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DEMINERALIZATION OF COAL BY STEPWISE BIOLEACHING: A STUDY OF SUB-BITUMINOUS INDIAN COAL BY FTIR AND SEM

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ABSTRACT

The effect of some filamentous fungi such as Aspergillus niger and penicillium spp on the bio-liquefaction of low rank Indian coals, its chemical composition, surface characteristics of the products and the microbial mechanisms of coal conversion were studied. Virgin and bio-liquefied/solubilized coal samples were characterized using FT-IR, Scanning electron microscopy. All the micrographs were bright field and reveal several features correspond to the mineral grains comprising of aluminium, silicates and calcites. The absence of some morphological features corresponds to inorganic elements in residual samples which confirm demineralisation. The change in absorption of mineral matter functional group of these coal samples were studied using Fourier transform infra red spectroscopy (FT-IR). The CHNS analysis of the inoculated mixed culture showed that the carbon content was increased by 20 wt% and calorific value was increased by 26.5 %. With the fungal strain penicillium spp 18.98 % increase in calorific value was observed. When coal was treated with penicillium spp, the oxygen content was further decreased to 19.139 wt% from an initial value of 31.107 wt% and carbon content was increased to 71.56 wt% from the initial value 60.12 wt%. From the proximate analysis it was found that the ash content decreased gradually and was a maximum of 73 % when treated with mixed culture. Amongst the fungal strains used, inoculated acclimatized mixed culture of Aspergillus niger, Penicillium spp and Penicillium spp had caused significant removal of mineral and ash phases along with increase of calorific value.

Keywords: biodegradation, coal characterization, FTIR, SEM.

INTRODUCTION

Heavy metal pollution is one of the most important environmental problems today. Conventional methods for removing metal ions from aqueous solution are chemical precipitation, filtration, ion exchange, electrochemical treatment, membrane technologies, adsorption on activated carbon, evaporation, etc. Volesky summarized the advantages and disadvantages of those conventional metal removal technologies [1, 2].

In recent years, applying biotechnology in controlling and removing metal pollution has gained importance in the field of metal pollution control. Alternative process is biosorption, which utilizes various natural materials of biological origin, including bacteria, fungi, yeast, algae, etc. These biosobents possess metal-sequestering property and can be used to decrease the concentration of heavy metal ions from ppm to ppb level. It is an ideal candidate for the treatment of high volume and low concentration complex industrial waste [3].

Interaction between micro organisms and the mineral surface occur at two levels [4-6]. The first level is physical sorption due to electrostatic forces. Microbial cell envelopes are positively charged leading to electrostatic interactions with the mineral phase due to the low pH usually occurring in leaching environments. This electrostatic force reduces the cohesive/adhesive force of mineral matter with coal matrix and loosely bound the minerals. The second level is characterized by chemical sorption where chemical bonds between cells and minerals might be established [6].Bio-leaching of metals from fly ash using *Aspergillus niger* has been reported by Deenan et al. [7] and Khin Moh Aung et al. [8]. It was also reported Oguz Bayraktar that when *A. niger* was used there was a significant decrease in dehydrogenation reactions i.e., the hydrogen to methane molar ratio and the coke yield [9]. Acharya et al. studied the reaction mechanism of bioleaching of manganese ore with manganese reducing fungi *Penicillium citrium* and various acids such as oxalic acid, citric acid and sulphuric acid [10]. The microbial solubilization of some Indian bituminous coal was carried out by Sharma and Wadhwa also revealed the removal of minerals by mixed culture of bacterium [11].

In the present paper attention is given to study the ability of fungal stains like *Aspergillus niger* and *Penicillium spp* on the bio leaching of minerals from coal. Change in surface morphology, functional groups and calorific value was also investigated.

