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POTENTIALS OF COAL INHABITATING MICROORGANISMS TO SOLUBILIZE FOSSIL COAL FOR THEIR CARBON REQUIREMENTS

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ABSTRACT

Coal is prime source of energy for domestic as well as for industrial use and has grat contribution in Indian economy. Chhattisgarh is one of the important states of India in terms of fissile coal reserve. Bacterial and fungal isolates from coal yard in Chhattisgarh coal mines area. They were screened for their ability to solubilize coal in vitro. The isolates were also tested for coal utilization for their carbon need. The fungal isolates exhibited significantly high rate coal solubilization. The bacterial isolate showed least solubilization of fossil coal.

Keywords: Coal solubilization, carbon, fossil fuel, bioremediation

INTRODUCTION

Coal is recognized as an important source of energy in India and the total estimated reserve of all types of coal include in lignite are 287 billion tones, which will last for more than 200 years and will full fill the power demand of India. Due to the organic origin of coal, it is susceptible to the transformation by several bacteria and fungi (Laborda *et al.*, 1997). Microbial degradation of coal has been considered as an economic and effective way of transforming macromolecules into simpler, low molecular weight products (Machnikowska *et al*, 2002). Low rank coals are a cheap carbon source and are a suitable model substrate for the bio depolymerization of macromolecules (Fuchtenbusch and Steinbuchel 1999, Dong *et al*, 2006) reported that the development of biological processes for fossil energy utilization has received increasing attention in recent years. Several fungi of the *Deuteromycetes* and *Basidiomycete*s are able to solubilize low-rank coal into chemically heterogeneous and complex liquefaction products like humic and fulvic acids (Cohen and Gabriele 1982, Willmann and Fakussaa 1997).

The ability of microorganisms to grow on low-rank coal (lignite) and to modify its physico-chemical properties was first reported in 1920. Several researchers have studied the biodepolymerization of low rank coal by different organisms like filamentous fungi (Faison 1992) *Streptomycetes* (Gupta *et al*., 1990) and *Basidiomycetes*, (Cohen and Gabriele 1982).

Bacterial and fungal coal solubilization will only occur in the presence of an enriched nutrient medium containing high nitrogen concentrations in the form of glutamate or ammonia (Hofrichter *et al*., 1997). While the presence of an additional carbon source is important for the growth of fungi, it was shown to cause inhibition of coal solubilization in studies by Holker and Hofter 2002).

Some microorganism secret biosurfactants for coal solubilization Biosurfactants can be used in coal dust control, enhancement of gas permeability of coal, removal of coal ash and bioremediation of soil and groundwater contaminated with hydrocarbon. Microorganisms producing biosurfactant can participate in oil degradation (Tripathi *et al*, 2009).

Several researchers have reported the appearance of 'black viscous liquid substances as breakdown products of microbial coal degradation and/ or modification is already reported (Ralph and Catcheside, 1994). To a large extent, this has influenced coal bioconversion research with the aim of recovering intermediate products such as humic acids, volatile fatty acids and low molecular weight specialized compounds such as methanol (Silva-Stenico *et al*., 2007) of particular interest, is the generation of Humic substances from coal, because they constitute a major component of the coal macrostructure and secondly they play an important role in agriculture.

A large number of fungi (Steffen *et al*, 2002), bacteria (Yuan *et al*, 2006) and actinomyctes (Quigley *et al*, 1989) have been identified for being capable of coal degradation/ solubilization.

Coal biodegradation is thought to involve a complex array of constituents and processes involving hydrolytic enzymes oxidative enzymes, acidic/alkaline substances, chelating agents, and surfactants. It is generally believed that oxidative enzymes are the primary factors in coal de polymerization. However, the exact pathway involved in coal degradation is dependent both on the strain used and the type of coal (Fakoussa *et al*, 1999)

Coal can serve as cheapest carbon source for microbial bio processing for production of some value added product from coal and some researchers also studied to produce liquid fuel from coal. Coals also serve as substrate for methanogenesis. Coal solubulizing microorganism can use in bioremediation of coal contaminated area around coal mines. The present study aimed to isolate microorganisms inhabitation coal contaminated field and water bodies and screen them to mobilize coal and produce some commercially important product from coal and clean coal contaminated field around coal mines.

