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**RESEARCH ARTICLE**

# BIOCHAR AS AN ALTERNATE CARRIER TO LIGNITE FOR THE PREPARATION OF BIOFERTILIZERS IN INDIA

**[1*]Saranya, K., [1]SanthanaKrishnan, P., [1]Kumutha, K. and [2]John French**

[1]Department of Agricultural Microbiology, Directorate of Natural Resource Management, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India- 641 003.
[2]Incrops Enterprise Hub, University of East Anglia, Norwich, United Kingdom - NR4 7TJ.

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**ABSTRACT**

Carrier based preparations of *Azospirillum lipoferum* (AZ 204) inoculant, developed using two different sources of biochar (acacia wood and coconut shell) were evaluated in comparison with lignite for their suitability as a best alternate to lignite for biofertilizer production. The survival of the microbial inoculant was estimated over a period of 180 days. Among the different carriers, coconut shell based biochar recorded a maximum population of log 10.79 cfu $g^{-1}$ of carrier on 180 days after inoculation with a maximum moisture content of 25.22%. It was also found that seedling vigour index of green gram (CO 3) was paramount in response to coconut shell based biochar. In addition, coconut shell based biochar was found to increase the survival of *Azospirillum lipoferum* upto 180 days (6 months) of storage period at a required population compared to acacia wood based biochar and lignite.



## INTRODUCTION

'Biochar' is a carbon rich product produced when burning organic matter like wood, grasses, manure or agricultural residues under conditions of low oxygen and it sounds more 'clean and green' (Lehmann, 2007). Low oxygen burning is referred to as pyrolysis. On a global scale, crop residue biomass represents a considerable problem as well as new challenges and opportunities. Bio-char based soil management systems acts as a potential technology to use carbon-rich residual waste to reshape agriculture, balance carbon and address nutrient depletion (Bird *et al.,* 1999). In India, especially Tamil Nadu, there are greater prospects for the production of biochar, since natural agricultural waste materials like coconut shell, acacia wood are available in about 130-150 million tonnes, which are simply dumped out every day without any usage (Shukla, 2010). Hence, there is an urgent need to make the best use of these valuable resources that are rich in nutrients, which are available cheap in abundance in the form of biochar. At present there is only a small scale biochar usage, there is also a need for medium scale biochar usage of (2-4 tonnes) by which the agricultural productivity, food and environmental security can be enhanced to a greater extent. Since 1971, lignite is being used as a carrier material instead of peat for the biofertilizer production in India and Tamil Nadu. For the last two decades, there are lots of inconveniences arising with this usage, because most of the lignite that are mined in India are used primarily for electricity generation; reserves available are scarce; lignite has lots of problems like storage, transport due to its high moisture content and environmental issues due to the high amount of $CO_2$ evolution causing global warming. So, there is a need for an alternate carrier for bioinoculant production, which can very well be 'Biochar', because of its growing prospects in India and added advantages that can boost up the human welfare and security economically by supporting sustainable agriculture. Moreover, biochar can act as a best substrate for the beneficial microorganisms, especially the heterotrophs as it holds high carbon content, good value of nutrients without any toxic substances. Because of all the supporting benefits and efficient chance of the utility of biochar for bioinoculant preparation in India, the present study was undertaken to assess the potential of biochar as a substrate for biofertilizer production as an alternate to lignite.

## MATERIALS AND METHODS

Authenticated strain of *Azospirillum lipoferum* (AZ 204), biochar and lignite were obtained from Department of Microbiology and Department of Bioenergy, Tamil Nadu Agricultural University, Coimbatore respectively for the conduct of the study. Physio-chemical properties of carrier materials were analysed by following standard protocols and the values are presented in Table 1.

