IMPACT OF BIOCHAR AND CSR-BIO APPLICATION ON METHANOTROPHS, MICROBIAL BIOMASS AND PADDY YIELDS IN SALINE SOIL

SUMMARY OF THESIS

Submitted to

Babasaheb Bhimrao Ambedkar University

(A Central University), Lucknow

BABASAHEB BHIMRAO AMBEDKAR UNIVERSITY

LUCKNOW

प्रज्ञा शील करुणा

ESTABLISHED 1996

For the Degree of

Doctor of Philosophy

In

ENVIRONMENTAL MICROBIOLOGY

Submitted By

Chhatarpal Singh

(Enrolment No 187/10)

Under the Supervision of

Dr. Jay Shankar Singh

Department of Environmental Microbiology

(School for Environmental Sciences)

Babasaheb Bhimrao Ambedkar University

(A Central University)

Vidya Vihar, Rae Bareli Road, Lucknow-226 025

Uttar Pradesh, India

2018

SUMMARY

India being an agriculture-dominant country produces more than 500 million tons of crop residues wastes annually. The huge crop residues of rice, wheat, cotton, maize, millet, sugarcane, jute, rapeseed-mustard, groundnut and other crops are typically burnt on agriculture farm sites across different states of the India. A large portion of unused crop residues are burnt every year in the fields primarily to clear the left-over straw and stubbles after the crop harvest. The problem is more severe in the irrigated paddy agriculture, particularly in the mechanized rice-wheat system of the north-west region of the country. Non availability of technically trained peoples, high cost of residue removal from the field and increasing use of combines in harvesting the crops, are main reasons behind burning of huge amount of crop residues at the fields. Burning of crop residues not only causes environmental pollution, but is also responsible for the loss of agriculturally important soil microbial community and abundance (Singh *et al*., 2017). Since, microbes are the crucial biological agents Therefore, appropriate management strategies of crop residues to agricultural use may assumed as a viable and sustainable option for enhancing the key beneficial microbial community in soils to agriculture and environment (Singh *et al*., 2011; Singh and Pandey, 2013; Singh, 2015). It is need of the hour to develop recent research efforts in conservation of agriculture-based crop management technologies which may be beneficial to enhance the beneficial soil microbial diversity and biomass (Singh and Gupta, 2018), agriculture soil fertility and crop productivity.

Sustainable degraded agriculture land management approaches such as organic farming, novel microbial inoculation with suitable bio-inoculant carriers have been considered as key tools for combating the loss of soil fertility and crop productivity. The inoculation of microbial bio-formulations, developed from agriculturally important microbes, in combination with suitable organic amendments has been demonstrated to speed up the restoration of degraded land soil fertility within short period of time (Singh *et al*., 2016). Evidently, the direct inoculation of beneficial microbial consortia in combination with suitable supporting soil amendments/carrier material can be a viable and new efficient tool to contribute significantly in enhancement of microbial density and biomass, which can help considerably to agro-ecosystem sustainability and crop productivity. Therefore, in this experiment, the CSR-BIO, a commercial bio-formulation consortia prepared from agriculturally beneficial microbes (Damodaran et

al., 2013), was used with cow dung manure (as carrier material) and RHB as soil conditioner. We hypothesized that the addition of RHB with CSR-BIO mixture will have positive effects on soil physico-chemical properties, methanotrophs abundance, SMB levels and paddy productivity. It may also be further assumed that the application of RHB in combination with novel microbial bio-formulation mixture (CSR-BIO developed by Damodaran *et al*., 2013, IISSR, Lucknow) would increase synergistically the soil available inorganic-N nutrients to paddy plants in nutrient deprived soils. However, the experimental evidences and answers for the above raised arguments and questions in field conditions are still to be investigated. Therefore, this study focused on impact of RHB application in combination of microbial bio-formulation-CSR-BIO (consortia of *Bacillus pumilus*, *Bacillus thurigensis* and *Trichoderma harzianam*) on soil physico-chemical properties, paddy crop yields, SMB, and methanotrophs diversity and abundance in paddy field.

