

Investigation on Biosolubilization of Coal by *Aspergillus Niger*

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Abstract: The effect of demineralization on Indian bituminous coal by filamentous fungus *Aspergillus niger* has been investigated. X-ray diffraction profile reveals the presence of inorganic components in the sample. Biosolubilised products were characterized by FT-IR, SEM-EDS and XRD. The study reveals that coal consist of a semi-crystalline turbostratic structure. The broad diffraction peak at 20~25.5° attributed to the crystalline carbon in the sample. Leaching process removed. Silicate, pyrite minerals and aluminates were removed considerably. The absence of morphological features corresponding to inorganic mineral grains confirms demineralization. The FTIR spectrum reveals the presence of aliphatic and aromatic stretching bands. During bio-solubilization the fungus produced acids like gluconic acid, oxalic acid and citric acid along with oxalates which are responsible for the demineralization in coal by the formation of mineral salts. EDS analysis reveals high percentage of carbon in bituminous coal than high volatile bituminous coal. The peak at 230-270 nm confirms the benzene-oxygen charge transfer in the sample.

Keywords: Biodegradation, Coal characterization, Fungi, *Aspergillus niger*

Introduction

Worldwide requirement for energy continues to increase due to the economic growth and industrialization of the developing nations. Fossil fuels contribute most of this energy demand. It is also imperative that the natural resources should be utilized most efficiently without polluting the environment. Among the natural resources, coal is the most important non-renewable energy source of fossil origin. Mineral matter and sulphur in coal place enormous limitations on its use. Conventional methods, to remove the mineral matters like precipitation, filtration, ion exchange, adsorption, electrochemical treatment, etc., are unproductive when the additive concentration is very low in nature and the by-products of any of these processes lead to another set of environmental pollutants.

In the present investigation attention is given to study the ability of fungal stains, *A. niger* in solubilizing minerals from coal. Change in surface morphology, functional groups and structure are investigated. The possible mechanism of demineralization with organic acid secreted by fungi is also discussed.

Experimental

Two coal samples (High volatile bituminous coal (K and B)) were crushed into small pieces approximately of 1 to 1.5 cms. Ten grams each of the crushed samples was added in 200 mL modified Czepek Dox medium with low sucrose (1%). Inoculum of the cultures were prepared in potato dextrose medium. Mycelial biomass along with the spores were washed using a buffer to eliminate the medium completely and inoculated to the modified Czepek Dox medium containing coal particles (in triplicate) and incubated for 10 days at room temperature under stationary condition. Culture filtrate, 1 mL each was drawn from the cultures at regular intervals for 10 days to check the pH and other components. After 10 days of incubation the cultures were decanted to obtain the residual coal particles. Decant was further centrifuged and separated into culture and filtrate and subjected to various analysis along with regular samples. The residual coal samples were thoroughly washed with deionised water, dried, powdered and subjected to SEM-EDS and proximate analysis.

The observation and quantification of minerals in bio-leached coals were carried out using a scanning electron microscope with an energy dispersive x-ray spectrum analyser (SEM-EDS) JEOL model JSM-6390 LV. The energy dispersive spectrum (EDS) was obtained by JEOL model JED-2300 ED spectrometer. The organic acids produced by *A. niger* and *Penicillium* spp. were determined by high performance liquid chromatography (HPLC) with UV detector at 210 nm for the organic acids. An Agilent Technologies 1200 series high performance liquid chromatograph with C18 column was used. The XRD data collection was performed by Bruker AXS D8 advance x-ray powder diffractometer. FTIR spectrum was recorded by using Shimadzu FTIR-8400 in the region 4000-400 cm^{-1} . UV-Vis-NIR spectrum was recorded using carry 500 spectrometer equipped with a praying Mantis diffuse reflectance accessory DRA.