***Corresponding author:*** saran.miles2go@gmail.com

**Treatment details**

T1- Acacia wood based biochar alone
T2- Acacia wood based biochar +*Azospirillum*
T3- Coconut shell based biochar alone
T4- Coconut shell based biochar + *Azospirillum*
T5-Lignite alone
T6-Lignite+*Azospirillum*

**Production of carrier based inoculums**

***Preparation of broth***

Nitrogen Free malic acid broth was inoculated with *Azospirillum lipoferum* (AZ 204) in 250 ml conical flask. It was allowed to multiply by incubating at 32°C in a shaker cum incubator at 100 rpm for 72 hours. The broth containing approximately 25.6 x $10^{11}$ cells $ml^{-1}$ broth was used for mixing with the carriers.

***Preparation of Carrier***

The carrier material collected were sundried, powdered and sieved through 150µ sieve, neutralized using lime if acidic and the neutralized carrier materials were packed in opaque low density polypropylene bags of thickness 75 µ and sterilized as per the procedure followed by Somasegaran (1985).

***Inoculation of broth culture in the sterile carriers***

Broth cultures having a cell load of $10^{11}$ cells $ml^{-1}$ broth in late log phage was inoculated in carrier aseptically until 35% moisture was obtained to the treatments which received *Azospirillum*. The treatments which donot receive *Azopsirillum* where added with sterilized broth of required quantity. The inoculant in 100 g was packed in opaque low density polypropylene bags of thickness 75 µ. The bags were thoroughly kneeded to ensure absorption of the liquid culture into the carrier.

***Analysis of the carrier based inoculant***

The inoculant packets prepared were stored at room temperature and analyzed for viable cell population, moisture content and seedling vigour at 15 days to one month interval up to six months of storage.

***Estimation of Azospirillum population***

The population of *Azospirillum* was enumerated by serial dilution of one gram of the samples upto $10^{-12}$, and plating in N-free malic acid agar medium by following drop plate technique (Somasegaran and Hoben. 1994), loading 10 µl per dilution. Dilutions from $10^{-6}$ and $10^{-12}$ were plated by dividing the petriplates and labeling the dilutions accordingly. The population of the inoculant in the inoculum packets was estimated at periodic intervals upto 180 days. The results were expressed as cfu $g^{-1}$ dry weight of the samples.

***Determination of moisture content of inoculant***

About 10g of the inoculant was drawn from each sample packet every time and taken in a pre-weighed clean petriplates. The contents were dried in a hot air oven at 80°C till constant weight is obtained. The plates were then removed, cooled to room temperature and weighed. The loss in weight was calculated and the moisture content was expressed in percent on oven dry basis.

***Vigor index test***

The green gram (CO 3) seeds were surface sterilized with 70% ethanol and then washed in sterile distilled water to remove the traces of ethanol. As per the treatment, about 2g of the inoculant was mixed with 50g of the seed and the treated seeds of about 10-15 were placed in moistened germination paper, rolled and incubated in growth chamber for an week and periodical recordings of the germination %, shoot length and root length was recorded and Vigour index was calculated by following formula.

Vigour index = Germination percent x (shoot length + root length)

## RESULTS AND DISCUSSION

***Survival of Azospirillum in carrier based inoculant***

The data presented in Table 2 and Plate 1 depict the survival of *Azospirillum lipoferum* (AZ 204) in carrier based inoculant prepared from different sources of biochar as well as lignite. The results indicated that inoculant prepared with coconut shell based biochar exhibited higher population of log 10.79 cfu $g^{-1}$ of carrier on the 180 days after inoculation followed by lignite of log 10.32 cfu $g^{-1}$ of carrier and acacia wood based biochar of log 10.02 cfu $g^{-1}$ of carrier . It was also observed that, the population of *Azospirillum lipoferum* increased up to 30 days after inoculation in coconut shell based biochar and lignite, where as in acacia wood based biochar inoculant, the population increased only up to 15 days of storage. When biochar (coconut shell) was used as carrier material, 14.22% reduction in *Azospirillum* population was observed after 180 days of incubation. In lignite also the reduction was to the tune of 17.37% of the orginial population after storing for 180 days.

