PRODUCTION AND UTILISATION OF FLY ASH BASED BIOFERTILIZERS IN PLANT GROWTH

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SUBMITTED BY

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CERTIFICATE

This is to certify that the project report entitled "**Production and Utilization of Fly Ash Based Biofertillizers in Plant Growth**" submitted by **ALISHA PRASAD** (109BT0557) in the partial fulfillment of the requirement for the degree of the Bachelor in Technology in Biotechnology Engineering, National Institute of Technology, Rourkela is an authentic work carried out by her under my supervision. To the best of my knowledge the matter embodied in the report has not been submitted to any other Institute/University for any degree.

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ABSTRACT

Fly ash an industrial waste resulting from combustion of pulverized coal can be used as an alternative for gainful and safe utilization of fly ashes on sustainable basis in agricultural sector to decimate the problem of land shortage. The research program consists of utilizing the mineralogical properties of fly ash such as porosity, low bulk density, presence of inorganic metal ions, and its macro nutrient property. Fly ash (pH: 6.5) with 200mesh size was treated by a technique called Bioleaching for extraction of low grade mineral concentrates from industrials wastes like fly ash by conversion of an insoluble metal into a soluble form using *Thyobacillus ferrooxidans* a gram -negative, rod shaped economic bacterium in the field of leaching. During their growth phase, these bacteria oxidize insoluble metal compounds especially FeS and converts it into soluble form in presence of the Fe (II)-oxidizing enzymes like *rusticyanin oxidoreductase*.

Research program results show the loss of trace elements through leaching, after which the leached water test analysis mainly focusing sulphur and iron tests were also done. Leached water showed high content of sulphur and iron content in it and the pH of fly ash was accounted to 8.5 after leaching. In order to check the construction formwork to some extent plant growth tests (includes plant height, thickness), was also undergone.

1. INTRODUCTION

Most of the industries in India have their own thermal power plant stations setup for generating power for industrial use and also for supply to other stations. All these thermal power plants use pulverized coal as a fuel source to generate power obtaining fly ash as a by-product. There are about 125 thermal power plants in India, which form the major source of fly ash in the country (Kumar and Singh, 2006). With the commissioning of super thermal power plants and with the increasing use of low grade coal of high ash content, the current production of fly ash is about 120 Million tonnes per year and is expected to reach around 170 Million tonnes by 2012 A.D (Kumar and Singh, 2006). This has posed a serious disposal and ecological problem in addition to occupying a large tract of scarce cultivable land.

Though the beneficial use of fly ash have been recognized in various areas like in concrete, brick making, soil stabilization treatment and other applications, only a small quantity of the total fly ash being generated is utilized in our country currently in such applications. Therefore, there is much need of research and development to utilize this waste for other application e.g. biofertilizer.

1.1 Fly ash a finely divided residue resulting from combustion of pulverized coal in thermal power plants comprising of fine particles rises with flue gases from the stock [1].The particle-laden flue gas from the boiler is captured by electrostatic precipitators a step before the flue-gases reach the chimneys of coal fired power plants, and together with bottom ash is removed from the bottom of the furnace, in this case jointly known as **coal ash**. The ESP works as a cleaning device, which utilizes electrical forces to separate dust particles from the flue gases [2].

Bottom Ash is the non combustible by product obtained during combustion in a furnace .The particles are coarser in comparison to fly ash and generally it does not rise with flue gases but they settle down at the bottom since they are heavier and are finally separated by ESP [1, 2].

1.2 DISADVATNAGE OF DUMPING FLY ASH IN INDUSTRY

Keeping in mind the global environment, industrial pollution is never limited to industrial areas. Traces of industrial pollutants has been identified in isolated human, animal, and plant populations accounting to the fact that they can travel immense distances due to climatic effects not only hurting the environment in a number of ways but also imparting negative impacts on human health and life.[3]

The industrial fly ash can act as a potent source of pollutant leading to an imbalance in the ecosystem by degrading air and thereby quality of life radically. This can have a vulnerable effect on the rural population residing in these areas earning their source of bread. An alternative can be adopted minimizing the pros and utilizing the cons on sustainable basis.

1.3 ADVATNAGES ASSOCIATED WITH FLY ASH FOR UTILISATION AS BIOFERTILISER

TABLE 1: Characteristics of fly ash (Mittra B. N, 2003)

Sl No.	PHYSICAL	CHEMICAL	BIOLOGICAL
1	Spherical in size, light weight leading to low bulk density, high porosity & water logging capacity. .	Elemental constituents of fly ash are: Al, Fe, Ca, C, Mg, K, Na, S, Ti, P, and Mn. Trace elements include :Mo, Se, Cd , and Zn Inorganic ions include: Ca, Na, Mg, K, Fe, S, and C.	Acts as a conditioner to arrest soil erosion.
2	Characteristic of fly ash for use as a biofertillizers is its porous nature which enhances its utility in water logging.	These account to provision of required salts for crop enhancing the growth.	Acts as good substrate for growth of various microorganisms in it by utilization of trace elements in it acting as a soil ameliorates.
3	Fly ash when added with soil particles changes the physical properties of soil like texture, bulk density, and water logging capacity.	With increase in the chemical properties like pH, a significant change in the soil moisture content, crop growth etc is observed.	

1.4 OBJECTIVE

1.4.1. To prepare biofertilizer from fly ash generated in industry

1.4.2. To use the prepared biofertilizer for plant growth

1.5. Scope of Work

Fly ash (pH: 6.5) a residue of pulverized coal generated in thermal power plants in today's modern industrial civilization is the most highlighted name in industrial sector. It has not only led to land occupancy but also a threat for land shortage thereby becoming a mecum to the society. Thus, we've come up posing an environmental challenge to use fly ash, and enhance it biologically for making biofertillizers to be used in agricultural sector, hereby acting as "**Quality Improvement Production**".

The two major processes carried out for this study are:

1. Bioleaching
2. Comparative growth study by plant growth

BIOLEACHING:

Fly ash(pH: 6.5)a byproduct of coal based thermal power plants due to its mineralogical property acts as a soil amendment for use in agricultural sector. Since fly ash contains toxic elements including trace elements in small amounts, first the fly ash(pH: 6.5) with 200mesh size is treated by a technique called 'bio-leaching' or 'bio-mining' for extraction of low grade metals and mineral concentrates. ***Thyobacillus ferrooxidans*** a gram negative, rod shaped bacteria due to its bio-hydrometallurgical property acts as an economic bacterium in the field of leaching. This is collected from sewages (SAIL Dump yard, Rourkela) then, followed by serial dilution and multiplied by media preparation which serves as nutrients for growth of the bacterium thereby

increasing the bacterial counts. After this bioleaching is carried out for extraction of unwanted metals present in the fly ash (pH: 6.5) stock.

