International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
Coden: IJARQG(USA)

Research Article

SOI: http://s-o-i.org/1.15/ijarbs-2016-3-1-6

Biosolubilization of lignite by marine soil microbes

A.Balasubramanian*

Department of Microbiology, Thiruvalluvar Arts and Science College, Kurinjipadi, Cuddalore, Tamilnadu, India.
*Corresponding author: *drbala.micro@gmail.com*

Abstract

Lignite an acidic compound cannot be converted to humic acid as such because humic acid requires alkaline condition for its production. So the lignite should be solubilized to give the alkaline condition here by we can get the humic acid. The chemical solubilization is not economically viable process, so only biosolubilization is the alternate method. Biosolubilization, this process deals with the solubilization of lignite to humic acid using microorganisms. Humic acids are applied as a fertilizer in the agricultural field; thereby we can increase our yield in the crop production. Humic is the final decomposed stage of organic matter. It consists of the numerous chemical substances such as water soluble sugars, amino acids, proteins, lignin, fats, carbohydrates and water in soluble humic acid.

Keywords: *:* Lignite, Mangrove forest soil sample, Sabourauds dextrose broth, Biosolubilization, *Penicillium* sp, Humic Acid.

Introduction

Humic acid is an inorganic fertilizer which is an important constituent of lignite. Humic acid has an extra ordinary character which is its water regaining capacity and also it can retain some elements which are essential for plants. Hence, it can act as a plant growth stimulator, as it has the ability to retain more amount of moisture in adverse conditions and so occupied an important place in agriculture.

Humates and Humic acid

Humic acid is a growth stimulant and a chelating agent. It will come from vegetation deposits that never heated up, cooperated and slowly carbonized to become coal. It will free up nutrients in the soil making them readily available for plants to take up. This is especially useful when dealing with clay soils as clay can “bind” nutrients preventing plants from taking them up.

Humic acid derivatives benefits for agriculture

University of California research scientists, scientists from other U.S. universities and European Scientists here agreed on these benefits from the soil humus formation.

- Improves soil physical properties.
- Holds exchangeable plant nutrients.
- Improves moisture conditions.
- Affects the release of plant nutrient through show decomposition of organisms.
- Improves trace element nutrition through halation.
- Has a growth promoting effects.
- Has a high Base Exchange capacity an improvement basis for soil fertility concepts.

Humates formation

Humic acids are reported to increase the permeability of plant membranes, so promoting the uptake of

nutrients. Sun states many investigators have observed a positive effect of humic substances on the growth of various groups of micro-organisms. They attribute this effect to the presence of iron in the humic acids or to their colloidal nature or they regarded humic substances as organic catalysts. There is evidence that same of the humate materials contain large population of micro-organisms that share the properties of both fungi and bacteria.

The coal humic fertilizers reactivated the bio-chemical processes in plants and increased the quality and yield of potatoes. Humic substances for many reasons, depending on soil and environmental conditions. It is clear however that they are a benefit to the farmer in western soil conditions and in similar soils in Ukraine and china. The consensus is that they work in law organic matter soils. Therefore, several research groups have investigated the ability of selected micro-organisms, above all filamentous fungi, to convert lava rank coal into water-soluble fungi. In most cases, however the soluble eat ion of coal is not accompanied by its depolymerisation (Chen, and Schnitzer, 1976).

Biosolubilization

Lignite an acidic compound cannot be converted to humic acid as such because humic acid requires alkaline condition for its production. So the lignite should be solubilized to give the alkaline condition here by we can get the humic acid.

The chemical solubilization is not economically viable process, so only biosolubilization is the alternate method.

Biosolubilization, this process deals with the solubilization of lignite to humic acid using microorganisms. Humic acids are applied as a fertilizer in the agricultural field; thereby we can increase our yield in the crop production.

Raw lignite has properties of moisture -50.00(%), ash -3.00 - 13.00(%). Volatile matter -20.00 - 25.00(%), fixed carbon -17.00 - 25.00(%), calorific value -2,600 - 2,800(%) (Kucher *et al*., 1997)

Humic substances

Humic is the final decomposed stage of organic matter. It consists of the numerous chemical substances such as water soluble sugars, amino acids, proteins, lignin, fats, carbohydrates and water in soluble humic acid.

