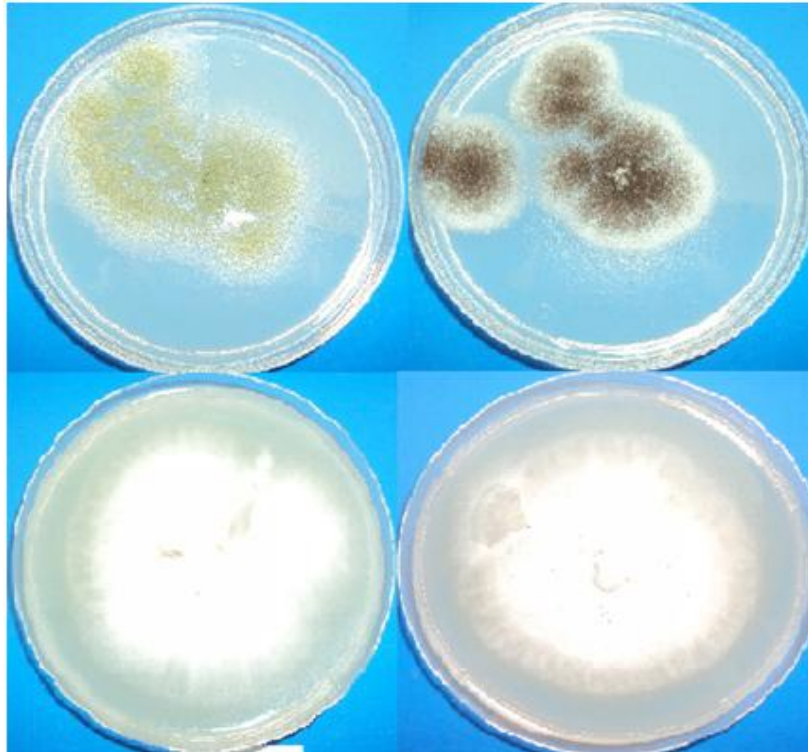


Fig 4.1: Fungal Isolates Obtained From Soil

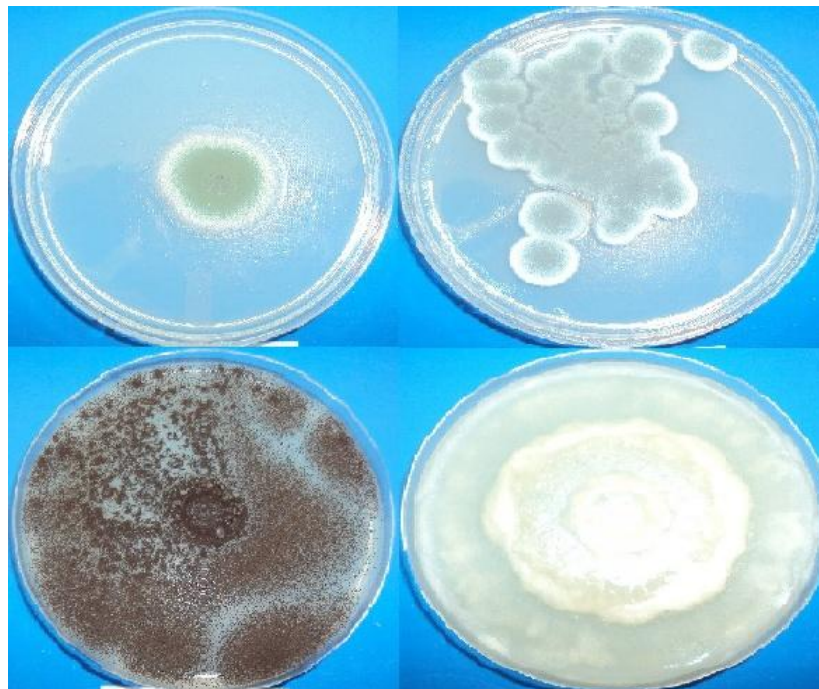


Fig 4.2: Fungal Isolates Obtained From WFS

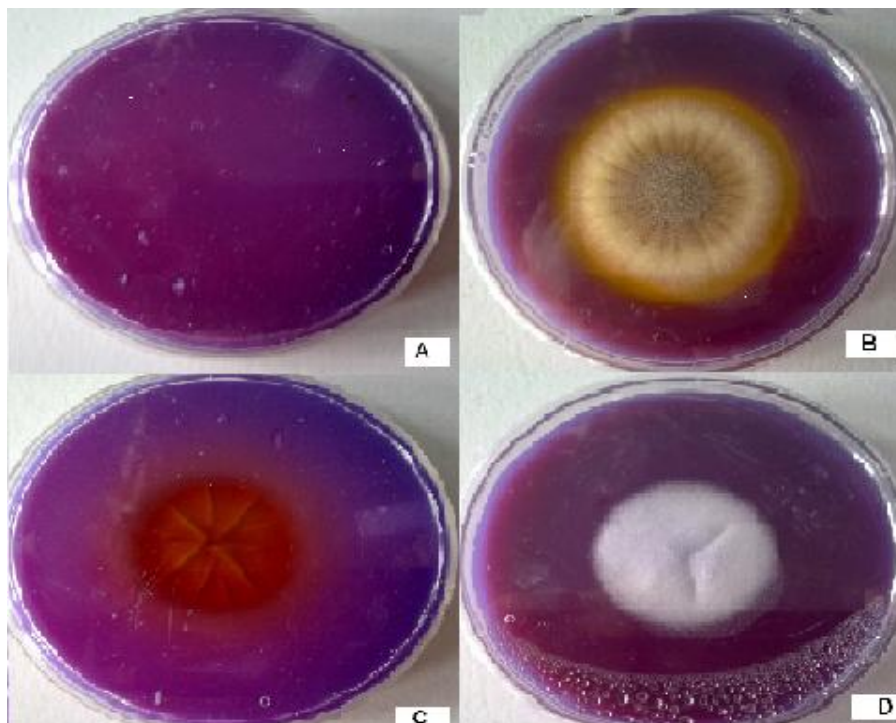


Fig 4.3: Yellow Zone Formation Around Fungal Colony (a) Control; (b) Yellow Zone Formation; (c) Inverted Plate; and (d) Absence of Zone Formation

Isolate SO₂, SA₂ & SA₄ were able to acidic metabolite production as it produced yellow zone on medium containing Bromo- cresol purple (Fig. 4.3). The diameter of yellow zone and that of fungal colony was measured. On the basis of acid coefficient (Q), fungal cultures SA₂ (Q= 1.3) and SA₄ (Q= 1) were found to be highly acidic in nature but isolate SO₂ (Q= 0.1) was found to be low in acid production. The formation yellow zone around the fungal colonies on medium containing bromocresol purple indicates the production of organic acid by fungal culture when sucrose was provided as carbon source. The presence of organic acid produced by fungal cultures changed from purple to yellow due to pH reduction. Similar type of acid activity by fungal cultures was observed by Gutarowska and Czyrowska (2009).

4.5 CHARACTERIZATION OF FUNGAL CULTURES

4.5.1 Macroscopic Features

For macroscopic studies, fungal cultures were observed on the basis of colony morphology, color, texture, elevation, appearance, and growth pattern of the fungal colonies on petri plates

containing PDA medium. The SA-4 fungal culture was found to appeared white colored colonies initially, and then quickly became black with the conidial production. Colony texture was appeared as downy to powdery when grown on PDA plates (Fig. 4.4). Reverse of colonies were found to be pale in color and production of radial fissures were also shown by both the fungal cultures. In isolate SA-2, colonies were found to be white initially and became bluish green with time due to production of conidia. Colony texture was found to be velvety and smooth when grown on PDA plates (Fig. 4.5).

4.5.2 Microscopic Features

In microscopic studies, fungal cultures were observed for structure of hyphae, shape and structure of conidiophores, color, diameter and pattern of conidia under compound microscope. Microscopic studies of SA-4 isolate showed that fungal hyphae were septate, hyaline and slightly brown in color. Conidiophores were found to be long with smooth walled biseriate large size globose vesicle. Metulae and phialides were found to cover the entire surface of vesicle and conidia were brown to black in color (Fig. 4.6). Microscopic studies showed that SA-4 fungal culture was likely to show the characteristic features of *Aspergillus* species.

SA-2 isolate showed septate hyphae with simple or branched conidiophores. Metulae were found to bear flask shaped phialides which formed brush like clusters. Conidia were round and appeared at the tip of phialides (Fig. 4.7). Microscopic studies showed that SA-2 fungal cultures were likely to show the characteristic features of *Penicillium* species.



Fig 4.4: Cultural Morphology of Fungal Isolate SA4



Fig 4.5: Cultural Morphology of Fungal Isolate SA2



Fig 4.6: Microscopic View of SA4 Isolate (100X Magnification)



Fig 4.7: Microscopic View of SA2 Isolate (100X Magnification)

4.5.3 Molecular Identification of Fungal Cultures

Molecular identification of fungal isolates was done by using 28S rDNA technique. Sequences were generated by using forward and reverse primers. Fig. 4.8 shows gel image of PCR amplicon for both the fungal cultures. The 28 S rDNA gene sequences were processed manually and analyzed at NCBI database using BLAST tool. BLAST analysis of the 28S rRNA gene sequence of the isolates (SA4 and SA2), revealed perfect match with that of *Aspergillus niger* type strain (100% similarity; Accession no. EF634376.1) and *Eupenicillium crustaceum* type strain (99% similarity; Accession no. AB000486.1) respectively. The alignment and comparison of the 28S sequence of the isolate (SA4 and SA2) to the published 28S rRNA sequences belonging to ten reference strains of phenotypically close species of *Aspergillus* and *Penicillium* confirmed the 100% correspondence to the *Aspergillus niger* and *Eupenicillium crustaceum* type strain respectively. 28S sequence-based phylogenetic tree showing the relationships between SA4 and SA2 isolate and 10 close strain types of *A. niger* and *E. crustaceum* respectively in Fig 4.9 and 4.10. The gene sequences of both the fungal cultures (*A. niger* and *E. crustaceum*) were submitted to Ben Bank NCBI and the accession numbers were assigned as JX233485 and JX233486 respectively.

The results of phylogenetic analysis suggested that strain SA4 and SA2 belongs to the genera *Aspergillus* and *Penicillium* respectively, formed a clade with *Aspergillus niger* isolate NRRL 35173 (Accession no. EF634376.1) with bootstrap value of 45 replicates and in case of SA2 formed a clade with *Eupenicillium crustaceum* (Accession no. AB000486.1) with bootstrap value of 105 replicates. Pair-wise sequence analysis indicated that the highest sequence similarity of SA4 isolates was with *Aspergillus niger* isolate NRRL 35173 (100% similarity) followed by *Aspergillus niger* isolate NRRL 326 (100%). In case of SA2 isolate, the highest similarity was with *Eupenicillium crustaceum* (99% similarity) followed by *Penicillium chrysogenum* strain CTSP F15 (99% similarity). Strain SA4 and SA2 can be differentiated from the other phylogenetic neighbors of previously reported *Aspergillus* and *Penicillium* species on the basis of molecular characterization.

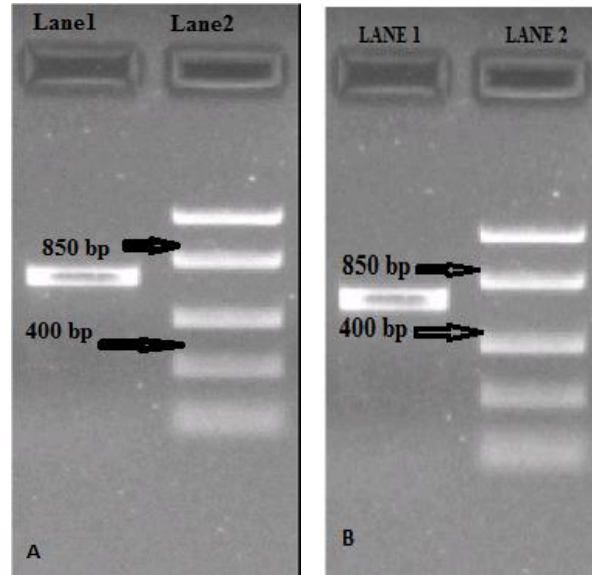


Fig 4.8: Gel Image of PCR Amplicon (A) *A. niger*; (B) *E. crustaceum*. Lane 1: D1/D2 Region of LSU (Large Subunit 28S rDNA) Amplicon Band; Lane 2: DNA Marker

Consensus Sequence of SA4 (604 bp)

GAACCTTAAGCATATCAATAAGCGGAGGAAAAGAAACCAACCGGGATTGCCTCAGTA
ACGGCGAGTGAAGCGGCAAGAGCTCAAATTTGAAAGCTGGCTCCTTCGGAGTCCGC
ATTGTAATTTGCAGAGGATGCTTTGGGTGCGGCCCCCGTCTAAGTGCCCTGGAACGG
GCCGTCAGAGAGGGTGAGAATCCCGTCTTGGGCGGGGTGTCCGTGCCCCGTGTAAAG
CTCCTTCGACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTTCA
TCTAAAGCTAAATACTGGCCGGAGACCGATAGCGCACAAGTAGAGTGATCGAAAGA
TGAAAAGCACTTTGAAAAGAGAGTTAAACAGCACGTGAAATTGTTGAAAGGGAAGC
GCTTGCGACCAGACTCGCCCGCGGGGTTTCAGCCGGCATTTCGTGCCGGTGTACTTCCC
CGTGGGCGGGCCAGCGTCGGTTTGGGCGGGCCGGTCAAAGGCCCTTGAATGTAGTG
CCCTCCGGGGCACCTTATAGCCAGGGGTGCAATGCGGCCAGCCTGGACCGAGGAAC
GCGCTTCGGCACGGACGCTGGCATAATGGTCGTAAACGACC

Consensus Sequence of SA2 (603 bp)

GCATATCAATAAGCGGAGGAAAAGAAACCAACAGGGATTGCCCCAGTAACGGCGA
GTGAAGCGGCAAGAGCTCAAATTTGAAAGCTGGCTCCTTCGGGGTCCGCATTGTAAT

TTGTAGAGGATGCTTCGGGAGCGGTCCCCATCTAAGTGCCCTGGAACGGGACGTCAT
AGAGGGTGAGAATCCCGTATGGGATGGGGTGTCCGCGCCCGTGTGAAGCTCCTTCG
ACGAGTCGAGTTGTTTGGGAATGCAGCTCTAAATGGGTGGTAAATTTTCATCTAAAGC
TAAATATTGGCCGGAGACCGATAGCGCACAAGTAGAGTGATCGAAAGATGAAAAGC
ACTTTGAAAAGAGAGTTAAAAAGCACGTGAAATTGTTGAAAGGGAAGCGCTTGCGA
CCAGACTCGCTCGCGGGGTTCAGCCGGCATTCTGTGCCGGTGTACTTCCCCGCGGGCG
GGCCAGCGTCGGTTTGGGCGGTCTGGTCAAAGGCCCTCGGAAGGTAACGCCCTAGG
GGCGTCTTATAGCCGAGGGTGCAATGCGACCTGCCTAGACCGAGGAACGCGCTTCG
GCTCGGACGCTGGCATAATGGTCGTAAACGACCCGTCTTG

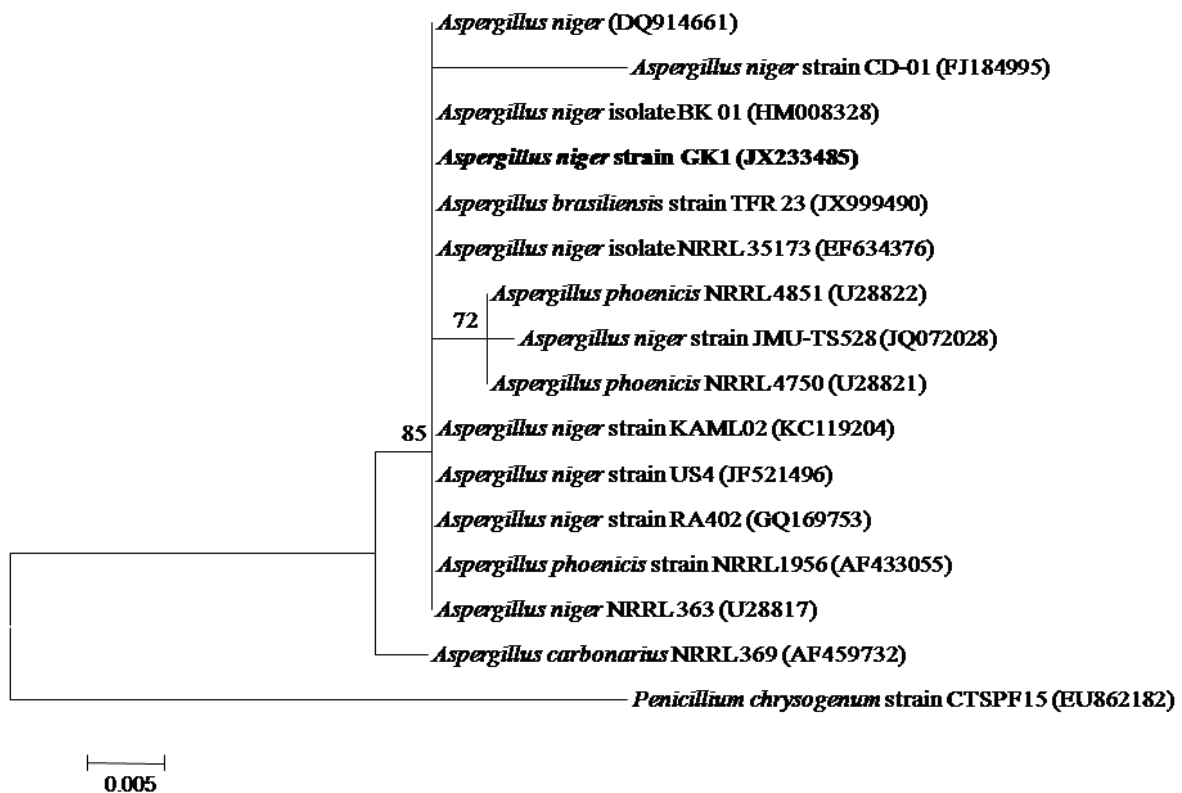


Fig 4.9: Phylogenetic Tree Showing the Relationships between SA4 Isolate and 10 Close Strain Types of *Aspergillus* species. Numbers over tree nodes are the bootstrap value. The scale bar at the bottom refers to branch length in terms of the number of steps in the tree construction.

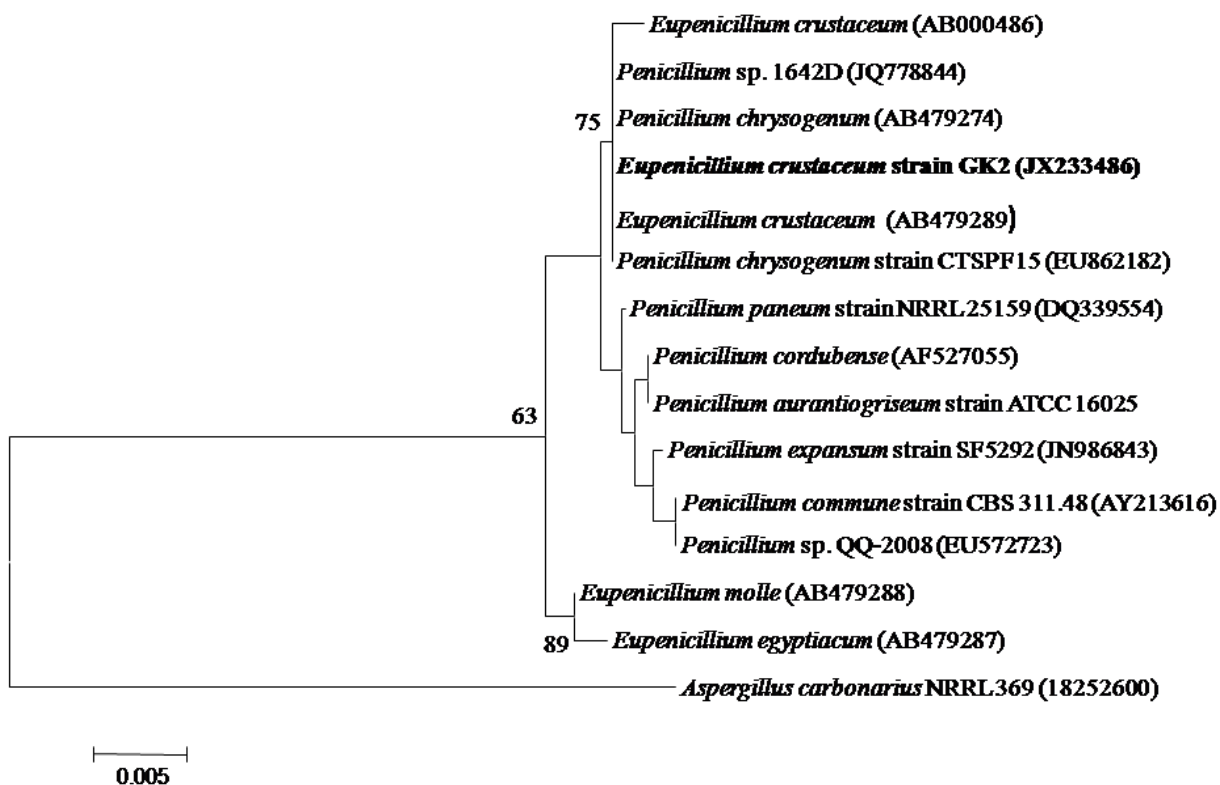


Fig 4.10: Phylogenetic Tree Showing the Relationships between *Eupenicillium crustaceum* strain GK2 and 15 Close Strain Types of *Penicillium* species. Numbers over tree nodes are the bootstrap value. The scale bar at the bottom refers to branch length in terms of the number of steps in the tree construction.

4.6 GROWTH AND PH PROFILE OF FUNGAL CULTURES

For growth studies, the fungal cultures were grown in PDB with pH at 5.6 ± 0.2 in shaking conditions at 120 rpm at 28 °C. In shake flask conditions, mycelium of *A. niger* (*Aspergillus niger*) were appeared as large beads of creamy white in color. At the end of the growth period, the color of the hyphae was changed to black color due to the formation of conidia. During the growth study of *E. crustaceum* (*Eupenicillium crustaceum*) under shake flask conditions, large number of tiny beads of light yellow color was appeared during growth period. Size and number of beads increased as the time progressed. The appeared hyphae were branched, scale like with

knobby ends. On the condensed mass, small hyphae appeared and the color of hyphae was changed from light yellow to bluish green at the end of the growth period. This bluish green color was of conidia because the hyphae are colorless in the inoculated flasks (Murad et al., 2003). At the end of growth period, decrease in pH due to organic acid production was observed in the range of 5.2-2.0 in fungal inoculated flasks.

4.6.1 Effect of Incubation on pH Profile

The fungal cultures were allowed to grow in minimal medium for a longer period of incubation in presence and absence of WFS and reduction in pH was observed. Reduction in pH was observed with increase in incubation days by both the cultures. The pH reduction was found to be less in flask inoculated with fungal cultures along with WFS. It shows that the presence of WFS hinders the growth of fungi and hence reduction of pH. Fig. 4.11 shows the reduction in pH with increase in incubation days in growth medium inoculated with *A. niger* in the presence and absence of WFS. Reduction in pH was observed as 5.8 to 4.1 after 4th day of incubation in medium (without WFS) but in medium containing WFS, reduction in pH was observed as 6.5 to 4.8. After 6 days of incubation, there was little increase in the pH level in flask containing medium and WFS. This was due to the complete utilization of carbon source by the fungal culture.

After 21 days of incubation the final reduction in pH was observed as 2.6 in flask containing minimal medium in the absence of WFS. Reduction in pH was observed as 3.8 in the medium containing WFS after 21 days of incubation. Similar pattern was also observed in case of *Eupenicillium crustaceum* (Fig. 4.12). It is clear that the pH decreases from the initial value with the increase in incubation period. The decrease in pH of the medium was mainly caused by the excreted metabolites produced by the fungi; the metabolites included H⁺ and organic acids (citric and oxalic) (Burgstaller and Schinner, 1993).