The statistical analysis revealed that, the reduction in the population of *Azospirillum lipoferum* was significant in all the three different sources of carrier material that were used. The significantly higher survival of microbal inoculant, when biochar was used as carrier material is confirmed by the findings of Ogawa (1986), Takagi (2003) and Beck (1991). Ogawa (1986) had used biochar as a carrier substrate for both *Rhizobia* and for Arbuscular Mycorrhizal (AM) fungus over the past 20+ years with excellent success. Additional studies conducted in Japan (Takagi, 2003) and in Syria by Beck (1991) had shown that biochar is a suitable carrier for the $N_2$-fixing root nodule bacteria *Rhizobium*, *Mesorhizobium* and *Bradyrhizobium*. They also reported that, it is not difficult to speculate on the variety of applications that biochar inoculant may have in agriculture and environmental remediation. Biochar will prove a most efficient inoculant delivery system and may also improve outcomes of bioremediation efforts by increased absorption of organic pollutants onto biochar impregnated with bacteria selected for their capacity to degrade the target pollutants. Moreover, coconut shell based biochar was acting as a best alternate to lignite compared to

**Table 1. Physio-chemical properties of the carrier materials used in the study**

| Properties (%) | Acacia wood based biochar | Coconut shell based biochar | Lignite |
|---|---|---|---|
| Water holding capacity | 200 | 430 | 198.9 |
| Moisture | 20-30 | 12 | 40-50 |
| Bulk density (g $cm^{-1}$ cube) | 1.62 | 1.22 | 1.66 |
| Porosity | 73.33 | 82.27 | 34.79 |
| Total surface area | 870.90 | 926.54 | 556.40 |
| Appearance | Black | Black | Brownish black |
| pH | 7.00 | 6.56 | 3.52 |
| Odour | Nil | Nil | Nil |
| Total Carbon | 84 | 86 | 65-70 |
| Total Hydrogen | 2.30 | 2.20 | 5.00 |
| Total Oxygen | 10.70 | 10.22 | 250.0 |
| Total Nitrogen | 0.01 | 9.42 | 0.98 |
| Total Ash | 3.24 | 3.00 | 7.21 |
| Toxic elements (oxides of Al, Fe, Ca, Si, Hg, Ar, Se) | Nil | Nil | Traces |

**Table 2. Survival of *Azospirillum* inoculum prepared using biochar and lignite carriers**

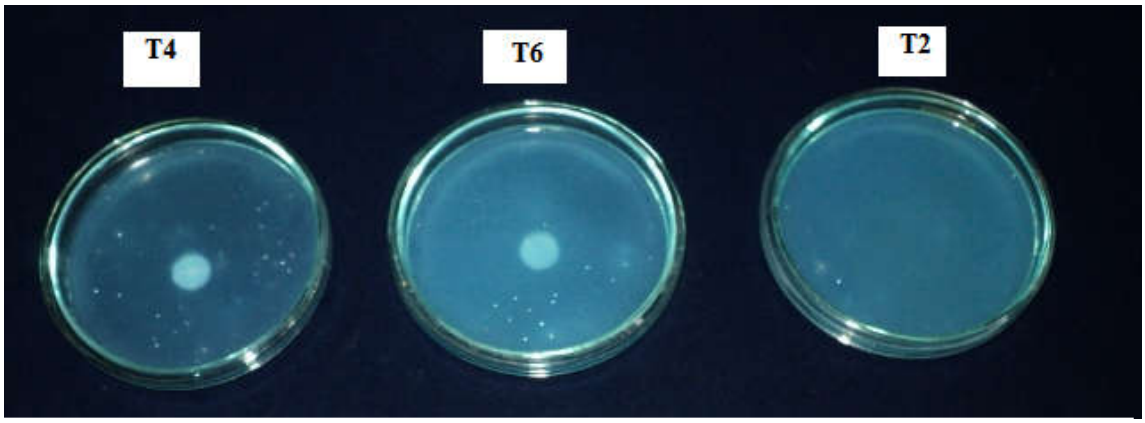
| Treatments | Population of *Azospirillum* inoculant (log cfu $g^{-1}$carrier) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Days after inoculation (DAI) | | | | | | | | |
| | 0 | 15 | 30 | 45 | 60 | 90 | 150 | 180 | Percent reduction in original population |
| Acacia wood based biochar alone | - | - | - | - | - | - | - | - | - |
| Acacia wood based biochar + AZ 204 | 12.23 | 12.33 | 11.65 | 10.77 | 10.71 | 10.43 | 10.08 | 10.02 | 18.07 |
| Coconut shell based biochar alone | - | - | - | - | - | - | - | - | - |
| Coconut shell based biochar + AZ 204 | 12.58 | 12.61 | 12.82 | 11.69 | 11.63 | 10.54 | 10.99 | 10.79 | 14.22 |
| Lignite alone | - | - | - | - | - | - | - | - | - |
| Lignite + AZ 204 | 12.49 | 12.51 | 12.66 | 11.61 | 11.22 | 11.49 | 10.49 | 10.32 | 17.37 |
| CD (*P*=0.05) | 0.08** | 0.02** | 0.58** | 0.09** | 0.15** | 0.03** | 0.53** | 0.10** | |