COMPARATIVE GROWTH STUDY BY PLANT GRWOTH:

Fly ash formulated into biofertillizers (pH: 8.5) containing 90% moisture content is mixed with soil in measurements by weight. 2 samples of fly ash to soil ratio by weight is taken and 1 sample of soil is taken as control for comparative growth analysis. The leached fly-ash (pH: 8.5) samples were weighed in different amounts in a ratio of 1:2, 1:2, and 1:1 respectively in kilograms along with the soil. The last sample taken was only soil. They were kept covered by a sack soaked in water for a period of 1-2 weeks allowing the microorganisms contained in both samples i.e. fly ash and soil to interact.

1.6. EXPERIMENTAL STUDY

To achieve the objectives outlined, a proper study plan was made for achieving the goals. The steps to be followed are given below:

1. **Collection of the fly ash samples**: Fly ash samples were collected from two different industrial plants namely:
    a. HINDALCO, Muri Works, Jharkhand, India.
    b. SAIL, RSP, Rourkela, Odhisha, India.

2. **Collection of the industrial-sewage samples:** For isolation of *Thyobacillus ferrooxidans* for bioleaching process, sewage wastes from industrial plants were collected from SAIL, RSP, Rourkela, Odhisha,India

2. LITERATURE REVIEW

2.1 MICROBIAL LEACHING (BIOLEACHING/BIOMINING) OF FLY ASH:

Bioleaching is a process for extraction of metals from their mineral source by oxidation of metals by naturally occurring microorganisms so as to extract certain elements from the material on filtration via water. Microbial technology renders helps in case of recovery of ores which cannot be economically processed with chemical methods, because they contain low grade metals. Bio-mining is, economically sound hydrometallurgical process with lesser environmental problem than conventional commercial application. It's an inter-disciplinary field that involves various fields like molecular biology, metallurgy and chemical engineering.[6, 7]

Bioleaching technique is helpful for recovery of copper, iron, cobalt, arsenic, zinc, silver etc.

'Bioleaching' which is one of the most used treatment technique for extraction of low grade mineral concentrates from mineral ore (red mud) and industrials wastes like fly ash by conversion of an insoluble metal (usually a metal sulfide, e.g., FeS_2) into a soluble form (usually the metal sulfate, e.g., $FeSO_4$) using microbes for gainful and safe utilization on sustainable basis. [5.6] The process of bioleaching involves judicial application of certain bacteria like of *Thyobacillus ferrooxidans, , Leptospirillum ferrooxidans, Acidithiobacillus ferrooxidans, Sulpholobus thermosulphidoxidans and Sulpholobus brierleyi* to the raw materials. During their growth phase, these bacteria oxidize insoluble metal compounds especially FeS and converts it into soluble form in presence of the Fe (II)-oxidizing enzymes like *rusticyanin oxidoreductase*. It's a safe and cost effective method of metal recovery and can be an attractive option to the industries. [6, 8]

2.2 MECHANISM

In bioleaching process, two major processes are involved namely: Biooxidation and Bioleaching. First the microorganisms involved in bioleaching interact with the samples, and the microbial oxidation process takes place at the exposed cell membranes of the bacteria. On interaction with the bacteria, the free electrons enter into the cell via. cell membrane pores and undergo biochemical process producing energy thereby reducing oxygen (O_2) to water (H_20) [8,9]. The

bacteria utilizes this energy to undergo the most critical reaction step in which the major reactant is produced when sulfides gets oxidized by a ferric iron**.**

2.3 MIROORGANISMS EMPLOYED IN LEACHING:

Bioleaching mainly involves conversion of solid metal values into their water soluble forms using typical microorganisms named ***Thyobacillus ferrooxidans*** and ***Thiobacillus thiooxidans***. [3, 5, 9]

Thyobacillus ferrooxidans is the most important genus of chemo-lithotrophs that metabolize sulfur and iron oxidizing it and producing sulphuric acid in the aqueous phase. The remaining solids are discarded. ***Thiobacilli*** can well adapt to wide variations of temperature and pH and can be readily isolated and enriched. These are generally isolated from industrial ponds, sewages, miming area, drainage effluents, rivers, canals etc.

2.4 MECHANISM BEHIND THE PICTURE:

Thyobacillus ferrooxidans and Thiobacillus ***thiooxidans*** derives energy from oxidation of Fe^{2+} or insoluble sulphur. Bioleaching involves two reaction mechanisms:

2.4.1 DIRECT BACTERIAL LEACHING:

In this method a physical contact exists between bacteria and ores thereby leading to oxidation of minerals following several enzymatically catalyzed steps.

A brief study of this method concludes the oxidation of pyrite to ferric sulphate. [3, 5]

1st Reaction: $2FeS_2 + 7O_2 + 2H_2O \rightarrow 2FeSO_4 + 2H_2SO_4$

In the above reaction, ferrous sulfide gets oxidized and gets converted to ferrous sulphate by *Thyobacillus ferrooxidans* utilizing trace amounts of oxygen from the surroundings for its energy source. Sulphuric acid is also formed as a by-product and is leached out in leached water. In this method, the bacteria are in direct contact with the ore i.e. the industrial fly-ash, so it's a fast and effective process of bio-leaching with high efficiency.

2.4.2 INDIRECT BACTERIAL LEACHING:

This method does not involve direct contact with minerals but leaching agents are produced by microorganisms which oxidize them [6].

1st Reaction: $FeS_2 + Fe_2SO_4 \rightarrow 3FeSO_4 + 2S$

In the above reaction, ferrous sulfide gets oxidized and free sulphur is formed. In the next reaction step, this free sulphur will undergo the mechanism process.

2nd Reaction: $2S + 3O_2 + 2H_2O \rightarrow 2H_2SO_4$

In the above reaction, the free sulphur reacts with utilizing trace amounts of oxygen present and gets reduced to yield sulphuric acid which is drained out from fly ash and sweeps in the leached water.

In the above reaction, the product obtained in leached water is sulphuric acid. If fly ash without treatment is added to the soil, these harmful acids can sweep through the soil crust and effect not only the crop growth but also disturb the ground water table. So prior to application of fly ash for plant growth, it has to be treated. This shows the effective bioleaching process.
Indirect bioleaching is another method of leaching, but the efficiency in comparison to the other one is comparatively less, since it does not involve direct contact with the ore. But this too can serve for the same purpose with a longer retention time of leaching.

Oxidation of ferrus (Fe^{2+}) to ferric (Fe^{3+}) by *Thyobacillus ferrooxidans* at low pH is given below [5. 6]:

1st Reaction: $4FeSO_4 + 2H_2SO_4 + O_2 \rightarrow 2\,Fe_2(SO_4)_3 + 2H_2O$

In the above reaction, ferrous sulfate reacts in the presence of sulphuric acid developing an acidic environment, and utilizing trace amounts of oxygen present for the oxidation to take place by *Thyobacillus ferrooxidans.* Basically in this reaction step ferrus gets oxidized to ferric ion.