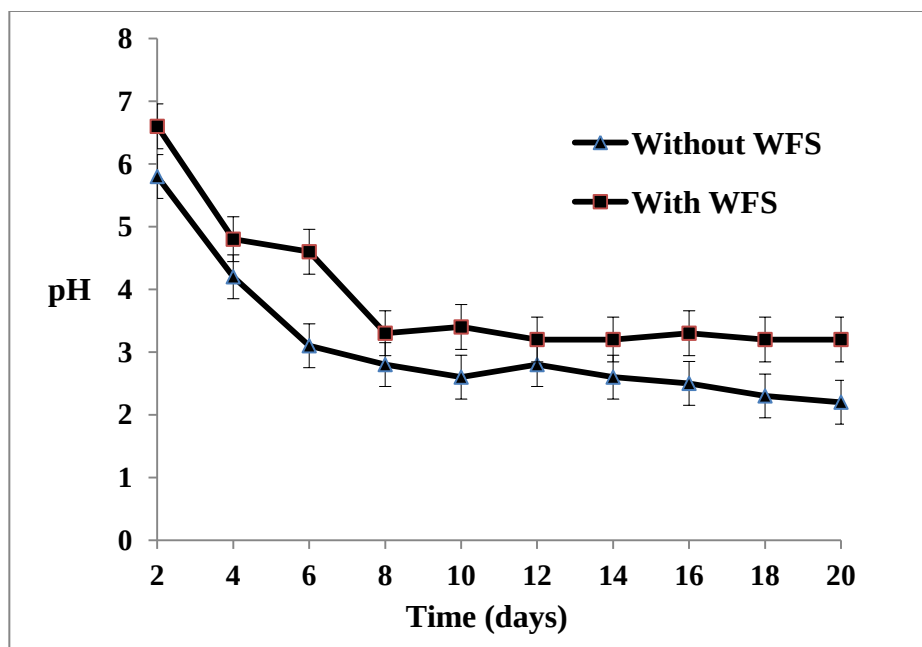


Fig 4.11: pH Profile with Varying Incubation Days (*Aspergillus niger*)

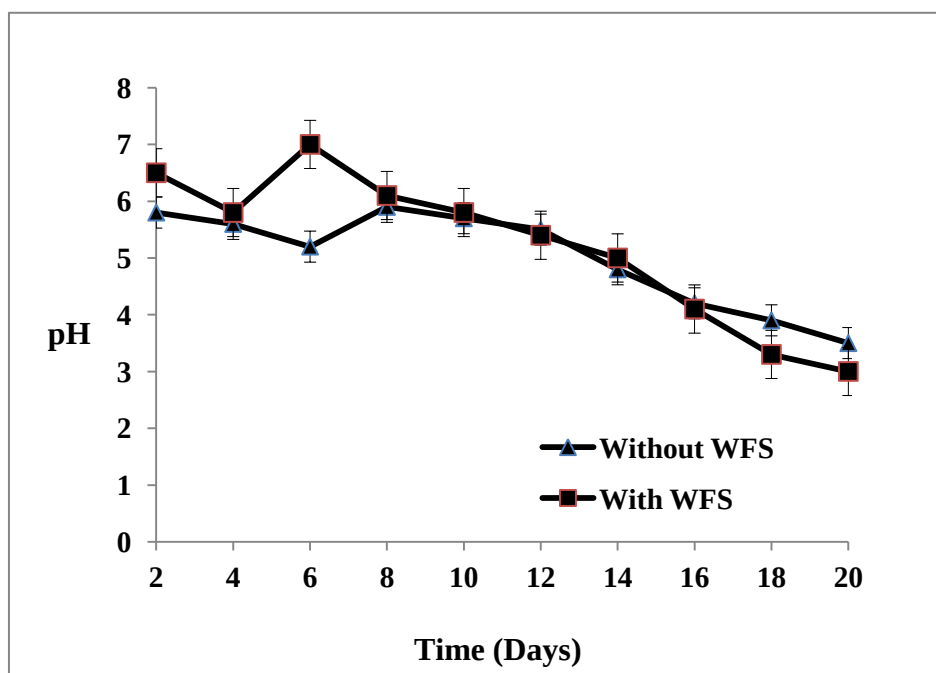


Fig 4.12: pH Profile with Varying Incubation Days (*E. crustaceum*)

4.6.2 Effect of Glucose Concentration on pH

In flask conditions, the fungal cultures were allowed to grow in minimal medium containing different concentration of glucose ranging from 1- 10% (w/v) for 7days (Fig. 4.13). In case of *Aspergillus niger*, pH was observed to be 3.6 at 1% glucose addition. It was observed that increase in glucose content favors the reduction in pH or in other words favor in acid production. Fig. 4.14 shows the reduction of pH is linear up till 5% glucose and after 6% decrease in pH is rapid and become constant at 9% and 10%. It indicates that further reduction of pH will occurred with increase in concentration of glucose. The range of pH reduction by *A. niger* is 3.6 to 2.4. At 10% glucose level, pH was reduced to 2.4 by *A. niger*.

In case of *Eupenicillium crustaceum*, pH was observed as 3.7 at the level of 1% glucose. Fig. 4.13, the pH decrease rapidly after 6% glucose and finally becomes 3.2 at 8%, 9% and 10%. Reduction of pH by *E. crustaceum* follows linear pattern and pH ranges between 3.7- 3.2.



Fig 4.13: Acid Formation with Varying Glucose Percentages

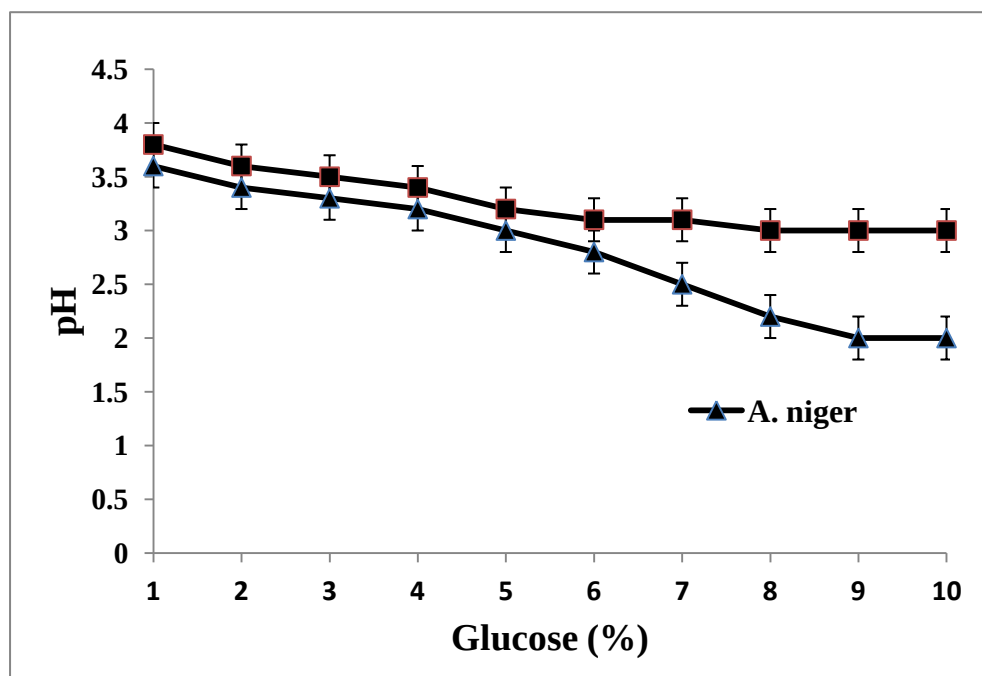


Fig 4.14: pH Profile with Varying Concentration of Glucose

4.6.3 Effect of Glucose Concentration on Biomass

For fungal biomass production, *A. niger* and *E. crustaceum* were allowed to grow in minimal medium containing various percentages of glucose at 28 °C for 7 days. Fig. 4.15 shows the biomass production by both fungal cultures with various percentages of glucose. It shows that with increase in percentage of glucose, there is increase in production of fungal biomass. In other words, presence of glucose favors the biomass production. In case of *A. niger*, there was constant increase in biomass production, where at 1% glucose level less than 0.1g of fungal biomass was observed. At 8% glucose level, production of biomass found to be 0.7g and after that little increase in production was observed at 10% glucose level i.e. 0.8g.

E. crustaceum was observed to be more efficient in production of biomass in presence of glucose as compared to *A. niger*. Increase in glucose level favors the biomass production of *E. crustaceum*. Initially the growth pattern for both the cultures was found to be similar, as both produce 0.3g of biomass at 5% glucose level. But at 6% glucose level, there is increase in biomass production by *E. crustaceum* i.e. 0.8 g. After 6% glucose level, little increase in biomass was observed.

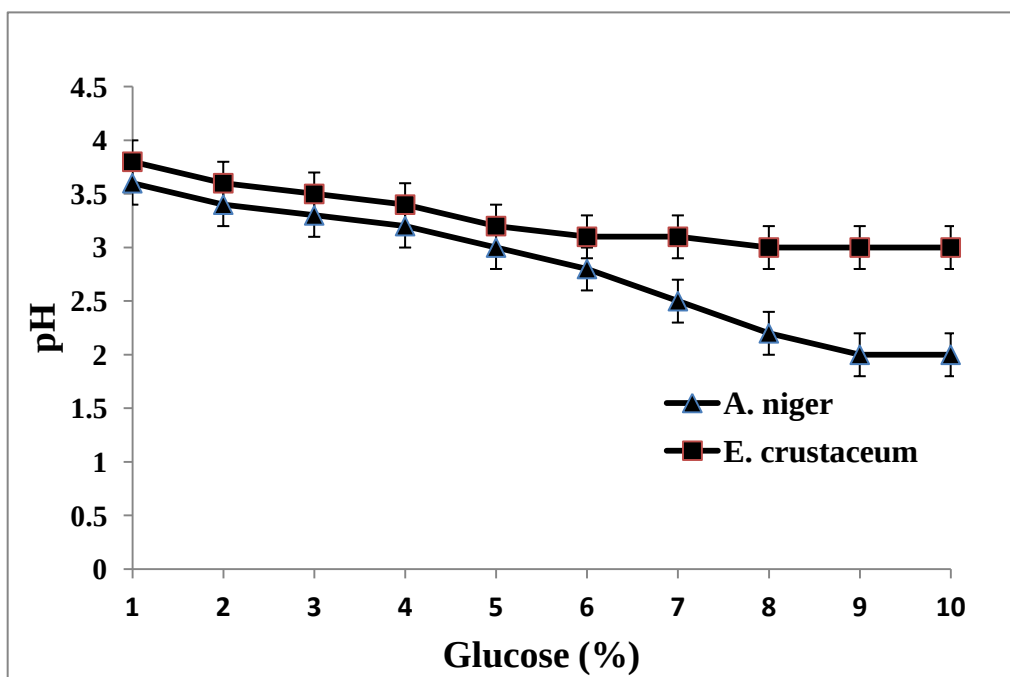


Fig 4.15: Biomass with Varying Percentages of Glucose

4.6.4 Effect of WFS Concentration on pH

Fungal cultures were grown in minimal medium containing various percentages of WFS (1-6%) and samples were taken after 4 and 7 days of inoculation. Fig. 4.16 shows the effect of various percentages of WFS on pH by both the fungal cultures. It was observed that the pH increased with the increase in percentage of WFS. In other words, acid production is decreased with increase in WFS percentage. This is mainly due to inhibition of fungal growth by the increase percentages of WFS, which was further due to the toxicity caused by the metals present in WFS.

In case of lower percentages (1% and 2%), the fungal cultures overcome the metal toxicity of WFS. After 4 days of incubation, there was no reduction in pH observed in various percentages rather it was observed that pH was found to be increase in higher percentages of WFS. After 7 days, reduction in pH was observed in 1% WFS as 1.9 and 2 by *A. niger* and *E. crustaceum* respectively (Fig. 4.17). Decrease in pH was also observed in 2% WFS as 3.5 by *A. niger* respectively. Little decrease in pH was observed in higher percentages of WFS.

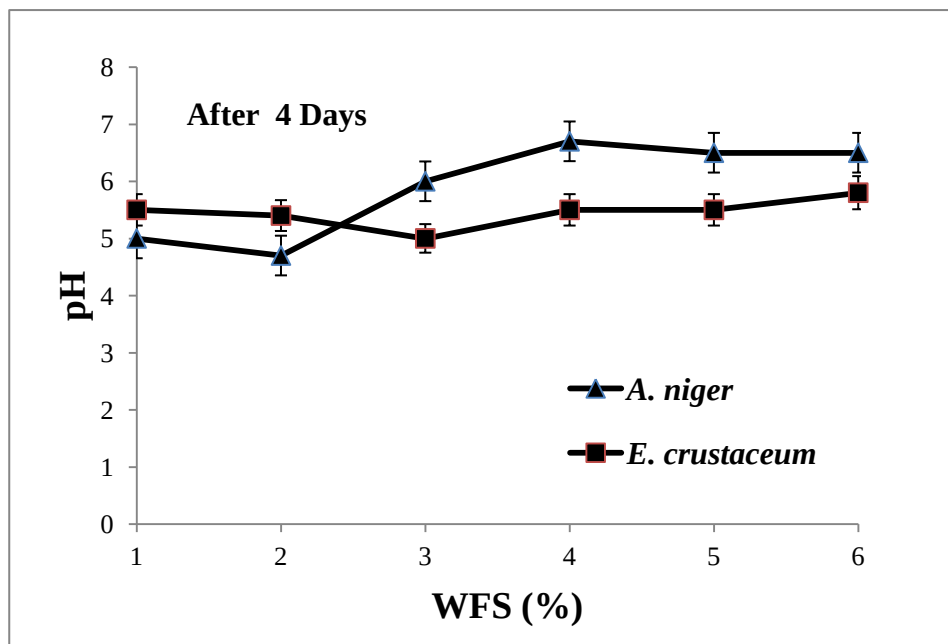


Fig 4.16: Effect of Various Percentages of WFS on pH (After 4 Days)

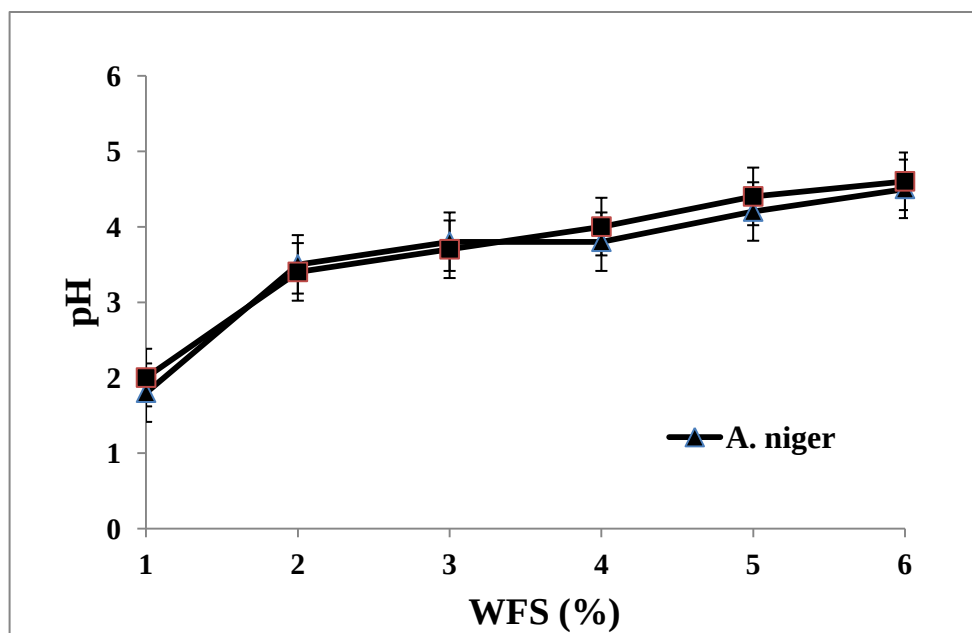


Fig 4.17: Effect of Various Percentages of WFS on pH (After 7 Days)

4.7 OPTIMIZATION OF FUNGAL INOCULUM

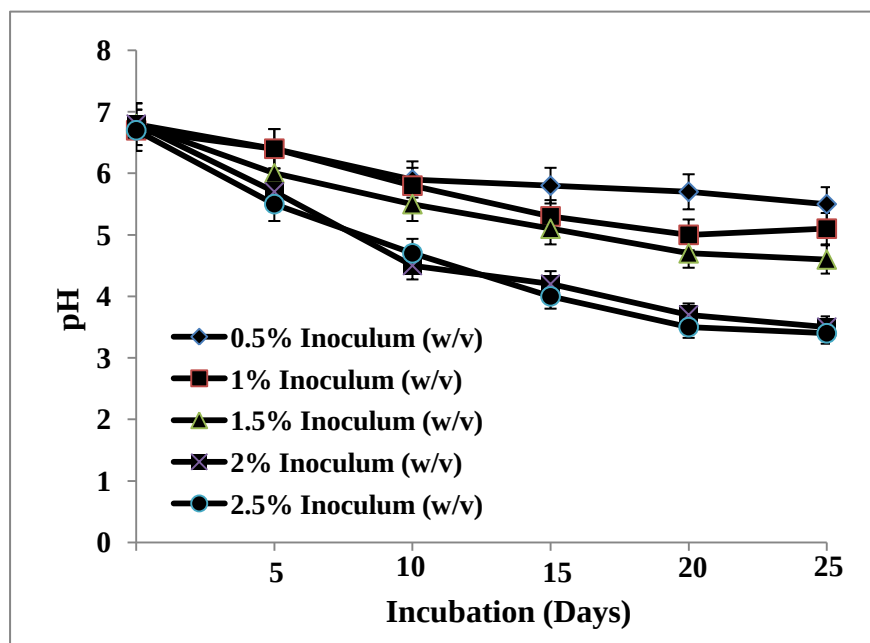


Fig 4.18: Reduction in pH by Various Percentage of Inoculum (*A.niger*)

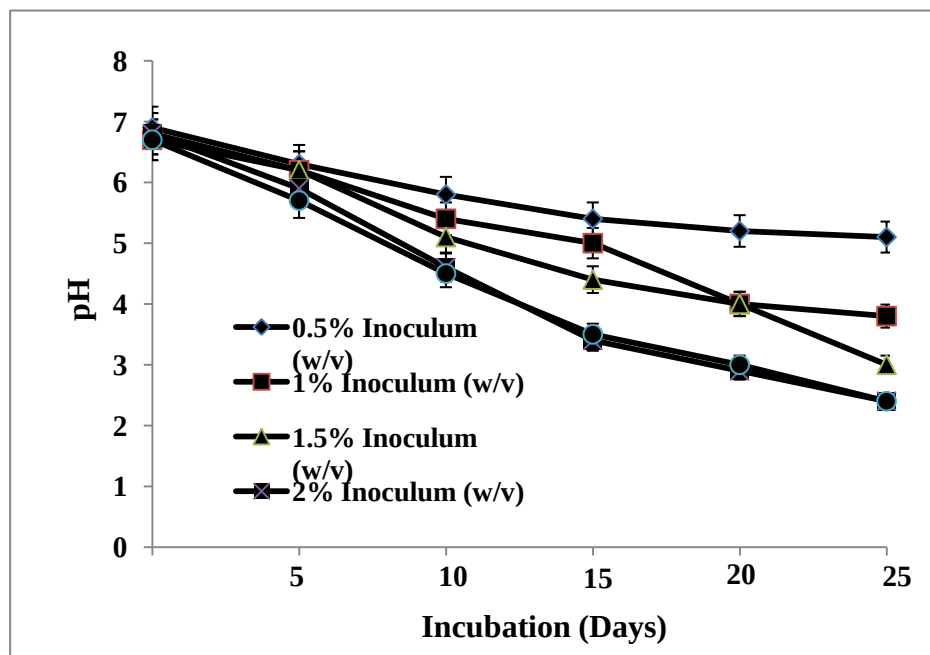


Fig 4.19: Reduction in pH by Various Percentage of Inoculum (*E. crustaceum*)

Table 4.3: Leachate Analysis of Fungal (*A.niger*) Inoculated WFS

Metal	WFS Untreated	Fungal Treated WFS (1.5% Fungal Inoculum)	Fungal Treated WFS (2% Fungal Inoculum)	Fungal Treated WFS (2.5% Fungal Inoculum)	WHO Standard limits (mg/l)	GWQS (mg/l)
Be	0.02±0.007	0.002±0.001	0.0005±0.0001	0.0004±0.0001	-	-
Ba	0.03±0.02	0.041±0.004	0.022±0.007	0.02±0.002	0.3	0.4
Cd	0.02±0.01	0.0079±0.002	0.0007±0.0002	0.0007±0.0001	0.003	0.0005
Cr	0.19±0.007	0.19±0.02	0.06±0.005	0.051±0.005	0.05	0.01
Co	0.01±0.003	0.008±0.004	0.0004±0.0001	0.0004±0.0001	-	-
Cu	0.051±0.011	0.031±0.004	0.02±0.002	0.023±0.001	2	-
Fe	0.25±0.04	0.09±0.02	0.06±0.001	0.058±0.006	-	0.15
Hg	0.1±0.08	0.082±0.03	0.54±0.02	0.54±0.01	0.001	0.0002
Li	0.07±0.02	0.037±0.002	0.03±0.001	0.028±0.002	-	-
Mo	0.10±0.08	0.08±0.01	0.01±0.002	0.01±0.001	0.07	-
Mg	0.05±0.003	0.036±0.01	0.025±0.003	0.02±0.01	-	-
Mn	0.14±0.06	0.13±0.002	0.036±0.001	0.03±0.001	0.5	0.02
Ni	0.05±0.04	0.041±0.002	0.016±0.001	0.015±0.002	0.02	-
Pb	0.19±0.014	0.12±0.004	0.03±0.002	0.02±0.004	0.01	0.001
Zn	0.31±0.11	0.30±0.03	0.07±0.003	0.066±0.01	3	2.5

Text in bold - Significant reduction in fungal treated samples as compared to standard limits

WHO- World health organization standard limits

GWQS- Ground water quality standards

Table 4.4: Leachate Analysis of Fungal (*E.crustaceum*) Inoculated WFS

Metal	WFS Untreated	Fungal Treated WFS (1.5% Fungal Inoculum)	Fungal Treated WFS (2% Fungal Inoculum)	Fungal Treated WFS (2.5% Fungal Inoculum)	WHO Standard limits (mg/l)	GWQS (mg/l)
Be	0.02±0.007	0.0001±0.0001	0.0001±0.00002	0.0001±0.00002	-	-
Ba	0.03±0.02	0.03±0.004	0.02±0.004	0.01±0.005	0.3	.4
Cd	0.02±0.01	0.004±0.002	0.0005±0.0002	0.0004±0.0002	0.003	0.0005
Cr	0.19±0.007	0.10±0.02	0.05±0.006	0.052±0.004	0.05	0.01
Co	0.01±0.003	0.005±0.004	0.0003±0.0001	0.0001±0.0001	-	-
Cu	0.051±0.011	0.022±0.004	0.01±0.003	0.01±0.001	2	-
Fe	0.25±0.04	0.08±0.02	0.04±0.02	0.045±0.03	-	0.15
Hg	0.1±0.08	0.08±0.03	0.05±0.02	0.05±0.01	0.001	0.0002
Li	0.07±0.02	0.03±0.003	0.02±0.007	0.022±0.004	-	-
Mo	0.10±0.08	0.06±0.02	0.004±0.002	0.004±0.001	0.07	-
Mg	0.05±0.003	0.03±0.01	0.02±0.01	0.01±0.01	-	-
Mn	0.14±0.06	0.11±0.002	0.01±0.004	0.015±0.001	0.5	0.02
Ni	0.05±0.04	0.033±0.002	0.013±0.006	0.011±0.004	0.02	-
Pb	0.19±0.014	0.10±0.004	0.01±0.008	0.01±0.004	0.01	0.001
Zn	0.31±0.11	0.28±0.03	0.05±0.02	0.05±0.01	3	2.5

Text in bold - Significant reduction in fungal treated samples as compared to standard limits

WHO- World health organization standard limits

GWQS- Ground water quality standards

Fungal growth was appeared on WFS surface after 30 days of incubation. Samples were taken from each tray (0.5%, 1%, 1.5%, 2% and 2.5%) and analyzed for pH and metal concentration reduction. It was found that reduction of pH at 2% and 2.5% inoculum level was more as compared to other percentages (Fig. 4.18 and 4.19). Also from metal reduction results, it was optimized that 2% fungal inoculum was found to be sufficient to inoculate the WFS for reduction of metal (Table 4.3 and 4.4).

4.8 CALCIUM OXALATE FORMATION BY *Eupenicillium crustaceum*

Fungal cultures were known to produce various types of minerals in the presence of different substrates. The ability of *Eupenicillium crustaceum* to produce mineral in broth containing urea- CaCl_2 was observed after 20 days of incubation.