EXPERIMENTAL

Bio Solubilization

Sub bituminous coal samples were collected from the sample sites of Godavari Coal fields and brought to the laboratory. 1g each of the samples were dried and powdered as particular size and inoculated to 200 ml minimal medium (**M_3** salts (mM), K_2HPO_4 - 2, KH_2PO_4 - 3, NaOH - 3.3, $NaHCO_3$ - 1, CH_3COONH- 1, $CaCl_2$ - 0.2, $FeCl_2$- 0.1, $MgCl_2$ -0.4, trace elements (μM)- Na_2EDTA-13, H_3BO_3-1.8, $CoCl_2$- 0.7.$CuSO_4$- 0.6, $(NH_4)_6Mo_7O_{24}$- 0.08, $MnCl_2$- 2.6, $ZnSO_4$- 8, pH - 6.4) without addition of any carbon source. *Aspergillus niger* and *Penicillium spp* were inoculated individually and in combination with both cultures. Inoculums of the cultures were prepared in potato dextrose medium. Spores as well as the mycelia were transferred to the minimal media containing coal particles and incubated for 10 days at room temperature under shaken condition. The mixed cultures were further acclimatized with the same amount of coal for the same time duration. Further, the acclimatized co-cultures were tested for their efficiency by inoculating them with the same amount of coal and the same incubation time. After 10 days of incubation the cultures were decanted to obtain the residual coal particles. The decant was further centrifuged and separated in to culture and filtrate. Filtrates were analysed for its pH and metabolites. The residual coal samples were thoroughly washed with deionised water, dried, powdered and subject to SEM, CHNS, proximate and FTIR analysis. The culture filtrates were plated on Nutrient and Potato dextrose agar plates to establish their purity. In addition, it was found that at the end of the experiment all fungal cultures were alive. This confirms that absorption process is biological and does not lead to the death of the organisms. The experiment is repeated thrice under same condition to check the repeatability. Elemental analysis was carried out using VarioEL III CHNS analyser. Calorific value (CV) of all the coal samples was determined using an IKA C5000 calorimeter.

FTIR Analysis

For analysis, about 20 g of the sample was crushed in to fine powder of about 5μm in size. 2 mg of the powdered sample was then mixed with KBr in the ratio 1:200 and ground for about 10 minutes. The grinding time and the sample to KBr ratio were kept constant for all the samples under study to ensure uniformity. The spectrum was recorded using Shimadzu FTIR-8400 spectrometer in the region 3500-500 cm^{-1} with a resolution of 4 cm^{-1}. The FTIR spectra of virgin sample and leached samples are shown in Fig.1.

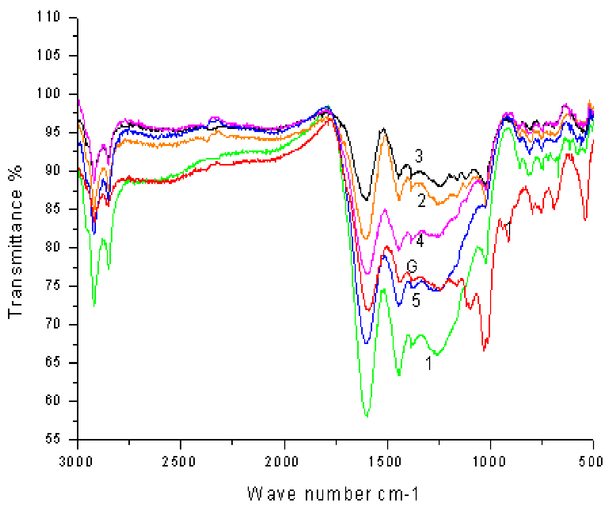

Fig. 1. FTIR spectra of virgin and bio leached samples. G-Virgin coal sample, 1- Coal sample inoculated with *Aspergillus niger*, 2- Coal sample inoculated with *Penicillium spp*, 3- Coal sample inoculated with mixed culture of *A. niger* and *Penicillium spp*, 4- Coal sample inoculated with 3 mixture, 5- Coal sample inoculated with acclimatized 4 mixed culture.

SEM Analysis

The SEM micrographs of the virgin and residual coal samples were obtained by Scanning Electron Microscope (SEM) model JSM 6390 from JEOL Company in Japan.

RESULTS AND DISCUSSION

Culture characteristics

Morphological analysis of the cultures, *Aspergillus niger* and *Penillium* spp exhibit no change in their morphology and other characteristics. The pH of the culture filtrates showed a significant variation than the control from an initial pH of 6.4 to final pH of 10.28. This variation must be due to the production of nitrogen compounds which lead to high alkalinisation of the medium. This result was in agreement with the findings of Jain and Sharma [12]. CHNS analysis of the samples also reported an increase of nitrogen content by about 103 % from initial value (Table 1).