COMPARISON OF BIOLEACHING WITH OTHER TECHNIQUES

In comparison to other techniques, bioleaching technique is better than other in every aspect. Few of the reasons to satisfy the reason is listed below: [9, 10]

1. **Economical aspect**: Cost effective process as it employs microorganisms for doing the leaching process, which requires no chemicals, or extra products for investment.
2. **Environment aspect**: Eco-friendly process, producing no toxic effluents, or by products which in comparison to chemical leaching may be produced in large scales.
3. **Ore concentration:** Bioleaching is effective in leaching poor ore concentrates which in case of chemical leaching is not attainable up to marks.

.

3. MATERIALS AND METHODS

For initiate the work under this project, we visited two industrial plants SAIL (Rourkela Steel Plant) and HINDALCO (Muri Works) for fly ash, and industrial sewage waste water sample collections.

The following methodology and techniques followed to achieve the objective of the project:

3.1 CONE QUARTER METHOD OF FLY ASH COLLECTION

After ESP completion, the fly ash is pumped by Fluidized Bed Reactors upstream and they are collected in trucks either for disposal to nearby mines, for making fly ash bricks and rest for dumping in the fly ash pond.

From these heaps, fly ash is collected by **'Cone & Quarter'** Method for study and analysis in the Laboratory.

In this method fly ash sample is collected from three portions of the heap fly ash storage, which is effective as all portions are included and mixed thoroughly for analysis. They are then divided into four parts and two of them are picked. This acts as the first collection. In the next go, the other two parts are picked. This serves as the second collection.

A simple layout of this method is illustrated below:

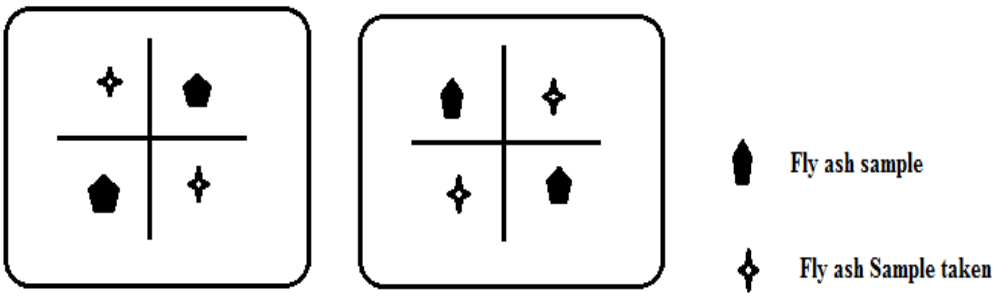

Fig: 1st Collection **Fig: 2nd Collection**

Fig 1: Cone &Quarter Method of FA Collection

3.2 MICROBIAL ANALYSIS OF SEWAGE WASTE

EXPERIMENT 1: MEDIA PREPARATION

MATERIALS REQUIRED:

1. Nutrient Agar Media 4 gm
2. Distilled water 200 ml

EQUIPMENTS:

1. Heating Mantle
2. Electronic Balance
3. Autoclave
4. Glassware's like beakers, measuring cylinder, glass rod, agar plates.
5. Spatula, cotton plugs, and Glass marking pen, aluminum foil.

METHOD:

1. 4 grams of Nutrient Agar Media was weighed in an Electronic Balance and the contents were put in a beaker.
2. 200 ml distilled water was added and stirred.
3. The mouth of the conical flask was closed with a cotton plug and it was covered with aluminum foil.
4. It was labeled with appropriate names and dates and autoclaved at 121℃ for 15 minutes.

EXPERIMENT 2: SERIAL DILUTION

MATERIALS REQUIRED:

1. Test tubes 10
2. Sewage waste sample 20ml

3. Fly ash sample 10g
4. Distilled water
5. Pipettes
6. Test tube stand

METHOD:

1. 10 grams of Sewage waste was weighed in an Electronic balance and transfer the contents were transferred into a clean dry test tube.
2. 10 clean test tubes were taken and marked serially 10^{0}, 10^{-1}, $10^{-2,}$ $10^{-3,}$ $10^{-4,}$ $10^{-5,}$ $10^{-6,}$ $10^{-7,}$ 10^{-8} ,10^{-9} and placed in order.
3. Oil sample was completely mixed with distilled water. This was the 1st dilution (10^{0}).
4. Now the test tube (containing Sewage waste) was placed in a test tube stand and allowed to sediment till faddy water settled above.
5. Carefully1ml of the faddy water was pipette out from the test tube avoiding the pipette to touch the soil surface. It was transferred to a new test tube with marking (10^{-1}).
6. 9ml of distilled water was added to it and again it was mixed thoroughly. This was the 2nd dilution.
7. The above steps were repeated similarly, with correct amounts until 10^{-9} dilutions.
8. After serial dilution was over the diluted sample of order $10^{-4 \text{ or }} 10^{-5}$ dilutions were kept aside for use in culturing and multiplication of bacterial strains present in Sewage waste sample on agar plates.

EXPERIMENT 3: GRAM STAINING

MATERIALS REQUIRED:

1. Crystal Violet
2. Grams Iodine

3. Alcohol
4. Safranin
5. Glass slides ,Spirit Lamp and Scalpel
6. Distilled water
7. Microbial Plates
8. Microscope
9. Laminar hood

METHOD:

1. The UV of the laminar hood was switched on for 20 minutes prior to work. After 20 minutes the UV was switched off and the blower and Light were put on and the floor was wiped with 70% ethanol towards the outward direction of the hood.
2. A clean glass slide was wiped with 70% ethanol and then heated over a spirit lamp held by the edges of the glass slide.
3. A scalpel was taken and heated over a spirit lamp till hot red.
4. Using the scalpel a clean colony was picked up slowly without disturbing the agar gel and it was smeared on the glass slide slowly making a light smear by adding a drop of distilled water for spreading.
5. It was kept for drying on a clean surface.
6. After this the glass slide was heated till the smear sticks on to the glass slide.
7. It was allowed to cool for a minute and the washed with running distilled water.
8. A drop of crystal violet was added to it.
9. After a minute gain wash it with running distilled water.
10. A drop of Grams Iodine was added to it .Again wash it with running distilled water.
11. After a minute a drop of Alcohol was added to it .
12. After a minute a drop of Safranin was added to it .

13. After a minute again it was washed with running distilled water and dried with tissue paper.
14. It was observed under a microscope under 10X magnification.