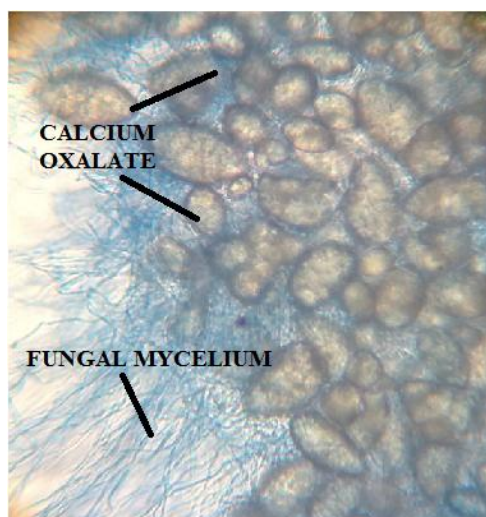


Fig 4.20: Microscopic Image of Fungal Mycelium and Calcium Oxalate Crystals

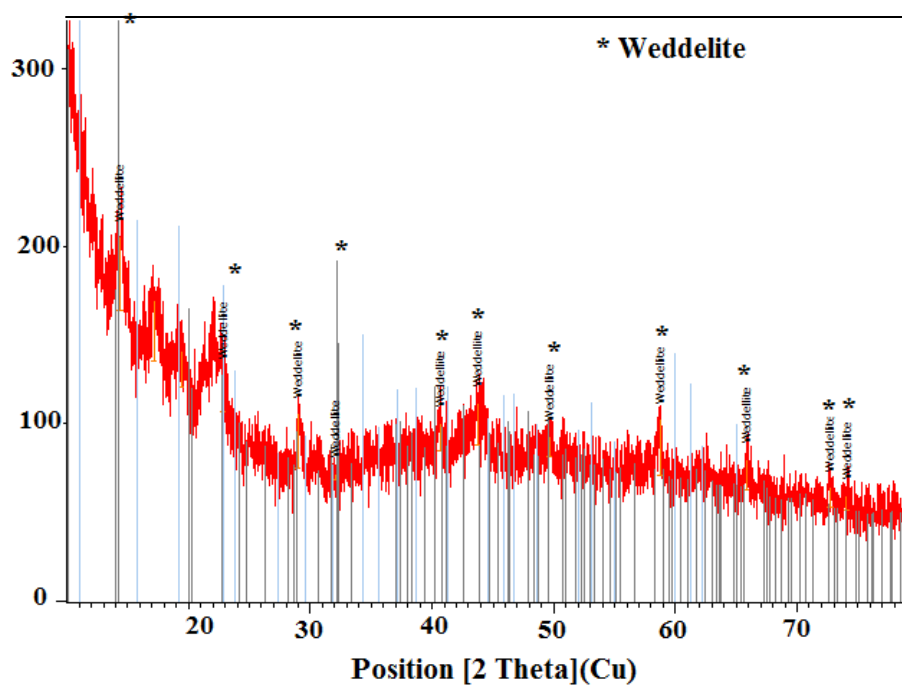


Fig 4.21 XRD Analysis Show Peaks of Weddellite (Calcium Oxalate)

Fig. 4.20 shows microscopic image of the fungal mycelium and calcium oxalate in broth. This broth containing fungal mycelium was filtered and then crushed to get fine powder. Peaks of calcium oxalate (weddellite) were observed in X-ray diffraction analysis. Fig 4.21 shows the XRD analysis of *Eupenicillium crustaceum* mycelium. Such type of results was not observed in case of *Aspergillus niger*. Similar results were reported by Burford et al. (2006). They investigated that the fungal cultures grown in laboratory microcosms showed biomineralization of fungal filaments and XRD showed that they were composed solely of calcite (CaCO_3) or of calcite with some calcium oxalate dehydrate ($\text{CaC}_2\text{O}_4 \cdot 2\text{H}_2\text{O}$).

4.9 PRODUCTION OF ORGANIC ACIDS BY FUNGAL CULTURES

Microbial oxidation of substrate is an acid producing system. During the growth studies of *A. niger* and *E. crustaceum*, substrate undergoes microbial oxidation which results in production of citric and oxalic, that play fundamental role in the environmental mobility of metals (Dronawat et al., 1995; Sayer and Gadd, 2001). Some of these acids act as complexing agent with two or more electron donors for metals. Citric and oxalic acid have chelating properties and have more affinity for Cr, Ni and Zn in the soil (Wasay et al., 2001).

To investigate the production of organic acids, flasks containing growth medium with WFS and without WFS were inoculated with *A. niger* and *E. crustaceum* separately and sample were analyzed by high performance liquid chromatography (HPLC) after 15 days. Samples were compared with the standard peaks of citric acid and oxalic acid (Figs. 4.22 and 4.23). Retention time, peak height and peak area of standard organic acids are shown in Table 4.5. It was observed from the HPLC data that major acid produced by *A. niger* and *E. crustaceum* was oxalic acid followed by citric acid. These acids are good metal chelators which can form complex with metal ions. Organic acid is called chelator if it has two or more electron donor groups. Hence one or more rings are formed and then the organic acid can be termed as chelating agent and resulting complex is termed as metal chelator (Denevre et al., 1996).

Table 4.5: Retention Time, Peak Height and Peak Area of Standard Organic Acids

Organic Acids	Retention Time	Peak Area (%)	Peak Height (%)	Concentration (ppm)
Standard Citric Acid	3.15	100	100	1000
Standard Oxalic Acid	2.9	96	94	1000
Sample without WFS (<i>A. niger</i>)	2.9	93.1	93.8	917
	3.3	3	2.6	214
Sample with WFS (<i>A. niger</i>)	2.9	27.4	20.4	883
	3.3	15.6	19.9	187
Sample without WFS (<i>E. crustaceum</i>)	2.9	80	79.9	950
	3.29	12.3	10.8	321
Sample with WFS (<i>E. crustaceum</i>)	2.9	79.9	69.9	916
	3.3	13.6	19.7	267

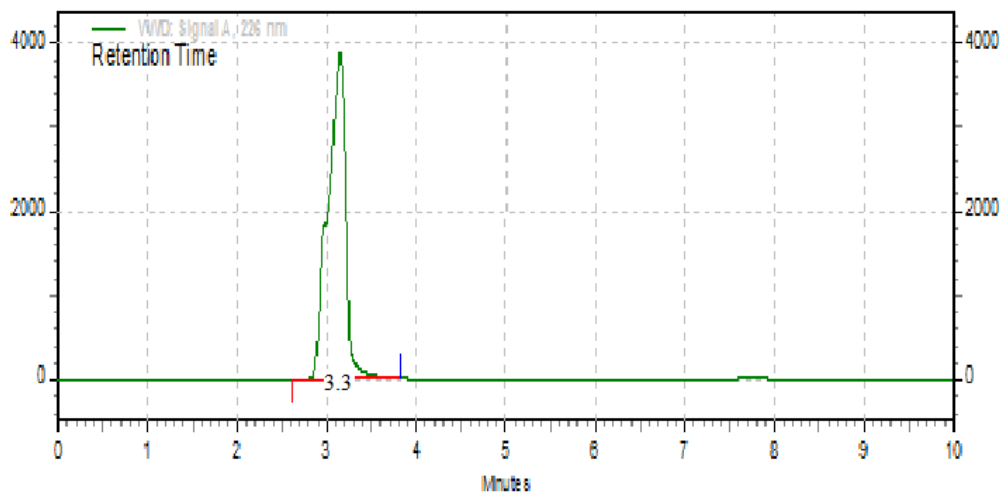


Fig 4.22: Peak of standard citric acid

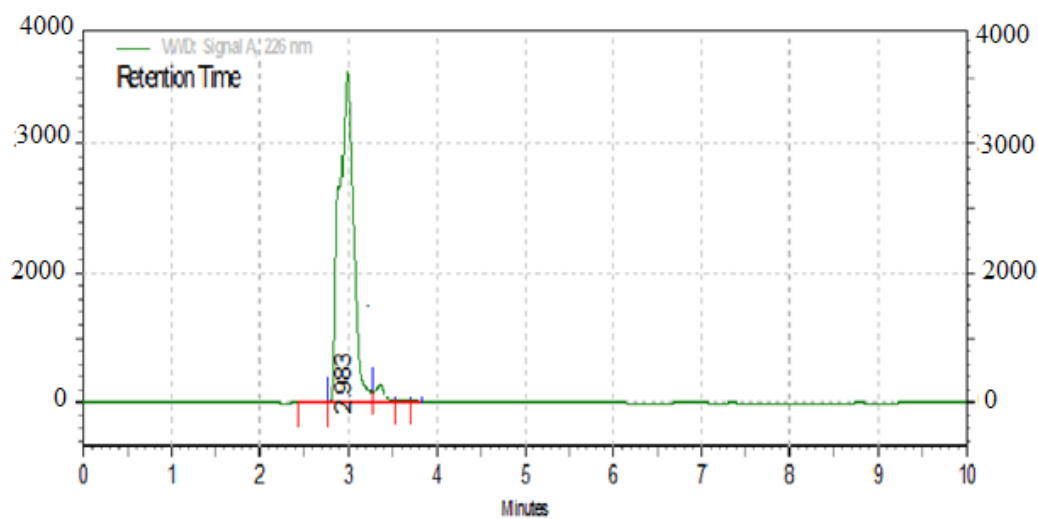


Fig 4.23: Peak of standard oxalic acid

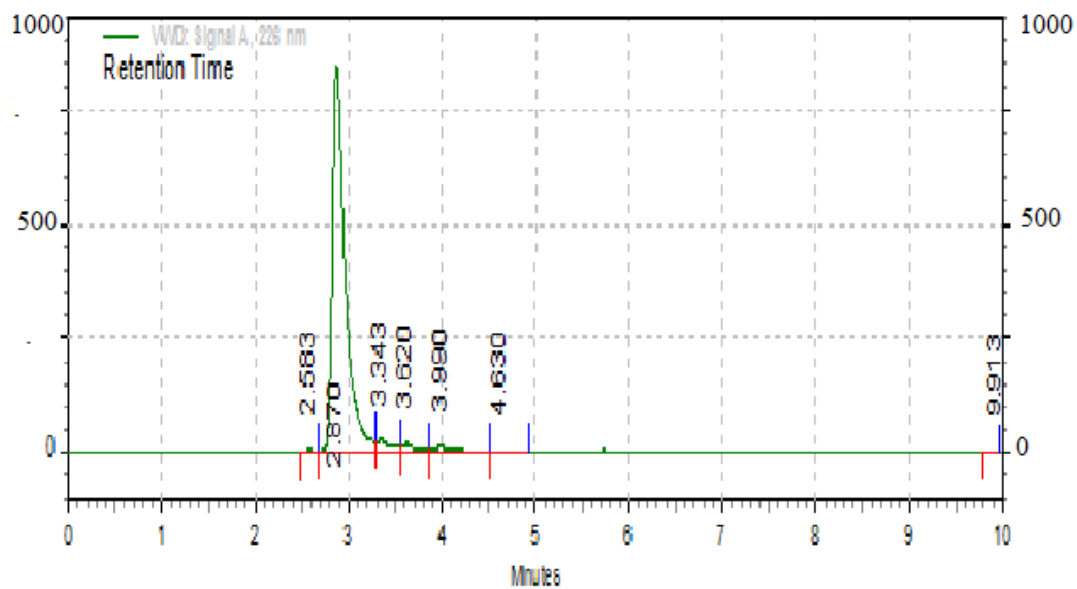


Fig 4.24: Peaks shown by the sample with *A. niger* (Without WFS)

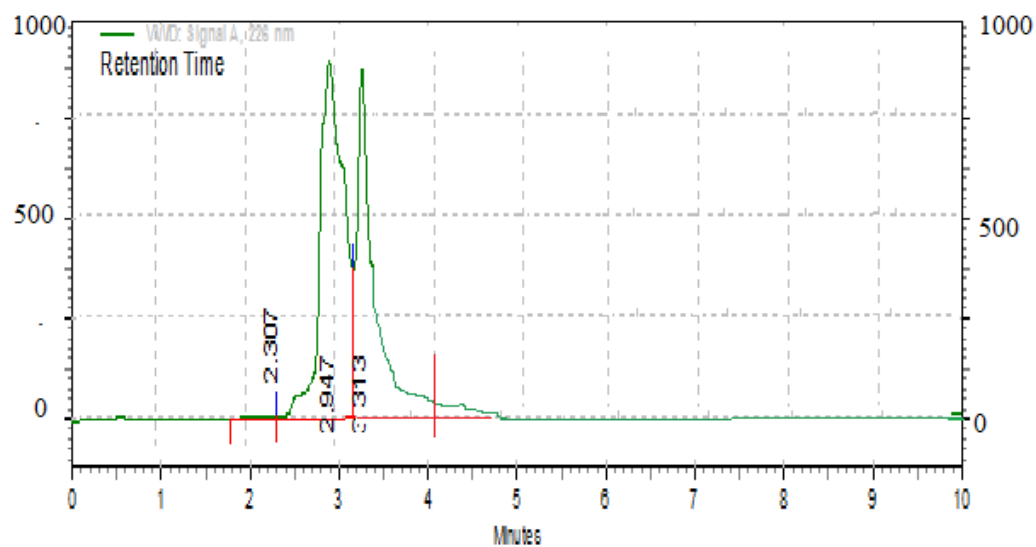


Fig 4.25: Peaks shown by sample with *A. niger* and WFS

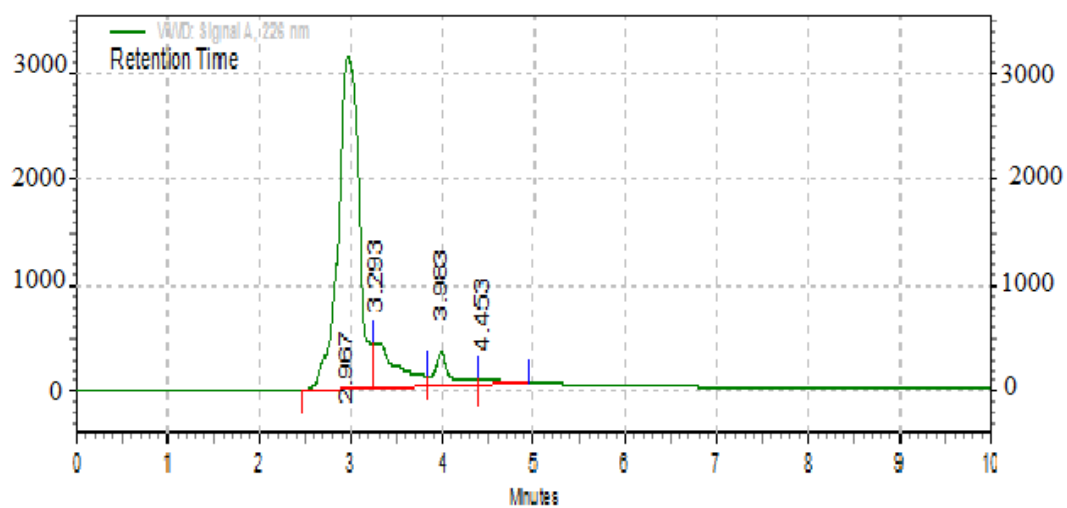


Fig 4.26: Peaks shown by sample with *E. crustaceum* (Without WFS)

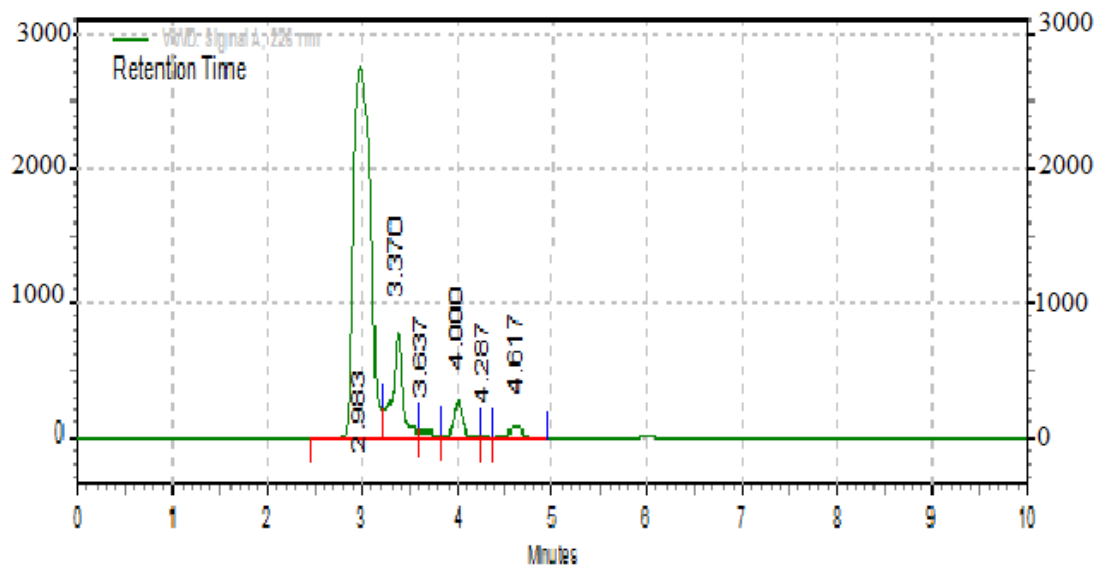


Fig 4.27: Peaks shown by sample with *E. crustaceum* and WFS

In samples containing WFS inoculated with *A. niger*, major peak was of oxalic acid along with minor peak of citric acid but in samples without WFS, oxalic and citric acid peaks were appeared as major peaks (Figs. 4.24 and 4.25). This suggests that presence of WFS inhibits the production of citric acid. Table 4.3 shows retention time, peak area and peak height of samples. Samples containing WFS inoculated with *E. crustaceum*, similar trend was observed as earlier in case of sample inoculated with *A. niger* (Figs. 4.26 and 4.27). It is well known that in the presence of toxic metals, the fungus ameliorates the toxic effects of metals through complexation or precipitation by excreted metabolites (Gadd, 1992). The higher accumulation of oxalic acid by *A. niger* and *E. crustaceum* in the presence of WFS is mainly due to the induction of the enzyme oxaloacetate hydrolase and the presence of heavy metals, such as Co, Mo and Ni.

4.10 METAL ANALYSIS OF LEACHATE

In this work, metal analysis of the leachate obtained from concrete mix made with untreated and fungal treated WFS (0%, 10%, 15% and 20%) was done by inductively coupled plasma mass spectrometry (ICP- MS). Fifteen metals (Ba, Be, Cd, Cr, Co, Cu, Fe, Hg, Li, Mo, Mg, Mn, Ni, Pb and Zn) were analyzed. Tables 4.6-4.8 show the metal analysis of leachate obtained from concrete mix made with untreated and fungal (*Aspergillus niger*) treated WFS (0%, 10%, 15%

and 20%). Utilization of WFS in concrete led to little increase in metal content in leachate obtained from concrete containing untreated WFS as compared to concrete without WFS. In concrete mix containing 10% WFS (untreated), the metal concentrations of Cd, Cr, Fe, Mo, Mn, Ni and Pb were 0.008mg/l, 0.12 mg/l, 0.20 mg/l, 0.02 mg/l, 0.06 mg/l, .018 mg/l and 0.087 mg/l respectively. When it was compared to concrete mix containing 15% WFS (untreated), it was found to be marginal increase in its concentrations i.e. 0.009 mg/l (Cd), 0.26mg/l (Fe), 0.08 mg/l (Mo), 0.08 mg/l (Mn), 0.02 mg/l (Ni) and 0.09 mg/l (Pb). Further, increase in metal concentrations were observed in leachate obtained from concrete mix containing 20% untreated WFS. Results showed that increase in various percentages of WFS contributes to the metal contamination in leachate. In casting process, organic additives and resins undergo thermal decomposition to produce number volatile and non- volatile organic compounds (Dungan and Reeves, 2005).

While most resin components are organic in nature, some phenolic resins are manufactured using divalent metal salts (e.g. based on Cd, Co, Cu, Mg, Ni, Pb, and Zn) to increase ortho orientation (Gardziella et al., 1999). Unfortunately, the presence of heavy metals in the resins can potentially lead to elevated concentrations within the WFS (Miguel et al., 2011). In general, the mean metal concentrations in the leachate obtained from concrete made with WFS were greater than those found in concrete without WFS. This result demonstrates that metals are being added to the sands when the resin binders are used to make the molds and cores and/or during the casting process. The largest difference in mean metal concentrations between the leachate of concrete with and without WFS was noted for Ni, Pb and Cr which can be attributed to the fact that waste sands were obtained from foundries that cast these metals.

WFS are used as an aggregate replacement in agricultural, geotechnical and construction purposes. Despite these benefits, the beneficial use WFSs has been constrained by many local and state governments due to uncorroborated concerns over potential contamination with metals. However, recent research has shown that the majority of foundry sands are a low contaminate waste with a low leaching potential when beneficially used in unencapsulated applications (Guney et al., 2006; Deng and Tikalsky, 2008). Tables 4.9, 4.10 and 4.11 present a comparison of mean concentrations of critical trace metals in leachate from concrete containing untreated and fungal (*E. crustaceum*) treated WFS and world Health organization (WHO) standard limits

and ground water quality standards (GWQS). It clearly shows that some of the metal concentrations such as Cd, Cr, Fe, Mo, Mn, Ni and Pb are higher in its concentration as compared to WHO and GWQS. These metals can be harmful to human and animal health if found at elevated concentrations in the environment (Adriano, 2011).

Inclusion of fungal treated WFS in concrete inhibit the leaching of these metals to the environment. Reduction of metal leaching is attributed due to the uptake or absorption of metals by fungal mycelium for its metabolic activity. Results show the reduction in the metal concentration in leachate obtained from fungal treated concrete. In *A. niger* treated concrete made with WFS (10%), the metal concentration in Cd, Cr, Fe, Mo, Mn, Ni and Pb were reduced from (.008 mg/l to .001 mg/l), (.12 mg/l to .06 mg/l), (.20 mg/l to .04 mg/l), (.02 mg/l to .005 mg/l), (.06 mg/l to .03 mg/l), (.018 mg/l to .017 mg/l) and (.08 mg/l to .01 mg/l) respectively. Likewise, in *E. crustaceum* treated concrete mix made with WFS (10%), the metal concentration in Cd, Cr, Fe, Mo, Mn, Ni and Pb were reduced from (.008 mg/l to .0009 mg/l), (.12 mg/l to .11 mg/l), (.20 mg/l to .003 mg/l), (.02 mg/l to .002 mg/l), (.06 mg/l to .01 mg/l), (.018 mg/l to .0006 mg/l) and (.08 mg/l to .01 mg/l) respectively. Metal reduction was observed to be more effective in concrete mix containing *E. crustaceum* treated WFS.

This was mainly because fungi removed both soluble and insoluble metal species from solutions and were also able to leach metal cations from solid waste (Burgstaller and Schinner, 1993). Sayer et al. (1997) suggested that fungi are capable of producing organic acids which can solubilize and complex metal cations. The production of organic acids provides anions and protons for metal leaching by acidolysis and complexolysis. Rezza et al. (2001) concluded that in acidolysis, the metals were solubilized through organic or inorganic acid produced by microorganism. In complexolysis, the anions form complexes with the metal cations led to produce metal complexes. Low pH favored acidolysis, with the release and enhanced mobility of free metal cations by protonation.