Results and Discussion

Characterization of organic acids in culture

The organic acids produced by *A. niger* was determined by high performance liquid chromatography (HPLC). Citric acid, oxalic acid and gluconic acid were mainly produced along with a variety of organic acids by the fungus *A. niger* using glucose as energy source. Decrease in pH of media was observed due to the organic acid production via incomplete oxidation of glucose by fungi. Unlike citric acid production where glucose was taken up by the organism, converted to citric acid and exported, gluconic acid was produced extracellularly. Glucose in the medium was oxidized in a two step reaction to gluconic acid through the action of glucose oxidase.

Characterization of the demineralized coal

Interaction between micro organisms and the mineral surface take place at two levels. The first level is physical sorption due to electrostatic forces. Microbial cell walls are positively charged leading to electrostatic interactions with the mineral phase due to the low pH usually occurring in leaching environments. This interaction reduces the cohesive/adhesive force of mineral matter with coal matrix and loosely binds the minerals.

The second level is chemical sorption where chemical bonds between cells and minerals might be established. In addition, extra cellular metabolites are formed and secreted during this phase in the near vicinity of the attachment. Low molecular weight metabolites produced by mineral oxidizers include, acids originating from the TCA cycle, amino acids, or ethanol-amine; whereas compounds with relatively high molecular weights include lipids and phospho lipids¹⁻³. In the presence of minerals, mineral oxidising micro organisms from the treated sample form a filamentous matrix similar to a bacterial glycocalyx suggesting the relative importance of this extra cellular silicate oxidising fungi for mineral extraction.

Spectroscopic properties

FT-IR spectrum of biosolubilized coal samples are illustrated in Figures 1 & 2. The spectra had general broad characteristics with sharp intense bands observed at 2920 and 2850 cm^{-1} in all the samples and it 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 Basaran *et al.*,¹. Of these, 2850 cm^{-1} band could be attributed to methoxy groups present in the samples. The sharpness of the bands in the samples indicated the probable age of the coal as it belongs to tertiary formations. In very low rank coals the intensity of 2920 cm^{-1} band was found to be very small due to the fact that the proportion of the aliphatic -CH_3 hydrogen being substituted by hydroxyl groups. The low intensity band at 1460 cm^{-1} was due to the scissoring (bending) vibration of the CH_2 group next to the carbonyl group⁴⁻⁶. Its first overtone was coupled with absorption due to aliphatic CH_3 vibration appearing at 2924 cm^{-1} as a weak band. It has been reported that sub-bituminous coal samples had large absorptivities for different aliphatic -CH bonds, the highest being most likely for -CH bonds in long chain methylene structure⁴⁻⁶. The findings also indicated that the absorptivities for aromatic -CH bonds were very small. The 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 cm^{-1} & 756 cm^{-1} due to aromatic -CH out of plane structure. The aromatic C=C stretching vibrations (1635-1600 cm^{-1}) showed strong absorption. This indicated that product had more carbon content. The reason could be a reduction of oxygen content via transformation of C=O to CH_2 or possibly due to decarboxylation of the matrix, which in turn would improve carbon and hydrogen content and hence calorific value. The bands at 1541 cm^{-1} and 1442 cm^{-1} were normally present in immature coals with more lignin content as reported by Manoj *et al.*, in low rank sub-bituminous coal⁷.