Since, application of RHB in combination with suitable commercialized microbial bio-formulation (CSR-BIO) as supporting amendment, from agriculture paddy filed conditions are lacking therefore, to find out the answers of above raised questions, the present doctoral research work has been carried out with the following objectives:

1. To assess the impact of CSR-BIO and Biochar application on soil physico-chemical properties of saline paddy fields.
2. To find out the influence of CSR-BIO and Biochar treatments on paddy yields.
3. To study the microbial biomass-C, -N and P variations as affected by CSR-BIO and biochar amendments.
4. To assess the influence of CSR-BIO and Biochar amendments on methanotrophs abundance and diversity.

Experimental design, treatments and paddy cultivation

A field experiment for two consecutive years (2015-2016) with paddy cultivation was carried out at Agriculture Research Farm of Babasaheb Bhimrao Ambedkar University located in Lucknow, Uttar Pradesh (India). Total 12 experimental plots each having dimension of 3×2 meter was established in completely randomized block design (RBD). Four treatments i.e. rice husk biochar (RHB), CSR-BIO (a commercial microbial bio-formulation), and RHB+CSR-BIO, including one control plot (without any treatment) was also established in triplicate. Except control plot, RHB and CSR-BIO were applied at a rate of 10 t ha^{-1} as described, respectively by Zhang *et al*. (2010)

and Damodaran *et al*. (2013). For this study, paddy (*Oryza sativa*) was selected as experimental crop. For the rice variety namely HUR 9-10 Hindu University Rice-9-10 was obtained from Department of Genetics and Plant Breeding, Institute of Agriculture Sciences, Banaras Hindu University (South campus), Mirzapur, Uttar Pradesh. The nursery of rice cultivar was prepared on 20 June during both the years 2015 and 2016. After 25 days of nursery growth, the paddy seedling was transfer to the experimental plots. Three hills (each with 2 seedlings) were transplanted on 25 July in both the years. Frequent irrigation (a water level of 6-12 cm) avoiding waterlogged condition, was provided throughout the crop cycle. At 105 day after transplantation (DAT), the matured paddy crop was harvested for the determination of selected paddy agronomic variables. The paddy agronomic variables such as panicle length (cm), tiller numbers ($plant^{-1}$), rice grain yield ($t\ ha^{-1}$) and paddy straw yield ($t\ ha^{-1}$) was determined according to Mahamud *et al*. (2013) and Amanullah and Inamullah (2016).

1. Impact of RHB and CSR-BIO and application on soil physico-chemical properties in paddy soils

The results showed that compared to treated plots, maximum electrical conductivity (EC) and pH, respectively was noted for untreated (control) plot in both the years. Across different treatments gravimetric soil moisture (GSM) and water holding capacity (WHC) were highest in RHB + CSR-BIO treated plots and lowest in control plots for each year. Compared to treated plots, bulk density (BD) was lowest in control plot. During both the years, among different treated plots the values of total-N, total-C and total-P were lowest in control plot than the treated soils. ANOVA revealed that studied soil physico-chemical characteristics such as EC, pH, SM, WHC, BD, total-N, C and -P varied significantly due to treatments. Across different treatments, inorganic-N (ammonium- and nitrate-N) levels and N-mineralization were minimum in control plots and maximum in RHB +CSR-BIO treated plots in both the years. Across different sampling dates, ammonium-N and nitrate-N levels were noted minimum on 35 DAT (tillering stage) and maximum on 105 DAT (maturity stage) in both the years. Across different sampling dates, N-mineralization was noted minimum on 0 DAT and maximum on 105 DAT (maturity stage) in both the years. ANOVA indicated significant difference in ammonium-N, nitrate-N and N-mineralization due to sampling dates for 2015 ($P = < 0.001$) and 2016 ($P = < 0.001$).