Table 4.6: Metal Analysis of Leachate Obtained from Concrete Mix Containing Untreated and Fungal (*A. niger*) Treated 10% WFS

Metals	Concrete Mix with 10% WFS (Untreated)	Concrete Mix with 10% WFS (Fungal Treated)	WHO Standard Limits (mg/l)	GWQS(mg/l)
Be	0.003±0.001	0.001±0.0006	-	-
Ba	0.04±0.005	0.04±0.018	0.3	0.4
Cd	0.008±0.001	0.001±0.0005	0.003	0.0005
Cr	0.12±0.011	0.06±0.011	0.05	0.01
Co	0.01±0.003	0.0005±0.0001	-	-
Cu	0.03±0.01	0.01±0.008	2	-
Fe	0.20±0.05	0.04±0.001	-	0.15
Hg	0.67±0.01	0.60±0.03	0.001	0.0002
Li	0.06±0.02	0.02±0.013	-	-
Mo	0.02±0.01	0.005±0.002	0.07	-
Mg	0.04±0.01	0.02±0.005	-	-
Mn	0.06±0.01	0.03±0.001	0.5	0.02
Ni	0.018±0.002	0.017±0.008	0.02	-
Pb	0.087±0.004	0.01±0.005	0.01	0.001
Zn	0.19±0.01	0.14±0.03	3	2.5

Text in bold - Significant reduction in fungal treated samples as compared to standard limits

WHO- World health organization standard limits;

GWQS- Ground water quality standards

Table 4.7: Metal Analysis of Leachate Obtained from Concrete Mix Containing Untreated and Fungal (*A. niger*) Treated 15% WFS

Metals	Concrete Mix with 15% WFS (Untreated)	Concrete Mix with 15% WFS (Fungal treated)	WHO Standard Limits (mg/l)	GWQS (mg/l)
Be	0.005±0.0008	0.00006±0.00002	-	-
Ba	0.04±0.008	0.02±0.003	0.3	0.4
Cd	0.009±0.002	0.0007±0.0001	0.003	0.0005
Cr	0.12±0.016	0.06±0.001	0.05	0.01
Co	0.002±0.0011	0.0003±0.0001	-	-
Cu	0.04±0.005	0.01±0.009	2	-
Fe	0.26±0.04	0.03±0.008	-	0.15
Hg	0.70±0.02	0.55±0.01	0.001	0.0002
Li	0.07±0.006	0.03±0.009	-	-
Mo	0.08±0.02	0.004±0.0005	0.07	-
Mg	0.04±0.009	0.02±0.001	-	-
Mn	0.08±0.001	0.01±0.004	0.5	0.02
Ni	0.02±0.005	0.01±0.001	0.02	-
Pb	0.09±0.001	0.01±0.009	0.01	0.001
Zn	0.22±0.07	0.07±0.002	3	2.5

Text in bold - Significant reduction in fungal treated samples as compared to standard limits

WHO- World health organization standard limits;

GWQS- Ground water quality standards

Table 4.8: Metal Analysis of Leachate Obtained from Concrete Mix Containing Untreated and Fungal (*A. niger*) Treated 20% WFS

Metal	Concrete Mix with 20% WFS (Untreated)	Concrete Mix with 20% WFS (Fungal Treated)	WHO Standard Limits (mg/l)	GWQS (mg/l)
Be	0.01±0.007	0.00005±0.00001	-	-
Ba	0.04±0.01	0.02±0.007	0.3	0.4
Cd	0.01±0.009	0.0007±0.0002	0.003	0.0005
Cr	0.17±0.06	0.06±0.005	0.05	0.01
Co	0.01±0.01	0.0003±0.0001	-	-
Cu	0.05±0.02	0.01±0.003	2	-
Fe	0.29±0.06	0.03±0.0006	-	0.15
Hg	0.71±0.01	0.54±0.02	0.001	0.0002
Li	0.08±0.007	0.03±0.004	-	-
Mo	0.12±0.05	0.01±0.001	0.07	-
Mg	0.05±0.02	0.01±0.005	-	-
Mn	0.12±0.02	0.01±0.001	.5	0.02
Ni	0.03±0.01	0.01±0.002	0.02	-
Pb	0.16±0.04	0.01±0.002	0.01	0.001
Zn	0.26±0.07	0.07±0.005	3	2.5

Text in bold - Significant reduction in fungal treated samples as compared to standard limits
WHO- World health organization standard limits;
GWQS- Ground water quality standards

Table 4.9: Metal Analysis of Leachate Obtained from Concrete Mix Containing Untreated and Fungal (*E. crustaceum*) Treated 10% WFS

Metals	Concrete Mix with 10% WFS (Untreated)	Concrete Mix with 10% WFS (Fungal Treated)	WHO Standard Limits (mg/l)	GWQS(mg/)
Be	0.003±0.001	0.00003±0.00002	-	-
Ba	0.04±0.005	0.03±0.008	0.3	0.4
Cd	0.008±0.001	0.0009±0.0005	0.003	0.0005
Cr	0.12±0.011	0.11±0.06	0.05	0.01
Co	0.01±0.003	0.0005±0.0002	-	-
Cu	0.03±0.01	0.001±0.002	2	-
Fe	0.20±0.05	0.003±0.08	-	0.15
Hg	0.67±0.01	0.55±0.09	0.001	0.0002
Li	0.06±0.02	0.03±0.005	-	-
Mo	0.02±0.01	0.002±0.0003	0.07	-
Mg	0.04±0.01	0.02±0.01	-	-
Mn	0.06±0.01	0.01±0.004	0.5	0.02
Ni	0.018±0.002	0.006±0.0007	0.02	-
Pb	0.087±0.004	0.01±0.03	0.01	0.001
Zn	0.19±0.01	0.06±0.02	3	2.5

Text in bold - Significant reduction in fungal treated samples as compared to standard limits

WHO- World health organization standard limits;

GWQS- Ground water quality standards

Table 4.10: Metal Analysis of Leachate Obtained from Concrete Mix Containing Untreated and fungal (*E. crustaceum*) Treated 15% WFS

Metals	Concrete Mix with 15% WFS (Untreated)	Concrete Mix with 15% WFS (Fungal treated)	WHO Standard Limits (mg/l)	GWQS (mg/l)
Be	0.005±0.0008	0.00005±0.00003	-	-
Ba	0.04±0.008	0.029±0.008	0.3	0.4
Cd	0.009±0.002	0.009±0.003	0.003	0.0005
Cr	0.12±0.016	0.12±0.05	0.05	0.01
Co	0.002±0.0011	0.001±0.0005	-	-
Cu	0.04±0.005	0.01±0.006	2	-
Fe	0.26±0.04	0.03±0.01	-	0.15
Hg	0.70±0.02	0.53±0.09	0.001	0.0002
Li	0.07±0.006	0.04±0.002	-	-
Mo	0.08±0.02	0.003±0.0004	0.07	-
Mg	0.04±0.009	0.02±0.01	-	-
Mn	0.08±0.001	0.01±0.003	0.5	0.02
Ni	0.02±0.005	0.005±0.001	0.02	-
Pb	0.09±0.001	0.01±0.01	0.01	0.001
Zn	0.22±0.07	0.07±0.02	3	2.5

Text in bold - Significant reduction in fungal treated samples as compared to standard limits

WHO- World health organization standard limits;

GWQS- Ground water quality standards

Table 4.11: Metal Analysis of Leachate Obtained from Concrete Mix Containing Untreated and Fungal (*E. crustaceum*) Treated 20% WFS

Metal	Concrete Mix with 20% WFS (Untreated)	Concrete Mix with 20% WFS (Fungal Treated)	WHO Standard Limits (mg/l)	GWQS (mg/l)
Be	0.01±0.007	0.00005±0.00002	-	-
Ba	0.04±0.01	0.03±0.01	0.3	0.4
Cd	0.01±0.009	0.0007±0.00005	0.003	0.0005
Cr	0.17±0.06	0.08±0.03	0.05	0.01
Co	0.01±.01	0.001±0.002	-	-
Cu	0.05±0.02	0.01±0.001	2	-
Fe	0.29±0.06	0.03±0.007	-	0.15
Hg	0.71±0.01	0.53±0.09	0.001	0.0002
Li	0.08±0.007	0.04±0.006	-	-
Mo	0.12±0.05	0.003±0.002	0.07	-
Mg	0.05±0.02	0.02±0.004	-	-
Mn	0.12±0.02	0.01±0.008	0.5	0.02
Ni	0.03±0.01	0.005±0.002	0.02	-
Pb	0.16±0.04	0.01±0.005	0.01	0.001
Zn	0.26±0.07	0.07±0.008	3	2.5

Text in bold - Significant reduction in fungal treated samples as compared to standard limits

WHO- World health organization standard limits;

GWQS- Ground water quality standards

4.11 EFFECTS OF FUNGUS ON CONCRETE PROPERTIES

4.11.1 Compressive Strength

Concrete cubes of M20 Grade (33.1 N/mm² at 28 days) were prepared to study the effect of fungus on compressive strength of concrete made with WFS. Compressive strength was investigated for concrete mixes made with WFS (untreated), without WFS (control) and fungal treated WFS at the age of 28 days. For fungal treatment of WFS, *Eupenicillium crustaceum* and

Aspergillus niger were used in concrete containing fungal treated WFS. At 28 days, the control mix (0% WFS) achieved compressive strength of 33.1 N/mm², whereas compressive strength of mixes containing 10%, 15% and 20% WFS (untreated) was 33.76, 29.30 and 27.80 N/mm², respectively. Inclusion of WFS as partial replacement of sand in concrete mixes demonstrated reduction in compressive strength. Decrease in compressive strength was observed in concrete mixes containing 15% and 20% WFS.

It was observed that compressive strength (33.76 N/mm²) of concrete containing 10% WFS was found be same as that of control concrete (33.1 N/mm²). This indicated that the particle size distribution of the concrete mix with 10% WFS has sufficient adherence than that of other concrete mixes. Similar results were reported by Siddique et al. (2009) and Guney et al. (2010), whereas compressive strength decreased with an increasing amount of foundry sand. The concrete with 10% waste foundry replacement indicated the optimum reallocation amount of waste foundry sand. The concrete with 10% waste foundry sand shows almost the same strength of control concrete mix; whereas the other entire waste foundry added mixtures exhibit lower values than that of the control.

Fungal (*E. crustaceum*) treated concrete exhibited compressive strength of 35, 35.20, 36.46 N/mm² with 10%, 15%, and 20% WFS, respectively. In case of *A. niger* treated WFS which exhibited compressive strength of 36.10, 32.81, 32.40 N/mm² with 10%, 15%, and 20% WFS. Fig. 4.28 shows the compressive strength of control (0% WFS), fungal (*E. crustaceum*) treated and untreated concretes containing (10%, 15%, and 20% WFS) There was significant increase in compressive strength of concrete containing fungal treated WFS as compared to untreated concrete. There was 24% increase in compressive strength at 20% replacement of fungal (*E. crustaceum*) treated WFS after 28 days as compared to untreated concrete. Marginal increase in compressive strength at 10% and 15% replacement of fungal treated waste foundry sand in concrete cubes is observed.

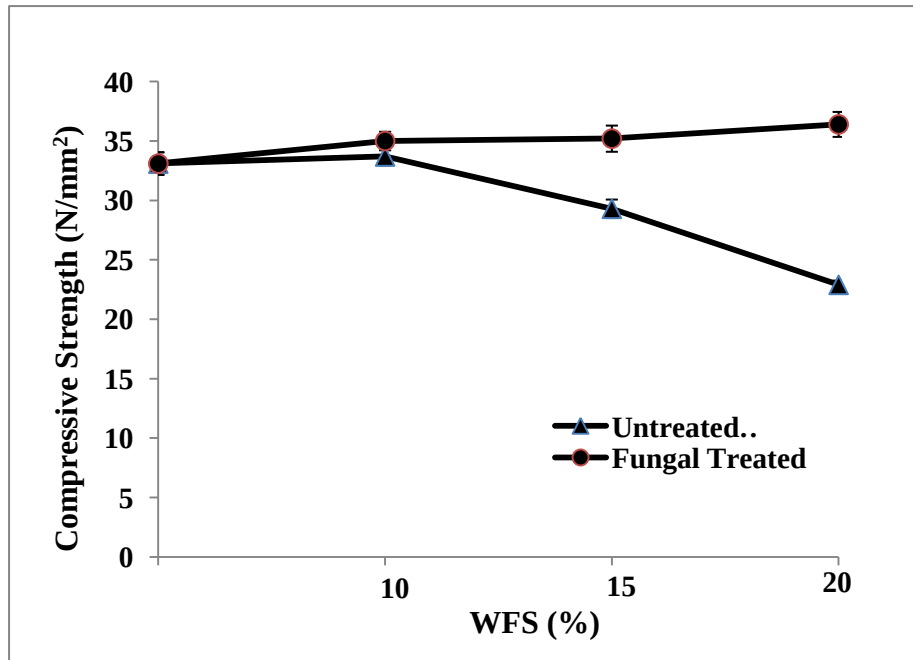


Fig 4.28: Compressive Strength of Concrete Containing Fungal (*E. crustaceum*) Treated and Untreated WFS

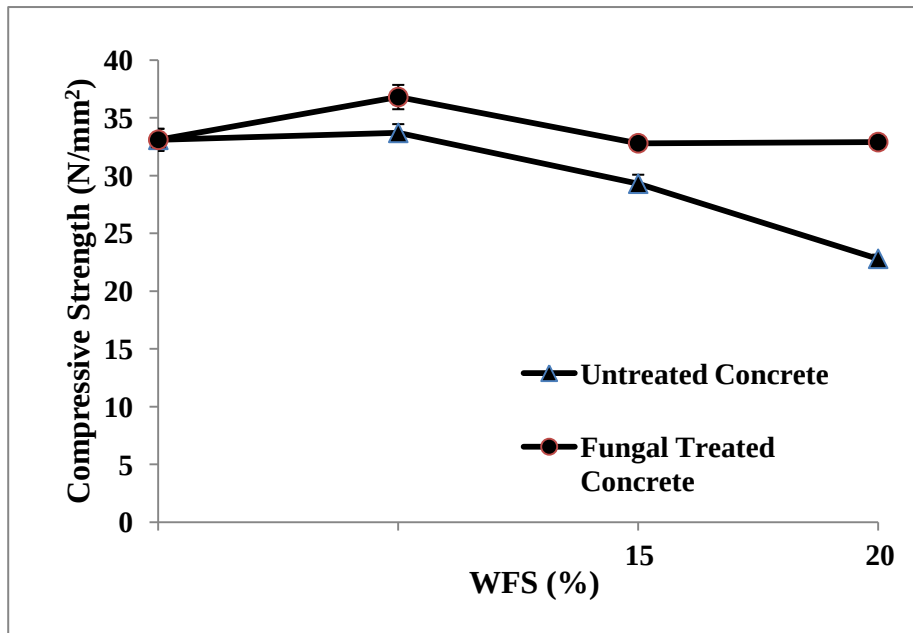


Fig 4.29: Compressive Strength of Concrete containing Fungal Treated (*A. niger*) and Untreated WFS

In case of concrete made with *A. niger* treated WFS, an increase of 15.6% in compressive strength with 20% WFS was observed (Fig. 4.29). *E. crustaceum* showed better results in compressive strength than *A. niger*. This improvement in compressive strength was probably due to deposition fungal biomineral within the pores of cement sand matrix, which plugs the pores within the concrete. Improvement in compressive strength by inclusion of microorganism in cement mortar was also studied by Ghosh et al. (2005). They used bacterial cultures in mortar and found 25% increase in 28 day compressive strength of cement mortar was achieved with the addition of about 10^5 cell/ml of mixing water

4.11.2 Water Absorption and Porosity

Use of fungal treated WFS in concrete led to decrease in water absorption as compared to control and concrete containing various percentages of untreated WFS. Reduction in water absorption was obtained as 16.6%, 20% and 44.4% in concrete made with fungal (*E. crustaceum*) treated WFS (10%, 15% and 20%) as compared to concrete made with untreated WFS (Fig. 4.30). Similarly, in case of *A. niger*, water absorption was obtained as 33.3%, 40% and 45% in concrete made with fungal treated WFS (10%, 15% and 20%) as compared to concrete containing untreated WFS (Fig. 4.31). This may be due to the high absorption capacity of WFS would leave the mix comparatively dry eventually hinders the strength gain but by inclusion of fungal treated WFS to concrete, led to decrease in water absorption capacity which ultimately increased the strength of concrete.

Figs. 4.32 and 4.33 shows reduction in porosity in concrete made with fungal (*Eupenicillium crustaceum* and *Aspergillus niger*) treated WFS as compared to concrete made with untreated WFS. Results showed that porosity led to decrease with the increase in WFS content in concrete made with untreated WFS as compared to control. It also showed that fungal treated WFS led to the further decrease in porosity of the concrete. In concrete containing fungal treated WFS, decrease in water absorption and porosity was probably due to deposition fungal biomass within the pores cement sand matrix, which plugs the pores within the concrete. Guney et al. (2010) determined the water absorption and void ratio of concrete containing WFS as partial replacement of fine aggregate. It was observed that water absorption ratio decreased for the specimens having waste foundry sand of 10% and 15%. This may be explained as the usage

waste foundry sand decreases the voids in the concrete, therefore water absorption values have tendency to decrease in the specimens with greater waste foundry sand than 5%.

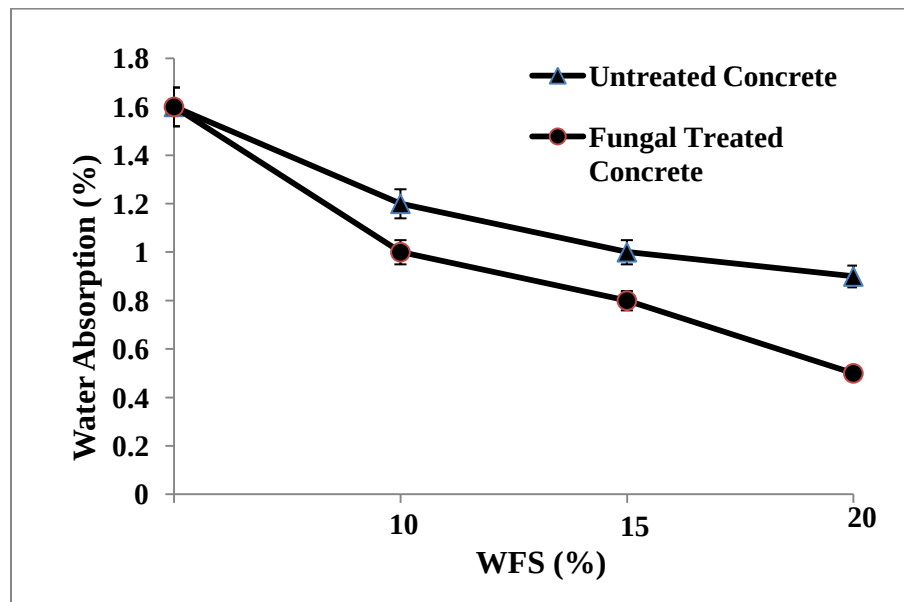


Fig 4.30: Water Absorption of Concrete Containing Fungal (*E. crustaceum*) Treated and Untreated WFS

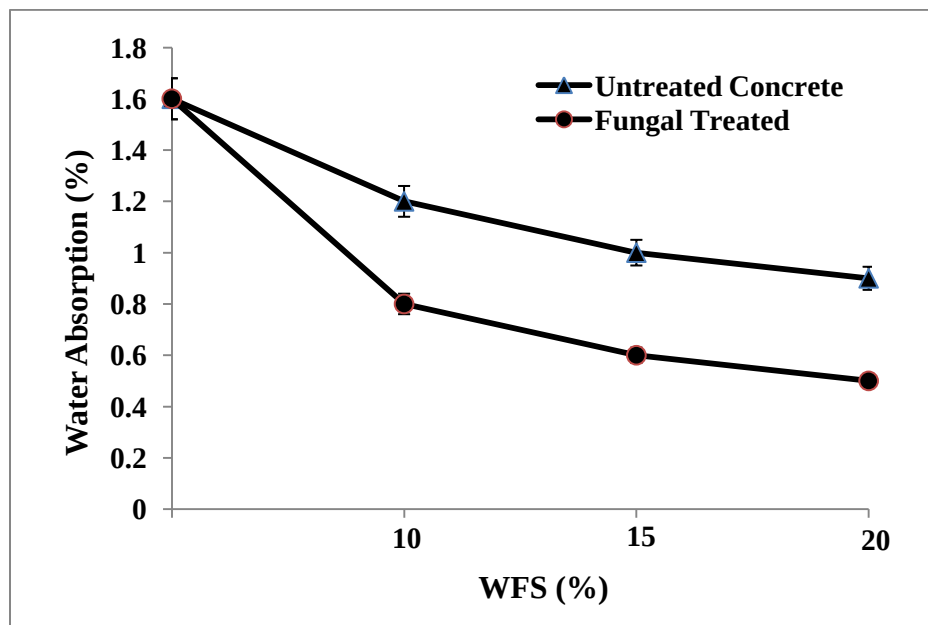


Fig 4.31: Water Absorption of Concrete Containing Fungal (*A. niger*) Treated and Untreated WFS

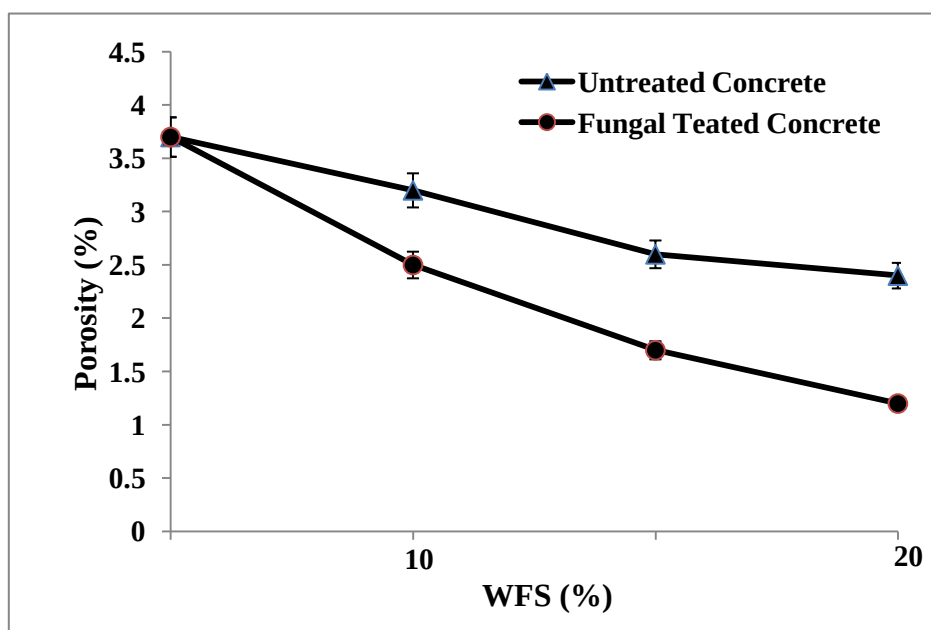


Fig 4.32: Percent Porosity of Concrete Containing Fungal (*E. crustaceum*) Treated and Untreated WFS

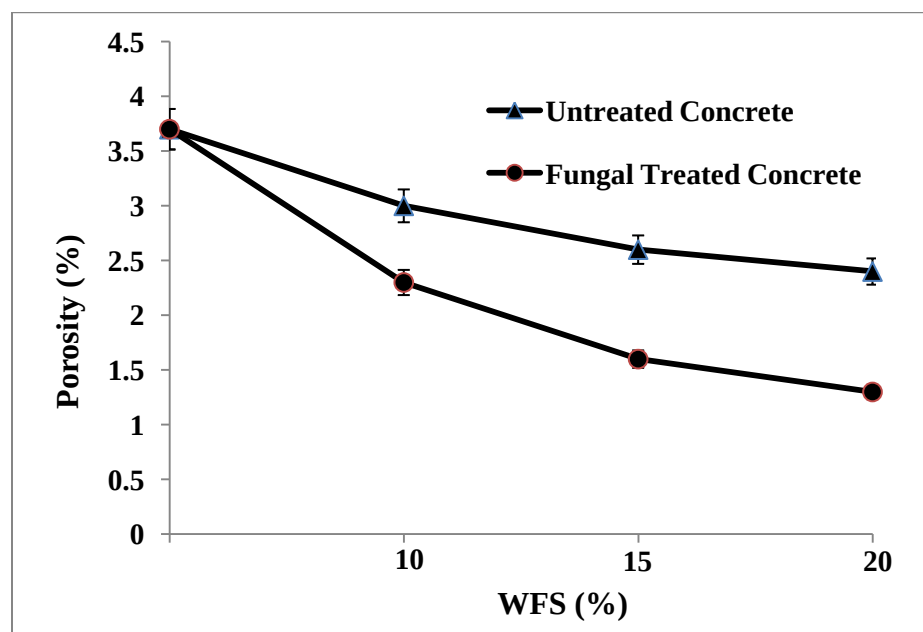


Fig 4.33: Porosity of Concrete Containing Fungal (*A. niger*) Treated and Untreated WFS

4.11.3 Statistical Analysis

To compare the results of concrete containing fungal treated WFS with concrete containing untreated WFS, t- test (unpaired) was applied. Results of compressive strength were obtained from concrete made with fungal treated WFS were significant at 95% confidence interval with ($p < 0.05$) as compared to concrete made with untreated WFS. In case of water absorption, results for concrete made with fungal treated WFS (15% and 20%) were significant at 95% confidence level with $p < 0.05$ as compared to control concrete. Results for the porosity of concrete containing fungal treated WFS were significant at 95% confidence level with $p < 0.05$ as compared to control concrete. This level of significance showed that use fungal treated WFS in concrete were effective for its strength improvement.