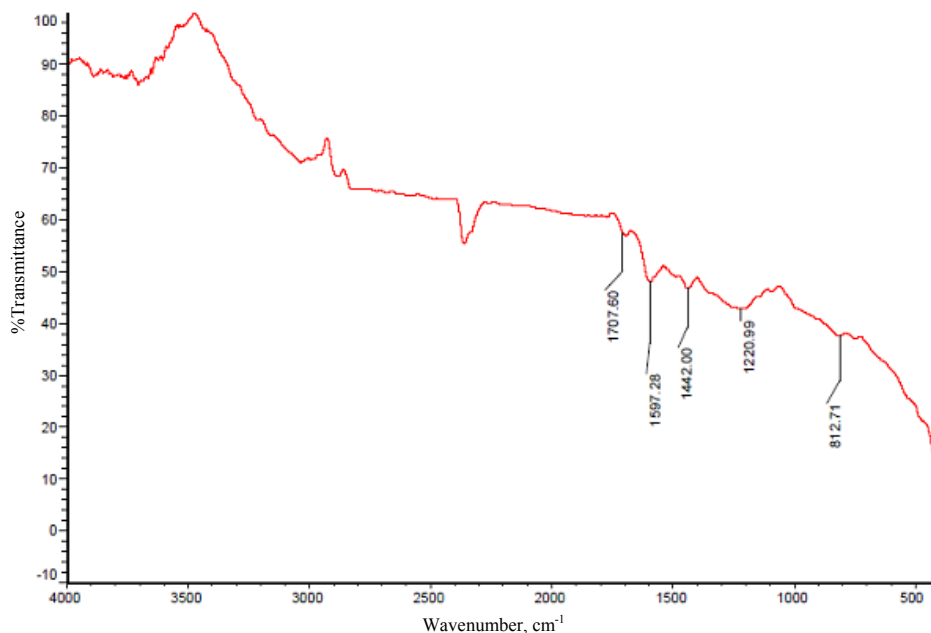


Figure 1. FT-IR spectra of bituminous coal (Kn)

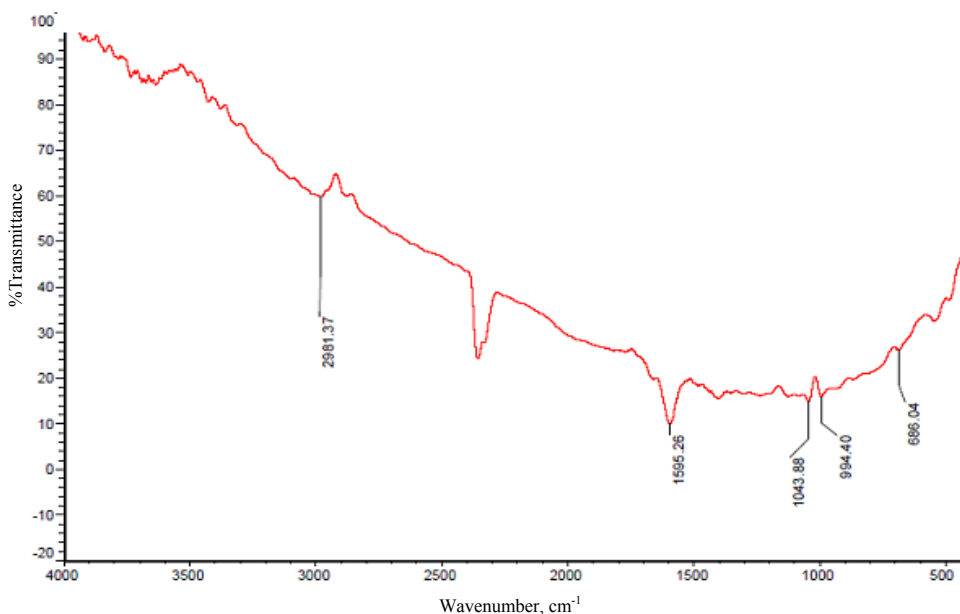


Figure 2. FT-IR spectra of high volatile bituminous coal (Bn)

The band at 1375 cm^{-1} can be due to absorption of methyl symmetric bending vibrations in tertiary butyl groups which are normally present in low rank coals. The intensity of this band decreased in the solubilized product. The FT-IR spectra of biosolubilized coal sample (Figure 1), upon comparing, were found that the hydroxyl stretching vibrations ($1410\text{-}1310\text{ cm}^{-1}$) decreased its intensity⁷.

SEM analysis of the sample

The SEM micrographs of the virgin and various leached samples are depicted in Figures 3 & 4. A bulk microstructure composed of homogeneously distributed network of small shining crystallites show the presence of minerals⁸. The bright luminosity was due to the presence of aluminium, potassium or sodium. The dark luminosity was mainly due to the presence of chalcophiles⁸. Etched pits, layers, some islands and hills & valleys could also be seen randomly distributed throughout the micrograph. These might have resulted from the calcinations of dolomite and calcites or their assemblages due to thermal shock during metamorphism.