This study demonstrates that RHB and CSR-BIO application improves the soil physico-chemical conditions that in turn enhance the availability of inorganic-N (ammonium- and nitrate-N) and rate of soil N-mineralization in paddy soil. The long term use of RHB may beneficial to enhance the soil nutrient status of nutrient deprived and saline soils. Thus, the plant residues after plant harvest such rice husk can be converted into RHB and in combination with CSR-BIO or other PGPR bio-formulations/amendments could be important strategies for enhancing the rate of soil N transformation and beneficial available N nutrients in poor paddy agriculture soil. This experiment was carried out only with the application of RHB and CSR-BIO, but other suitable organic amendments, green manures, farm yard manure (FYM) derived from crops residues may be used for restoration of soil fertility of disturbed soils.

2. Influence of RHB and CSR-BIO treatments on paddy yields

The results suggest that variation in paddy agronomic variables (variables such as panicle length, tiller number, rice grain and paddy straw yield) due to RHB and CSR-BIO treatments for two consecutive years (2015 and 2016) are statistically significant. All the selected paddy agronomic parameters were found greater in treated plots (maximum in RHB + CSR-BIO treatment) compared to untreated (control) plot. It was interesting to note that among the various selected paddy agronomic variables the influence of RHB treatment was more effective for rice grain yield.

All the selected paddy agronomic parameters (panicle length, tiller number, rice grain and paddy straw yield) were found greater in treated plots (maximum in RHB + CSR-BIO treatment) compared to untreated (control) plot. ANOVA showed significant difference ($P = < 0.001$) in paddy agronomic variables due to treatments. The percent increase in panicle length, tiller number, rice grain yield and paddy straw yields in RHB + CSR-BIO treated plot was comparatively than other treatments for both the years. Further, it was interesting to note that among the various selected paddy agronomic variables the influence of RHB treatment was more efficient for rice grain yield. The high levels of soil organic carbon accumulation in RHB amended plots in present study can enhance N efficiency and increase paddy productivity in nutrient poor paddy soils of tropical regions. Though, the effect of biochar on the selected paddy agronomic variables (tiller numbers and panicle length) is difficult to elucidate because there is insufficient information based on the present experiment or previous studies. However, the positive effects of RHB treatments on paddy yields in present study may be attributed to the nutrients directly available to the paddy plants by the RHB because of having sufficient trace elements (Table 3.1 Chapter 3). Further, the increase in paddy yield witnessed is basically due to increase in nutrient mobilization to the paddy crop plants from the rhizosphere soil which has been enabled by the inoculated microbial consortia present in CSR-BIO to harness the available nutrients from the soil strata in field condition.

When the data were pooled across different treatments and sampling dates, the paddy agronomic variables shoed negative relationship with inorganic-N (ammonium- and nitrate-N) contents and positive with SMB-C, -N and -P. The results showed that SMB levels in the paddy soil were drastically reduced on 35 (tillering stage) and 65 (flowering stage) days after transplantation (DAT). It is assumed that the paddy plant during these days (an active crop growth period) may possibly require a greater amount of soil available nutrients to support the vegetative growth of crop plants. This could be the reason for a significant reduction in SMB quantity (Figure 3) during active paddy growth periods (tillering and flowering stage). During active paddy growth period, a strong demand of soil nutrients by the crop plants may compete for available soil nutrients with microbial community and consequently led to a reduced SMB levels. The higher levels of SMB-C, -N and -P in this study on 105 DAT (crop maturity stage) could be due to a reduced demand of available soil nutrients by the inactive paddy crop growth conditions. Furthermore, the situation of higher SMB levels at the paddy crop maturity sampling date (105 DAT) could have also arisen because of greater microbial nutrient N-immobilization due to reduced available-N nutrients demand by matured paddy plants. These situations make easy availability of nutrients to micro-flora and consequently a higher SMB build-up. In view of the above arguments, when the pooled data across different sampling days were considered for correlation analysis, a positive relationship between SMB and paddy plant growth parameters might be expected as given in below table.

Table. Linear regression parameters, correlation coefficient and significance levels for the relationships of paddy agronomic variables (*Y*) and soil variables (*X*) in rice husk biochar (RHB) and CSR-BIO treated agriculture soil.