4.12 X-RAY DIFFRACTION (XRD) ANALYSIS

XRD technique was used to determine the state of chemical combination of the constituent components involved and phases present in the system. The fine powdered samples of control (concrete without WFS), concrete with untreated WFS and concrete with fungal (*A. niger* and *E. crustaceum*) treated WFS were analyzed by XRD and the peaks were identified on the basis of JCPDS data file. XRD analysis of concrete containing WFS showed some extra peaks of calcium silicate (Gyrolite) with high intensities as compared to control.

Fig. 4.34 shows the intensity peaks of calcium silicate, calcium hydroxide and silicon dioxide in control. Silicon dioxide was found as major phase present with intensity of 5000 cps (count per second) in control sample. Peaks of SiO_2 indicated the presence of free silica. Peak intensities of calcium silicate were found to be less in control. In concrete made with WFS (10%), intensity of calcium silicate phase was found to be higher as compared to control. In concrete containing fungal (*E. crustaceum*) treated WFS (10%), peaks of calcium aluminum silicate (Gismondine) were found at intensity of 11000 cps where as in concrete made with *A. niger* treated WFS (10%), peaks intensities of Gismondine were also higher as compared to concrete made with untreated WFS (Fig. 4.35). An elaborate search on peaks (comparing the values of $2\theta/d/I/I_0$) for JCPDS data file exclusively showed that these new peaks were matched with peaks of calcium aluminium silicate hydrate ($\text{CaAl}_2\text{Si}_2\text{O}_8 \cdot \text{H}_2\text{O}$). Peaks of calcium aluminum silicate hydrate in

concrete containing fungal treated WFS showed more intense as compared to concrete made with untreated WFS.

Similarly, the peak intensities of calcium silicate and calcium aluminium silicate were found higher in concrete containing fungal treated WFS (15% and 20%) as compared to concrete containing untreated WFS (Figs. 4.36 and 4.37). In concrete containing untreated WFS (10%, 15% and 20%) samples, peak intensities of calcium silicates were found to decrease with the increase in WFS percentage. In other words, an increase in percentage of WFS in concrete led to reduce the C-S-H gel formation as silica in the foundry wasn't react properly to form the C-S-H gel. XRD results showed an increase in free silica with increase in percentage of WFS. The fungal cultures were capable to increase the ability of WFS and cement to react properly by forming new phases of minerals. This led to increase in C-S-H gel formation as silica present in WFS was consumed to form C-S-H gel which in turn hardened the concrete. XRD confirmed that free silica was decreased with increase in percentage of WFS in concrete made with fungal treated WFS. Earlier Gosh et al. (2008) investigated the effects of incorporating a facultative anaerobic hot spring bacterium on the microstructure of a cement–sand mortar. X-ray diffraction (XRD) study confirmed the formation of new phases of silicates within the matrix of such mortar material, which causes an improvement in the strength of the material.

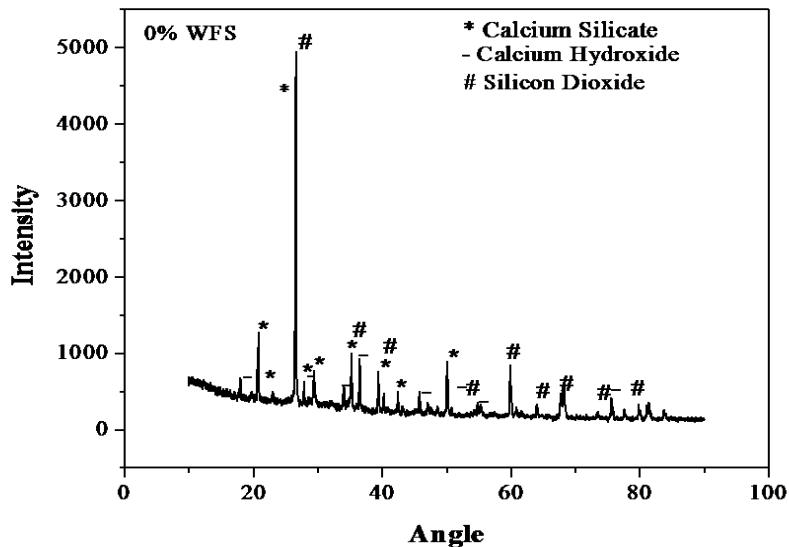
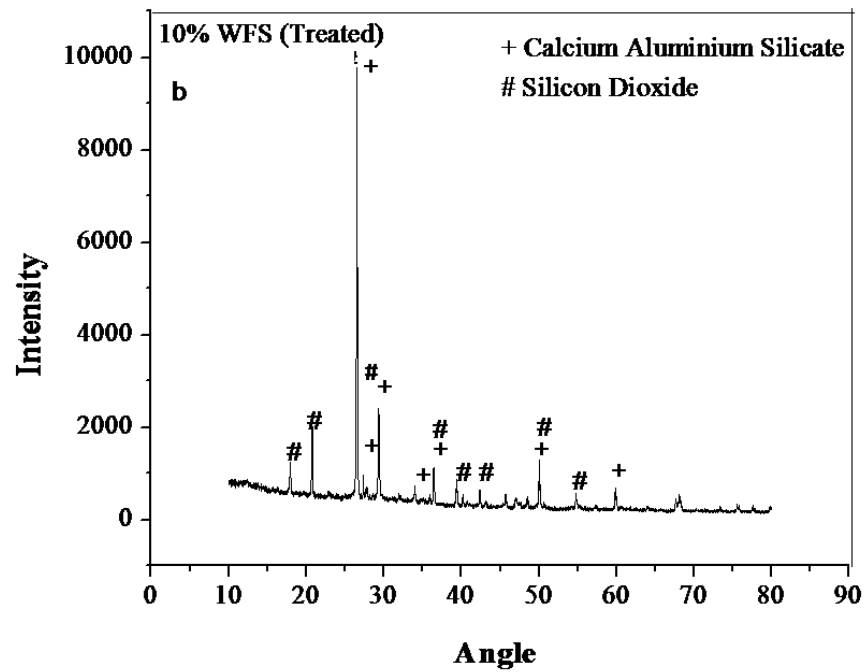
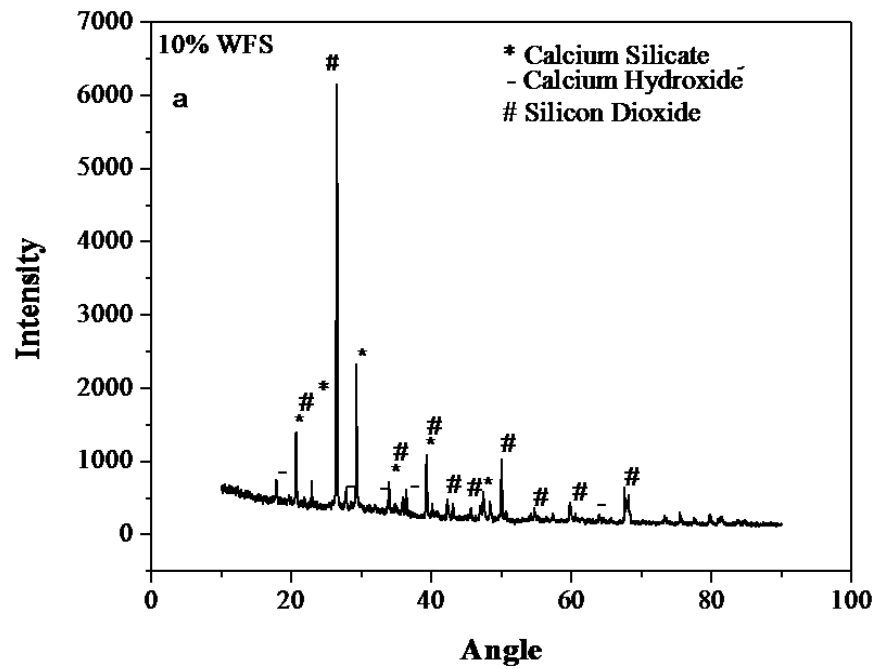


Fig 4.34: X-Ray Diffraction Shows Concrete without WFS (control).



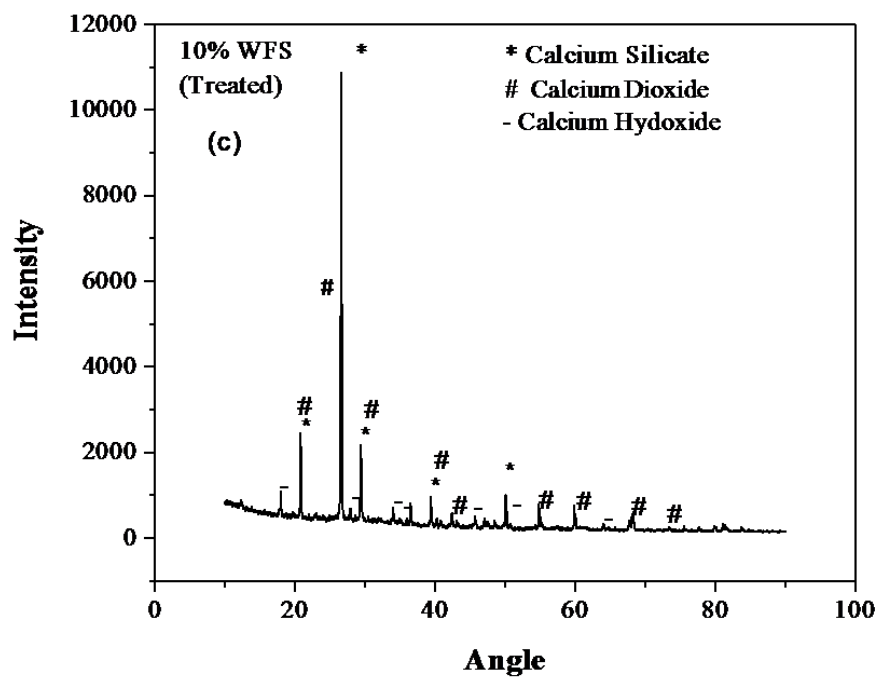
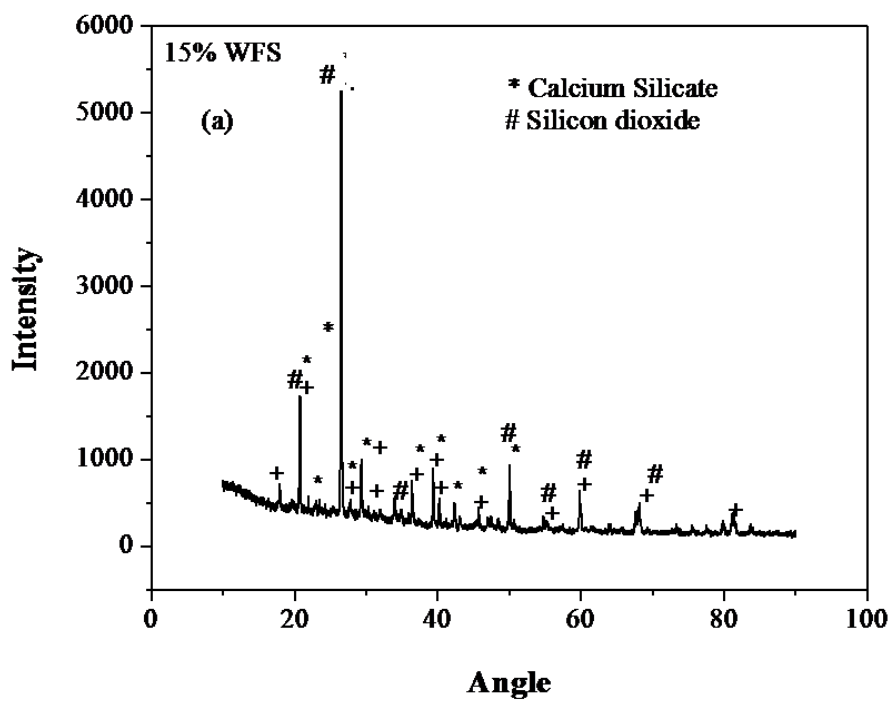


Fig 4.35: X- Ray Diffraction Shows Concrete Containing 10% WFS, (a) Untreated WFS, (b) *A. niger* Treated WFS and (c) *E. crustaceum* Treated WFS



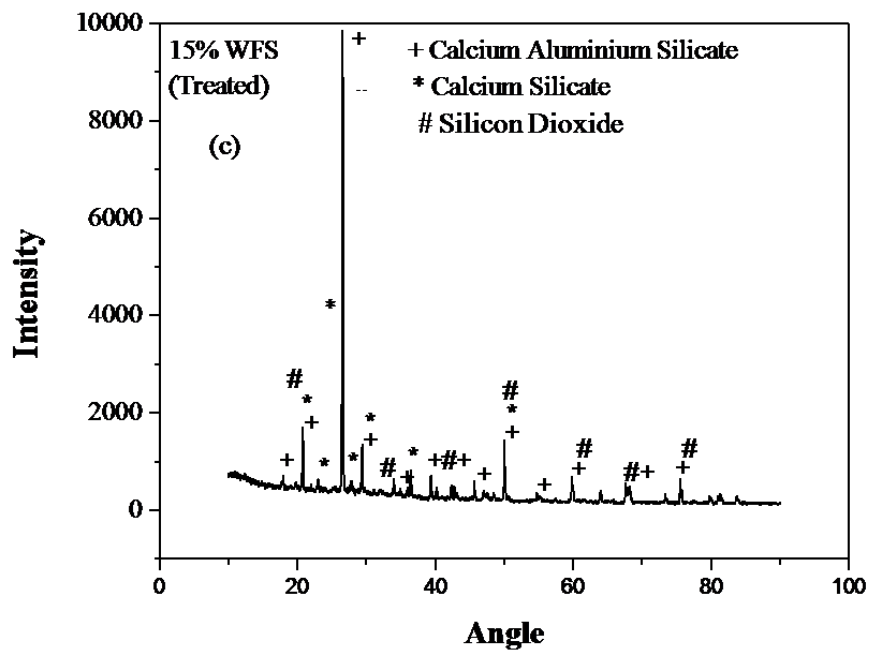
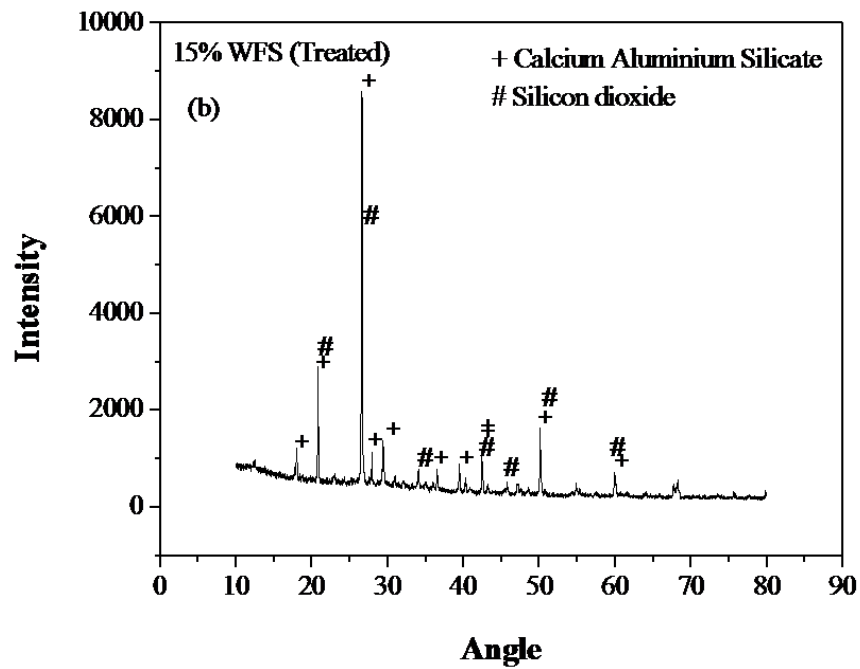
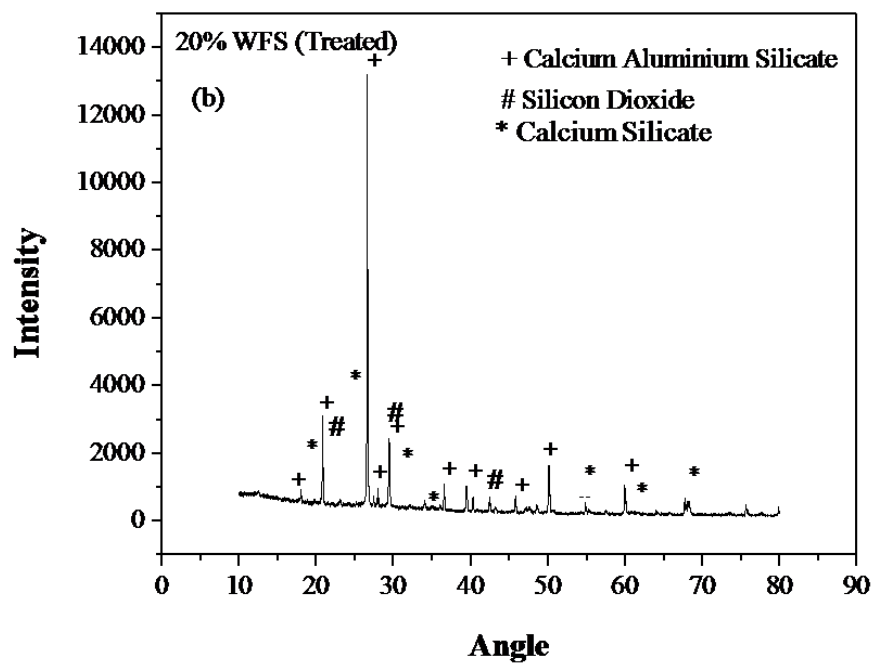
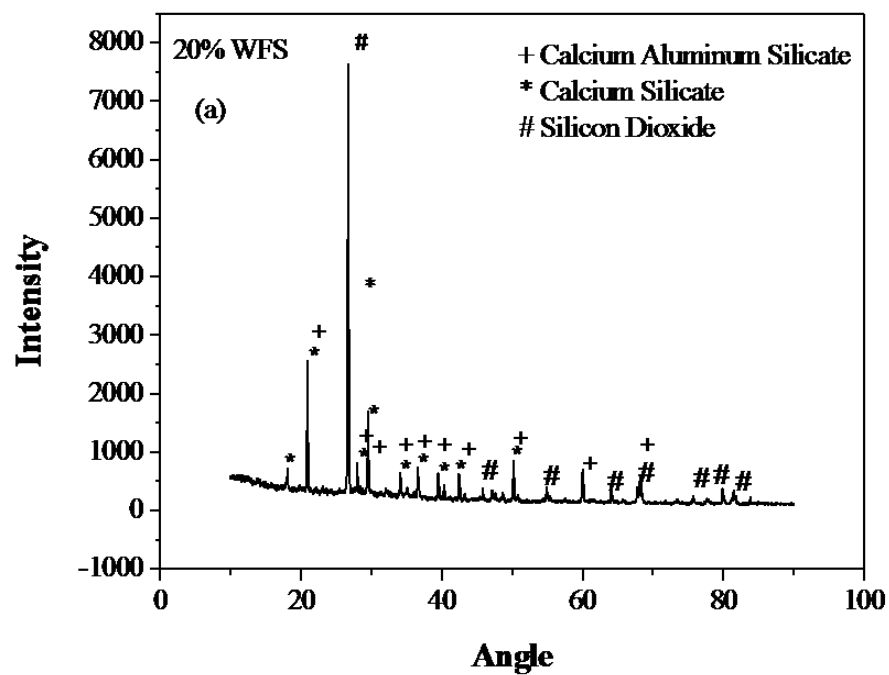


Fig 4.36: X- Ray Diffraction Shown by Concrete Containing 15% WFS (a) Untreated WFS, (b) *A. niger* Treated WFS and (c) *E. crustaceum* Treated WFS



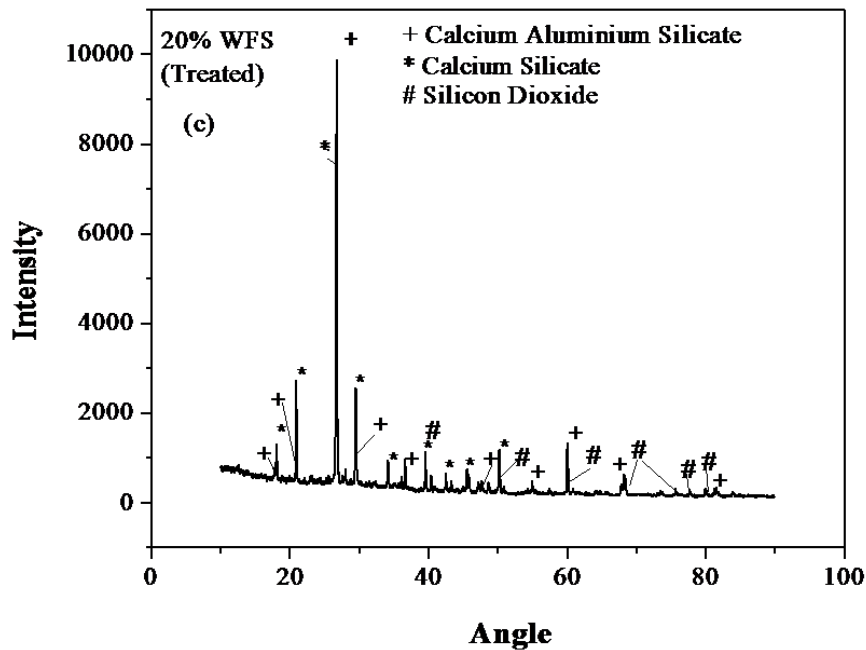


Fig 4.37: X- Ray Diffraction Shows Concrete Containing 20% WFS (a) Untreated WFS, (b) *A. niger* Treated WFS and (c) *E. crustaceum* Treated WFS

4.13 SCANNING ELECTRON MICROSCOPY (SEM) ANALYSIS

Scanning electron microscopy (SEM) images showed the surface view of hardened concrete and changes occurred in surface morphology of hardened concrete after addition of fungal treated WFS to the concrete. Microstructure and morphology of the hydrate mixes were observed on fractured surfaces. Fractured small samples were mounted on the SEM stubs with gold coating. Strength properties mainly depend on calcium–silica–hydrate (C–S–H) phase present in hardened concrete. Size and shape of the particles, distribution of particles, particle concentration, particle orientation, topology of the mix, composition of the dispersed/continuous phases and the pore structure were some of the factors which influence the behavior of C-S-H phases.

Surface morphology showed the number of pores present on the surface of hardened concrete. In control sample, the concrete surface appeared as uneven, rough with large number of cracks and porous empty spaces.