Structure of humic acid

Humate is defined as the salts of humic acids. According to Obreza,*et al.,* (1977) humate materials are widely distributed organic carbon containing compounds found in soils. Humic acid comes from vegetation deposits that have heated up, compacted and slowly carbonized to become coal.

Properties of humic substances

Humic Acids

The fraction of humic substances that is not soluble in water under acidic conditions (pH<2) but is soluble at higher pH values. They can be extracted from soil by various reagents and which is insoluble in dilute acid. They are dark brown to black in colour (Singhal, and Pramod kumar 1986).

Formation and chemical nature of humic acid

The genesis of the humic materials is a two stage process involving the predominantly microbial degradation of organic polymers to monomeric constituents such a phenols, quinones, amino acids and sugars and the subsequent polymerisation of these by spontaneous chemical reactions, auto oxidation and oxidation catalysed by microbial enzymes such as laccase, polypenol oxidase and peroxidase. The aromatic ring structure that serves as building blocks of the humic acid core may orginate from the microbial degradation of lignin or may be synthesised by various micro organisms from other carbon substrates (Ghosh, and Schnitzer, 1979).

Interestingly, humic acid is present upto 50 to 60% in the Neyveli Lignite. Chemical extraction of humic acid from lignite has been done and a pilot plant for its production has been established. (Hayase *et al.*, 1983)

Fisher *et al*., 1960 later tested a penicillium species on a soft brown coal and described evidence of growth on the coal .A report by Cohen and Gabriele established the potential for application of fungi for bioconversions of solid coal to solubilized products.

Characters of humic acid

Humic acid increases nutrient uptake, drought tolerance and seed germination. It increases the microbial activity in the soil making it an excellent

roots stimulator. It increases the availability of nutrients that are already in your soil and will naturally aerate the soil from the inside.

It has been used by nursery men, landscapers, golf courses and athletic fields for years. These professionals have found that humic acid saves them on fertilizing and watering rates.

The most common salts responsible for salinity are chlorides, sulfates, and carbonates of sodium, potassium, calcium, and magnesium. Most microorganisms inhibiting the sea are capable of be halophilic. It contrast to the microorganisms from lakes and rivers , which can't grow at a salt concentration of more than 1%, those marine halophiles are capable of growing best at 3.5-5.0%
Many reports in the literature indicate the extraction of humic acid. The available literatures are presented under the following heads to serve as a background for the present study.

An exhaustive study has been conducted for the production of humic acid from Neyveli Lignite with the objective of utilizing humic acid as an effective fertilizer for the plant growth. The physico chemical characteristics of Lignite used for extraction of humic matters are deal with in detail. In line with the optimum parameters such as lignite site distribution, temperature, pressure and duration of reaction have been evaluated. A series of experiments have been carried out for the purpose of determining the effect of above parameters on the rate of organic matter transfer in to the medium of varying.

Developed a new theory in this field which highlights the significante of exchange capability and absorption property of humic matter. His conclusion was that humic matter originated under the action of micro organisms (Manoharan *et al*., 1989).

One of the important benefits realised from using manure is that manures are parent materials for the synthesis of Humic acid derivatives (HAD). Humic acid derivatives are mixtures of Humic acid. Humic acid and fulvic acid and products of organic matter transformations by the soil micro organisms. Humic acid derivatives have several known benefits to agriculture. (Schnitzer and Chen. 1976).

Humified organic matter is also known to increase microbial growth and activity. Visser (1985a) pointed out that humic acids, if added to selective media, could increase the growth of a wide range of taxonomic and functional groups of soil bacteria and he hypothesized that a modification of cellular activity and growth might be promoted by humic substances through their influence on cell membrane permeability or on nutrient absorption (Visser 1985 b).

Materials and Methods

The development of new technologies for applying molecular methods to environmental studies, effect of bacteria and different fungal on solublization of lignite into humic acid requires a wealth of several materials and methods.