3.3 BIOLEACHING OF FLY ASH via ***Thyobacillus ferrooxidans***

MATERIALS REQUIRED:

1. Fly ash samples-SAIL, HINDALCO
2. ***Thyobacillus ferrooxidans*** inoculums
3. Filter paper
4. Filter Funnel
5. Centrifugal pump
6. Glass apparatus like conical flasks, beakers, glass rod etc.
7. Distilled water
8. Electronic Balance

METHOD:

1. Fly ash was sieved in 200 meshes sized sieve and 500 grams of it was weighed in an Electronic balance and the contents were transferred into a clean dry beaker.
2. A filter funnel was taken , cleaned thoroughly, dried and placed on a conical flask containing 200 ml ***Thyobacillus ferrooxidans*** inoculums.
3. The mouth of the conical flask was closed with cotton plug and covered with aluminum foil.
4. It was labeled with appropriate names and dates and incubated in a shaker incubator at 60°C for 1 week.
5. The smell and color change were checked every two days after incubation.

6. After 7 days the water was separated from the fly ash by using a funnel placing a filter paper over it. The leached water was kept aside for characterization and analysis.

3.4 MIXING OF FLY ASH AND SOIL TOGETHER FOR BIOFERTILIZER FORMULATION

MATERIALS REQUIRED:

1. Bioleached Fly ash samples.
2. Soil samples

METHOD:

1. Bioleached fly ash samples were weighed in three different weights 500g, respectively and the contents were transferred in an aluminum foil basket.
2. Now soil sample were weighed in three different weights 500g, 500g, 1000g respectively.
3. The 1st two samples were transferred in the same aluminum foil basket along with the bioleached fly ash sample. The last sample was placed separately in another basket.
4. All the 1st two samples were mixed thoroughly along with the soil adding some water to it. Also the soil sample were mixed separately breaking all the lumps making it finer.
5. All the samples were labeled with appropriate names, weights and dates.
6. The basket was covered leaving some space for air entry and stored in a cool place for biofertillizers formulation for 6-7 days.

3. 5 CHECKING FOR PLANT GROWTH

MATERIALS REQUIRED:

1. 3 marigold plant of same height

METHOD:

1. The marigold plant was taken and their height was measured and noted down.

2. The plant were planted in the three baskets and water was added so as to maintain some moisture content for growth.
3. The samples were labeled with appropriate names, seeding dates and keep it under sunlight for checking the growth.

4. RESULTS AND DISCUSSION

4.1 Morphological characteristics of fly ash

The morphology

The morphology of the fly ash samples were studied by SEM (scanning electron microscope). For this, specimens of fly ash (powder) from both HINDALCO and SAIL were pressed to a grid and coated prior to scanning. The micrograph pictures presented below depict the typical microstructure of the fly ash samples.

Fig. 2 (a) is a SEM image of SAIL-Fly ash showing its characteristic morphology. In the picture we can see number of spherical shaped particles of different sizes with varying diameters. The image was taken in X500-50µm range at 15kV. Inside the sphere we can see some more hollow shaped particles in the interiors.

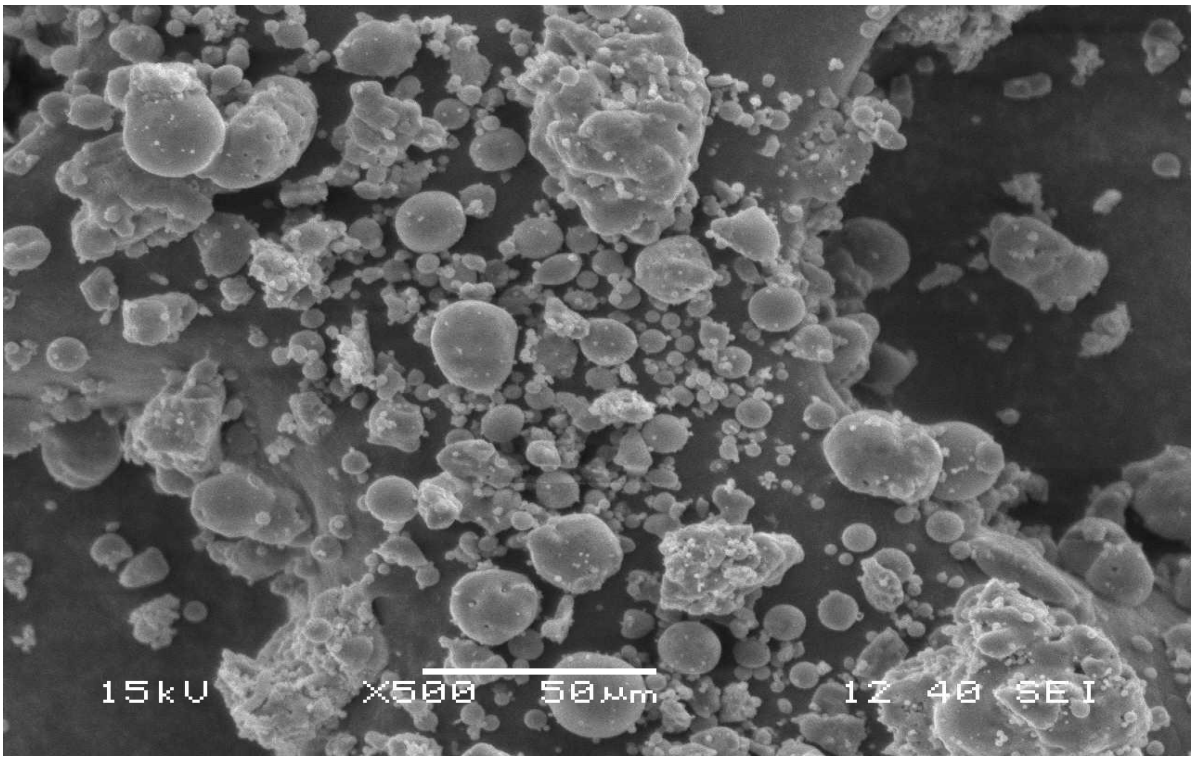

Fig. 2 (a): SEM image of SAIL-Fly ash

Fig. 2 (b) is a SEM image of one of the interior hollow particles of SAIL-Fly ash taken in X500-10µm range at 15kV.