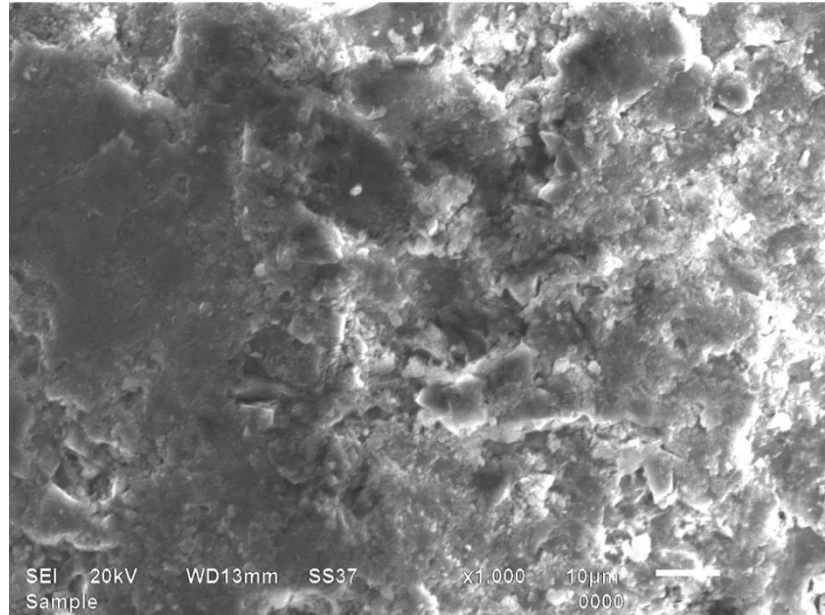


Fig 4.38: SEM Image of Concrete without WFS (Control)

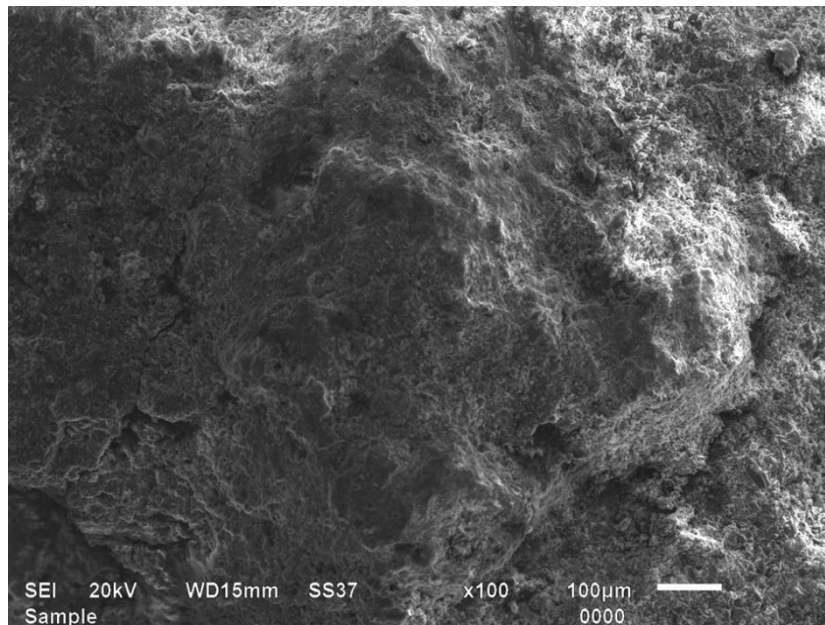


Fig 4.39: SEM Image Shows Concrete containing WFS (10%)

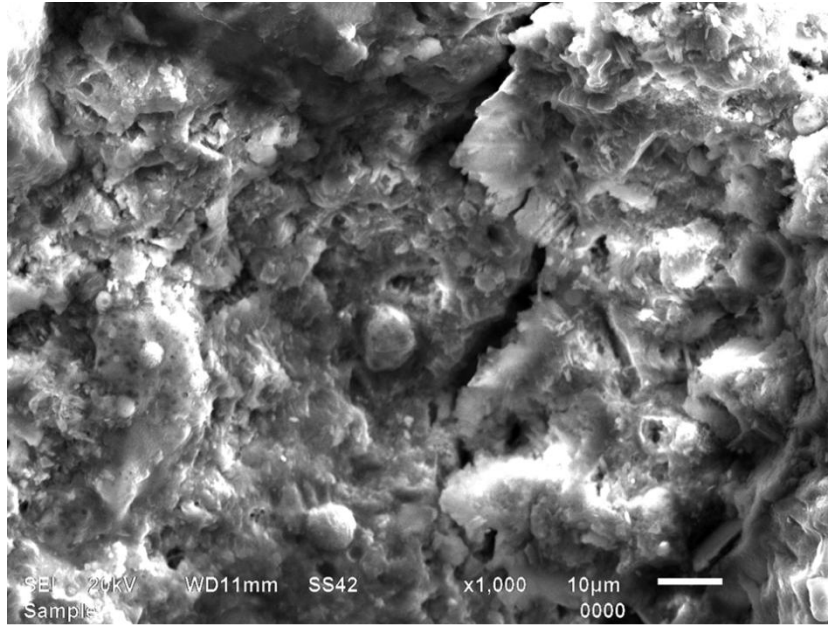


Fig 4.40: SEM Image Shows Concrete Containing *A. niger* Treated WFS (10%)

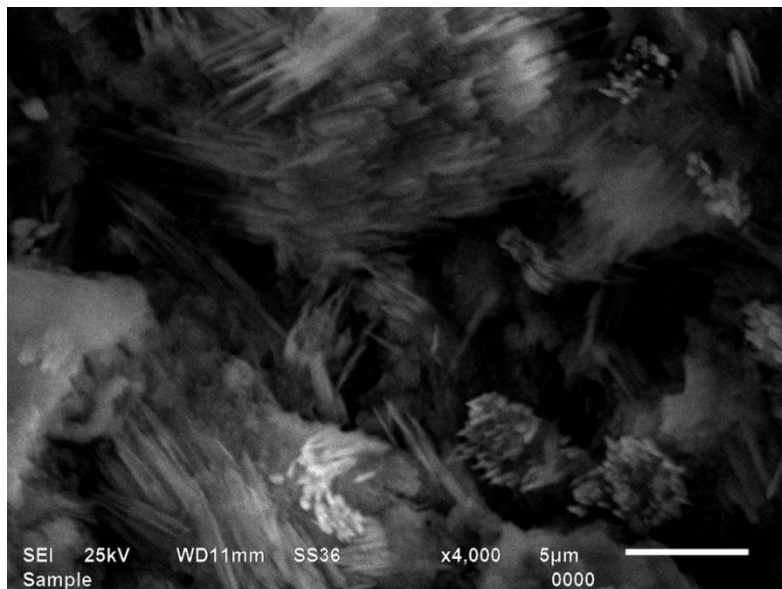


Fig 4.41: SEM Image Shows Concrete Containing *E. crustaceum* Treated WFS (10%)

These porous spaces led to increase the absorption capacity of concrete and hence reduce its strength (Fig. 4.38). In concrete made with untreated WFS (10%) sample, the surface appeared similar to control samples as 10% addition of WFS was quite less to show the visible difference. With the increase in WFS percentage, concrete surface showed visible difference as compared to control (Fig. 4.39). This was mainly due to filling of WFS particles within the pores of concrete. In concrete made with fungal treated WFS, fungal inclusions led to decrease the empty pore spaces as fungal mycelium firmly binds to the WFS particle within the pore spaces (Figs. 4.40 and 4.41).

Fig. 4.42 shows the smooth surface of concrete containing WFS (15%) as compared to control. SEM image clearly shows the binding of fungal treated WFS in concrete pores. In some particular areas in Fig. 4.43, the fungal mycelium binds and encircles the empty pore spaces. Fungal mycelium appeared to form a mesh or network inside the pores of concrete. Fig. 4.44 shows the formation of rod shaped biomineral on the surface of pores. Formation of calcified filaments inside the pores was due to the fungal activity. The newly formed biomineral plugs the pores of treated concrete which act as filling material in cracks and hence reduce the water absorption and porosity. Similarly, Kolo and Claeys (2005) reported that fungi can induce the formation of bio-cement between the grains in the form of bridging meniscuses cement as biomineralized fungal hyphae.

In concrete containing untreated WFS (20%), the surface appeared as more compact than control due to the increase in WFS percentage in concrete (Fig. 4.45). In concrete made with fungal (*A. niger*) treated WFS sample, newly formed mineral was deposited on fungal hyphae of mycelium (Fig. 4.46). Kolo and Claeys, (2005) suggested that biomineral form dense encrustations on fungal hyphae endogenously and exogenously. Fig. 4.47 shows the fine network of biomineralized fungal filaments spread all over in concrete made with *E. crustaceum* treated WFS (20%). Earlier Hillgartner et al. (2001) reported that the newly formed biomineral was produced by reaction of fungal organic acid with calcium or metal released from the substrate.

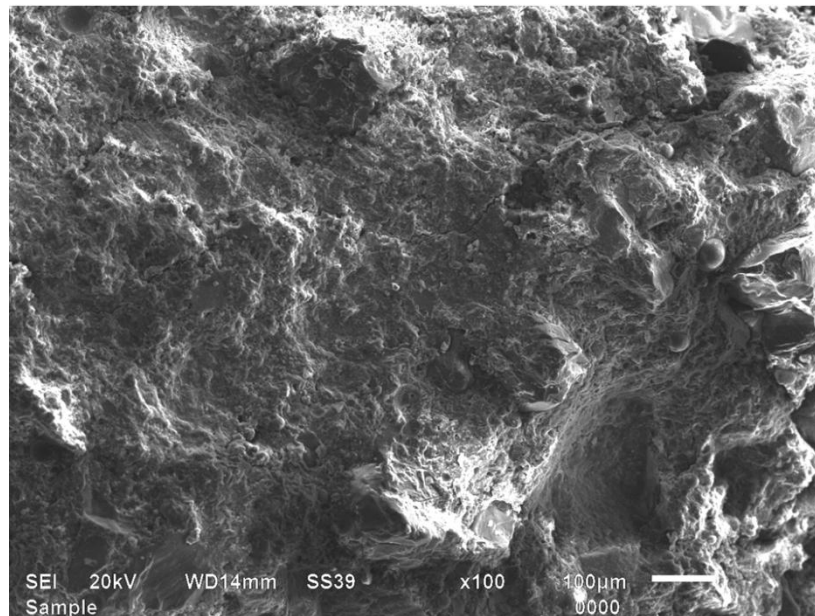


Fig 4.42: SEM Image Shows Concrete Containing WFS (15%)

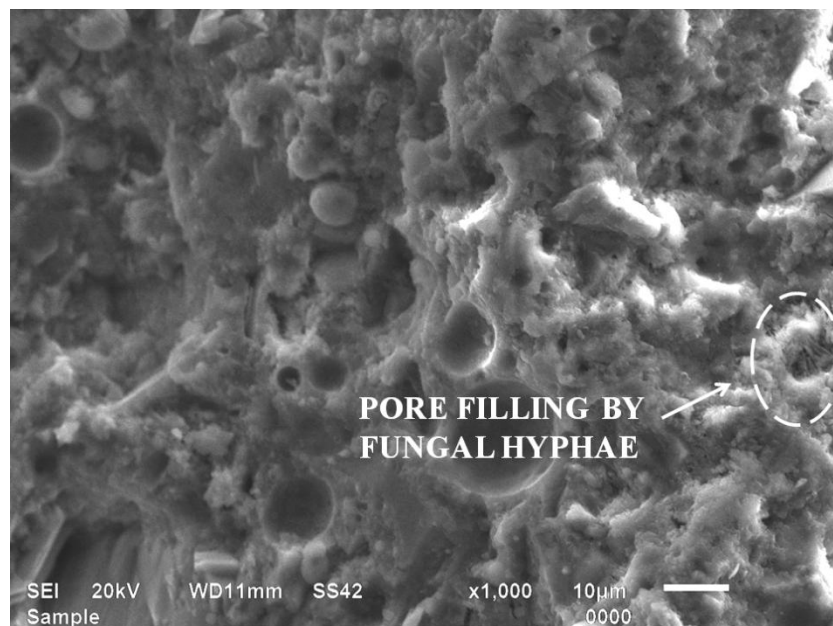


Fig 4.43: SEM Image Shows Concrete Containing *A. niger* Treated WFS (15%)

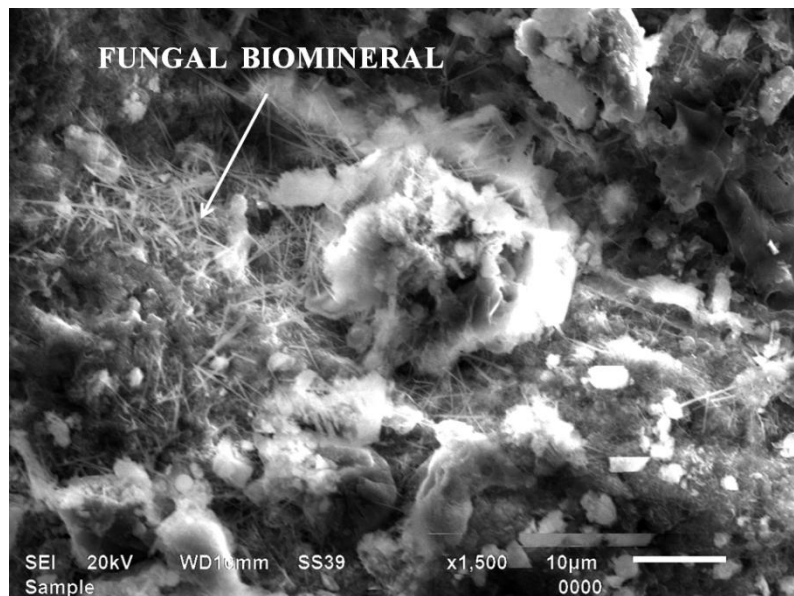


Fig 4.44: SEM Image Shows Concrete Containing *E. crustaceum* Treated WFS (15%)

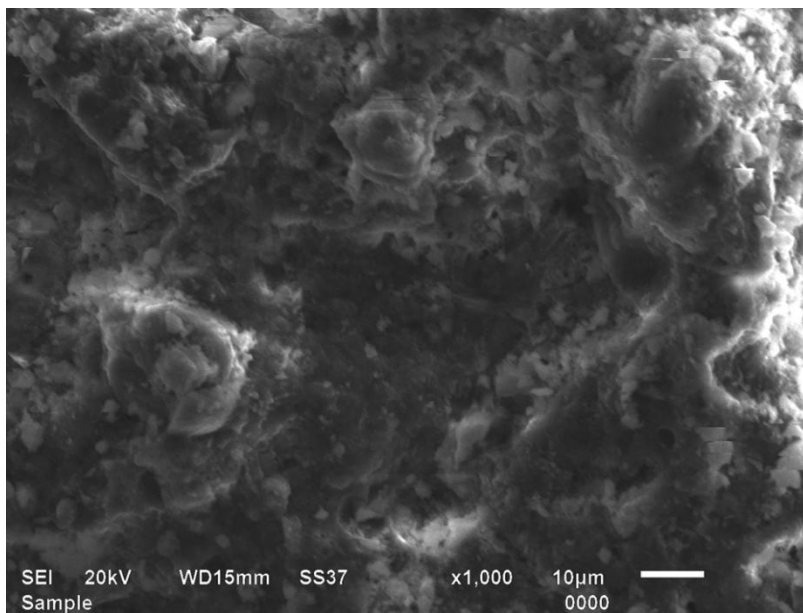


Fig 4.45: SEM Image Shows Concrete Containing WFS (20%)

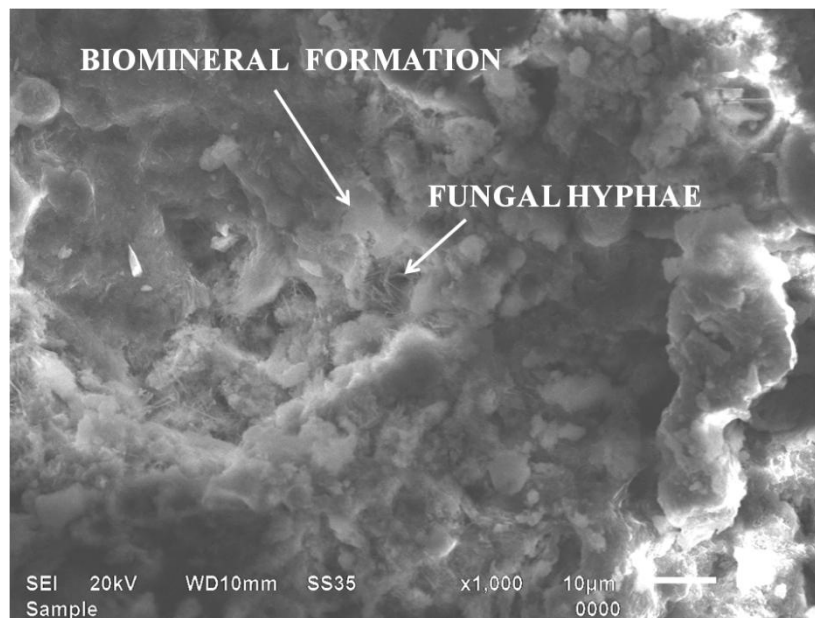


Fig 4.46: SEM Image Shows Concrete Containing *A. niger* Treated WFS (20%)

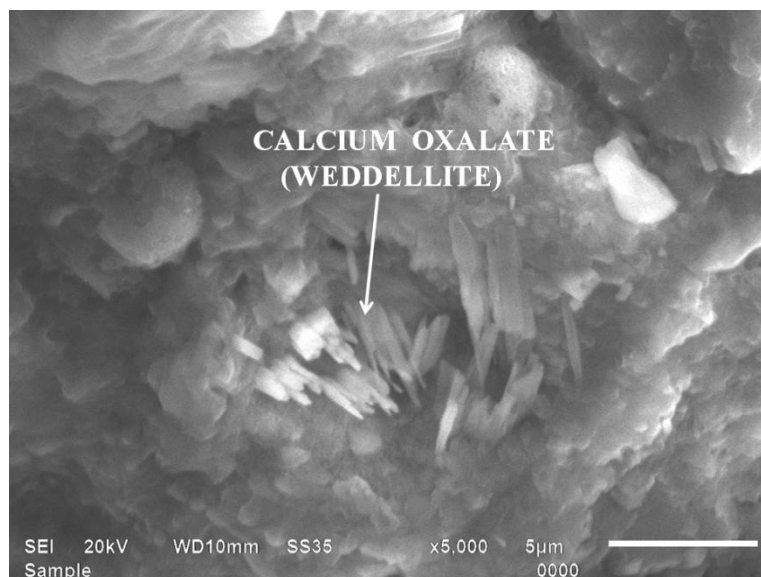


Fig 4.47: SEM Image Shows Concrete Containing *E. crustaceum* Treated WFS (20%)

4.14 ENERGY DISPERSIVE SPECTROSCOPY (EDS) ANALYSIS

Energy dispersive x-ray spectroscopy (EDS or EDX) is a simple technique to identify and percent estimation of elemental composition of as little as a cubic micron of materials. Broken pieces of concrete cubes containing untreated and fungal treated WFS (0%, 10%, 15% and 20%) were analyzed for their elemental percentages after 28 days of curing. EDS showed the variation in percentage of elements in concrete containing untreated and fungal treated WFS. In control, elemental percentages showed the presence of calcium (2.26%), Aluminium (27.1%) and silica (56.8%). These results showed the presence of free silica (SiO_2) in control sample which indicates silica is not consumed properly to form the good C-S-H gel (Fig. 4.48).

Iron was found as 2.7% in WFS. In concrete made with untreated WFS (10%), there was marginal increase in calcium (5.6%) as compared to control. Free silica (13.9%) was found to be less as compared to control which showed that silica was consumed in formation of C-S-H gel (Fig. 4.49). In concrete made with *A. niger* treated WFS, there was increase in calcium content (24.1%) and decrease in silica content (8.9%). These results showed that Ca/Si ratio increased within the CSH gel of concrete mix by fungal treatment (Fig. 4.50). Similarly, high level of calcium content in concrete made with fungal treated samples confirmed the biomineral formation by *E. crustaceum* (Fig. 4.51).

With increase in percent inclusion of WFS in concrete, there was increase in percent iron content as WFS was brought from ferrous based foundry (Figs. 4.49, 4.52 and 4.55). So, it was quite possible that iron levels were found to be increase with increase in percentage level as compared to control. In concrete made with fungal treated WFS, it was reduced to 4.1% by *A. niger* and whereas 1.7% in case of *E. crustaceum*. The reason behind the reduction was mainly due to the ability of fungal cultures to solubilize the complex metal compounds so that metals can be easily leached out or may be absorbed by the fungal mycelium for their metabolic activity.

In concrete made with fungal treated WFS (15% and 20%), there was drastic increase in calcium content as shown in Figs. 4.53 and 4.54 in case of *A. niger* treatment whereas Figs. 4.56 and 4.57 in case of *E. crustaceum* treatment. Earlier it was reported that the formation of different types of biominerals by fungi were generally controlled by metal type and availability in the mineral substrate (Arocena et al., 2003). These results indicated that the release of calcium in the

form of biomineral fill the pores of concrete, resulted in reduction of porosity. These results showed the high Ca/Si ratio in case of treated samples as compared to control and untreated samples.

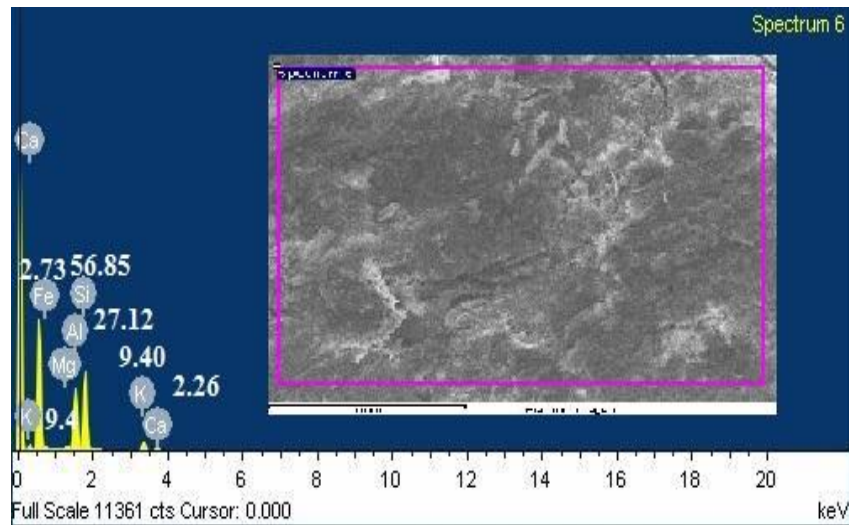


Fig 4.48: EDS Image of Concrete without WFS

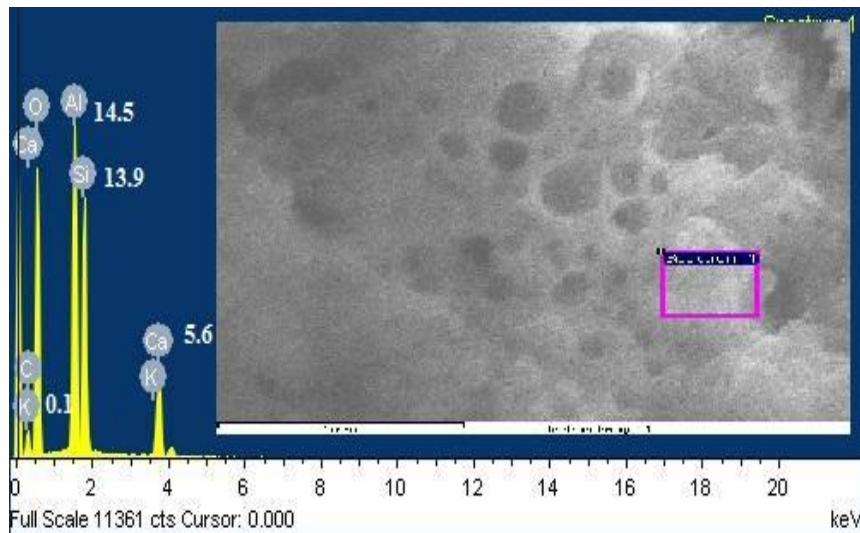


Fig 4.49: EDS of Concrete Containing WFS (10%)

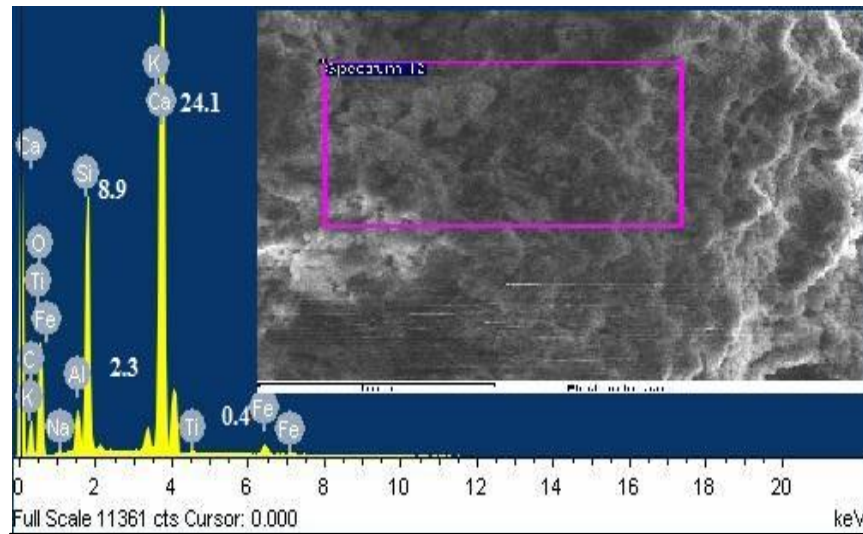


Fig 4.50: EDS Shows Concrete Containing Fungal (*A. niger*) Treated WFS (10%)