The coal samples that subjected to bio-leaching with *A.niger* exhibited sub-rounded grains on the micrograph. Some cracks were also observed on the surface (Figure 3). The size of the sub-rounded sphere was about $10\text{ }\mu\text{m}$. This is an indication of change in mineral structure.

Figure 3 SEM Image of bituminous coal sample inoculated with *A. niger* (Kn). In the micrograph of leached sample (Figure 4), the surface portrayed grinding, collision and precipitation due to weathering. The number of shining grains decreased which led to the dark surface in addition to the small pores. The size of luminous region measured about $5\text{ }\mu\text{m}$ and this size reduction of the luminous region confirmed the process of demineralisation.

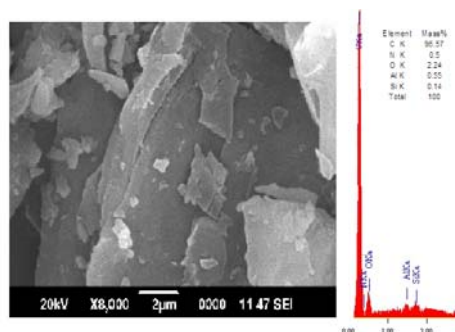


Figure 3. SEM image of bituminous coal sample inoculated with *A. niger* (Kn)

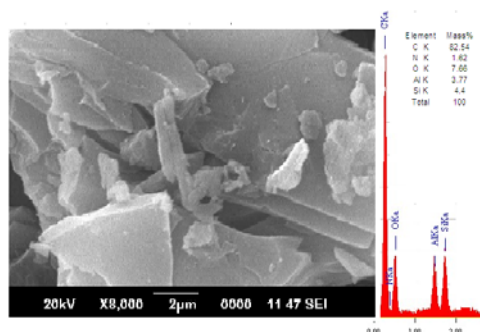


Figure 4. SEM image of high volatile bituminous coal sample inoculated with *Aspergillus niger* (Bn)

X-ray diffraction analysis

The XRD spectrum with bioleached sample is presented in Figure 5. The profile exhibited strong diffraction peaks with various diffraction intensities suggesting that Indian coal contains lots of crystalline material. The 2θ peaks at ~ 12.5 , 20.5 and 33.3° were assigned to the mineral kaolinite. The diffraction peaks at 26.6° which was assigned to the quartz diffraction, reveals that the coal sample had a significant amount of quartz. The weak diffraction peaks at 29.3° provide an evidence of the presence of dolomite in the coal sample. The sharp peaks were formed due to the inorganic components such as kaolinite, pyrite and quartz, whereas the strong diffraction peak at $20\sim 25^\circ$ was due to the crystalline carbon in the sample. The broad diffraction peaks were assigned to the high content of the minerals overlapping with weak amorphous coal⁹⁻¹¹.

The existence of crystallites in coal structure is confirmed by the appearance of the peak corresponding to 002 and 110 reflections in XRD spectrum of coal¹¹. These observations suggest that the crystallites in all coal samples had intermediate structures, the so called turbostratic structure or random layer lattice structure between graphite and amorphous state⁹⁻¹⁰. The ratio of γ - band to Π - band (or I20/I26) is a measure of disorder in the amorphous carbon^{9,12}. When the ratio is low, better is the ordering of carbon layers in the coal sample as it approaches the graphitic like carbon.

