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Review Paper

Microbial technology for revegetation in overburden dumps of coal mined area of Assam, India – a review

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Abstract

Activities of open cast coal mining create large area of overburden dump (OBD) in Makum Coalbelt, Assam. These OBDs are highly acidic and low in organic carbon, nutritional status and microbial activity. The natural process of re-establishment of vegetation on the OBDs is very slow. However, research works indicated that application of microbial including arbuscular mycorrhiza (AM) fungi, phosphate solubilizing bacteria (PSB), lime and organic amendments (FYM) can enhance plant growth on OBDs. Application of natural forest topsoil could stimulate plant growth through improvement of microbial activity in the inert OBDs.

Keywords: Arbuscular mycorrhiza (AM) fungi, coal mine overburden dump (OBD), microbial, FYM and lime and revegetation.

Introduction

Coal mining activity destroys overlaying vegetation and creates overburden dumps (OBDs). OBD spoils are comprised of rocks, shale; coarse tailings which spread over a thousand hectares of lands. Although, plant species invade in these OBDs, their abundance and density remain low, particularly during initial years following creation of OBDs. Heterogeneity of physicochemical and biological properties of OBD spoils is the principal cause of sporadic plant growth. Need for research to develop suitable techniques to reclaim the OBDs and to establish sustainable and functional plant community as well as to recover the disturbed ecosystems and communities from its status of degradation[1]. Restoration of these spoils is essential because a large area of derelict land in Assam and Meghalaya of North East India has been created by opencast coal mining of natural forest area[2].

Arbuscular mycorrhizal (AM) fungi association with root of naturally occurring plant species of the OBDs of Tikak Colliery, Makum Coalbelt and adjacent natural forest was studied[3]. Role of different microbial agents on re-establishment of vegetation on orphan mine sites have been reported in different parts of world[4-9]. AM fungi have been reported as an important component of soil microflora for exploitation in the rehabilitation of disturbed soils[10,11].

The importance of other soil microorganisms like *Rhizobium* and fluorescent Pseudomonas (FP) in reclamation and revegetation of wasteland has also been recognized[12]. In general, these beneficial soil microorganisms are known to interact in exhibiting their beneficial effect on plant growth[13]. Dual inoculation of AMF and PSB in acid laterite soils of very low P content can stimulate of plant growth[14]. Therefore, combined application of beneficial microorganisms may be an effective method for reclamation of mine spoils to promote plant growth. Lime and organic matter are also found to be important agents in stimulating vegetation growth in such spoils[15,16]. Biological activities in coal mine spoils are very low and addition of nutrient, organic matter, decomposer and symbiotic microorganisms has been found to be effective in enhanced microbial activities in mine spoils[4].

Application of fertilizer, peat or sewage to a sub-alpine coal mine spoil during initial growing seasons could cause alteration of the microbial composition of the spoil materials[5].

Coal mine spoils of different parts of the world vary in their physicochemical and biological properties. These mine spoils are also created over different geographical and climatic conditions. Therefore, it is necessary to develop of suitable package for their reclamation in a location specific manner. Locally available resources and locally adopted superior strains of soil microorganisms can be used in reclamation efforts of coal mine spoils with native plant species[17].

In this article, how coal mine activities affected physico-chemical and biological properties and effectiveness of microbial i.e. AMF and fluorescent Pseudomonas inoculants along with a few amendments have been discussed with special reference to the revegetation of coal mine spoils with native plant species of Makum Coal Belt, Assam.

Coal mining reduces nutritional status of overburden dumps (OBDs)

Coal mining activities in upper Assam drastically altered all physico-chemical characteristics of the natural forest soils and reduced them into just about 3 folds in 1-year OBD spoils[18]. However, a distinct trend within the natural recovery pattern of physico-chemical parameters was marked for the OBDs with an improvement of the status of organic carbon, N, P and K with raised the age of the OBDs. An OBD once it's is created at the start contained elemental sulphur (S) having spoils and weathered gradually due to exposure to air during the subsequent years following the dumps created. The highest amount of H^+ ions produced up to 5 years of OBD age. From fifth years onward, sulphur is reduced and H+ ions are leached out, after which rise in pH was observed. The recovery pattern and process seem to take many years to get original state like adjoining natural forest sites[18]. Several studies encountered similar observation. For example, the study in the Singrauli Coal field areas of Madhya Pradesh estimated that total N recovery might take about 200 years to reach the level of native forest soil[19] or about 2160 years in American spoils[20]. The study on accumulation of the N in the OBD spoils revealed that it reached almost double in 4-8 years (40 kg ha^{-1}) and the subsequent 8-15 years increase of nitrogen in the spoils was 90 kg ha^{-1} in coal mine overburden[21]. It was also observed that about 26.5 per cent increase in total N between 5 to 20 years in coal mine overburden[22]. In an another study of Dhanpuri in Madhya Pradesh opined for introduction of suitable plant species as a viable alternative to enrich the phosphorus level of coal mine overburden dumps and it was recorded for three folds increased in the soils of planted sites than that of unplanted sites[23].