MATERIALS AND METHODS

Sample collection

Coal sample was collected from Gataura, Bilaspur and water sample was collected from Balrampur coal mines of Chhattisgarh.

Isolation of microorganism

One gram of coal contaminated soil sample and 1 ml of water sample was added in a test tubes containing 9 ml of sterile distilled water.

Tubes were serially diluted and 10^{-4} dilution from sample was spread on LBA plates. On another set of plates containing BHM with coal as sole carbon source, 10^{-1}dilution of sample was spread. The plates were incubated at 37 °C for 48 hours and the cultures obtained were maintained on LBA media.

Screening for coal solublization activity

Coal solubilization ability of the isolates was performed in Yeast extract mannitol broth. Flasks containing YEM with and without coal (1% w/v) were inoculated with equal number of overnight grown culture and incubated at 37°C on shaking at 200 rpm for 7 days. Culture from each flask was centrifuged at 10,000 rpm for 5 min to pellet the cells and the cell free culture supernatant was used for spectrophotometric detection of coal solubilization by taking spectrum against control between range of 200 nm to 800nm, YEM without coal and YEM with coal and without inoculums was use as control (Willmann and Fakoussa, 1997).

Coal solubilization ability of the isolate was also detected in complete nutrient media. Flasks containing media with and without coal (1% w/v) were inoculated with equal number of overnight-grown culture and incubated at 37°C. The broth centrifuged and the cell free supernatant was used for spectrophotometric detection of coal solubilization by taking spectrum agains control between range of 200nm to 800nm. (Laborda *et al*., 1997).

Coal solublization study in cell free extract

Coal solubilization in cell free extract was tested as follows. Five ml of culture supernatant and 20mg of coal powder were kept on a laboratory shaker for 1 hour at room temperature. Then it was centrifuged for 20 min at 10,000 rpm to separate insoluble coal material. Spectrum of the supernatant was taken as compare to the control (Yuan *et al*., 2006).

Morphological characterization of fungal strain: The morphology of Fungal isolates were examined under microscope by staining with lectophenol and cotton blue dye.