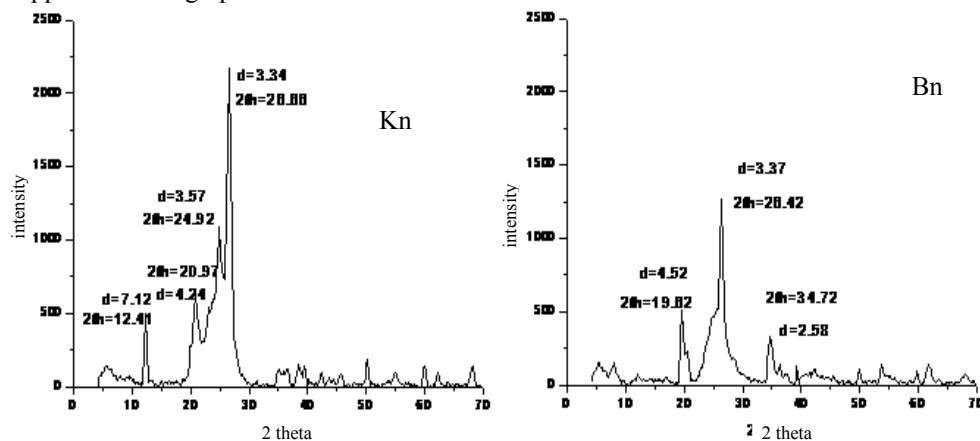


Figure 5. X-ray diffraction profile of bioleached samples

UV Vis-spectroscopy analysis of demineralized coal

The electronic absorption spectra of bituminous coal and high volatile bituminous coal were measured by the diffuse reflectance spectrometry in the UV-Visible and near IR regions (Figure 6 and Figure 7). The general shape of the spectrum was characteristic for hydrocarbons with a single benzene ring. The two principal bands that were characteristic for the naphthalene systems (220 & 280 nm) were masked by the absorption regions of monoaromatic rings; which indicated that naphthalenoid hydrocarbon was very low¹³. The absorption of benzene-oxygen (1/1) system was found to be lying between 240-270 nm. This was due to the benzene –oxygen charge transfer band which showed a red shift with HF leaching. The sample was transparent in the region 475 nm to 650 nm and near IR region. There were small distinct absorption peaks in the UV region and 680 nm. The intensity of the broad absorption in the visible (680 nm) and near IR region (759 nm) were attributed to the $\Pi-\Pi^*$ electronic transitions of the poly nuclear aromatic hydrocarbons. Intensity of this band increased with chemical leaching due to increase in electronic transitions.

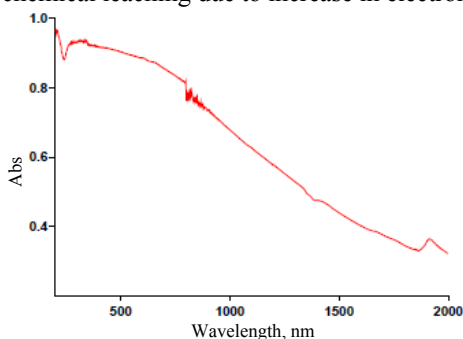


Figure 6. UV Vis-NIR spectrum of Bn sample

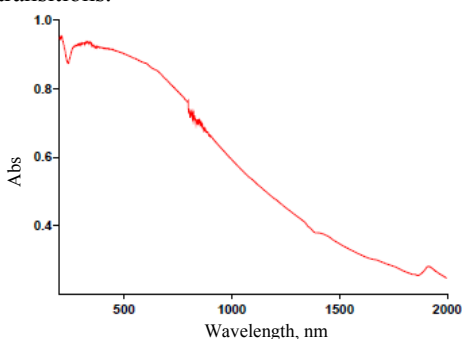


Figure 7. UV Vis-NIR spectrum of Kn sample

Conclusion

On comparing the FT-IR spectrum of both samples, it was found that, fungal leaching is most effective in the aliphatic and aromatic region. Vibrations of “aromatics” structure of graphite-crystalline or non-crystalline were responsible for the absorption at 1600 cm^{-1} region. The high background intensity of the diffraction profile reveals amorphous carbon embedded in coal structure. HPLC analysis confirms the secretion of oxalic acid, gluconic acid and citric acid. SEM-EDS analysis confirmed removal of Silicates, Aluminates, Sulphur and Calcites from the sample. FTIR spectra reveal that coal contains aliphatic $-\text{CH}_2$ and $-\text{CH}_3$, aromatic $\text{C}=\text{C}$ and $-\text{CH}$ bonds in their macromolecular network.

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