Sample Collection

The mangrove forest soil sample beneath the root surface was collected in different location of sedimentation about 50cm to 75cm depth from the surface water level and also river soil samples were collected.

Total microbial count

Soil sample was serially diluted up to 10^{-8} dilution. From that tubes, 5^{th} dilution tube was taken for the pour plate technique. It plated then incubated for 24 hours at 37^{0} C.

The next day the plates were examined for the colony morphology of bacteria, fungi and actinomycetes. Actually these type of tests were performed for the examination of nutrient status, soil fertility etc.

Then the observed plates were taken again they were made to plate on the following culture plates to isolate the cultures: Bacteria-Nutrient Agar, Fungal-Sabourauds Dextrose Agar, Actinomycetes-Glycerol Yeast Extract Agar. The plates were plated and then the result was noted.

Microbial status

The collected soil samples were serially diluted upto 10^{-8} dilution. Higher dilution was used for bacteria; next lower dilution was used for fungi & Actinomycetes. A Liquots were taken from the diluted samples in sterilized petriplates and plates were poured with Nutrient agar, Potato Dextrose agar and Glycerol Yeast Extract agar for the isolation of bacteria, fungi & Actinomycetes respectively (Kieft *et al.*, 1987).

The plates were incubated for 2-7 days and observed for colony morphology and colony count. The results were recorded and tabulated.

pH

For the same sample the pH was calculated using the pH meter. The pH meter was standardised using the standard buffer solution and then the pH of the sample was determined.

EC

Electrical conductivity of the sample was determined using electrical conductivity meter.

Determination of soil pH and EC

Whey 10g of air dry soil and transfer it to a 100ml beaker. Add 30ml of distilled water, stir well with a glass rod and kept it for 1 hour with frequent stirring. Measure the pH in a pH meter, and measure the EC meter.

Estimation of humus substances in soil
(Obreza *et al.,* 1977)

The differential solubility of humic acid in alkali and acid was followed for separating them into empirical groups.

Acid washed (0.1 N HCl) 40g soil was mixed with 200ml of 0.5N sodium hydroxide in a poly ethylene centrifuge bottle and then centrifuged. The dark for 12 h on a mechanical shaker and then centrifuged. The dark coloured supernatent liquid was filtered and the pH of the filtrate was adjusted to 1.0 with concentrated HCl.

The supernatant liquid in this acidified extract was siphoned off. The precipitated humic acid was re dissolved in 0.5 N. NaOH and re precipitated with concentrated HCl and centrifuged again and this purification procedure was repeated several times.

The humic acid obtained was washed with distilled water until it was free of chloride and dried to get a fine powder. This was weighed and reported as percentage of humic acid on moisture and ash free based and also as percentage of organic matter.

Estimation Of available nitrogen in soil
(Subbish and Asija – 1956)

Equipment and Apparatus

A Kjeldehl distillation set measuring cylinder, pipette, conical flask, heater or a burner.

Reagents

0.32 % Potassium permanganate solution: Dissolve 3.2 g of potassium permanganate in distilled water and made up the volume to one liter.
2.5 % Sodium Hydroxide solution: Dissolve 25 g of sodium hydroxide pellets in distilled water and make up the volume to one liter.

Procedure

20 gm of soil is weighted and is treated with excess of alkaline potassium Permanganate and distilled potassium permanganate is a mild oxidizing agent in an alkaline medium. The organic matter present in the soil is oxidized by the nascent oxygen, liberated by potassium permanganate in the presence of sodium hydroxide. The ammonia released is distilled and absorbed in a known volume of a standard acid, the excess of which is titrated with a standard alkali, using methyl red as an indicator. Nitrogen estimated by this method was considered hydrolysable nitrogen or potentially available nitrogen (Uhlen, 1991).

Estimation of available phosphorus
(Subbish & Asija – 1956)

This method has found widely applicable in slightly acid, neutral, alkaline and calcareous soils.