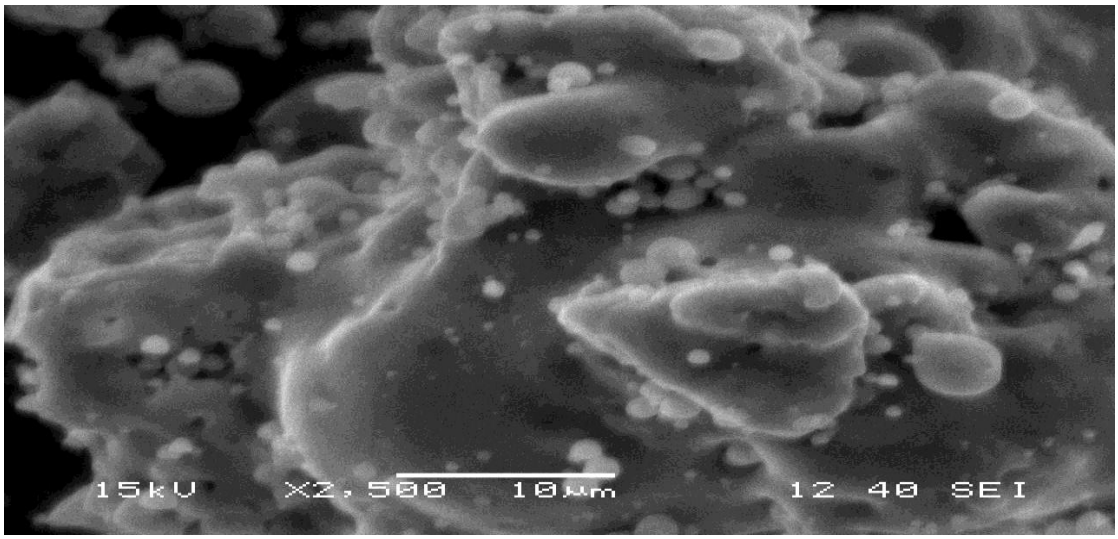

Fig. 2 (b): SEM image of one of the interior hollow particles

Fig. 3(a) is a SEM image of HINDALCO-fly ash showing its characteristic morphology. In this picture we can see particles with varying shapes unlike SEM image of SAIL-Fly ash. In this case also the image was taken in X500-50µm range at 15kV .In the interior of the particles we can see some hollow shaped particles.

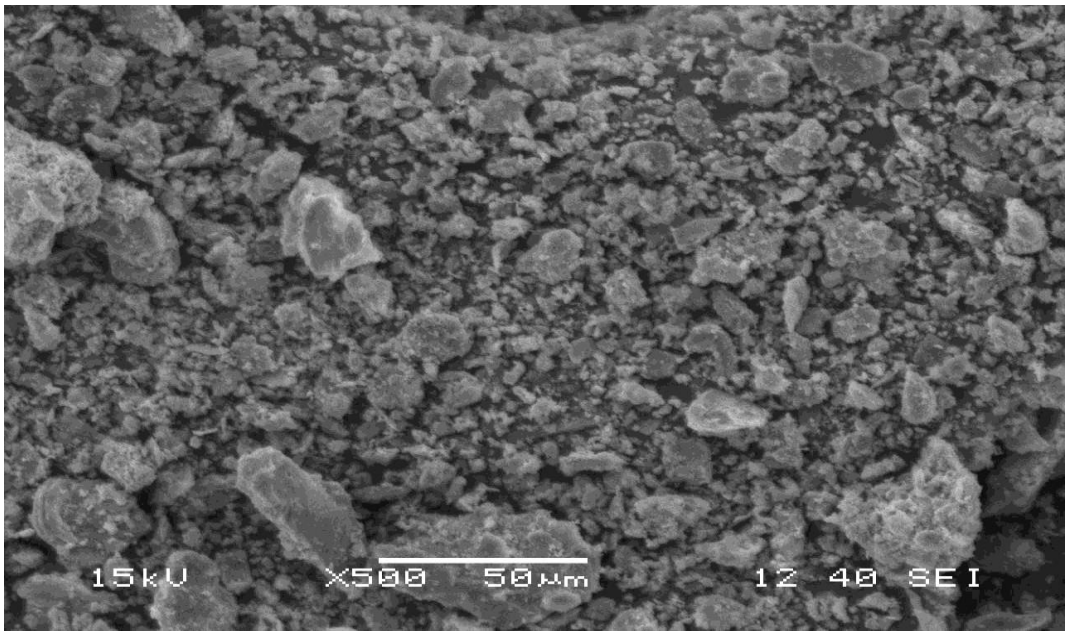

Fig. 3(a): SEM image of HINDALCO-fly ash

Fig. 3 (b) is a SEM image of one of the interior hollow particles of HINDALCO-Fly ash taken in X500-10µm range at 15Kv.

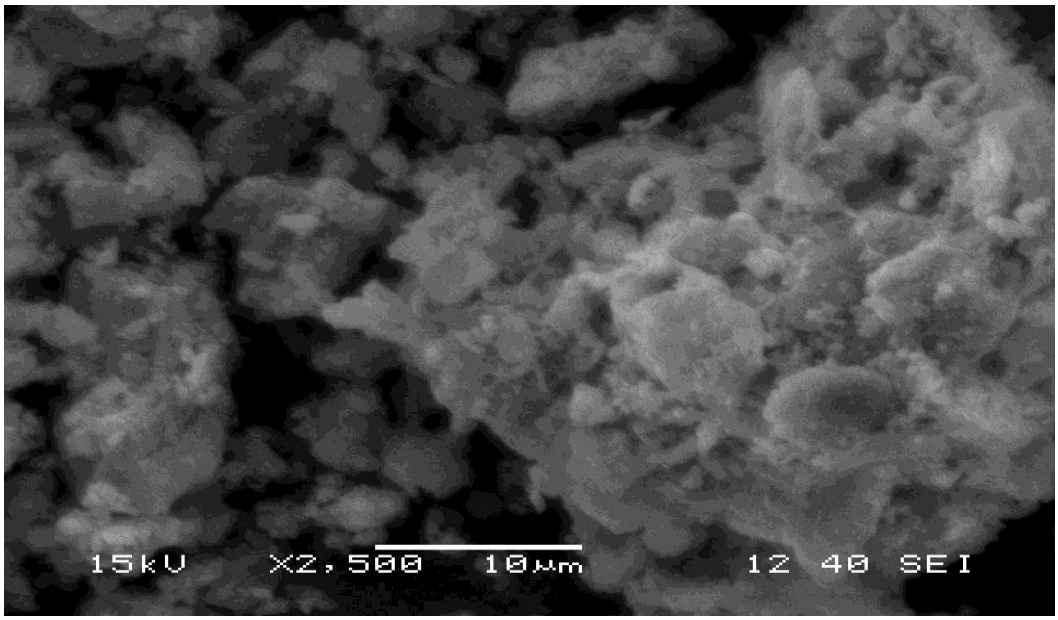

Fig. 3 (b): SEM image of one of the interior hollow particles

For characterization and study of fly ash properties, fly ash samples after collection by 'Cone and Quarter method' were taken and analyzed in the Lab by **TGA Analyzer**

4.2 Physical characteristics of fly ash

TABLE 2: Physical Characteristics of Coal, fly ash and Bed ash by **TGA Analyzer .**

SAMPLES	LOCATION		MESH SIZE (in μ)		MOISTURE (in %)	
	HINDALCO	SAIL	HINDALCO	SAIL	HINDALCO	SAIL
COAL	2	2	1.2512	1.2241	3.02	2.85
	3	3	1.4001	1.3745	3.11	2.89
	4	4	1.3941	1.3861	4.98	3.12
AVERAGE CONTENT			**1.3474**	**1.328**	**3.70**	**2.95**
FLY ASH	5	5	1.2254	1.3174	2.85	3.59
	6	6	1.4362	1.4790	2.89	3.24
	7	7	1.3432	1.4021	3.13	4.09
AVERAGE CONTENT			**1.3349**	**1.3395**	**2.95**	**3.64**

For determining the physical characteristics, the fly ash and coal samples of SAIL and HINDALCO were taken in a tablet holder punched and kept in a rack in line. As the circular rack moved, corresponding results accounting to it were displayed.