CHNS & Proximate Analysis of of Biosolubilized Coal Sample

Table 1 shows C, H, N, S and O contents in raw coal sample and its biosolubilized products. When raw coal was treated with *aspergillus niger* alone, the carbon content increased from 60.12 to 72.05 wt % where as the hydrogen content was decreased from 6.840 to 2.980 wt %. Sulphur content did not change where as oxygen content decreased from 31.107 wt % to 21.50 wt %. The calorific value was also decreased. When coal was treated with *penicillium spp*, the oxygen content was further decreased to 19.139 wt % and carbon content to 71.56 wt %. Other elements did not show any change. When the sample was treated with mixed culture of *aspergillus niger* and *penicillium spp* (3 and 4), slight change in calorific value was observed. But when the coal was solubilized with inoculated acclimatized mixed cuture (5) carbon content was increased to 72.40 wt % accompanied by a reduction in oxygen content to 17.273 wt %. Further, the calorific value also increased by 26.5% than the raw sample. This significant increase of calorific value, suggesting that oxidation has happened to the coal organic structure during bio leaching. The alkalinization of the media was also observed from an initial pH of 6.4 to final pH of 10.28. The nitrogen content inncreased to 2.414 wt % from 1.469 wt %. This might be due to the production of ammonia by fungi and high alkalinisation of the medium. Leaching with *Penicillium spp* also showed an increase of calorific value of 18.98 % from the raw coal. Proximate analysis of the sample reported an increase fixed carbon content of about 51 % after leaching. Ash content

Table 1. Proximate and ultimate analysis of the virgin and leached sample.

Sample	G	1	2	3	4	5
Proximate analysis, wt %						
Fixed carbon, %	45.66	64.09	68.12	68.01	68.55	68.89
Volatile matter	36.90	20.82	24.36	24.36	24.61	24.46
Ash	12.87	11.39	3.86	3.95	3.34	3.26
Moisture	4.57	3.70	3.66	3.68	3.50	3.39
CHNS analysis, wt %						
C	60.12	72.05	71.56	71.22	71.50	72.40
H	6.84	2.98	6.23	4.96	5.02	7.16
N	1.47	2.98	2.49	2.66	2.64	2.41
S	0.46	0.49	0.59	0.55	0.57	0.76
O, % by diff.	31.11	21.50	19.14	20.62	20.27	17.27
Calorific value, MJ/kg	25.18	25.17	29.97	27.83	28.07	31.86

was decreased to 3.06 wt % from an initial value of 12.87 wt %. Percentage of volatile matter remains constant at 24 % for almost every sample except *aspergillus niger* leached sample. Moisture content showed marginal decrease from that of virgin sample.

FT-IR Spectra of the Raw Coal Sample and Bioliquefied /Solubilized product

FT-IR spectrum of biosolubilized coal samples are illustrated in Fig. 1 as spectrum G and 1-5. The peaks of solubilized products were compared with FTIR spectrum of the virgin coal sample to establish the solubilization. Sharp intense bands observed at 2920 and 2850 cm^{-1} in all the samples could be attributed to the presence of valency oscillations of-CH_3 and -CH_2 aliphatic groups. This might also arise due to the stretching vibration from –CHO group as reported by Yesim Basaran and Denizli [13]. Of these 2850 cm^{-1} band could be attributed to methoxy groups present in the samples. The sharpness of the bands in the studied samples indicates that the probable age of the coal as belonging to tertiary formations.The medium intensity band at 1460 cm^{-1} was due to the scissoring (bending) vibration of the CH_2 group next to the carbonyl group. Its first overtone was coupled with absorption due to aliphatic CH_3 vibration appearing at 2924 cm^{-1} as a strong band. Methylene groups often absorb more strongly when they are attached directly to a carbonyl group [14]. Sobkowiak et al. reported that sub bituminous coal samples had large absoptivities for different aliphatic –CH bonds, the highest being most likely for –CH bonds in long chain methylene structure [15]. In the present study, sample showed very strong absorption at 2920 cm^{-1} and 2850 cm^{-1} which was assigned for aliphatic –CH_3 and –CH_2 groups respectively and weak absorption bands in the region 930 -756 cm^{-1} was due to aromatic –CH out of plane structure. From this information we can conclude that the sample is moderately ranked sub bituminous coal. The bands at 2800-2920 cm^{-1} (C-H aliphatic stretching) and 1375-1465 cm^{-1} (C-H aliphatic bending) were more intense in Aspergillus treated sample (Spectrum 1).