Y-variables	*X*-variables (µg g^{-1}dry soil	a	b	N	R^2
Panicle length (cm)	Ammonium-N	-0.1454	8.208	12	+ 0.8407**
	Nitrate-N	-0.1063	6.2462	12	0.7653**
	SMB-C	13.615	12.989	12	0.6544*
	SMB-N	5.5753	83.341	12	0.7502**
	SMB-P	1.5278	15.031	12	0.9547**
Tiller number ($plant^{-1}$)	Ammonium-N	-0.1137	7.4575	12	0.8381**
	Nitrate-N	-0.0823	5.6752	12	0.7464**
	SMB-C	11.092	46.531	12	0.7077**
	SMB-N	4.5389	58.888	12	0.8102**
	SMB-P	1.2042	7.3682	12	0.9665**
Rice grain yield (t ha^{-1}	Ammonium-N	-0.3997	6.335	12	0.551*
	Nitrate-N	-0.2177	4.5697	12	0.2782^{NS}
	SMB-C	52.819	99.208	12	0.8539**
	SMB-N	20.592	33.135	12	0.8873**
	SMB-P	4.3081	4.2099	12	0.6582*
Paddy straw yield (t ha	Ammonium-N	-0.2309	6.5405	12	0.3648^{NS}
	Nitrate-N	-0.1004	4.4786	12	0.1174^{NS}
	SMB-C	35.667	30.733	12	0.7728**
	SMB-N	13.656	57.838	12	0.7746**
	SMB-P	2.5355	1.6155	12	0.4526 *

* P = < 0.05; ** P < 0.001; NS= Not significant; SMB= soil microbial biomass

The paddy plant growth parameters (number of tillers and panicle length) and yields (rice grain and paddy straw) enhancement, following to RHB and CSR–BIO application, could be attributed to the synergistic effects of combined amendments on soil nutrients availability to paddy crop plants in nutrient poor soils. The RHB generation from paddy rice husk and its application with beneficial microbial inoculants may be a sustainable crop residues waste management option to enhance the nutrient status and paddy productivity of nutrient poor agriculture soils.

3. Impact of RHB and CSR-BIO treatments on soil microbial biomass (SMB)-C, -N and -P in paddy field condition

Across the treatments highest quantity of SMB-C, -N and -P was observed in RHB + CSR-BIO treated plots compared to other treatments. Data showed that variations in SMB-C, -N and -P due to treatments were significant ($P = < 0.001$). Across different sampling dates, the SMB-C, -N and -P was recorded minimum on 65 DAT (tillering stage) and maximum on 105 DAT (maturity stage). ANOVA indicated significant differences ($P = < 0.001$) in SMB-C, -N and -P quantity due to treatments and sampling dates in both the years 2015 and 2016.

The result suggests that RHB and CSR-BIO added alone or in combinations to the soil contributes significantly to the enhancement of soil physico-chemical properties and therefore, microbial biomass in nutrient poor paddy agriculture soils of tropical regions. The results also revealed that RHB amendments notably enhanced the SMB. The RHB, having large surface area, pore size and nutrient elements provide favourable soil conditions for the growth and multiplication of microbial communities and consequently higher SMB levels, in nutrient deprived paddy agriculture soil. The RHB generation from paddy rice husk and its application with beneficial microbial inoculants may be a sustainable crop residues waste management option to enhance the nutrient status, microbial community of nutrient poor agriculture soils. It is suggested that, use of chemical fertilizers could be reduced to enhance the economic benefits and agriculture soil health because of RHB and CSR–BIO application. Available N immobilization and its release by SMB is an adaptation to provide nutrients to plants in nutrient limited ecosystems. The results of this investigation are based on short-term duration so, larger scale paddy cultivation experiments under field conditions, are required to verify the mechanisms involved in RHB-microbes interactions for soil fertility improvement and microbial community compositions.

