Optimization of humic acid by *Trichoderma viridi* and it's effect on sorghum plant

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ABSTRACT

Trichoderma viridi is a major biocontrol agent against a wide range of phytopathogenic organism in economic important crops and extensively used in various parts of the world and known to degrade complex organic molecules into simpler solubilize forms which helps to improve soil fertility. Humic acid is an organic fertilizer which enhances the plant growth. The aim of the present study is to solubilize lignite to humic acid by *T.viridi* under defined conditions. The solubilization efficacy of *T.viridi* was carried out in Czapex Dox broth containing different concentration of lignite such as 1, 1.5, 2, 2.5 and 3% inoculated with 0.6 ml spore suspension under shaking condition at 30°C for 7 days. The humic acid was obtained at 2.5% concentration and comparatively very less amount of humic acid in other respective concentrations. The media was optimized for evaluation of enhanced solubilization by Placket Burman Model reveals 0.2% of humic acid at 2.5% of lignite supplemented media. The media optimization was especially concerned with addition of various nutrient sources such as Glucose and Yeast extract. Moreover plant growth promoting efficacy of humic acid derived from lignite solubilization with *T.viridi* was done is sorghum plants grown in pots reveals that *T.viridi* solubilized humic acid recorded maximum plant growth than synthetic humic acid and untreated control. From this study it is clear that biosolubilization of humic acid enhances plant growth and biocontrol efficacy against phytopathogenic organism.

Key Words: *Trichoderma viridi*, Lignite, Biosolubilization, Humic acid.

INTRODUCTION

Millions of years takes for formation of ligite in the earth reserves, but degradation of lignite is have short time process .Ligninolytic fungi have the ability to degrade low-rank coal (lignite), where magenese peroxidase has an important role in degrading the low rank coal. Lignite and wastes of coal industry are promising sources for biologically active compounds humic acids 30 to 40% and fulvic acid. Several lignin degrading organisms have also been shown to solubilize oxidized lignite, including *Phanerochaete chrysosporium* (Scott and Lewis, 1987), *Streptomyces setonii* (Millard and Burr, 1926) and *S. viridosporus* (Strandberg and Lewis, 1987). Ward (19852) was able to isolate a range of lignite degrading fungi from a naturally exposed lignite seam. In this study *Trichoderma viride* was able to degrade low rank coal – lignite. In view of the fact that 1982 when Cohen and Gabriele first reported that fungi could grow directly on and metabolize naturally occurring coal, biological conversion of low-rank coals by bacteria, fungi, or preparations of the enzymes they produce has been the subject of intensive research. North American lignite's were found to be much less susceptible to solubilization by both these and other fungi (Ward, 19851 and Scott *et al.,* 1986). Recently Gutiérrez-Miceli *et al*. (2007) developed liquid formulation using vermicompost leachate having high concentration of humic acid which enhanced the growth of sorghum (*Sorghum bicolor* (L.) Moench). In the present investigation, we designed a method to increase humic acid production and recorded its effect on sorgum plant.

MATERIALS AND METHODS

Sample collection and isolation of fungi

Lignite was collected from Neyveli Lignite Corporation, Ltd, Neyveli, Tamil Nadu, and India.According to standard methods and the collected sample was kept in ice bag, brought to the laboratory. Fungi were isolated from the collected lignite sample adopting standard methods and the fungal colonies were identified based on cultural and morphological characteristics. Pure culture was maintained on czapex Dox agar slants.

BIOSOLUBILIZATION ASSAY

Respective fungal isolates were inoculated into liquid media with different concentration of lignite 1, 1.5, 2, 2.5

and 3% and the inoculated flasks were kept under shaking condition at 37 C for seven days. After the incubation period, the broth was filtered through Whatman No. 1 filter paper and the filtrate was used for solubilization assay. To the collected filtrate, 1 ml volume of concentrated HCL added, and stirred with magnetic stirrer. Formation of dense precipitate indicated solubilization of lignite to humic acid (Stevenson, 1982). The fungi which showed positive result for solubilization was used for further study. To increase solubilization rate, the lignite was treated with different concentration of nitric acid 5,10,15,20 and 25 % nitric acid and the biosolubilization was performed with fungal isolate which solubilized lignite as described earlier. Nitric acid treatment carried out to remove excess phenolic compounds which affect solubilization rates.

PLACKET BURMAN DESIGN

Placket Barman design was performed to optimize the media for enhanced solubilization rate. The media with different concentration of Glucose (1gm to 2 gm) , yeast extract (0.2 gm to 0.3 gm), lignite (1.5gm to2.5gm), Dipottasium hydrogen phosphate (0.02mg to 0.05mg), Potassium orthophosphate (0.02 mg to 0.04mg), magnesium sulphate (0.01 mg to 0.03mg) and Calcium chloride (0.01mg to 0.02 mg) concentration. The media was inoculated with 0.5 ml of *T.viridi* spore suspension and solubilization assay was carried out as described earlier.