From the above results, it was observed that though the fly ash generated after complete burning from same grade of coal (class-C), account to variation in mesh size and moisture content. The mesh size of HINDALCO-coal is coarser as compared to SAIL-coal, which results in higher moisture holding capacity of HINDALCO-coal than SAIL-coal. Since volatility is co-related to moisture content in coal, lesser the moisture content better is the efficiency in combustion of coal, generating more of fly ash. On the contrary-wise more the mesh size of fly ash, better would be its usage in relation to biofertillizer for crop growth as it would increase the water logging capacity. Also more the moisture content in fly ash better it is considered with more interaction of the microorganisms increasing the bio-oxidation process by *Thyobacillus ferrooxidans* enhancing the leaching process.

From the average values so obtained, it's clear that SAIL-coal has lesser moisture content as well as finer granules; fly ash so obtained by combustion from it will have increased moisture content which will tend to be a better potent for biofertillizer production in comparison to HINDALCO samples.

4.3 Chemical characteristics of fly ash

TABLE 3: Chemical Characteristics of fly ash

SL NO	TEST PARAM-ETERS	BEFORE LEACHING (by serial dilution of fly ash)			AFTER LEACHING (using leached out water of fly ash)		
		HINDALO	SAIL	INFERENCE	HINDALCO	SAIL	INFERENCE
1	pH	8.37 (Basic)	8.02 (Basic)	Low pH content	4.80 (Acidic)	4.52 (Acidic)	High pH content
3	Turbidity	0.355 (absorbance)	0.567	Less turbid	0.773	0.981	More turbid due to bio-oxidation
4	Iron Test(Fe^{2+})	0/0219	0.0267	Low iron content but more than in drinking water.	0.7694	0.8933	High content of iron in leached water
5	Sulphur Test	0/0116	0.0134	Low sulphur content but more than in drinking water.	0.3752	0.4561	High content of sulphur in leached water

The chemical tests including test parameters like pH, Turbidity, Iron test and Sulphur tests were done two times: Before leaching and after leaching for both HINDALCO and SAIL samples respectively.

1. **BEFORE LEACHING:** The fly ashes prior to leaching for chemical characterization were undergone serial dilution to measure the different parameters. This was accounted to be the reference values for comparative analysis with data obtained from leached water testing after bio-leaching.

2. **AFTER LEACHING:** the fly ashes after leaching with *Thyobacillus ferrooxidans* were taken and the leached water from it was separated and collected for analysis checking the degree of leaching.

From the above values noted as shown in Table 1.2, it was observed that there was an increase in leached water pH, turbidity and the Iron and Sulphur content also showed drastic increase from the reference values taken. The conclusions that can be drawn from these values are explained below:

a. **pH**: increase in pH in the leached water shows that effective mechanism undergone by *Thyobacillus ferrooxidans* draining out maximum of the elemental constituents of fly ash (as listed in Table 1.1) by bio-oxidation process.

b. **Turbidity**: the leached water turbidity increases in comparison to the reference values, and since turbidity deals with the measure of clarity in water, from this it can be predicted that this may be due to some growth of organic matter occurred during the bioleaching or may be because of bio-oxidation of certain hydroxides etc. also presence of high ionic concentrations which imparts a red-coloration adds up to the increased turbidity in water.

c. **Iron test:** To determine the presence of iron in leached water, 1, 10-phenanthroline method was used involving the principle of iron to be reduced to its ferrous state by boiling hydrochloric acid (11N) along with hydroxylamine chloride solution. The molecules of phenanthroline chelate each atom of ferrous ion to form an orange-colored red complex. For the same, standard solution and stock solution were prepared and its absorbance at 510 nm was measured. To measure the unknown iron content in the leached water sample, the standard solution along the x-axis versus stock solution along y-axis was extrapolated.

Figure 4: shows graph for iron test through the origin, absorbance taken at 510 nm.

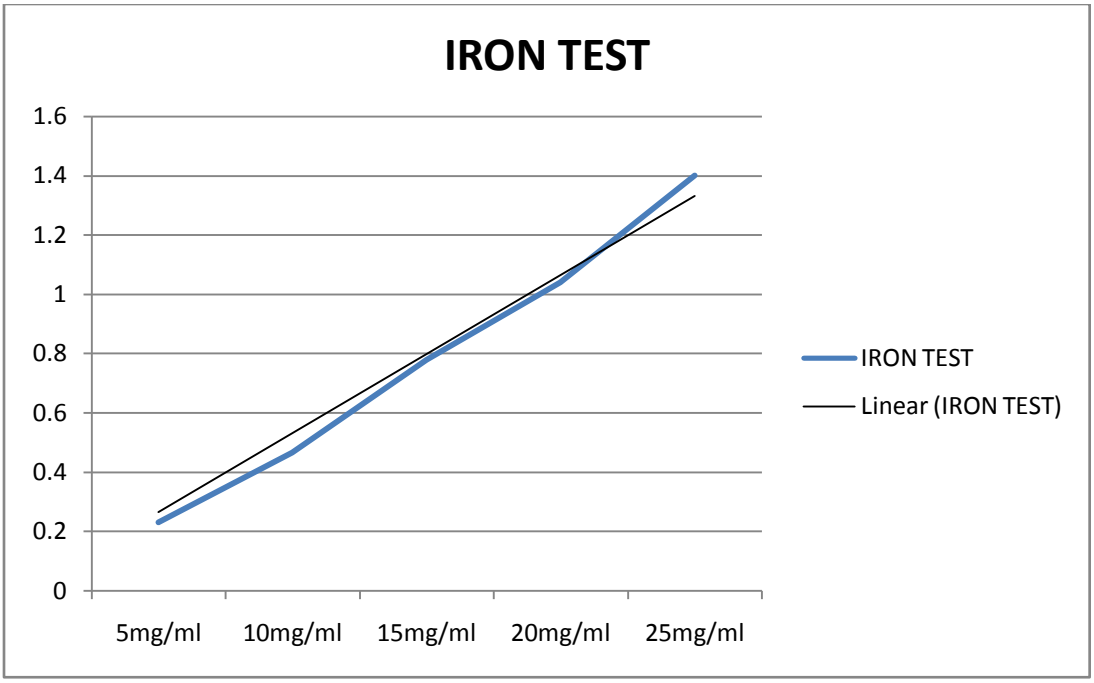

Figure 4: Absorbance of standard solution versus strength of sulphur content in mg/ml