The sample were shaken with 0.5M $NaHCO_3$, at nearly constant pH of 8.5 in 1:20 ratio, for half an hour in the presence of **Darco**-G 60 (which absorbs the dispersed organic matter in the sample and thus helps giving a clear extract) and the extract is obtained by filtering the suspension. Phosphorus in the extract is treated with ammonium molybdate (a complexing agent), (yellow coloured). Then the phosphomolybdate is reduced by the use of $SnCl_2$ (a reducing agent). As a result of this reduction, some of Mo^{6+} is converted to Mo^{3+} and for Mo^{5+} and the complex assumes the characteristic blue colour. The intensity of the blue colour obtained is proportional to the quantity of P, entering into the reaction, yielding the complex known as "Molybdenum blue". This

colouration permits P to be quantitatively determined with a satisfactory precision.

Determination of available potassium in soil
(Subbish & Asija – 1956)

The method is based on the principle of equilibrium of soils with an exchanging cation made of the solution of neutral Ammonium acetate, in a given soil, solution ratio. During the equilibrium, ammonium ions exchange with the exchangeable K ions of the soil. The K contents in the equilibrium solution are estimated with a flame photometer. Since NH_4^+ holds highly changed layers together just as K, the release of the fixed k, in an exchangeable form, is retarded during NH_4 OAC extracted.

Estimation of available sodium in soil
(Baruhh & Bhabukar – 1954)

The method was based on the principle of equilibrium of soils with an exchanging cation made of the solution of neutral Ammonium acetate, in a given soil, solution ratio. During the equilibrium, ammonium ions exchange with the exchangeable Na ions of the soil. The Na contents in the equilibrium solution were estimated with a flame photometer. Since NH_4^+ holds highly changed layers together just Na, the release of the fixed Na, in an exchangeable form and retarded during NH_4 OAC extracted.

Estimation of organic carbon
(Dalal & Mayer – 1986)

1 gm of soil was treated with an excess volume of standard K_2 Cr_2O_7 in the presence of Con. H_2SO_4 and the organic carbon in the soil was thus oxidised to CO_2. The highest temperature, attained by the heat of dilution reaction, produced on the addition of H_2SO_4 was approximately 120°C which was sufficient to oxidize the active form of the soil organic carbon but not the mere inert form of carbon that may be present. The excess of $K_2Cr_2O_7$ not reduced by the organic matter is titrated back against a standard solution of Ferrous Ammonium Sulfate (FAS) in the presence of NaF or Phosphoric acid (Uhlen, 1991).

Extraction of Humic Acid

Estimation of Humic Acid using biosolubilization process can be done by the following 2 process.

1. Shake flask Method
2. Respirometer Method

Preparation of Broth culture

The isolated cultures of bacteria were inoculated into Nutrient broth to get more amount of inoculum.
The isolated cultures of fungi are inoculated into Sabourauds Dextrose broth to get more amount of inoculum.
The broth was prepared and dispersed, into 4 clean conical flasks and sterilized by autoclaving at 121^{o}C temperature at 15 lbs pressure for 15 minutes.
After sterilization the broth is allowed to cool. The isolated colonies of various fungal strains are inoculated into each of the 4 flasks containing the broth and incubated for 48 hours.
Actinomycetes broth was prepared, which was sterilized at 121^{o}C,
15 lbs pressure for 15 minutes. After cooling of which the isolated stain of actinomycetes was inoculated into the broth and incubated for 72 to 90 hours.

Inoculation of broth culture to lignite

To the Sabourauds Dextrose Broth prepared, 10% of lignite was weighed and added. The flasks containing of lignite was sterilized at 121^{o}C at the pressure for 15 minutes.

After sterilization the broth was cooled to room temperature and inoculated with 5 ml of the fungal cultures under specific conditions. Forget a control maintained. The flasks are then incubated in a shaker.

Procedure

1. The incubated cultures were tested for the presence of humic acid by soluble ratio of the lignite by the different micro organism on the 7th, 14th, 21st and 28th day.
2. The pH, electrical conductivity of the culture was also determined on the respective days using pH meter and EC water.
3. 30ml of each broth culture was taken from which extract was prepared.
4. From the extract the humic acid content was estimated.
5. The pH of the extract was determined with a pH meter.