Coal mining reduces the active population of soil microorganisms

Arbuscular mycorrhizal fungi (AMF) status in rhizophere soils of nineteen species planted on four overburden dumps (OBDs) of Tikak Colliery, North East Coalfields, Margherita, Assam, including thirteen naturally occurring plant species were investigated and found positively mycorrhizal[24,25]. The study also revealed that coal mining reduces the mycorrhizal status as well as species diversity in younger OBDs. The nature could recover arbuscular mycorrhizal status and species diversity on the OBDs with time, through increase in age of OBDs and found to be higher in plant rhizosphere of OBD spoils even in comparison to natural forest soils[25].

Similarly, the study of soil microbial biomass carbon (MB-C) of the opencast coal mining area of Tikak Colliery, Margherita, also revealed that one third of the MB-C reduced in OBD spoils due to mining in comparison to the Natural forest soil adjacent to the mined area[18]. The reduction of microbial activity indicates that the mining reduced active microbial population. The study of actinomycetes and bacteria of non-vegetated, vegetated and undisturbed sites in coal mine area of West Verginia reported that their numbers increased in mine spoils during vegetation progressed[26]. The lowest number of fungal genera was recorded in the un-vegetated area from strip-mined habitats of Ohio, USA[27]. The microbial populations in coal mine overburden spoils were observed to increase sharply in relation to vegetation development in Gevra Colliery, Bilaspur, M.P. The increase was directly proportionate with an increase in age of vegetation on the overburden[28,29]. They also obtained a positive correlation between the number of organisms and organic carbon and total nitrogen in spoil material. The microbial biomass and nutrient availability in dry tropical forest of the Vindhyan Hill Region of M.P. was studied for the effect of coal mining and observed that microbial contributed substantially to the N and P requirements of growing vegetation as a functional index of soil redevelopment after mining[19].

AM fungi and other amendments for revegetation in coal mine

Application of amendments such as fresh organic matter and lime could increase moisture holding capacity of soil and decreased soil acidity resulting in better activity of AM fungi for successful revegetation[30]. Establishment and growth enhancement of the plant in coal mine waste amended by only the topsoil was reported by many workers[31-33]. Moreover, sufficient caution have to be taken during application of organic and inorganic amendments as mycorrhizal colonization in plant roots and its contribution to plant growth may be affected by the organic and inorganic amendment[34]. Furthermore, application of limestone and NH_4NO_3 was observed to increase more than 90% of AM fungal infection with a striking improvement in growth and biomass production of three legume crops[35]. While grown maize and soybean in two tropical acid soils the application of lime was recorded for increased in AM fungi colonization and simultaneous enhancement of shoot-dry weight, total root-length of the maize and soybean crops[36]. Moreover, several research findings supports lime application for improve the status of exchangeable Al and /or Mn and increased root growth[37-40]. AM colonized seedlings of *Artiplex canescens* planted onto a 4 year old coal mine spoil were found superior in growth and survival compared to the non-mycorrhizal plants[41]. AM fungal inoculation of red maples in anthracite wastes stimulated plant growth, when P was supplied externally[42]. However, traditional application of organic matter such as compost, yard waste and municipal waste locally available may also be instrumental for promotion of the plant cover and also for better crop biomass[15]. They also reported that these amendments, even improved soil reaction, available nitrate, ammonia, phosphate as well as cation exchange capacity (CEC) of mine spoils. Application of P fertilizer and sewage amendment to sub alpine coal mine spoils did not decrease in AM fungi development[43]. They apprised that the initial application of an amendment to coal mine spoils could have significant effects over the time not only on the development of AM fungi but also brought about adequate success in revegetation programme. Some workers studied the growth response of AM fungal status on plant in disturbed sites by transferring topsoil from vegetation community of adjoining areas to the rooting zone of the transplanted cuttings of seedlings[44]. Topsoil addition had favorable effect on plant growth in coal mine-disturbed sites. Soil transfer could be a simple method of stimulating plant growth in disturbed sites[45]. It was suggested that soil transfer from adjacent natural native communities, augmented plant growth and establishment due to addition of adapted mycorrhizal fungi in the form of mycelia, spores and /or infected plant roots as well as bacteria and other microorganisms to the rhizosphere in spoils. Soil microorganisms have interactive effect in stimulating plant growth. For example, bacteria may help or hinder mycorrhizal formation[46]. Similarly, the treatment of AM fungi along with phosphate solubilizing bacteria in combination could be a deciding factor for improvement of more plant growth in comparison to the treatment with either organism alone[47].