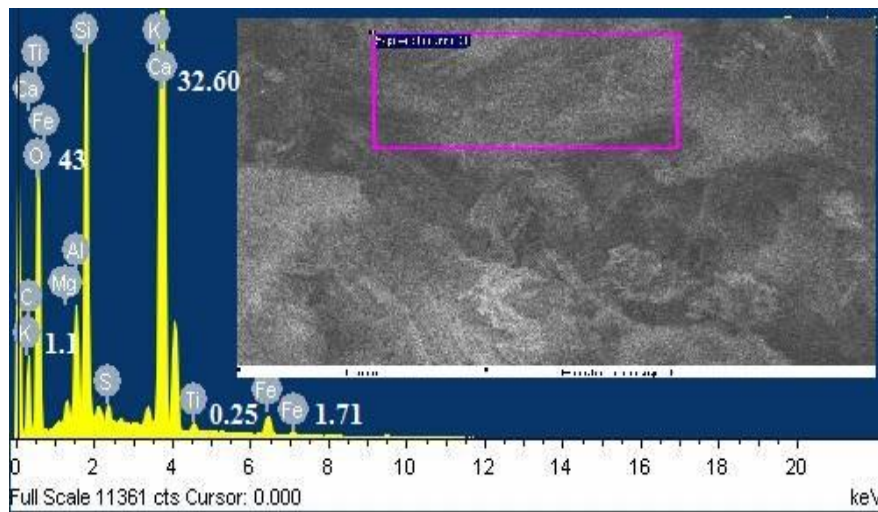


Fig 4.51: EDS Shows Concrete Containing Fungal (*E. crustaceum*) Treated WFS (10%)

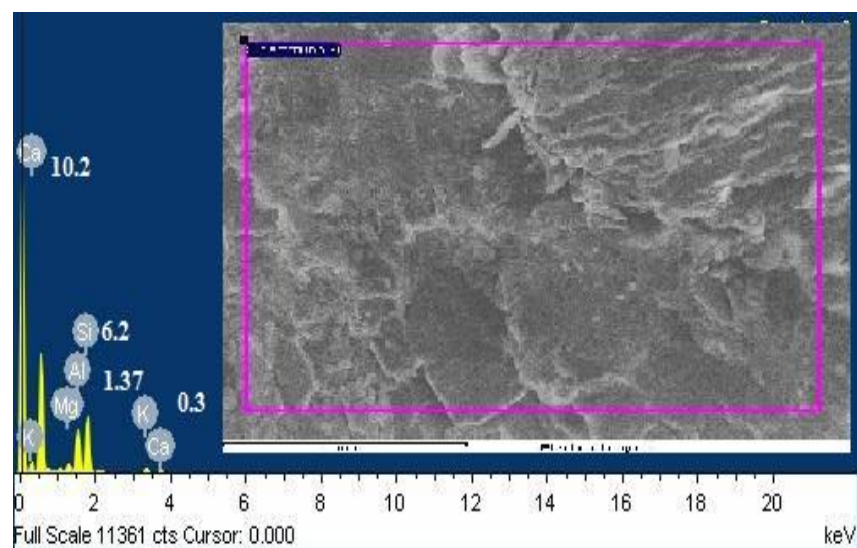


Fig 4.52: EDS Shows Concrete Containing WFS (15%)

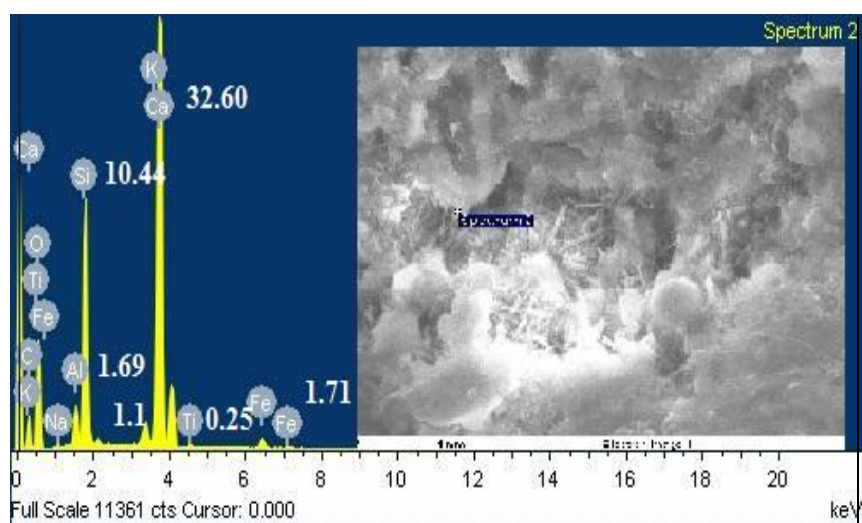


Fig 4.53: EDS Shows Concrete Containing Fungal (*A. niger*) treated WFS (15%)

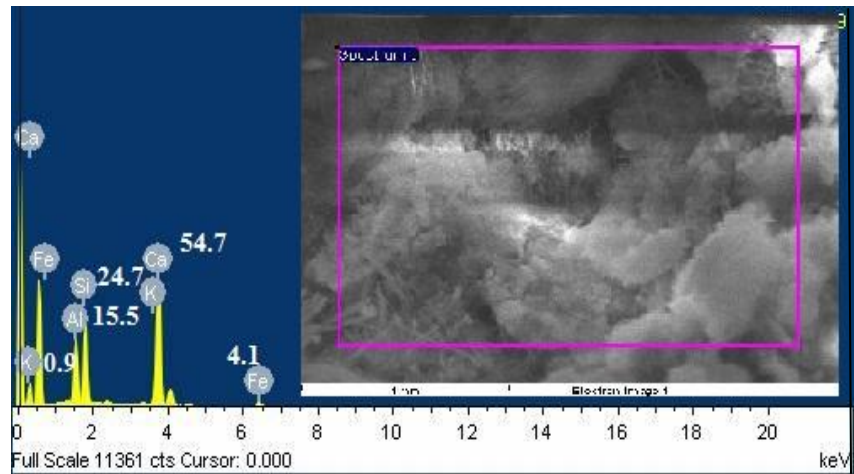


Fig 4.54: EDS image shows concrete containing fungal (*E. crustaceum*) treated 15% WFS

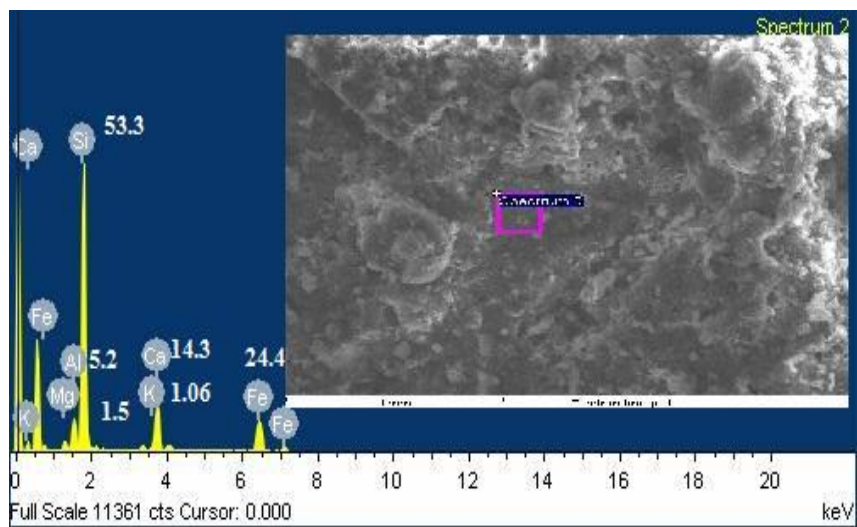


Fig 4.55: EDS Shows Concrete Containing WFS (20%)

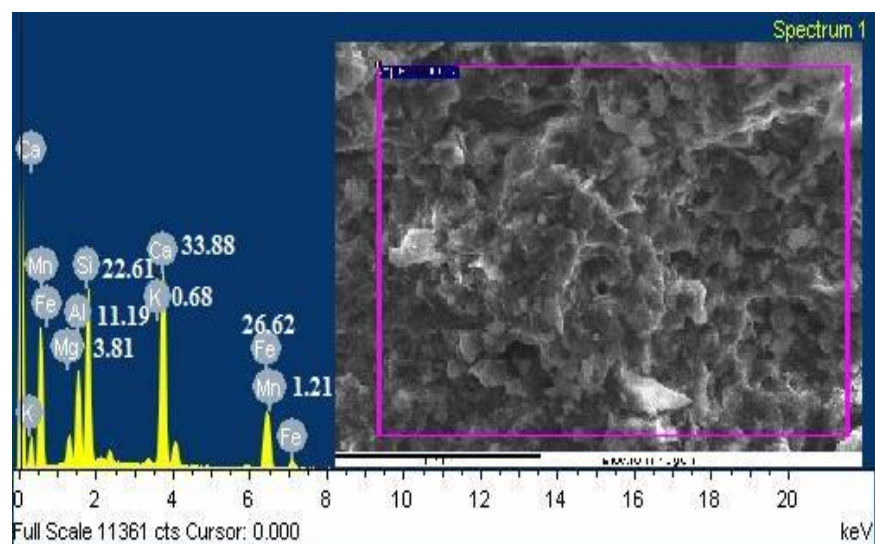


Fig 4.56: EDS Shows Concrete Containing Fungal (*A. niger*) Treated WFS (20%)

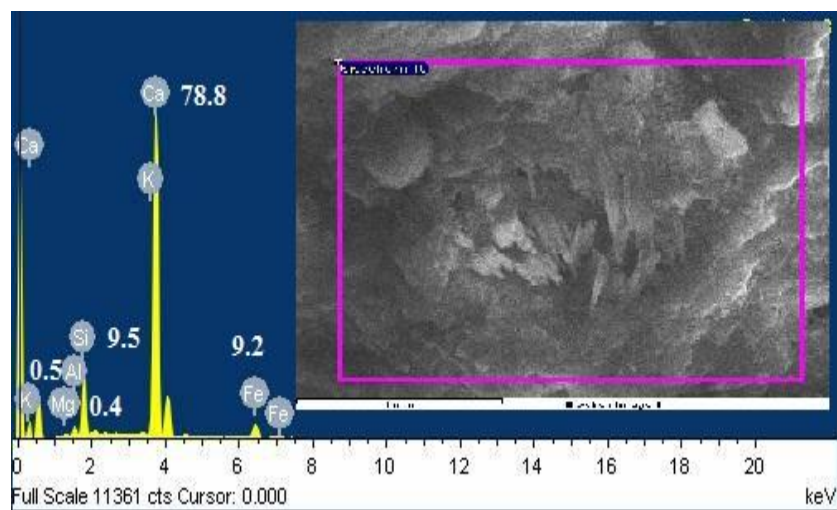


Fig 4.57: EDS Shows Concrete Containing Fungal (*E. crustaceum*) treated WFS (20%)

CHAPTER – 5

CONCLUSIONS

The present work investigated the physical and chemical properties of waste foundry sand. Fungal cultures were isolated for treatment of WFS for its utilization for concrete. Leachate and concrete properties (compressive strength, water absorption and porosity) were analyzed for untreated and fungal treated WFS as partial replacement of sand (0%, 10%, 15% and 20%). On the basis of the results from the present study, following conclusions are drawn.

5.1 ANALYSIS OF WFS

- i. Waste foundry sand is a byproduct of metal foundries which contains many heavy metals (Cd, Cr, Pb, Hg and Li) and other toxic substances.
- ii. Specific gravity was observed as 2.16 and bulk relative density was 2255.9 kg/m³.
- iii. Water absorption was found to be observed as 6.4 and fineness modulus was obtained as 2.07. The pH of waste foundry sand was 6.8.
- iv. WFS contained high silica content (83.3%) whereas iron oxide (5.39%) and strontium oxide were in lesser quantities.

5.2 ISOLATION OF FUNGAL CULTURES

- i. Eight fungal cultures were isolated (SO1, SO2, SO3 and SO4) isolated from soil and (SA1, SA2, SA3 and SA4) isolated from WFS. SA2 (Q= 1.3) and SA4 (Q= 1) were screened out as high acid producers as compared to other isolates.

- ii. Morphological and microscopic characteristics of SA4 and SA2 fungal isolates were found similar to the characteristics of *Aspergillus* sp. and *Penicillium* sp. respectively.
- iii. Fungal culture SA4 was identified as *Aspergillus niger* strain GK1 (Accession No. JX233485) and SA2 as *Eupenicillium crustaceum* strain GK2 (Accession No. JX233486) respectively.

5.3 ORGANIC ACID PRODUCTION

- i. The major acids produced by *Aspergillus niger* and *Eupenicillium crustaceum* were oxalic acid followed by citric acid.
- ii. Oxalic and citric acid peaks were appeared as major peaks in medium without WFS but in medium containing WFS, citric acid appears as minor peak. This suggested that presence of WFS inhibits the production of citric acid.

5.4 LEACHATE STUDIES

- i. Waste foundry sand contains leachable metals including Ba, Be, Cd, Cr, Co, Cu, Fe, Hg, Li, Mo, Mg, Mn, Ni and Zn. Results showed the reduction in the metal concentration in leachate obtained from fungal treated concrete.
- ii. In *Aspergillus niger* treated concrete made with WFS (10%), the metal concentration in Cd, Cr, Fe, Mo, Mn, Ni and Pb were reduced from (.008 mg/l to .001 mg/l), (.12 mg/l to .06 mg/l), (.20 mg/l to .04 mg/l), (.02 mg/l to .005 mg/l), (.06 mg/l to .03 mg/l), (.018 mg/l to .017 mg/l) and (.08 mg/l to .01 mg/l) respectively. Likewise reduction was also observed in leachate obtained from 15% and 20% fungal treated WFS containing concrete.
- iii. In case of *Eupenicillium crustaceum* treated concrete made with WFS (20%), the metal concentration in Cd, Cr, Fe, Mo, Mn, Ni and Pb were reduced from (.01mg/l to .0007mg/l), (.17 mg/l to .08mg/l), (.29 mg/l to .03mg/l), (.12 mg/l to .003 mg/l), (.12 mg/l to .01 mg/l), (.03 mg/l to .005 mg/l) and (.16 mg/l to .01 mg/l) respectively. Similarly reduction was also observed in leachate obtained from 10% and 15% fungal treated WFS containing concrete.

5.5 CONCRETE PROPERTIES

5.5.1 Compressive Strength

- i. The control mix (0% WFS) showed compressive strength of 33.1 N/mm^2 , whereas concrete mixes containing 10%, 15% and 20% WFS (untreated) exhibited compressive strength of 33.76, 29.30 and 27.80 N/mm^2 respectively at the age of 28 days.
- ii. Decrease in compressive strength was observed in 15% and 20% WFS containing concrete. It was observed that compressive strength in 10% WFS containing concrete was found be same as control concrete.
- iii. Fungal (*Eupenicillium crustaceum*) treated concrete, showed the compressive strength as 35.01, 35.20, 36.46 N/mm^2 in concrete containing WFS at various percentages of 10%, 15%, and 20% respectively.
- iv. In case of *Aspergillus niger* treated WFS containing concrete, compressive strength were 36.10, 32.81, 32.40 N/mm^2 at various replacement levels (10%, 15%, and 20%).

5.5.2 Water Absorption and Porosity

- i. Use of fungal treated WFS in concrete led to decrease in percent water absorption and porosity as compared to control and untreated concrete containing various percentages of WFS.
- ii. Reduction in water absorption was obtained as 16.6%, 20% and 44.4% in concrete made with fungal (*Aspergillus niger*) treated WFS (10%, 15% and 20%) as compared to untreated concrete whereas in case of *Eupenicillium crustaceum*, water absorption was obtained as 33.3%, 40% and 45% in fungal treated concrete made with WFS (10%, 15% and 20%) as compared to concrete made with untreated WFS.
- iii. Percent porosity led to decrease with increase in WFS percentage in concrete containing untreated WFS as compared to control. It also showed that the utilization of fungal treated WFS in concrete led to the further decrease in porosity.

- iv. There was reduction in porosity as 17%, 35% and 50% in concrete made with fungal (*Aspergillus niger*) treated WFS at various percentage levels (10%, 15% and 20%) as compared to concrete made with untreated WFS. Similarly, porosity was reduced as 23%, 38.4% and 50% in concrete made with fungal (*Eupenicillium crustaceum*) treated WFS at various percentage levels (10%, 15% and 20%) as compared to concrete made with untreated WFS.

5.6 X- RAY DIFFRACTION STUDIES

- i. XRD analysis of concrete samples with or without fungal treated waste foundry sand showed extra peaks of calcium aluminum silicate hydrate (Gismondine) in fungal treated WFS containing concrete as compared to untreated WFS containing concrete.
- ii. An elaborate search on peaks (comparing the values of $2\theta/d/I/I_0$) for JCPDS data file exclusively showed that these new peaks match with peaks of calcium aluminium silicate hydrate ($\text{CaAl}_2\text{Si}_2\text{O}_8 \cdot \text{H}_2\text{O}$).

5.7 SEM AND EDS STUDIES

- i. SEM confirmed the large number pores present on the surface of control concrete which led to increase the porosity and hence decrease in strength of concrete.
- ii. In concrete made with fungal treated WFS, there was formation of calcium oxalate by the fungal activity which plugs the pores of treated concrete and hence porosity was reduced.
- iii. EDS (Energy dispersive spectroscopy) showed high level of calcium content (78.8%) in treated concrete samples which confirmed that the biomineral formed by the fungal (*Eupenicillium crustaceum*) is highly calcified as compared to the untreated and control samples.

REFERENCES

- Abichou T, Edil TB, Benson CH, Bahia H (2004). Beneficial use of foundry by-products in highway construction. In: Geotechnical engineering for transportation projects. Geotechnical Special Publications, ASCE, Reston; 126: 715-722.
- Abin L, Coto Y, Bosecker K (2002). Screening of fungi with capacity for organic acid production. Review of Biology; 16: 69-71.
- Adriano DC (2001). Trace elements in the terrestrial environments: biogeochemistry, bioavailability, and risks of heavy metals, second ed. Springer-Verlag, pp. 867, New York.
- Aggarwal P, Siddique R, Aggarwal Y, Gupta SM (2008). Self-compacting concrete- procedure for mix design. Leonardo Electronic Journal of Practices and Technologies; 12: 15-24.
- Ahmad I, Zafar S, Ahmad F (2005). Heavy metal biosorption potential of *Aspergillus* and *Rhizopus* sp. isolated from wastewater treated soil. Journal of Applied Science and Environment Management; 9: 123 – 126.
- American Foundry men's Society, AFS (1991). Alternative utilization of foundry waste sand. Final Report (Phase I) prepared by American Foundry men's Society Inc. for Illinois Department of Commerce and Community Affairs, Des Plaines.
- Arocena JM, Zhu LP, Hall K (2003). Mineral accumulations induced by biological activity on granitic rocks in Qindhai Plateau, China, Earth Surf. Processes Landforms; 28: 1429–1437.
- Arwidsson Z, Johansson E, von Kronhelm T, Allard B, Patrick VK (2009). Remediation of metal contaminated soil by organic metabolites from fungi I—production of organic acids. Water Air Soil Pollution; 205: 215-226.
- ASTM D 3987 (1985). Standard test method for shake extraction of solid waste and water. In: Annual book of ASTM standards, vol. 04, American society for testing and materials, Philadelphia.

- ASTM D 4874 (1995). Standard test method for leaching solid in a column apparatus. In: Annual book of ASTM standards, vol. 04, American society for testing and materials, Philadelphia.
- ASTM C 642 (1997). “Standard test method for density, absorption and voids in hardened concrete”. 100 Barr Harbor Drive, West Conshohocken, PA 19428- 2959, United States.
- ASTM C 128 (2001). Standard test method for density, relative density and absorption of fine aggregate, 100 Barr Harbor Drive, West Conshohocken, PA 19428- 2959, United States.
- Bakis R, Koyuncu H, Demirbas A (2006). An investigation of waste foundry sand in asphalt concrete mixtures. Waste Management Research; 24: 269-274.
- Bhat ST, Lovell CW (1996). Design of flowable fill: waste foundry sand as a fine aggregate. Transportation Research Record; 1546: 70-78.
- BIS: 516 (1959). Indian Standard Methods of Test for Strength of concrete. Bureau of Indian Standards. New Delhi, India.
- BIS: 383 (1970). Specifications for coarse and fine aggregates from natural sources for concrete. Bureau of Indian standards. New Delhi, India.
- BIS: 8112 (1989). Specifications for 43-Grade Portland cement, Bureau of Indian Standards. New Delhi, India.
- BIS: 456 (2000). Plain and Reinforced concrete Code of Practice (Fourth Edition). Bureau of Indian Standards. New Delhi, India.
- Bishnoi NR, Garima (2004). Fungus: An alternative for bioremediation of heavy metal containing wastewater. Journal of Scientific and Industrial Research; 64: 93-100.
- Bosecker K (1987). Microbial Leaching. In fundamentals of biotechnology, ed by P. Prave, U. Faust, W. Sittig and D.A. Sukatsch, pp. 662-683, New York, VCH.
- Bosecker K (1997). Bioleaching: metal solubilization by microorganisms. FEMS Microbiology Reviews; 20: 591-04.

- Bosshard PP, Bachofen R, Brandl H (1996). Metal leaching of fly ash from municipal waste incineration by *Aspergillus niger*. *Environmental Science and Technology*; 30: 3066-3070.
- Braganca SR, Vicenzi J, Guerino K, Bergmann CP (2006). Recycling of iron foundry sand and glass waste as raw material for production of whiteware. *Waste Management*; 24: 60-66.
- Brandl H, Krebs, Brombacher C, Bosshard PP, Bachofen R (1997). Microbiological systems for metal recycling. In *Proceedings of R'97-Recovery, Recycling, Re-integration*, Geneva; 4: 16-20.
- Brandl H (2001). Microbial leaching of metals. In *biotechnology*, vol.10, ed by H.-J.Rehm and G. Reed, pp. 191-224, Weinheim, Wiley-VHC.
- Bredberg K, Karlsson HT, Holst O (2004). Reduction of Vanadium (V) with *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. *Bioresource Technology*; 92: 93-96.
- Briand L, Thomas H, de la Vega AA, Donati E (1999). Vanadium recovery from solid catalysts by means of *Thiobacilli* action. In *biohydrometallurgy and the environment toward the mining of the 21st century*, vol. A, ed by R. Amils and A. Ballester, Elsevier; 263-72.
- Bright H (1994). Biosensors for the future. *Naval Research Reviews*; 46: 48–49.
- Brierley JA (1990). Biotechnology for the extractive metals industries. *The Journal of the Minerals, Metals & Materials Society*; 42: 28-30.
- Brombacher C, Bachofen R, Brand H (1997). Biohydrometallurgical processing of solids: A patent review. *Applied Microbiology and Biotechnology*; 48: 577-87.
- Burford EP, Kierans M, Gadd GM (2003a). Geomycology: Fungi in mineral substrata. *Mycologist*; 17, 98–107.
- Burford EP, Fomina M, Gadd GM (2003b). Fungal involvement in bioweathering and biotransformation of rocks and minerals. *Mineralogical Magazine*; 67: 1127– 1155

- Burford EP, Hillier S, Gadd GM (2006). Biomineralization of Fungal Hyphae with Calcite (CaCO_3) and Calcium Oxalate Mono- and Dihydrate in Carboniferous Limestone Microcosms. *Geomicrobiology Journal*; 23: 599-611.
- Burgstaller W, Strasser H, Wobking H, Schinner F (1992). Solubilization of zinc oxide from filter dust with *Penicillium simplicissimum*: bioreactor leaching and stoichiometry. *Environmental Science and Technology*; 26: 340-346.
- Burgstaller W, Schinner F (1993a). Leaching of metal with fungi. *Journal of Biotechnology*, 27: 91-116.
- Burgstaller W, Schinner F (1993b). Metal leaching with fungi. In: biohydrometallurgical technologies, ed by A.E. Torma, J.E. Wey and V.L. Lakshmanan, pp. 325-333. The Minerals, Metals & Materials Society.
- Cachim PB (2009). Mechanical properties of brick aggregate concrete. *Construction and Building Materials*; 23: 1292–1297.
- Carey PR, Sturtz G (1995). Sand binder systems part IV urethane binders. *Foundry Management and Technology*; 123: 25-29.
- Castro IM, Fietto JKR, Vieira RX, Tropia MJM, Campos LMM, Paniago EB, Brandao RL (2000). Bioleaching of zinc and nickel from silicates using *Aspergillus niger* cultures. *Hydrometallurgy*; 57: 39-49.
- Chakradhara RM, Bhattacharyya SK, Barai SV (2011). Influence of field recycled coarse aggregate on properties of concrete. *Materials and Structures*; 44: 205-220.
- Chen J, Blume HP, Beyer L (2000). Weathering of rocks induced by lichen colonization—A review. *Catena*; 39: 121– 146.
- Clark TR, Ehrlich HL (1992). Copper removal from an industrial waste by bioleaching. *Journal of Industrial Microbiology*; 9: 213-18.
- CWC, Fact Sheet (1996). Managing partner of the recycling technology assistance partnership (ReTAP) under U.S. EPA, Beneficial Reuse of Spent Foundry Sand (IBP-95-1).