The above graph shows the tremendous increase in iron content in the leached water sample as the concentration of the stock solution increases. This shows that there is a high amount of iron present in leached water indicating the effective leaching draining out all the toxic metals by bio-oxidation.

d. **Sulphur test:** To determine the presence of sulphur in leached water, turbidimetric method was used based on the principle of precipitation of sulphate after adding barium. For the same, standard solution and stock solution were prepared and its absorbance at 510 nm was measured. To measure the unknown sulphur content in the leached water sample, the standard solution along the x-axis versus stock solution along y-axis was extrapolated. Fig5. shows the sulphur test through the origin, absorbance taken at 495 nm.

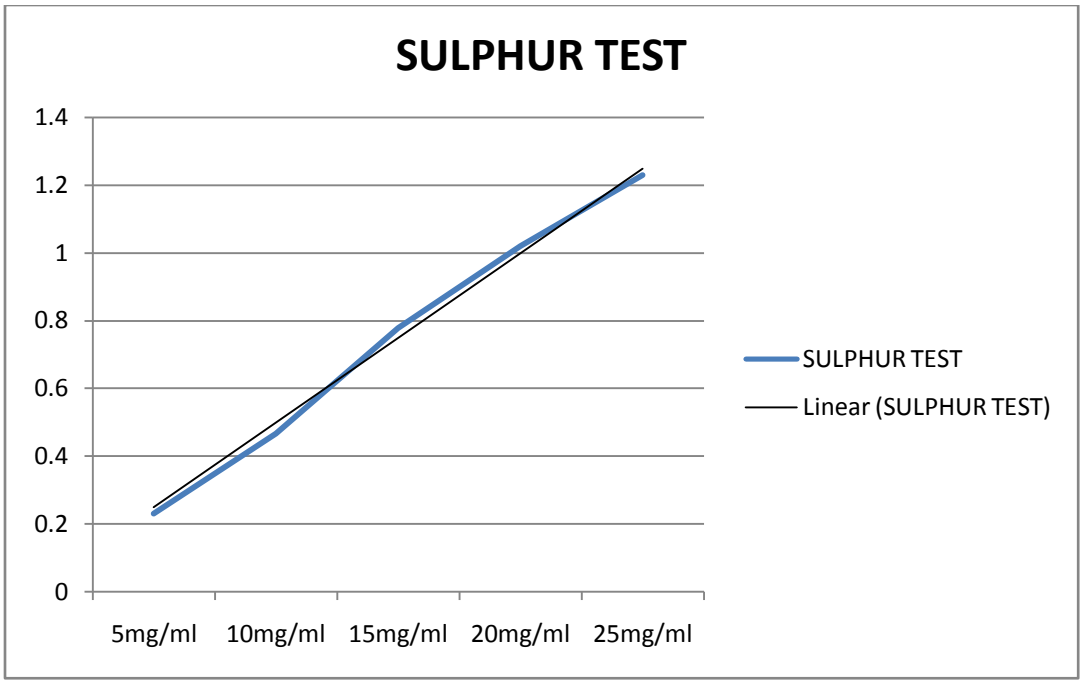

Figure 5: Absorbance of standard solution versus strength of iron content in mg/ml

4.4 Checking presence of ***Thyobacillus ferrooxidans*** in sewage waste

Thiobacillus ferrooxidans a gram negative rod shaped bacteria is important in our context as it acts as the missile in bioleaching process triggering the bio-oxidation mechanism. These are commonly found in sewage wastes, drainage systems, and in water sediments etc. The *Thiobacillus* are beneficial in recovering metals such as sulphur, iron etc by the bio-oxidation process involving conversion of an insoluble metal (usually a metal sulfide, e.g., FeS_2) into a soluble form (usually the metal sulfate, e.g., $FeSO_4$).

For checking the presence of *Thiobacillus ferrooxidans***,** as per chapter 3 materials and methods**,** microbial analysis of sewage waste , serial dilution , media preparation followed by gram staining were done. Reddish colored colonies of *Thyobacillus ferrooxidans* were observed indicating the presence of iron.

Fig. 4 is a microscope image of *Thyobacillus ferrooxidans* in 100µm range. In the image we can see rod shaped filled white structures spread in large density. These are the *Thyobacillus* found in sewage wastes.