Soil microorganisms other than AM Fungi in revegetation of coal mine OBDs

The importance of microorganisms in soil formation and revegetation through their activities as decomposers and nutrient cyclers, Nitrogen fixers has been widely recognized[48]. The symbiotic association of nitrogen fixing trees (NFT) and *Rhizobium* bacteria or *Frankia, Actinomycetes* as one of ameliorates was reported successful in the rehabilitation programme of coal mine areas[49]. The study revealed that micro-symbionts of NFTs are not only important as play a key role in supply of nutrients to their host but also they could enhance the biomass accumulation in their hosts significantly. Micro-symbionts protect the host from various stress such as desiccation[50], heavy metal toxicity[51], bring success in the rehabilitation[52] and produce hormones that help in seed germination[53] and root initiation[54]. Therefore, development of most appropriate micro-symbionts in root and rhizophere of their host on the mined wastes would be an important element of a successful rehabilitation effort.

AM fungi, nitrogen fixtures and revegetation of coal mine spoils in India

Studies on revegetation of coal mine spoils and their AM fungi status in India are limited. There was a successful plantation of 13 species of plant in coal mine overburdens of Madhya Pradesh[55]. The pits for plantation in these overburden dumps were filled with soils brought from adjacent natural Sal forest. However, AM fungal status of these coal mine overburdens was not studied. The effectiveness of nitrogen fixing tree (NFT) s with *Rhizobia* and AM fungi association in stimulation of plant growth was reported in coal mine spoils[49]. AM fungal status in the succession of plant communities in coal mine overburden dumps of any region of the country was scanty except work on the natural recovery pattern of plant, microbial and nutritional status of coal mine spoils with age[29]. Plantation of leguminous species sapling was done on overburden spoils of coal mine dumps of SECL, Bilaspur (M.P.) amended with topsoil of nearby area and FYM after inoculating with mycorrhizae-*Rhizobium* and non-leguminous plants were inoculated with mycorrhizae- *Azotobacter*[7]. Though these amendments they increased soil stability and plant growth.

Revegetation using AM fungi and other beneficial microorganisms in Makum Coalbelt

Coal mining activities in Makum Coal belt, Assam have created a large area of wasteland in the form of OBDs over natural forest**.** Coal mine overburden spoils of Tikak Colliery are characterized by poor to moderate strength of rooks represented by mudstone, clay, sandy clay, siltstone with sandy shale and carbonaceous shale. pH of the spoils ranges from 2.5 –5.5. Barren OBDs are devoid of soil beneficial microorganisms for establishment of plant growth[56].

In 1-and 5-year coal mine overburden dump (OBD) spoils of Tikak Colliery, Makum Coalbelt, Assam, the effect of application of arbuscular mycorrhiza (AM) fungi, phosphate-solubilizing bacteria (PSB), other bacteria and fungi (GBF) and amendments with farm yard manure (FYM), lime, topsoil was studied in a pot experiment for on biomass production of *Crotalaria striata* DC[17]. Apart from that the study also evaluated the contribution of different treatments and amendments on status of physicochemical and microbial properties of colliery spoils[17]. According to the experimental findings, AM fungi could colonized in plant roots and also was instrumental for enhancing the above ground biomass in inoculated pots. The number AM fungal spores multiplied many folds in the colliery spoils at harvest in the inoculated pots in 1-year OBD spoil in comparison to the original number of spores inoculated to them. The treatment combination of Lime, AM fungi and FYM had favorable effects on biomass production, both in 1-year and 5-year OBD spoils. Dual inoculation of AM fungi with PSB was also found most effective on plant growth. Interestingly, plant growth was marked as poor in 5-year OBD spoil in comparison to 1-year OBD spoil irrespective of all the treatment combinations applied. Such poor growth of plants in 5 year OBD spoils could be a reflection of more harsh condition for plant growth in 5-year OBD spoils, because of presence of very high level of H+ ions in the spoils. However, the amendment of natural forest topsoil was also effective in improving plant growth in coal mine spoils. Similarly, in accordance to the variations shown in the rate of plant growth enhancement by different treatments, however, similar trend was also revealed in case of improvement of physico-chemical and biological properties of colliery spoils. In other words, the performance of best treatment combinations for plant biomass production was also as equally superior in improving status of physico-chemical and biological properties of colliery spoils[17].