Estimation of humic acid content in lignite extract

1. The lignite extract prepared from the isolated broth incubated for 7, 14, 21 and 28 days may contain some amount of humic acid in soluble forms.

2. To estimate the amount of humic acid present in the broth, 30ml of the broth culture was taken.
3. From this 30ml extract was prepared by filtration.
4. To the extract concentrated hydrochloride acid (HCl) was added.
5. Humic acid if present precipitates with concentrated HCl.
6. The precipitate was then filtered and weighed from which the amount of humic acid solubilization from lignite by different strains could be estimated (Schisler and linderman 1989).

Aerobic / Anaerobic respirometer

Challenge AER 200 aerobic / anaerobic respirometer precisely, accurately measures oxygen consumption or gas production in small culture vessels or large laboratory scale reactors. Oxygen uptake in aerobic application is measured as low as 0.06 mg and at rates as high as 600 mg/hr.

Procedure

1. Potato Dextrose broth was prepared to which different concentration of lignite was added in 500 ml bottles.
2. The bottles were marked for the same concentration (5 gm lignite).
3. To the bottles the sub cultured strains were added.
4. A magnetic stirrer was added to the bottle and the bottle was screwed tight with a lid a tube containing 5ml KOH was inserted for absorption of CO_2.
5. The bottles were the incubated in the respirometer for 10 days at a temperature of 30^oC.
6. The respirometer records the uptake of oxygen by automated data collection i.e., through the computer.
7. The humic acid content pH and EC of the samples were determined on the 10^{th} day.

Results and Discussion

Biosolubilization of Lignite by using marine soil organisms, collected from mangrove forest. During biosolubilization of lignite the humic acid is a product which is growth factor for plants. Microbial cultures collected from marine environments such as bacteria, fungi, Actinomycetes which has ability to solubilize lignite into humic acid. Marine organisms have high pH tolerance and secrete intracellular and extracellular enzymes. This property of the marine organisms solubilize the lignite to form humic acid. However using of chemical method to solubilize lignite many disadvantages occurs. Such as emission of gas and effluents hazardous to the environment and also expensive.

Hence using biotechnical methods, such as application of bacteria and fungi the lignite can be converted to valuable product humic acid.According to the test result, using of bacteria, fungi and Actinomycetes, Fungi were given highest solubilization of lignite and form humic acid.

Results obtained from total microbial count (Table-I) 10^5 dilution shows bacteria were high count compare to fungal and actinomycetes. Results from microbial status production of Biosolubilized product humic acid fungi gives high amount (0.103%) compare to bacteria and actinomycetes. Hence fungi gave best result for biosolubilization of lignite to humic acid.

Estimation of soil sample, (Table-II) mangrove soil and river soil sample the pH shows in an alkaline condition. This is considerable result microorganisms able to solubilize lignite to humic acid in mangrove and river soil sample.

Microorganisms isolated from mangrove and river soil sample and test for solubilize lignite to humic acid in laboratory condition by lignite using in the culture medium. Soil microorganisms solubilise lignite to humic acid by using their enzymes. Biosolubilization of lignite by mangrove and River soil organisms is measured by different parameters such as estimation of N, P, K, (Table - III,IV&V) sodium and soil organic carbon and organic matter (Table-VI&VII). It was measured by different parameters such as Shaker culture method (Table-VIII), Biosolubilization of lignite using different fungi (0.09) halophilicorganisms. Electrolytic Respirometer such as bacteria, fungi, actinomyces and oxygen consumption. Above these test results *Penicillium* Sp were given best result for biosolubilization of lignite.

Mangrove and River soil sediment microorganisms such as bacteria, fungi, actinomyces were vital role in solubilization of lignite to humic acid. Humic acid maintain waste regaining capacity and some elements are essential for plants. Hence, it can act as a plant "growth stimulators". So it plays an important role in agriculture.