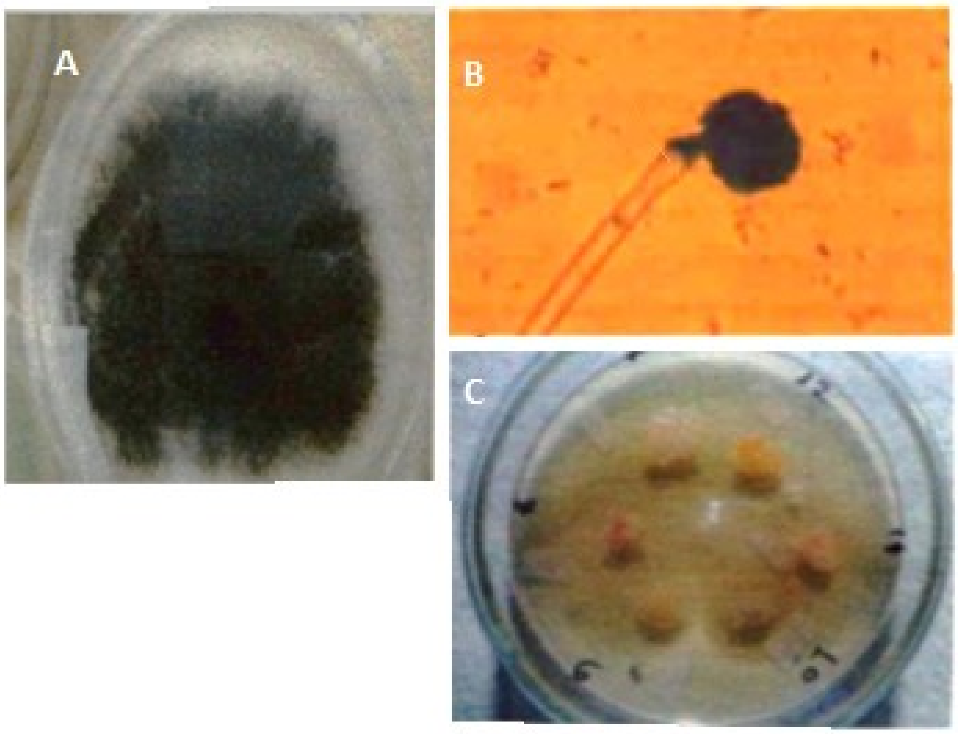

Figure 1: Plates of isolates (A) Fungal culture maintained in nutrient plate (B) Microscopic picture of one of the fungal isolate (C) Bacterial isolates maintained in nutrient plate

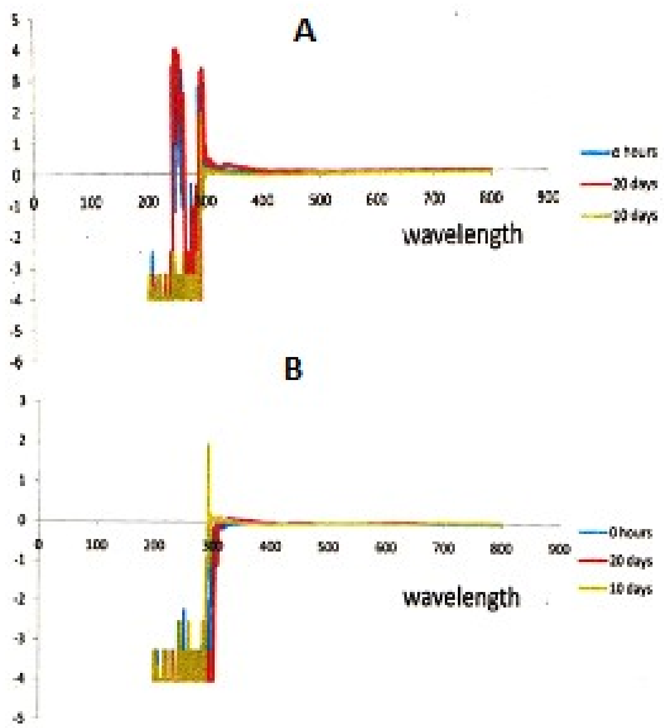

Figure 2: Spectrum analysis with Bacterial Isolate no 1 (A) Without inoculum (B) With inoculum