4. Influence of CSR-BIO and Biochar amendments on methanotrophs abundance and diversity

During the two consecutive years (2015-2016) of paddy crop cycle, the average methanotrophs populations were counted highest in the soil of RHB + CSR-BIO treated plots for both the study years 2015 ($54.75 \pm 0.98 \times 10^{-5}$ cells g^{-1} dry soil) and 2016 ($57.25 \pm 0.88 \times 10^{-5}$ cells g^{-1} dry soil). Across different sampling dates the maximum methanotrophs abundance was noted at 65 DAT compared to control plot during both the years. At all the soil sampling days the RHB+CSR-BIO amended plot showed higher number of methanotrophs population compared to other treatments. The ANOVA showed that differences in methanotrophs population due to treatments were statistically significant (P=<0.001) for both the study years. The higher methanotrophic bacterial community in RHB+CSR-BIO treated soil could be due to the development favourable soil conditions because of higher organic contents and optimum soil moisture that may support the growth and multiplication of methanotrophic. The variations in soil physico-chemical properties due to RHB and CSR-BIO amendments could be one of the major reasons for the variations in methanotrophic abundance across the treatments. While unfavourable soil in control plot may suppress the methanotrophs population growth. Beneficial microbial agents present in CSR-BIO formulation might create beneficial conditions to enrich the soil physico-chemical environment therefore; a higher number of methanotrophs might be build up in nutrient rich soil. The increase in methanotrophs abundance in RHB treated soil could be due to the improved soil physico-chemical properties as well as nutrient sources because of favourable soil conditions that supports the growth and multiplication of methanotrophs. Further, the lager pore size of RHB could provide shelter to soil methanotrophs and protects them from soil microbes feeding (predator) such as protozoa, nematodes, etc. It is suggested that RHB may optimize the soil aeration and moisture conditions which favours the colonisation of methanotrophs. It is expected that long term application of RHB in soil could promote activity, growth and community structure of methanotrophs (Bender and Conrad 1995; Henckel, 1999). So, increasing methanotrophic abundance and diversity in paddy soils due to application of RHB can contribute to enhance the CH_4 consumption.

To find out the impact of different treatments in paddy soil, the correlation analysis was performed between methanotrophs number and some relevant soil properties such as soil moisture, WHC, soil pH, total-C, ammonium- and nitrate-N. A positive significant correlation between methanotrophs number and soil moisture (N=12; R^2 = 0.6734; P=<0.001) showed that soil moisture is an important factor that regulates growth and multiplication of methanotrophs in dry tropical paddy soils. It has been also reported that methanotrophs are very sensitive microbes affected by soil moisture conditions (Singh *et al.,* 2010). This indicates that adequate soil moisture could be a necessary factor for the optimal functioning of methanotrophs in paddy soil. The information available about the impact of soil moisture on methanotrophs in RHB treated paddy soil indicate that soil moisture could be a very crucial parameter that may govern the community composition of these unique group of bacteria and their role in methane sink activity from upland paddy soil.

The DGGE profile of the study soil samples showed variations in methanotrophic community composition in treated and control soils. It is clear from the occurrence of more diverse bands in RHB treated soil attributed to a greater methanotrophic community diversity compared to control soils. Since, all the methanotrophic genera in this study were identified as the type-I methanotrophs (*Gammaproteobacteria*) it may be proposed that type-I methanotrophs are predominant in paddy soils. The CP1 band-1 and band-4 of RHB treated soil showed highest similarity with the two methanotrophic genera of family Methylococcaceae. So, both these bands may belong to the methanotrophic genera such as *Methylomonas*, *Methyloglobulus* and *Methylobacter*. However, CP1 band-5 revealed highest closeness to the methanotrophic genera *Methylobacter*. Likewise in control soil CP2 band-1 was closely related to only single methanotrophic genera *Methylosarcina*. In this study, the differences in banding patterns observed in the DGGE profile, suggesting that RHB treatments in paddy soil altered the methanotrophic community structure. Therefore, the long term application of RHB and other organic amendments in upland paddy soil could be a beneficial option to enhance the methanotrophic abundance and diversity in disturbed saline soils. This study revealed that the long term application of RHB in paddy fields could suppress the emission of CH_4 due to increasing the diversity and abundance of methanotrophs in amended soil. But the study relating RHB and methanotrophs in paddy fields, regarding CH_4 reduction and higher crop production is ambiguous so there is the need to further study because paddy agriculture is contribute major share in the production CH_4 of total anthropogenic sources.