Table-I **Total microbial count**

Sample	Bacteria	Fungi	Actinomycetes
M1	122	23	4
M2	106	27	5
M3	117	18	3
R	110	12	7

M1, M2, M3- Mangrove forest soil, R-Rives Soil

Table-II **Estimation of soil sample**

Sample	pH	EC ms/cm
M1	7.18	3.00
M2	7.30	2.44
M3	7.28	2.52
R	8.72	0.96

Table-III **Available Nitrogen**

Nutrients	Soil sample	Kg/ha
Available Nitrogen	M1	17.248
Available Nitrogen	M2	48.608
Available Nitrogen	M3	14.56
Available Nitrogen	R	14.112

Table - IV **Available Phosphorus**

Nutrients	Soil sample	Kg/ha
Available Phosphorus	M	5.9
Available Phosphorus	M	7.5
Available Phosphorus	M	17.3
Available Phosphorus	R	38.4

Table-V **Available Potassium**

Nutrients	Soil sample	Kg/ha
Available Potassium	M1	588
Available Potassium	M2	296.8
Available Potassium	M3	425.6
Available Potassium	R	1400

Table-VI **Available Sodium**

Nutrients	Soil sample	Kg/ha
Available Sodium	M1	3791.2
Available Sodium	M2	1568
Available Sodium	M3	2441.6
Available Sodium	R	481.6

Table-VII **Soil organic carbon and organic matter**

Soil Sample	Organic carbon (%)	Organic matter (%)
M1	2.49	4.13
M2	1.05	1.81
M3	2.50	4.31
R	0.27	0.46

Table-VIII **Shaker culture method**

Sample	7th day		14th day		21st day	
	pH	**EC** ms/cm	**pH**	**EC** ms/cm	**pH**	**EC** ms/cm
HF1	4.58	15.20	3.08	15.85	4.47	16.23
HF2	2.95	15.86	2.95	16.74	3.10	16.47
HF3	4.11	16.20	2.91	15.85	2.87	16.23
RF	3.72	16.60	2.75	16.07	3.93	17.42
FC	4.32	17.27	4.17	15.23	4.76	15.22

FC = Fungi control, HF = Halophilic Fungi, RF = River Fungi

Table- IX **Respirometer**

Biosolublisation of lignite using different halophilic Fungal Strains (*Penicillium* Sp)

Name of the sample	pH	EC **ms/cm**	Bio-Solublizing product broth
HF1	4.52	17.9	0.06
HF2	4.34	18.1	0.04
HF3	4.46	17.4	0.02
HF4	4.17	17.9	0.03
Control	5.20	17.5	0.009

C = Fungi control, HF = Halophilic Fungi

Hence organisms isolated from soil sediment and allow solubilizing lignite to humic acid in numerous amounts for direct use in agriculture. According to the results among microorganisms fungi has high ability to solubilize lignite to humic acid.

Summary

Marine pollution is defined as the discharge of waste substances into the sea resulting in harm to living resources, hazards to human health, hindrance to fishery and impairment of quality of sea water for use.

Trees reduce atmospheric pollution by absorbing polluting gases and collecting suspended particulate matter in air. But in coastal areas, only few plants are grown because of high salt content of the soil. To overcome this condition, we can apply the biosolubilized product humic acid which is a 'Plant Growth Stimulation'. Humic acid reduces high salt content of the soil and increases humus content in soil. Humus (organic matter) is important for soil because it increase both aeration and hydration. Thus, by applying humic acid as a fertilizer to plants which are unable to grow in coastal area? (Eg) Trees of all kinds, lawns, shrubs house plants, vegetables and flower gardens. For transplanting use a root dip or soak, spray right on the soil to naturally build the humus content. A tree absorbs pathogenic microorganisms and other toxic substances emitted by decayed fish on their surface. So the people in coastal areas are not affected by marine pollutants.