EFFECT OF HUMIC ACID ON SORGHUM PLANT GROWTH

The media which showed maximum solubilization rate was selected for this study. The broth was filtered through Whatman No.1 filter paper, the collected filtrate was sprayed on 20 DASE (Days after seedlings emergence) of sorghum plants grown in pots. Five replications including control was also maintained the total height and weight of treated plants was recorded after 20 days of treatment.

RESULT AND DISCUSSION

Fungal isolates

Trichoderma viridi and *Aspergillus sp* were isolated from the collected lignite samples. Few *Streptimyces sp* was also recorded. Among the isolates, *T.viridi* was found to be predominant species followed by *Aspergillus sp.* The solubilization of lignite was effected at ambient temperature and pressure with the aid of some potent fungal strains isolated from soil samples collected from Neyveli lignite mines (Tripathi and Jain, 2010).

Solubilization

All the isolates belong to *T.viridi* was found to be solubilized lignite to humic acid at all the tested concentration and maximum yield of humic acid (1.2 %)was recorded at 2.5% concentration (Table 1) followed by 2.0% of lignite which yielded 0.92% of humic acid.The pH was increased from 5.0 to 6.0 after the solubilization. Moreover, the effect of nitric acid on solubilization reveals 10% of nitric acid treated lignite showed maximum humic acid yield (1.8%). (Table 2) followed by 15% nitric acid treated lignite (0.97%). Changes in pH were also observed at respective treatment. *Aspergillus fumigatus* showed maximum (22.3%, w/w) solubilization, and other isolated microbes, e.g., *Fusarium udum, Fusarium solani, Aspergillus oryzae*, and *Aspergillus sydowii*, which showed much less solubilization, being in the range of 4.5-16.8% (w/w). The process parameters were optimized in respect of media, initial pH, incubation period, and substrate concentration for maximum yield of the product (Tripathi and Jain, 2010)

Table 1. Yield of humic acid (%) at various concentration of lignite by *T.viridi* and changes in pH

% of Lignite	Yield of humic acid (%)	Initial pH	pH After 3 Days
1.0	0.6	4.1	5.0
1.5	0.8	4.0	5.5
2.0	0.92	4.5	5.2
2.5	1.2	4.6	6.8
3	0.5	4.3	4.2

PLACKET BURMAN MODEL

In order to get more solibilization of lignite, the medium was subjected to design by placket barman model (Table 3). In this study seven variables were used. Among these variables Glucose, yeast extract and lignite was positive effect significant. Dipottasium hydrogen phosphate, potassium orthophosphate, calcium chloride and magnesium sulphate was negative effect.

HUMIC ACID ON SORGHUM PLANT GROWTH

Distinct difference recorded in plant weight and height

Table 2. Yield of humic acid (%) at various concentration of nitric acid treated lignite

Concentration of Nitric acetic acid	% of lignite	Yield of HA(%)	Initial pH	pH after 3 days
5	2.5	0.6	4.5	5.8
10	2.5	1.8	4.3	6.5
15	2.5	0.7	4.1	5.5
20	2.5	0.34	3.8	5.0
25	2.5	0.1	3.5	4.2

Table 3. Results of placket n burman model

Trials	glucose	yeastextract	linite	KH_2PO_4	K_2HPO_4	$CaCl_2$	$MgSO_4$	YIELD
1	H	L	H	L	H	L	H	1.4
2	L	H	H	H	L	H	L	1.6
3	L	L	H	H	H	L	H	1.8
4	H	L	L	H	H	H	L	1.1
5	L	H	L	L	H	H	H	1.2
6	H	L	H	L	L	H	H	2.0
7	H	L	H	H	L	L	H	1
8	L	L	L	L	L	L	L	0.98

H = High concentration, L= Low concentration

was recorded **.** 54 cm height and 101 gm total weight was recorded in treated plants. Control was recorded only 46 cm height and 90 g. of weight. Treated plants with humic acid gave lower total phenol and ortho – dihydroxy phenols would promote the growth of plants .*Trichoderma viridi* is a major biocontrol agent against a wide range of phytopathogenic organism in an economic important crop. In this study the organism was solibilized lignite and converted to humic acid. The major advantage of this study, the lignite solibilized broth was filtered and without sterilized. So that the organism will be acting as biocontrol agent for crop and the humic acid was used for plant growth supplements.

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