- Cwirzen A (2010). Controlling physical properties of cementitious matrixes by nanomaterials. *Advanced Materials Research*; 123-125: 639-642.
- Cyr M, Coutand M, Clastres P (2007). Technological and environmental behavior of sewage sludge ash (SSA) in cement-based materials. *Cement and Concrete Research*; 37: 1278-1289.
- Dayton EA, Whitacre SD, Dungan RS, Basta NT (2010). Characterization of physical and chemical properties of spent foundry sands pertinent to beneficial use in manufactured soils. *Plant Soil*; 329: 27-33.
- Deng A, Tikalsky PJ (2008). Geotechnical leaching properties of flowable fill incorporating waste foundry sand . *Waste Management*; 28: 2161-2170.
- Deng A (2009). Contaminants in waste foundry sand and its leachate. *International Journal of Environment and Pollution*; 38: 425-443.
- Denevre O, Garbaye J, Botton B (1996). Release of complexing organic acids by rhizosphere fungi as a factor in Norway spruce yellowing in acidic soils. *Mycological Research*; 100: 1367-1374.
- Diwaniyan S, Kharb D, Raghukumar C, Kuhad RC (2010). Decolorization of synthetic dyes and textile effluents by basidiomycetous fungi. *Water, Air and Soil Pollution*; 210: 409-419.
- Douglas SK (2003). Toxicity characteristic leaching procedure and iron treatment of brass foundry waste. *Environmental Science and Technology*; 37: 367-371.
- Dronawat SN, Svihla CK, Hanley TR (1995). Production of gluconic acid by *Aspergillus niger*. *Applied Biochemistry Biotechnology*; 51: 347-354.
- Dungan RS (2008). The characterization of trace metals and organics in spent foundry sands over a one- year period. *Journal of Residuals Science and Technology*; 5: 111-125.
- Dungan RS, Dees NH (2009). The characterization of total and leachable metals in foundry sands. *Journal of Environmental Management*; 90: 539-48.

- Dungan RS, Reeves JB (2005). Pyrolysis of foundry sand resins: a determination of organic products by mass spectrometry. *Journal of Environmental Science and Health*; 40: 1557-1567.
- Dungan RS, Kim JS, Weon HY, Leytem AB (2009). The characterization and bacterial communities in soils blended with spent foundry sand. *Annals of Microbiology*; 59: 239-246.
- Dutton MV, Evans CS, Atkey PT, Wood DA (1993). Oxalate production by basidiomycetes including the white rot species *Coriolus versicolor* and *Phanerochaete chrysosporium*. *Applied Microbiology and Biotechnology*, 39: 5–10.
- Ehrlich HL (1992). Metal extraction and ore discovery. In *encyclopedia of microbiology*, vol 3, ed by J. Lederberg, pp. 75-80, Academic Press, San Diego.
- Ehrlich HL (1997). Microbes and metals. *Applied Microbiology and Biotechnology*; 48: 687-692.
- Ellouze M, Aloui F, Sayad S (2007). Detoxification of Tunisian landfill leachates by selected fungi. *Journal of Hazardous Materials*; 150: 642-648.
- Engroff EC, Fero EL, Ham RK, Boyle WC (1989). Laboratory leachings of organic compounds in ferrous foundry process waste. Final Report to American Foundry men's Society; Des Plaines, Ill, USA.
- Environmental Protection Agency (1998). Back Document for Proposed CPG III and Draft RMAN III. EPA Report EPA530-R-98-003.
- Etxeberria M, Pacheco C, Meneses J M, Beerridi I (2010). Properties of concrete using metallurgical industrial by-product as aggregate. *Construction and Building Materials*; 24:1594-1600.
- Ezzouhri L, Castro E, Moya M, Espinola F, Lairini K (2009). Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier, Morocco. *African Journal of Microbiology Research*; 3: 35-48.

- Federal Highway Administration (1998). User guidelines for waste and by-product materials in pavement construction. Federal Highway Administration, Turner-Fairbank Highway Research Center.
- Federal Highway Administration (2004). United States Department of Transportation, Foundry Sand facts for Civil Engineers, Report No. FHWA-IF-04-004, USA, 80.
- Felsenstein J (1985) Confidence limits on phylogenies: An approach using the bootstrap. *Evolution*; 39:783-791.
- Fero RL, Ham RK, Boyle WC (1986). An investigation of ground water contamination by organic compounds leached from iron foundry solid wastes. Final Report to American Foundrymen's Society, Des Plaines, Ill, USA.
- Fiore S, Zanetti MC (2007). Foundry wastes reuse and recycling in concrete production. *American Journal of Environmental Sciences*; 3: 135-142.
- Gadd GM (1992). Metals and microorganism: a problem definition. *FEMS Microbiology Letters*; 100: 197-203.
- Gadd GM (1999). Fungal production of citric and oxalic acid: Importance in metal speciation, physiology and biogeochemical processes. *Advances in Microbial Physiology*; 41: 47–92.
- Gadd GM, Sayer JA (2000). Influence of fungi on the environmental mobility of metals and metalloids. In: *Environmental microbe-metal interactions*, ed by D.R. Lovley, pp. 237-25, ASM Press, Washington DC.
- Gadd GM, Burford EP, Fomina M, Harper FA, Jacobs H (2002). Fungal influence on metal mobility: Mechanisms and relevance to environment and biotechnology, paper presented at 7th International Mycology Congress, Oslo University, Oslo.
- Gadd GM (2004). Mycotransformation of organic and inorganic substrates. *Mycologist*; 18: 60-70.

- Gardziella A, Pilato LA, Knop A (1999). Phenolic resins: chemistry, applications, standardization, safety and ecology, second ed. Springer, New York.
- Ghosh P, Mandal S, Chattopadhyaya BD, Pal S (2005). Use of microorganisms to improve the strength of cement mortar. *Cement and Concrete Research*; 35: 1980- 1983.
- Ghosh P, Mandal S, Pal S, Bandyopadhyaya G, Chattopadhyaya BD (2006). Development of bioconcrete material using enriched cultures of novel thermophilic anaerobic bacteria. *Indian Journal of Experimental Biology*; 44: 336-339.
- Ghosh P, Chattopadhyaya BD, Mandal S (2008). Use of hot spring bacteria for remediation of cracks and increment of durability of structures. *Indian Concrete Journal*; 82: 11-16
- Goyal N, Jain SC, Banerjee UC (2003). Comparative studies on the microbial adsorption of heavy metals. *Advances in Environmental Research*; 7: 311-319.
- Guney Y, Aydilek AH, Demirkan MM (2006). Geoenvironmental behavior of foundry sand amended mixtures for highway sub bases. *Waste Management*; 26: 932–945
- Guney Y, Sari YD, Yalcin M, Tuncan A, Donmez S (2010). Re-usage of waste foundry sand in high-strength concrete. *Waste Management*; 30: 1705–1713
- Gutarowska B, Czyżowska A (2009). The ability of filamentous fungi to produce acids on indoor building materials. *Annals of Microbiology*; 59: 807-813.
- Hahn M, Willscher S, Straube G (1993). Copper leaching from industrial wastes by heterotrophic microorganisms. In *biohydrometallurgical technologies*, ed by A.E.Torma, J.E. Wey and V.L. Lakshmanan, pp 99-108. The Minerals, Metals & Materials Society.
- Ham RK, Boyle WC, Fero RL (1987). Evaluation of organic compounds in groundwater at ferrous foundry waste landfills. *American Foundrymen's Society*; 95: 693-694.
- Ham RK, Boyle WC, Blaha FJ (1990). Comparison of leachate quality in foundry waste landfills to leach test abstracts. *Journal of Hazardous and Industrial Solid Waste Testing and Disposal*; 6: 29–44.

- Hawksworth DL, Mueller GM (2005). Fungal communities: their diversity and distribution. In: Dighton J, Oudemans P, White J (eds.). The fungal community: its organization and role in the ecosystem, pp. 27–37, Marcel Dekker, New York.
- Hillgartner H, Duparez C, Hug W (2001). Microbially induced cementation of carbonate sands: Are micritic meniscus cements good indicators of vadose diagenesis?. *Sedimentology*; 48: 117– 131.
- Henry J, Heinke G (1996). *Environmental Science and Engineering*, Prentice Hall, ISBN 0-13-120650-8.
- Hoffland E, Kuyper TW, Wallander H, Plassard C, Gorbushina AA, Haselwandter K, Holmström S, Landeweert R, Lundström US, Rosling A, Sen R, Smits MM, Patrick AW, van Breemen N (2004). The role of fungi in weathering. *Frontiers in Ecology and Environment*; 2: 258– 264.
- Hughes MN, Poole RK (1991). Metal speciation and microbial growth – the hard (and soft) facts. *Journal of General Microbiology*; 137: 725- 734.
- Iram S, Ahmad I, Javed B, Yaqoob S, Akhtar K, Kazmi MR Badar-uz-Zaman (2009). Fungal tolerance to heavy metals. *Pakistan Journal of Botany*; 41: 2583-2594.
- Javed S, Lovell CW (1994). Use of foundry sand in highway construction. Joint Highway Report No. C-36-50N. Indiana, USA: Department of Civil Engineering, Purdue University.
- Ji S, Wan L, Fan Z (2000). The toxic compounds and leaching characteristics of spent foundry sands. *Water Air and Soil Pollution*; 132: 347-364.
- Johnson CK (1981). Phenols in foundry waste sand modern casting. American Foundry men's Society.
- Jones B, Renaut RW, Rosen MR (1997). Biogenecity of silica precipitation around geysers and hot springs vents, North Island, New Zealand. *Journal of Sedimentary Research*; 67: 88– 104.

- Kapoor A, Viraraghavan T (1995). Fungal biosorption – An alternative treatment option for heavy metal bearing wastewaters: A review. *Bioresource Technology*; 53: 195-206.
- Kendall DS (2003). Toxicity characteristic leaching procedure and iron treatment of brass foundry waste. *Environment Science and Technology*; 37: 367-371
- Khatib JM, Ellis DJ (2001). Mechanical properties of concrete containing foundry sand. *ACI Special Publication*; 200: 733-748.
- Khatib JM (2005). Properties of concrete incorporating fine recycled aggregate. *Cement and Concrete Research*; 35: 763-769.
- Kimura M (1980). A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. *Journal of Molecular Evolution*; 16: 111-120.
- Kleven JR, Edil TB, Benson CH (2000). Evaluation of excess foundry system sands for use as subbase material. *Transportation Research Record*; 1714: 40-48.
- Kolo K, Claeys P (2005). In vitro formation of ca-oxalates and the mineral glushinskite by fungal interaction with carbonate substrates and seawater. *Biogeosciences*; 2: 451– 497.
- Konhauser KO, Ferris FG (1996). Diversity of iron and silica precipitation by microbial mats in hydrothermal waters, Iceland: implications for Precambrian iron formations. *Geology*; 24: 323–326.
- Kovacevic ZF, Sipos L, Briski F (2000). Biosorption of chromium, copper, nickel and zinc ions onto fungal pellets of *Aspergillus niger* 405 from aqueous solutions. *Food Technology and Biotechnology*; 38: 211–216.
- Kraus RN, Naik TR, Ramme BW, Kumar R (2009). Use of foundry silica-dust in manufacturing economical self-consolidating concrete. *Construction and Building Materials*; 23: 3439-3442.
- Krebs W, Brombacher C, Bosshard PP, Bachofen R, Brandl H (1997). Microbial recovery of metals from solids. *FEMS Microbiology Reviews*; 20: 605-17.

- Landeweert R, Hoffland E, Finlay RD, Kuyper TW, Breemen NV (2001). Linking plants to rocks: Ectomycorrhizal fungi mobilize nutrients from minerals. *Trends in Ecology Evolution*; 16: 248-254.
- Lindsay BJ, Logan TJ (2005). Agricultural reuse of foundry sand. *Journal of Residual Science and Technology*; 2: 3-12.
- Lundgren DG, Valkova-Valchanova M, Reed R (1986). Chemical reactions important in bioleaching and bioaccumulation. In workshop on biotechnology for the mining, metal-refining and fossil fuel processing industries, ed by H.L. Ehrlich and D.S. Holmes; pp. 7-22, John Wiley & Sons, New York.
- Makela M, Galkin S, Hatakka A, Lundell T (2002). Production of organic acids and oxalate decarboxylase in lignin-degrading white rot fungi. *Enzyme Microbial Technology*; 30: 542–549.
- Mast DG, Fox PJ (1998). Geotechnical performance of a highway embankment constructed using waste foundry sand. In: *Recycled materials in geotechnical applications*. Geotechnical Special Publications. ASCE, Reston; 79: 66-85.
- Metal World (2007). Foundry Cluster in India- An Overview; 17-18
- Miguel RE, Banda Noriega RB, Porta AA (2011). Analysis of heavy metals in leachate from spent foundry sands in micro, small and medium-sized companies in Argentina. Paper Presented at the 15th Congress of Brazilian Foundry Association, Sao Paulo, Brazil.
- Miguel RE, Ippolito JA, Leytem AB, Potra AA, Noriega RBB, Dungan RS (2012). Analysis of total metals in waste molding and core sands from ferrous and non-ferrous foundries. *Journal of Environment Management*; 110: 77-81.
- Mishra S, Jyot J, Kuhad RC, Lal B (2001). In situ bioremediation potential of an oily sludge-degrading bacterial consortium. *Current Microbiology*; 43: 328–335.
- MNR (1992). Mineral aggregate conservation Reuse and recycling. Report prepared by John Emery Geotechnical Engineering Limited for Aggregate and Petroleum Resources Section, Ontario Ministry of Natural Resources, Queen's Printer for Ontario.

- MOEE (1993). Spent foundry sand. Alternative uses study. Report prepared by John Emery Geotechnical Engineering Limited for Ontario Ministry of the Environment and Energy and the Canadian Foundry Association, Queen's Printer for Ontario.
- Modak JM, Natarajan KA, Saha B (1996). Biosorption of copper and zinc using waste *Aspergillus niger* biomass. Mineral and Metallurgical Processing; 13: 52-57.
- Muller B, Burgstaller W, Strasser H, Zanella A, Schinne F (1995). Leaching of zinc from an industrial filter dust with *Penicillium*, *Pseudomonas* and *Corynebacterium*: citric acid is the leaching agent rather than amino acids. Journal of Industrial Microbiology; 14: 208-212.
- Mulligan CN, Galvez-Cloutier R, Renaud N (1999). Biological leaching of copper mine residues by *Aspergillus niger*. In biohydrometallurgy & the environment toward the mining of the 21st century, Part A, ed by R. Amils and A. Ballester, pp. 453-461. Elsevier Science B.V, Netherlands.
- Mulligan CN, Cloutier RS (2003). Bioremediation of metal contamination. Environmental Monitoring and Assessment; 84: 45-60.
- Murad A, El-Holi K, Al-Delaimy S (2003). Citric acid production from whey with sugars and additives by *Aspergillus niger*. African Journal of Biotechnology; 2: 356-359.
- Naik TR, Patel VM, Parikh DM, Tharaniyii MP (1994). Utilization of used foundry sand in concrete. Journal of Materials in Civil Engineering; 6: 254-263
- Naik TR, Singh SS, Ramme WB (2001). Performance and leaching assessment of flowable slurry. Journals of Environmental Engineering; 127: 359-368.
- Naik TR, Kraus RN, Chun YM, Ramme WB, Siddique (2004). Precast concrete products using industrial by-products. ACI Materials Journal; 101: 199-206.
- Newnham RE (1997). Molecular mechanisms in smart materials. Materials Research Society Bulletin; 22: 20-33.

- NEN 7341 (1993). Determination of the leaching behavior of granular materials: Availability test. Netherlands Normalization Institute, Delft
- NORD TEST (1995). Solid waste, granular inorganic material: column test. Nord test method NT ENVIR 002, Espoo, Finland.
- Rakshvir M, Barai SV (2006). Studies on recycled aggregates-based concrete. *Resources, Conservation and Recycling*; 50: 71-81.
- Ramachandran SK, Ramakrishnan V, Bang SS (2001). Remediation of concrete using microorganisms. *ACI Materials Journal*; 98: 3–9.
- Ramakrishnan V, Deo KS, Duke EF, Bang SS (1999). SEM investigation of microbial calcite precipitation in cement. In: *Proceeding of the international conference on cement microscopy*, Las Vegas, Nevada, 406-416.
- Raoa A, Jhab KN, Misra S (2007). Use of aggregates from recycled construction and demolition waste of concrete. *Resources, Conservation and Recycling*; 50: 71-81.
- Reddia LN, Riecka GF, Schwabb AP, Chouc ST, Fan LT (1996). Stabilization of phenolics in foundry waste using cementitious materials. *Journal of Hazardous Materials*; 45: 89-106.
- Ren WX, Li PJ, Geng Y, Li XJ (2009). Biological leaching of heavy metals from a contaminated soil by *Aspergillus niger*. *Journal of Hazardous Materials*; 167:164–169.
- Rezza I, Salinas E, Elorza M, Tosetti MS, Donati E (2001). Mechanisms involved in bioleaching of an aluminosilicate by heterotrophic microorganisms. *Process Biochemistry*; 36: 495–500
- Riediker S, Ruckstuhl S, Suter MJF, Cook AM, Giger W (2000). p-Toluenesulfonate in landfill leachates: leachability from foundry sands and aerobic biodegradation. *Environmental Science and Technology*; 34, 2156-2161.
- Saitou N & Nei M (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Molecular Biology and Evolution*; 4: 406-425.

- Sahu AK, Kumar S, Sachan AK (2003). Crushed stone waste as fine aggregate for concrete. *The Indian Concrete Journal*; 77: 845- 847.
- Santurde RS, Andres A, Viguri JR, Raimondo M, Guarini G, Zanelli C, Dond M. (2011). Technological behavior and recycling potential of spent foundry sands in clay bricks. *Environmental Management*; 92: 994-1002.
- Sanyal A, Rautaray D, Bansal V, Ahmad, Sastry M (2005). Heavy-metal remediation by a fungus as a means of production of lead and cadmium carbonate crystals. *Langmuir*; 21: 7220-7224.
- Sayer JA, Raggett SL, Gadd GM (1995). Solubilization of insoluble metal compounds by soil fungi: development of a screening method for solubilizing ability and metal tolerance. *Mycological Research*; 99: 987-993.
- Sayer JA, Gadd GM (1997). Solubilization and transformation of insoluble inorganic metal compounds to insoluble metal oxalates by *Aspergillus niger*. *Mycological Research*; 101: 653–661.
- Sayer JA, Gadd GM (2001). Binding of cobalt and zinc by organic acids and culture filtrates of *Aspergillus niger* grown in the absence or presence of insoluble cobalt or zinc phosphate. *Mycological Research*; 105: 1261-1267.
- Schmidt W, Brouwers HZH, Kuehne HC, Meng B (2011). Influence of environmental temperatures on the performance of polymeric stabilizing agent in fresh cementitious materials. *Key Engineering Materials*; 499: 97-104.
- Schinner F, Burgstaller W (1989). Extraction of zinc from industrial waste by a *Penicillium* sp. *Applied Environmental Microbiology*; 55:1153-56.
- Seidel A, Zimmels Y, Armon R (2001). Mechanism of bioleaching of coal fly ash by *Thiobacillus thiooxidans*, *Chemical Engineering Journal*; 83: 123-130.
- Shi C, Qian J (2003). Increasing coal fly ash use in cement and concrete through chemical activation of reactivity of fly ash. *Energy Sources*; 25: 617-628.

- Siddique R, Gupta R, Kaur I (2007). Effect of spent foundry sand as partial replacement of fine aggregate on the properties of concrete. 22nd International Conference on Solid Waste Technology and Management, Widener University, Philadelphia, USA.
- Siddique R, de Schutter G, Noumowe A (2009). Effect of used-foundry sand on the mechanical properties of concrete. *Construction and Building Materials*; 23: 976-980.
- Siddique R, Kaur G, Rajor A (2010). Waste foundry sand and its leachate characteristics. *Resources, Conservation, and Recycling*; 54: 1027-1036.
- Siddique R, Kadri EH (2011). Effect of metakaolin and foundry sand on the near surface characteristics of concrete. *Construction and Building Materials*; 25: 3257-3266.
- Singh G, Siddique R (2012a). Abrasion resistance and strength properties of concrete containing waste foundry sand (WFS). *Construction and Building Materials*; 28: 421-426.
- Singh G, Siddique R (2012b). Effect of waste foundry sand (WFS) as partial replacement of sand on the strength, ultrasonic pulse velocity and permeability of concrete. *Construction and Building Materials*; 26: 416-422.
- Scott R, Singh SP (2011). High performance silica fume concrete and some applications in India, *Proceedings of the International UKIERI Concrete Congress – New Developments in Concrete Construction*, 8-10 Ma, pp. 217-238, IIT Delhi.
- Skinner WCH, Jahren HA (2003). Biomineralization. In: Schlesinger WH, editor. *Treatise on Geochemistry*; 8:117–184.
- Tamura K, Dudley J, Nei M, Kumar S (2007). MEGA4: Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Molecular Biology and Evolution*; 24:1596-1599.
- Travis J (1997). Microchips covered with DNA emerge as powerful research tools. *Science News*; 151: 144–146.

- Tuason MMS, Arocena JM (2009). Calcium Oxalate Biomineralization by *Piloderma fallax* in Response to Various Levels of Calcium and Phosphorus. *Applied Environmental Microbiology*; 75: 7079-7085.
- Valix M, Usai F, Malik R (2001). Fungal bioleaching of low grade laterite ores, *Materials Engineering*; 14: 197-203.
- Van der Sloot HA (1998). Quick techniques for evaluating the leaching properties of waste materials: Their relation to decisions on utilization and disposal. *Trends in Analytical Chemistry*; 17: 298-310.
- Verrecchia EP, Dumont JL, Verrecchia KE (1993). Role of calcium oxalate biomineralization by fungi in the formation of calcretes : A case study from Nazareth, Israel. *Journal of Sedimentary Research.*; 63: 1000-1006.
- Velosa AL, Cachim PB (2009). Hydraulic-lime based concrete: Strength development using a pozzolanic addition and different curing conditions. *Construction and Building Materials*; 23: 2107–2111.
- Vipulanandan C, Weng Y, Zhang C (2000). Designing flowable grout mixes using foundry sand, clay and fly ash. *Advances in Grouting Modification, Geotechnical Special Publications*. ASCE, Reston; 104: 215-233.
- Volesky B (1991). Biosorption by fungal biomass. In *biosorption of heavy metals*, ed by B. Volesky, pp. 140-168, CRC Press, Canada.
- Wasay SA, Barrington SF, Tokunaga S (1998). Using *Aspergillus niger* to bioremediate soils contaminated by heavy metals. *Bioremediation Journal*, 2: 183-190.
- Wasay SA, Barrington SF, Tokunaga S (2001). Organic acids for the in situ remediation of soils polluted by heavy metals: soil flushing in columns. *Water Air Soil & Pollution*; 127: 301-314.

- Winkler ES, Kosanovic B, Genovese T, Roth I (1999). A survey of foundry participation in the Massachusetts beneficial use determination process. Chelsea Centre for Recycling and Economic Development, University of Massachusetts, MA.
- Winkler ES, Bolshakov AA (2000). Characterization of foundry sand waste. Chelsea Centre for Recycling and Economic Development, University of Massachusetts, MA.
- Yestis U, Ozcengiz G, Dilek FB, Ergen N, Erbay A, Dolek A (1998). Heavy metal biosorption by white-rot fungi. *Water Science and Technology*; 38: 323- 330.
- Zafar S, Aqil F, Ahmad I (2007). Metal tolerance and biosorption potential of filamentous fungi isolated from metal contaminated agriculture soil. *Bioresource Technology*; 98: 2557-2561.