**Table 3. Moisture content of *Azospirillum* inoculum prepared using biochar and lignite carriers**

| Treatments | Moisture content (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Days after inoculation (DAI) | | | | | | | | |
| | 0 | 15 | 30 | 45 | 60 | 90 | 150 | 180 | Percent reduction in original moisture |
| Acacia wood based biochar alone | 35.90 | 29.62 | 22.72 | 20.66 | 17.40 | 15.97 | 14.22 | 12.01 | 66.54 |
| Acacia wood based biochar + AZ 204 | 35.40 | 30.66 | 26.30 | 24.82 | 19.78 | 16.50 | 15.30 | 13.22 | 62.71 |
| Coconut shell based biochar alone | 36.70 | 34.50 | 31.92 | 28.10 | 25.70 | 23.80 | 21.20 | 20.52 | 44.08 |
| Coconut shell based biochar + AZ 204 | 38.10 | 34.82 | 32.80 | 29.10 | 27.50 | 26.30 | 26.10 | 25.22 | 33.80 |
| Lignite alone | 36.60 | 32.10 | 31.80 | 26.90 | 22.10 | 21.90 | 20.17 | 20.12 | 45.58 |
| Lignite + AZ 204 | 38.90 | 33.40 | 32.70 | 28.90 | 26.70 | 26.20 | 25.10 | 24.22 | 37.73 |
| CD (P=0.05) | 1.03** | 0.06** | 0.80** | 0.10** | 0.09** | 0.10** | 0.11** | 0.19** | |

**Table 4. Vigour index of green gram (CO3) in response to biochar and lignite based *Azospirillum* inoculants**

| Treatments | Seedling vigour | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Days after inoculation (DAI) | | | | | | | |
| | 0 | 15 | 30 | 45 | 60 | 90 | 150 | 180 |
| Acacia wood based biochar alone | 2643 | 2227 | 2635 | 2442 | 2562 | 2213 | 2543 | 2502 |
| Acacia wood based biochar + AZ 204 | 2720 | 2535 | 2885 | 2777 | 2800 | 2502 | 2622 | 2540 |
| Coconut shell based biochar alone | 3379 | 3380 | 3230 | 3820 | 3922 | 3378 | 3297 | 3580 |
| Coconut shell based biochar + AZ 204 | 3834 | 4202 | 4199 | 4002 | 4012 | 4216 | 3721 | 3987 |
| Lignite alone | 3124 | 3333 | 3390 | 3290 | 3362 | 3256 | 3052 | 2860 |
| Lignite + AZ 204 | 3685 | 4237 | 4145 | 3942 | 3946 | 4310 | 3595 | 3922 |
| CD (P=0.05) | 16.89** | 6.88** | 22.96** | 16.73** | 16.43** | 6.72** | 16.89** | 8.76** |




T2 - Acacia wood based biochar +*Azospirillum;* T4 - Coconut shell based biochar+ *Azospirillum ;* T6 – Lignite + *Azospirillum*

**Plate 1. Growth of *Azospirillum* enumerated from different carriers on 180$^{th}$ day after inoculation**

acacia wood source biochar because of its higher surface area and total nutrient availability which supported the viability and sustenance of the microbe over a period of time.