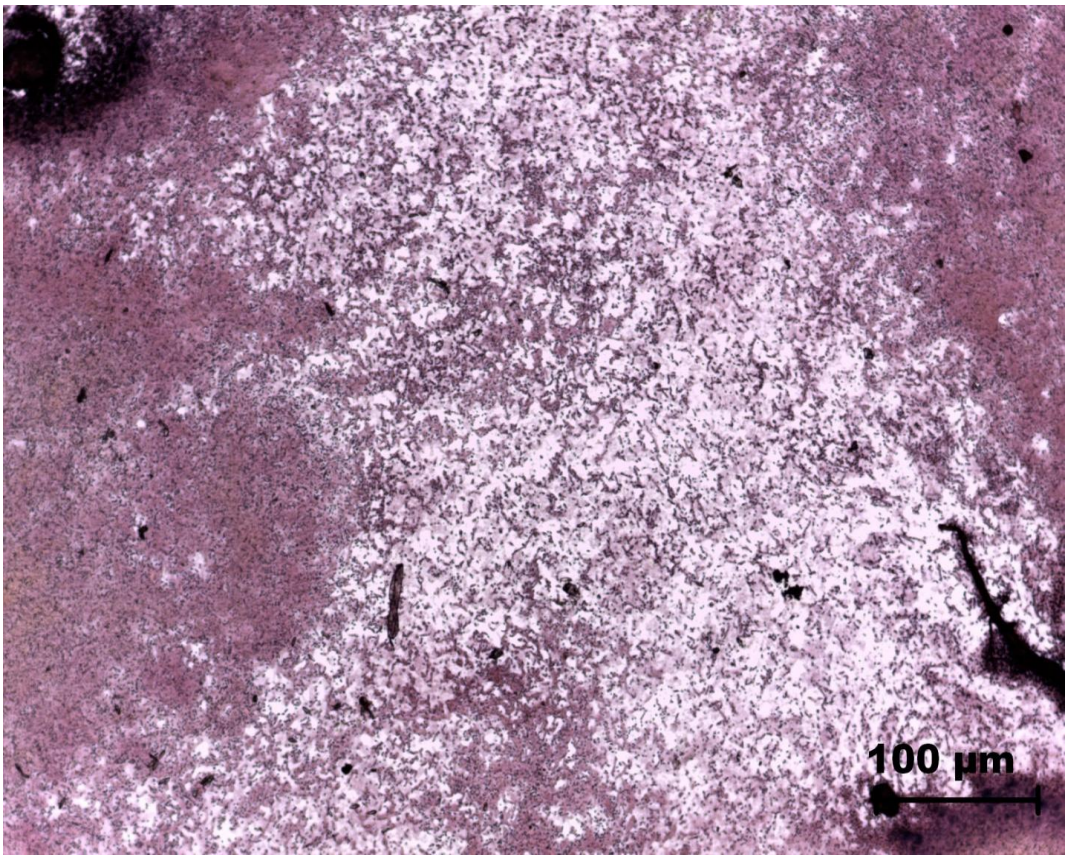

Fig. 4 Microscope image of *Thyobacillus ferrooxidans*

4.5 BIOLEACHING OF FLY ASH via *Thyobacillus ferrooxidans*

After growth of ***Thyobacillus ferrooxidans,*** leaching of fly ash was carried out as mentioned in chapter 3 materials and methods, using the inoculums of ***Thyobacillus ferrooxidans.*** Leaching was carried out for 7 days in a shaking incubator.

Fig 7 (a): Bio-leaching of SAIL-fly ash (from 11.02.2013-18.2013). On the 3rd day, a slight reddish color change was observed, indicating the presence of iron in it. On the 7th day, on opening the plug, of the conical flask, smell similar to decay of organic matter was observed, indicating the microorganism's interaction. Pictures shown below of fly ash samples inside the shaking incubator were taken on the 7th day.

Fig 7 (a): Bio-leaching of SAIL-fly ash

Fig 7(b): Bio-leaching of HINDALCO-fly ash (from 08.02.2013-17.02.2013). On the 5^{th} day, a slight reddish color change was observed, indicating the presence of iron in it. The reason for taking more time than SAIL–fly ash may be due to the difference in fly ash samples .also from the physical characterization of fly ash in TGA Analyzer, it was found that the fly ash granules of HINDALCO were coarser than SAIL-fly ash indicating. So more the surface area presented in case of SAIL-fly ash, better and faster is the bio-oxidation by ***Thyobacillus ferrooxidans.*** On the 9^{th} day, on opening the plug, of the conical flask, smell similar to decay of organic matter was observed, indicating the microorganism's interaction. Pictures shown below of fly ash samples inside the shaking incubator were taken on the 9^{th} day.

Fig 7 (b): Bio-leaching of HINDALCO-fly ash

4.6 MIXING OF FLY ASH AND SOIL TOGETHER FOR BIOFERTILIZER FORMULATION

After successful bioleaching and testing of leached water, the bio-leached fly ash samples were mixed with soil in different proportions and kept aside for 6-7 days. After 6-7 days, a slight color change was observed, the fly ash were darker in color in comparison to the un-leached fly ash**.** This gave a rough indication that microorganisms present both in soil and fly-ash samples have interacted**.**

4.7 CHECKING FOR PLANT GROWTH

After production of fly ash based biofertillizer, this was used for checking plant growth. For this purpose we planted marigold plant in three samples of different ratios 1:2, 1:2, and 1:1 respectively in kilograms along with the soil. The last sample taken was only soil. They were kept covered by a sack soaked in water for a period of 1-2 weeks allowing the microorganisms contained in both samples i.e. fly ash and soil to interact. After 2 weeks, we observed a slight

change in color and texture of the samples that is basically the fly ash mixed in soil turned darker in color. This indicated the interaction of microorganisms of soil and leached fly ash.

After this, the samples were transferred in pots, and marigold plants were planted to check the growth profile in each case.

Figure 8(a): **SAIL-Fly ash plant growth**. The marigold plant was planted on 8th April, and the growth was observed on the 5th day, i.e. on 13th April. By the 10th day, good growth was observed. Pictures taken below are of the 10th day i.e. 18.04.2013.

Figure 8(a): **SAIL-Fly ash plant growth**

Figure 8(b): **HINDALCO-Fly ash plant growth**. The marigold plant was planted on the same day as SAIL-Fly ash i.e. on 8th April. In comparison to SAIL-Fly ash, the growth was delayed by 2 days after the latter one i.e. on 7th April. Full plant growth was also observed late almost after 13days prior to planting. Picture taken below of HINDALCO-Fly ash is on 21st April.

Figure 8(b): **HINDALCO-Fly ash plant growth**

THREADING METHOD TO CHECK PLANT GROWTH

To check the plant growth for comparative study of both the samples, the plant growth was checked concerning two parameters: Height of plant and Thickness of plant. This was done by threading method.

In this method a thread was used to wind up the plant with the help of a thread and the range so covered by the thread was measured using a scale .the dada was so collected for both the samples on different dates and based on this portfolio, comparison was done.

Table 4: Analysis after plant growth shows the data so measured on different dates.

TABLE 4: Analysis after plant growth

SAMPLES	DATED	HEIGHT (in cm)	THICKNESS (in cm)
SAMPLE 1 (SAIL-Fly ash)	**08.04.2013**	-	-
	13.04.2013	2.5-	0.5
	18.04.2013	5.5	0.9
SAMPLE 2 (HINDALCO-Fly ash)	**08.04.2013**	-	-
	16.04.2013	1.7	0.3
	21.04.2013	4.8	0.6

From the data so noted above we observe a good and faster growth in SAIL-Fly ash than the HINDALCO-Fly ash. This concludes to our experimentation that, SAIL-Fly ash is better and is more efficient for plant growth than the other one, though HINDALCO-Fly ash can also serve the same purpose but with less productivity.

So, fly ash in general can be used in agricultural sector for crops though the growth pattern may vary depending on various factors like coal grade, fly ash quality, like mesh size, moisture content, and amount of trace elements etc. keeping in mind these factors, fly ash can be used for different crops, thereby utilizing this industrial waste, acting as a soil ameliorate in agricultural sector.

5. CONCLUSION

Physical, chemical and morphological characteristics of the fly ash from different industrial plants, gave us instincts relating to differences in fly ash belonging from the same coal family grade. Also results obtained from leached water tests gave us a substantial evidence of the authenticity of the lab work. Plant growth results proved that fly ash can be used as a biofertillizer after proper treatment via. bioleaching by the beneficiary effect of ***Thyobacillus ferrooxidans*** and its metal extracting nature and comparative study on this basis was also successful.

The research has opened doors for further work in the field of bio-leaching utilizing it to a vast expense for managing industrial wastes.

The aims of the discipline of Bio-leaching might someday completely tackle the three basic problems, shortage of land, pollution of the environment and diminishing quality of human health.

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