It is compatible with Golden Harvest Natural Fertilizer and Most other fertilizers. Can be used with enzymatic & biological formulas too. Mixing ratio is 128:1. One ounce Humic acid per one gallon of water or other solution used for spray in to the Marine or all plants/ Humic acid has a growth promoting effect, high base exchange capacity and an important basis for soil fertility concepts. Humic acid is a biostimulant and can be used on turf, trees, shrubs, bushes, beds, gardens and even on indoor plants. All most has been used by Nurserymen, Landscapers half courses and athletic fields for years

Conclusion

From the above study it is concluded the Humic Acid can be extracted from the *Penicillium* sp isolated from the mangrove ecosystem. Biosolubilization is a process which helps for this bio technical conversion. Humic acid is above stimulate and can be used on turf, trees, shrubs, bushes, beds, gardens and even on indoor plants. Almost anything that grows will benefit from Humic Acid.

Humic acid increases nutrient uptake, drought tolerance and seed germination. It increases the microbial activity in the soil making it an excellent root stimulator. Humic acid increases the availability of nutrients that are already in our soil and will naturally aerate the soil from the inside. It also will help to lower the ph of the soil and will flush high levels of salts out of the root zone.

References

Baruh and Bhabukar. 1954 The text book of soil analysis. Pp 169-171.

Chen,Y. and Schnitzer, M. 1976 Soil Humic Acid derivatives soil Sci soc of Am J 40: 866-872.

Cohen MS., Gabriele PD (1982) Degradation of coal by the fungi polyporus persicolor and poria monticola. Appl Environ Microbiology 44: 23-24.

Dalal, R.C. and R.J. Mayer. 1986. Long-term trends in fertility of soils under continuous cultivation and cereal cropping in southern Queensland. II. Total organic carbon and its rate of loss from the soil profile Aust. J. Soil Res. 24:281-292.

Fischer and Fuchs and Rogaff, 1960 . Isolation and Application of Coal Solubilizing microorganisms. Bioprocessing and Biotreatment of coal. Pp 42:303-304.

Ghosh,K and Schnitzer, 1979 Electron Microscopic study of soil Humic Acid. J Soil Sci. 30 :735

Hayase,K and Tsubota,H. 1983. Sedimentary Humic Acid and fulvic acid as surface active substances. Geochim, Cosmochim.Acta. 47:947- 952.

Kieft Tl., Soroket E., Firestone MK (1987) Microbial biomass response to a rapid increase in water potential when dry soil is wetted. Soil Biol Biochem 19:119-126.

Kucher,M. and Donaldwise,L. 1997 Bio processing and Bio treatment of coal Pp 42:303-319

Manoharan,V. and Gowrisankaran,S. 1989 Seminar on Humic Acid in Agriculture. 173-178.

Obreza, A.H., Webb, R.G. and Biggs, R.H. 1977 on humate and humic acid. J . soil Sci. 38 : 667-678

Singhal, R.N. and Pramod kumar. 1986 Electron Microscopic study of soil Humic Acid. J in Soc soil Sci 40: 556-558.

Subbiah and Abija 1956. The text book of soil analysis. Pp 146-149

Schnitzer, M. & Glebova, G.L. (1972) Agri. Chem. 7, 131.

Singhal, R.M., Rawat, V.R.S., Pramod kumar, Sharma, S.D. & Singh, H.B. (1986) Indian Forester 112(9), 819.

Schisler DA, linderman RG (1989). Influence of humic-rich organic amendments to coniferous nursery soils on Douglas-fir growth, damping-off and associated soil micro organisms. Soil Biol Biochem 21:403-408.

Uhlen, G. 1991. Long-term effects of fertilizer, manure, straw and crop rotation on total N and total C in soil. Acta Agric. Scand. 41:119-127.

Visser SA (1985a) Effects of humic acids on numbers and activities of micro organisms within physiological groups. Org Geochem 8:81-85.

Visser SA (1985 b). Physiological action of humic substances of microbial cells. Soil Biol Biochem 17:457-462.

Access this Article in Online	
	Website: www.ijarbs.com
Quick Response Code	Subject: Marine Microbiology

How to cite this article:
A.Balasubramanian. (2016). Biosolubilization of lignite by marine soil microbes. Int. J. Adv. Res. Biol. Sci. 3(1): 38–46.