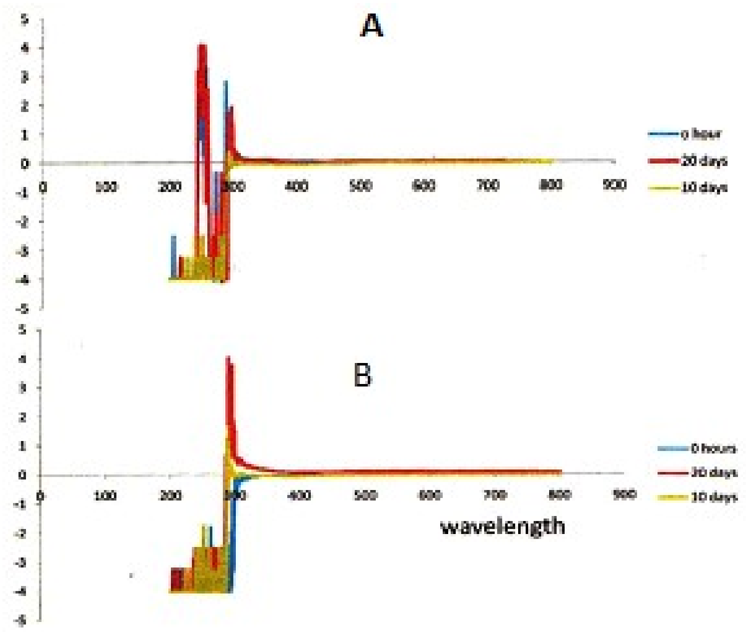

Figure 3: Spectrum analysis with Bacterial Isolate no 2 (A) Without inoculum (B) With inoculum

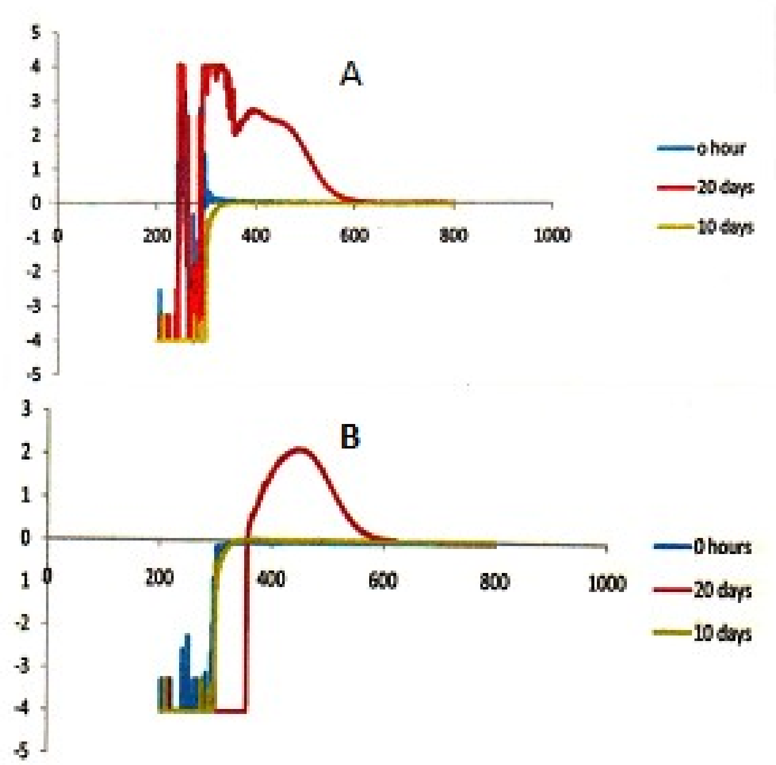

Figure 4: Spectrum analysis with fungal Isolate (A) Without inoculum (B) With inoculum