Effectiveness of AM fungal inoculum and lime, FYM and the topsoil from the 10 year OBD spoils in promotion of growth of two herb species *Crotalaria striata* and *Mimusa pudica* was tested in the harsh environment of 1 and 5 year OBD spoils in a pot experiment[56]. Results of the pot experiment suggested that 5-year OBD spoils was harsher than the 1-year OBD spoils, where the plant biomass production, and AMF populations were less as well as values of soil chemical parameters were lower than that of 1-year OBD spoils. The main reason for low level of biomass production in 5-year OBD could be the low pH. Under condition of low soil pH, activities of both plant species and the soils inhabiting plant growth-promoting microorganisms were also lower[57]. This, in turn resulted in low plant biomass production in 5-year OBD spoils. Application of AMF inoculum and other amendment viz. lime, FYM and topsoil in different combination increased plant biomass production in both the OBDs spoils (1 and 5 year) under pot culture condition. Application of lime in improving the acidity and activities of AMF in mine spoils has also been found by other workers[36]. Combine application of Lime, AMF, FYM treatment was the best among different treatments in increasing both the plant biomass production and improvement of soil quality[56]. Amendment of topsoil in coal mine spoils of both 1 and 5 year OBD spoils was useful in increasing the plant biomass production and root length colonization. The topsoil amendment from natural forest soil might have added beneficial microorganisms and infective AMF propagules in the form of spores, infected root segments and fungal hyphae[24].

The topsoil from 10 year OBD soils was also a useful treatment in increasing biomass production. Therefore, the effect of there five treatments on a native leguminous plant was tested subsequently in a 5 year old OBD field at Tikak colliery. The results of the field experiment also indicated that plant growth could be enhanced in the harsh environment such as 5 year OBD by using lime, AMF and topsoil as amendment. The reason for better growth of plant in topsoil-amended soil was investigated in a pot experiment. It was hypothesized that topsoil contained beneficial microorganisms such as Fluorescent Pseudomonas (FP are known for plant growth promoting activity) which stimulated plant growth in OBD spoils. This inoculum was used in different treatment combination to test their effect on growth of *Crotalaria striata* DC. The result of this experiment was the best also for Lime, AMF, FYM treatment combination for plant growth. The same was also obtained in the field experiment also with the best treatment combination[25].

The beneficial micro flora of the topsoil of natural forest adapted well and manifested its effect. Inoculation of *C. striata* with AMF isolates along with the FP isolates of 10 year OBD was observed as beneficial for its growth[56]. As pure culture, large population of fluorescent Pseudomonas (FP) was introduced and these cells perhaps could out complete the indigenous microflora and colonize in the rhizosphere of *C. striata*. Subsequently, the plant obtained the benefit from the FP, and this was manifested in higher level of biomass production. FP isolates of natural forest soils were also equally effective in increasing biomass production by *C. striata*. Beneficial effect of some bacterial like FP in plant growth promotion alone or with AMF in dual inoculation is well known[58-61]. Mechanisms such as root growth enhancement through hormonal effect enhanced nutrient uptake and disease suppression are well reported in literature for growth enhancement of plant due to inoculation of FP[62]. But, the mechanisms of enhancement of *C. striata* growth by the FP isolates of the natural forest and 10 year OBD spoils observed in that study could not be ascertained by the researcher. That growth increment may also be achieved because of inoculation of other bacteria and fungi isolates from the natural forest soils and 10 year OBD spoils which was applied with the AMF inoculum. But the effect of bacteria and fungi isolates of 10 year OBD was not as conspicuous as that of natural forest soil[17].

The combined effect of fluorescent Pseudomonas (FP) and AMF of natural forest was much better in biomass production than that topsoil of natural forest and or 10 year OBDs and even FP and AMF of 10 year OBD spoils[24]. However, effect of topsoil in biomass production was better than AMF treatment. Increase in AMF status of plant and soil FP in AMF and FP treatment was an indication of synergistic effect of these soil microorganisms.

Conclusion

Coal mine OBDs of Makum Coal belts, Assam represent varying degree of hostility since its creation with time to plant growth and the harshest conditions was recorded in 5-year OBD among the different aged OBDs. This was evident from presence of elemental sulphur contamination in spoils and the consequent response of OBD spoils to plant growth and biomass production in both pot experiments and field trial. AM fungi component of the plant rhizosphere soils was also observed to change depending upon the age of the OBDs. The research results implicate that revegetation in OBD spoils could be enhanced by application of AMF inoculum under both pot and field conditions. Application of lime and FYM along with AMF inoculum was very effective in enhancing plant growth of OBD spoils. Topsoil from revegetated 10-year OBD or adjacent natural forest could also serve as an amendment for plant growth in OBD spoils. The beneficial effect of topsoil could be due to the presence of beneficial microorganisms including AMF, PSB and fluorescent Pseudomonas. Therefore, this two important treatments, i.e. combination of Lime, AMF and FYM and topsoil from either 10-year or natural forest can be taken up for revegetation of OBDs.

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