Upon comparing the C=C band at 1600 cm^{-1}, in Fig.1, spectrum corresponds to *Aspergillus niger* (Spectrum 1) and *Aspegillus niger + Penecillium spp* (acclimatized- Spectrum 5) showed maximum intensity of absorption at 1600 cm^{-1} and least for spectrum 3. The aromatic C=C stretching vibrations (1635-1600 cm^{-1}) showed strong absorption. This indicated that product had more carbon content. This result was in confirmation with the CHNS analysis. The possible reason could be a reduction of oxygen content via transformation of C=O to CH_2 or possibly via decarboxylation of the matrix, which in turn would improve carbon and hydrogen content and hence calorific value. Up on comparing the spectrum, it is observed that all the samples showed a remarkable absorption near 1440 cm^{-1} with maximum absorption for spectrum 1, spectrum 5 and the least for spectrum 3. This indicated the strong presence of methylene and methyl groups in the sample. It was inferred that *Aspergillus niger* could enhance the methylene absorption than *Pencillium spp* and mixed culture. The bands at 1541 cm^{-1} and 1442 cm^{-1} was normally present in immature coals with more lignin content as reported by Andrew G et al in low rank Greek coals [20]. This was shifted from strong absorption to weak intensity in the spectra of solubilized product of spectrums of 3 and 2. This revealed the effectiveness of mixed culture and penicillium spp in solubilizing the lignin content. The band at 1375 cm^{-1} was due to absorption of methyl symmetric bending vibrations in tertiary butyl groups. This band was normally present in low rank coals. The intensity of this band decreased in the solubilized product.

On comparing the silicate band at 1010 cm^{-1}, 1030 cm^{-1} and 1090 cm^{-1}, spectrum 5 (Fig. 3) showed maximum removal of minerals with bio leaching. *Penicillium spp* and mixed culture were effective in solubilizing silicates minerals than *aspergillus niger*. The removal of silicates was due to the alkalinization of the medium as reported by Avakyan [21]. It was in conformation with the observed result of change in pH (Initial value of 6.4 to final of 10.28).

SEM analysis of the sample

The SEM micrographs of the virgin and variously leached samples were provided in Figs. 2-5. Fig. 2 represents the SEM image of the virgin coal sample. A bulk microstructure composed of homogeneously distributed network of small shining crystallites showed the presence of minerals. In the matrix, luminous as well as non luminous features could be seen. These fea-

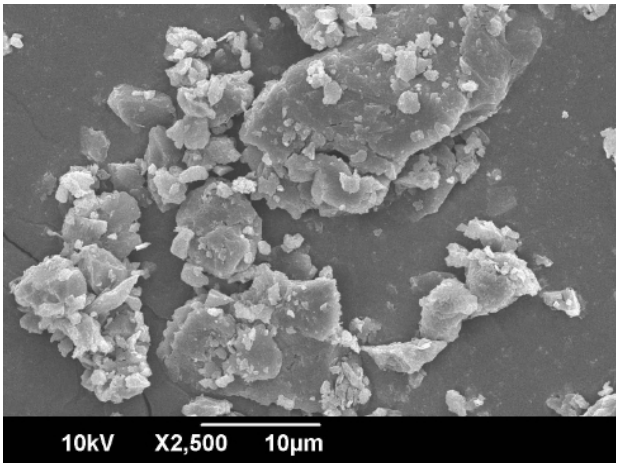

Fig. 2. SEM micrograph of virgin coal sample.

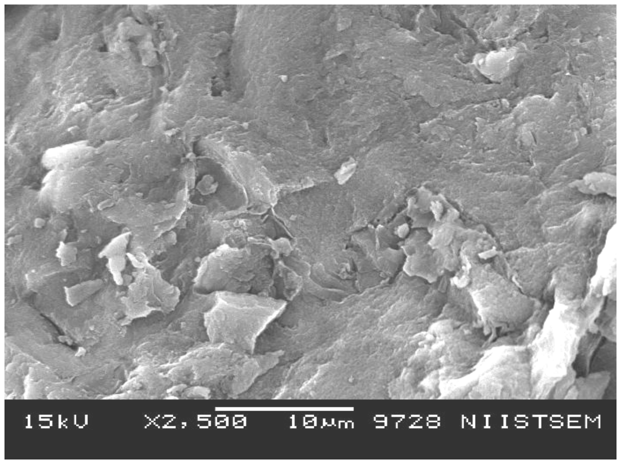

Fig. 4. Coal sample inoculated with *penicillium spp*.

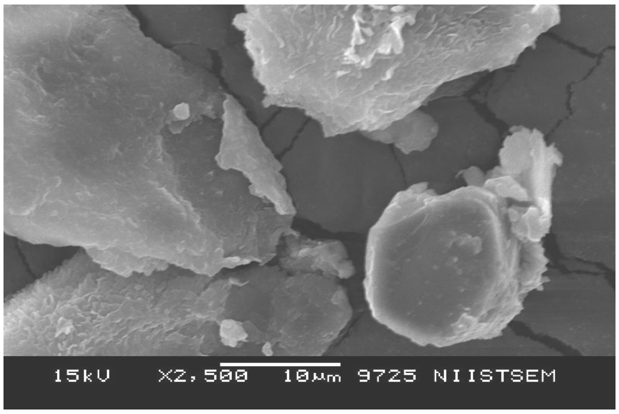

Fig. 3. Coal sample inoculated with *aspergillus niger*.