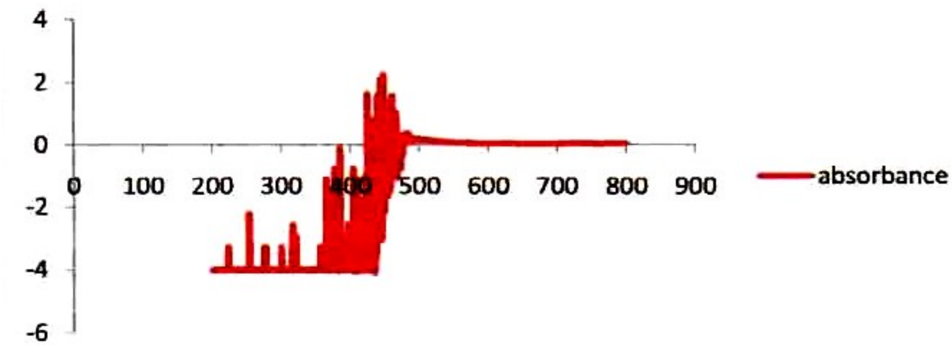

Figure 5: Spectrum analysis of coal solubilization by cell free extract

DISCUSSION

Coal is not inert by microbial attack some microorganism can used coal as sole carbon source (Fakoussa, 1999) Microbial degradation of coal has been considered as an economic and effective way of transforming macromolecules into simpler, low molecular weight products (Machnikowska *et al*., 2002). A large number of fungi (Steffen *et al*., 2002), bacteria (Yuan *et al*., 2006) and actinomyctes (Quigley *et al.,* 1989) have been identified for being capable of coal degradation/solubilisation. This microorganism can solubilize coal in medium and color change of medium was occur and cell free culture supernatant of media show absorbance at 450 nm in spectrophotometer. Some microorganism requires organic nitrogen source such as peptone for coal solublization and some microorganism also require additional carbon sources. solublization of hard coal by bacteria is rarely obtain.

In the study coal sample from Gataura and water sample from Balrampur coal mines of Chhattisgarh and inoculated this sample on NAM and BHM containing coal media (for the enrichment isolation) respectively (Figure 1.). Eighteen different bacterial isolate were isolated from coal sample collected from Gataura and this 18th isolate were not able to solublize coal in YEM. Previously, Tripathi *et al*., 2009 isolate bacteria from Indian coal bed of Jharia and ability of isolate to solublise coal was detected in YEM broth and isolate solublise coal by secreting biosurfactants that lower the surface tension of coal and solublize in medium. Only fungal isolate isolated from water sample collected from Balrampur coal mines was able to solublize coal and in spectrum analysis show maximum absorbance at 447 nm that value is very near the standard value that was describe by Tripathi *et al*., 2009. Bacterial isolates isolated from water sample collected from Balrampur coal mine do not show coal solubilization in complete medium with organic nitrogen source, Hofrichter *et al*., 1997 reported that bacterial and fungal coal solubilization will only occur in the presence of an enriched nutrient medium containing high nitrogen concentrations in the form of glutamate or ammonia. Bacterial isolate no.1 isolated from water sample collected from Balrampur coal mines (Figure 2.), show maximum absorbance at 290 nm after 10 day of incubation and bacterial isolate no.2 show maximum absorbance at 323 nm after 20 days of incubation this is may be due to bacterial strain secret some component in response to coal (Figure 3). Coal may be contain some compound that toxic to bacteria so bacterial strain secret some component in response to coal for their survival. Bacterial community of Gataura and Balrampur coal field may be not able to solublize coal in comparison to fungal isolate that is characterized as *Aspegillus* species and was able to solublize coal and give absorbance at 447 nm that is near the standard value 450 nm (Fugure 4). Cell free culture supernatant of fungal strain also show coal solublization and after 1 hour of incubation with coal powder show maximum absorbance at 448 nm that is near the standard value 450 nm (Figure 5.). Bacterial strain do not show good result than fungul isolate because fungal species have more ablility to degrade lignolitic waste. In comparison to bacteria fungul species also have more ablility to break polyphenolic compound in comparison to bacteria so fungal isolate show good result than bacteria in microbial coal solublization. Yuan *et al*., 2006 reported that cell free culture supernatant of fungus *Penicillium decumbens* strain P6 also show coal solubilization activity.

CONCLUSION

The result presented show that isolated fungus can solubilize coal in complete media whereas bacterial samples were not able to solubilize coal. It's because fungus is more evolved to degrade polyphenolic compound such as lignolytic waste of plant origin and bacteria with coal solublizing activity is difficult to isolate. Due to structural similarity in plant lignin and coal fungus is able to degrade coal in complete media. May be hard coal is not easily degradable by bacteria than as compared to fungi. Fungus requires complete media for their growth and activity because coal has complex organic material that is not easily degraded by microorganism.

In future coal solubilizing microorganism can used in bioremediation of coal contaminated land around coal field. Coal solubilizing microorganism also enhance methenogenesis in coal. It serve as cheapest raw material for any microorganism for production of some value added product from coal.