### *Effect of different carrier on the moisture content of Azospirillm inoculant*

The effect of different carriers on the moisture content of *Azospirillum lipoferum* inoculant was studied and the result of the study presented in Table 3 revealed that the moisture content of *Azospirillum* inoculant ranged from 35.40% to 38.90% at the initial stage and thereafter a gradual decline in moisture content was noticed in all the treatments. Moisture content was significantly influenced with the use of different carrier materials. When carriers were inoculated with *Azospirillum* broth, coconut shell based biochar retained the higher moisture content of 25.22% at 180 days after storage, which was significantly higher than lignite based *Azospirillum* inoculant, where it was 24.22%. A drastic reduction in moisture was observed in acacia wood based biochar to the level of 12.01%. When carriers alone were added with the sterile broth, the similar trend was noticed. The magnitude of moisture reduction was calculated for all the carriers used. It was observed higher in acacia wood based biochar (62.71%), followed by lignite based inoculant (37.73%) and the reduction level was lesser in coconut shell based biochar inoculant (33.80%).

The results prove that biochar has a high moisture holding capacity due to slow release of moisture from its sponge like matrix (Peitikeinen *et al*, 2000) which is the main fact that is instrumental for the higher survival rate of microbial inoculant compared to lignite over a period of even six months and more. Moreover the reduced particle size and higher surface area of biochar especially produced from coconut shell dramatically act as a best nutrient rich substrate for the microbes (Lehmann *et al.,* 2002). Higher survival of *Azospirillum* under treatment recieving coconut shell based biochar was due to its higher water potential. The decrease in the water potential of lignite and acacia wood based biochar over time affected the survival of *Azospirillum* (Griffith and Roughley, 1992).


T1 - Acacia wood based biochar alone; T2 - Acacia wood based biochar; +*Azospirillum;* T3 - Coconut shell based biochar alone ; T4 - Coconut shell based biochar+ *Azospirillum ;* T5 - Lignite alone ; T6 – Lignite + *Azospirillum*

**Plate 2. Effect of different carrier based *Azospirillum* inoculant on the seedling vigour of Green gram (CO 3)**

### *Effect of Azospirillum inoculants on the vigour index of green gram (CO 3)*

The vigour index was calculated at 7 days after treatment of *Azospirillum* inoculant with green gram. The results from the table 4 predict that among the *Azospirillum* inoculant stored up to 180 days, the vigour index was maximum (3987) in green gram inoculated with *Azospirillum* which received coconut shell based biochar, followed by the treatment receiving lignite + *Azospirillum* (3922), where it was minimum (2502) in green gram inoculated with acacia wood based biochar alone. It was observed that all the treatments differed significantly in their seedling vigour when inoculated with different sources of carrier material especially with *Azospirillum* (Plate 2). The results are in conformity with the findings of Dhanapal *et al.,* 1978 who reported that the seedling growth and vigour increases due to inoculation of *Azospirillum* in crop like pearl millet and sorghum. Higher seedling vigour of microbial inoculants treated with coconut shell based biochar is due to the physicochemical properties of biochar (Table 1) that favours the survival of microbes that in turn increases crop growth as reported by Steiner *et al.,* 2004. Biochar is not only a nutrient rich substrate that act as a good microbial habitat and increase their survival, but it also add pointer to crop

growth by enhancing the plant microbial interaction as reported by Warnock *et al.,* 2007.

**Conclusion**

Based on the results of this study, biochar can be used as a best alternate carrier material to lignite for the preparation of microbial inoculant. The use of coconut shell based biochar was found to increase the survival of microbial inoculant upto six months since it is free from toxic elements and it is highly ecofriendly by adding to the carbon sequestration and it reduces the global warming; doesnot require any sterilization process as that of the other carrier since it is already heated up and is free from contaminants; available in plenty, everywhere. Moreover, biochar does not have any storage problem due to its limited moisture content. It does not have any blending problem as that of the lignite since it is free from waxy contents and acts as an efficient plant health assurance when used for field application.

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