The present study showed a higher methanotrophic bacterial abundance and community in RHB treated soil compared to control plots of saline paddy field. The variations in soil physico-chemical properties due to RHB amendments has been found as major reasons for the variations in methanotrophic abundance across the treatments. The unfavourable soil conditions in control plot suppressed the number of methanotrophs. The improved soil physico-chemical properties as well as nutrient sources in RHB treated soil supported the growth and multiplication of methanotrophs. The lager pore size of RHB provide shelter to soil methanotrophs and protects them from soil microbes feeding (predator) such as protozoa, nematodes, etc. and therefore, a higher methanotrophs population build up.

Our study demonstrated that the abundance and methanotrophic microbial community are apparently affected RHB treatments in paddy soils and hence that bacterial abundance and diversity has to be taken into account in the global biodiversity debate. Alteration in soil physico-chemical characteristics due RHB treatments are crucial factors that may provoke shifts in methanotrophs abundance and community compositions in paddy up land soils. Type-I methanotrophs has been found as the dominant bacterial group in the paddy soil of present study. However, to confirm this, extensive ecological experiments have to be carried out with pure cultures of methanotrophs isolated from upland paddy soils differing in methanotrophs abundance and diversity. The plant residues based biochar and fertilizer addition studies have to be carried out in paddy soil habitats with dominance of methanotrophs types (type-I, type-II, etc.) and of, where methane consumption has to be linked with diversity by using advanced tools and techniques. Finally it can be said that biodiversity conservation and land use policy makers should also consider methanotrophic abundance and diversity, which is apparently important for global green house gases particularly methane reduction in soils.

Bibliography

Singh, C., Tiwari, S., Boudh, S., and Singh, J.S. 2017. Biochar application in management of paddy crop production and methane mitigation In: Agro-Environmental sustainability: managing environmental pollution. Singh, J.S., and Seneviratne, G. (eds.). Springer. pp. 123-146.

Singh, J.S., Pandey, V.C., and Singh, D.P. 2011. Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. *Agric. Ecosyst. Environ.* 140, 339-353

Singh, J.S., and Pandey, V.C. 2013. Fly ash application in nutrient poor agriculture soils: impact on methanotrophs population dynamics and paddy yields. *Ecotoxicol. Environ. Saf.* 89, 43-51.

Singh, J.S. 2015. Microbes: the chief ecological engineers in reinstating equilibrium in degraded ecosystems. *Agric. Ecosyst. Environ.* 203, 80-82.

Singh, J.S., and Gupta, V.K. 2018. Soil microbial biomass: A key soil driver in management of ecosystem functioning. Sci. Total Environ. 634, 497-500.

Singh, J.S. Abhilash, P.C., and Gupta, V.K. 2016. Agriculturally important microbes in sustainable food production, *Trend Biotechnol.* 34, 773-775.

Damodaran, T., Rai, R.B., Jha, S.K., Dhama, K., Mishra, V.K., Sharma, D. K., Singh, A. K., and Himanshu, D., 2013. Impact of CSR-Bio- An eco-friendly bio-growth enhancer on increasing the profitability of horticultural crops to small and marginal land holders. *Int. J. Curr. Res.* 5, 2682-2685.

Mahamud. J.A, Haque, M.M., and Hasanuzzaman, M. 2013. Growth dry matter production and yield performance of transplanted Aman rice varieties influenced by seedling densities per hill. *Int. J. Sustain. Agric*. 5, 16-24.

Amanullah, and Inamullah. 2016. Dry Matter Partitioning and Harvest Index Differ in Rice Genotypes with Variable Rates of Phosphorus and Zinc Nutrition. *Rice Sci.* 23, 78-87.

Bender, M., and Conrad, R. 1995. Effect of CH4 concentrations and soil conditions on the induction of CH4 oxidation activity. *Soil Biol. Biochem.* 27, 1517-1527.

Henckel, T., Friedrich, M., and Conrad, R., 1999. Molecular analyses of the methane oxidizing microbial community in rice field soil by targeting the genes of the 16s rRNA, particulate methane monooxygenase, and methanol dehydrogenase. *Appl. Environ. Microbiol*. 69, 1980-1990.