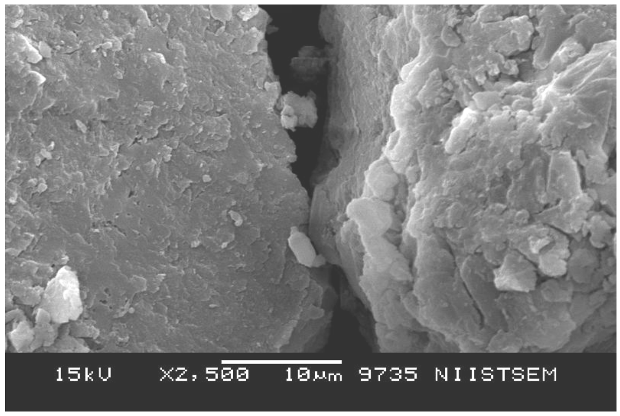

Fig. 5. SEM micrograph of inoculated acclimatized mixed culture.

tures indicate the presence of minerals distributed in the organic matrix. Many fissures, cleats, cracks and veins were also observed. The bright luminosity was due to the presence of aluminium,potassium or sodium. The dark luminosity was mainly due to the presence of chalcophiles [22-24]. Etiched pits, layers, some islands and hills & valleys could also be seen randomly distributed throught the micrograph.These might had resulted from the calcinations of dolomite and calcites or their assemblages due to thermal shock during metamorphism. It was inferred that the coal under study contain large proportins of silica, calcium carbonates and dolomite, as well as some proportions of elements such as aluminium, potassium and sulphur. It was reported by manoj et al in the case of sub bituminous coal [23].

To remove the minerals and enrich the coal in usable carbon, bio leaching was performed with *Aspergillus niger*. In the present investigation sub-rounded grains were observed in sample treated with *Aspergillus niger*. Some cracks were also observed in the surface (Fig. 3). The size of the sub-rounded sphere was about 10 μm. There was indication of change in mineral structure.

In the micrograph of *Penicillium spp* leached sample (Fig. 4), surface portrayed grinding, collision and precipitation due to weathering in the study area. The number of shining grains was decreased and surface looks dark. Some small pores were observed on the surface. The size of luminous region is about 5 μm and this size reduction of the luminous region confirmed demineralisation had taken place.

The sample was further inoculated with acclimatized mixed culture and the micrograph is shown in Fig. 5. Surface portrayed many cracks and concoidal fracture. This was due to change in mineral structure. The size of the shining object on the surface was reduced to about 2 μm. The examined surface consists of a number of small objects of 0.5 μm to few micrometers in size. There were small pores also observed on the surface. This might be due to the oxidation of the coal matrix.

CONCLUSIONS

On comparing the spectrum of samples with different leachants, it was inferred that coal undestudy had more lignin content which usually present in immature coals. The intensity of this band was least when treated with *Aspergillus niger* and mixed culture of *Aspergillus niger* and *Pencillium spp*. The fungal leaching was most effective in the region of aromatic, out of plane C-H bending with different degrees of substitution. The mineral band due to silicates decreased its intensity when treated with fungal culture. Inoculated mixed culture showed an increase of 26.5 % in calorific value and the carbon content increased by about 20 wt %. Oxygen content was decreased from an initial value of 31.107 wt % to a final value of 19.139 wt %. The nitrogen content was increased by 103 % during bio leaching along with high alkalinization of the media. On comparing the micrograph, it was found that the surface had undergone drastic change with bio leaching. Cracks and small pores due to oxidation had been observed on the surface of leached samples. The size of the mineral grains also decreased to less than 2 μm in dimension. On comparing the micrographs, it was found that mixed culture were better leachant for removing mineral phase. The proximate analysis showed a systematic decrease of ash content with fungal leaching. Ash content was decreased by about 73% from initial value when leached with acclimatized mixed culture of *aspergillus niger* and *penicillium spp*. Comparing FTIR spectrum, SEM micrograph, Proximate and Ultimate analysis, it could be concluded that *Penicillium spp* and Fungal culture (Mixture of *Penicillium spp* and *Aspergillus niger*) caused significant removal of ash and mineral matter while improving the calorific value.

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