REFERENCE

[1] Cohen, M. S. and P. D. Gabriele (1982) Degradation of coal by the fungi polyporous versicol or and poria placenta. *Appl. Environ. Microbiol*. 44: 23-27.

[2] Dong L.H, Yuan Quan, Yuan Hong Li (2006), Changes of chemical properties of humic acids from crude and fungal transformed lignite. *Fuel* 85:2402-2407

[3] Faison, B. D. (1992) In Biotransformation of Low Rank Coals. CRC Press, Boca Raton, FL. pp. 1-26

[4] Fakoussa, R. M. and P. J. Frost (1999) In vivo -decolorization of coal derived humic acids by laccase-excreting fungus Trametes versicolor. *Appl. Microbiol. Biotechnol*. 52: 60-65.

[5] Fuchtenbusch A. and Steinbuchel A. (1999) Biosynthesis of polyhydroxy-alkanoates from low-rank coal liquefaction products by *Pseudomonas oleovorans* and *Rhodococcus ruber*. *Appl. Microbiol. Biotechnol*. 52: 91-95.

[6] Gupta R. K, Deobald L. A, Crawford DL (1990), Depolymerization of lignite coal by Pseudomonas strain 07. *Appl Biochenz Biotechnol* 24/25:899-907.

[7] Hofrichter M, Fritche W (1997), Depolymerization of low rank coal by extracellular fungal enzyme systems. II. The ligninolitic enzymes of the coal-humic-acid-degrading fungus *Nematoloma frowardii* b19. *Appl. Microbiol Biotechnol* 47:419-424.

[8] Holker, U. and M. Hofer (2002) Solid substrate fermentation of lignite by the coal solubilizing mould, *richoderma atroviride*, in a new type of bioreactor. *Biotechnol. Lett.* 2: 1643-1645.

[9] Laborda F, Ferna'ndez M, Luna N and Monistrol I. F, (1997). Study of the mechanisms by which microoganisms solubilize and/or liquify spanish coal. *Fuel Processing Technology*. 52:95-107.

[10] Machnikowska H, Pawelec K, Podgo'rska A (2002), Microbial degradation of low rank coals. *Fuel Process Technol* 77: 17-2

[11] Quigley, D. R., B. Ward, D. L. Grawfordd, H. J. Hatcher, and P. R. Dugar (1989) Evidence that microbially produced alkaline materials are involved in coal biosolubilization. *Appl. Biochem. Biotechnol*. 20/21: 753-763.

[12] Ralph, J. P. and D. E. A. Catcheside (1994) Decolourisation and depolymerisation of solubilized low-rank coal by the white rot basidiomycete *Phanerochaete chrysosporium*. *Appl. Microbiol. Biotechnol*. 42: 536-542.

[13] Silva-Stenico ME, Vengada jellum CJ, Janjua HA, Harrison STL,Burton SG, Cowan DA (2007), Degradation of low rank coal by *Trichoderma atroviride* ES11. *J Ind Microbiol Biotechno.* 134:625-631.

[14] Steffen, K. T., A. Hatakka, and M. Hofrichter (2002) Degradation of humic acids by the litter-decomposing basidiomycete *Collybiadryophila Appl. Environ. Microbiol*. 68: 3442-3448.

[15] Tripathi, R. C., Jain, V. K. and Tripathi, P. S. M. (2009) Fungal Biosolubilization of Neyveli Lignite into Humic Acid Energy Sources, *Part A: Recovery, Utilization, and Environmental Effects*. 32(1):72-82.

[16] Willmann, G. and R. M. Fakussaa (1997) Biological bleaching of water soluble macromolecules by a basidiomycete strain. *Appl. Microbiol. Biotechnol.* 47: 95-101

[17] Yuan H-L, Yang JS, Wang FQ, Chen WX (2006), Degradation and solubilization of Chinese lignite by Pcnicillium sp. P6. *Appl Biocheni Alicrobial.,* 42:52-55.