

Microbial consortia for bioremediation of agricultural pollutants

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With the fast growing world population a huge portion of agricultural land is being depleted each year by industries and urban encroachments, which in turn is creating food scarcity. After Green Revolution the promotion of high-yielding varieties of crops has led to large-scale use of chemicals as pesticides. The increased demand of agro-products in changing regional climate has resulted in an increase in consumption and application of pesticides. The extensive use of pesticides leads to an accumulation of a huge amount of pesticide residues in the environment, therefore causing a substantial environmental health hazard due to uptake and accumulation of these toxic compounds in the food chain and drinking water. The hazardous effects of pesticides draw attention towards its removal from the environment. Although, various conventional methods are able to degrade persistent pollutants hazardous to human health as well as the environment, but they are less efficient. Bioremediation is an option that offers the possibility to destroy or render harmless various contaminants using natural biological activity. The microbial action of bacteria, fungi or their consortia in the environment causes the natural degradation of the pesticides which might convert parent compounds to intermediates or comparatively less toxic compounds. As such, it uses relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on-site.

Key words: Agricultural pollutants, Bioremediation, Microbial consortia, Pesticides

THE population explosion induces human civilization to produce more food from available limited resources. Depletion of agricultural land becomes a matter of concern due to urbanization and industrialization which in turn results in food scarcity. Moreover, crop production is severely affected by crop enemies like pathogens, insects, weeds, nematodes etc. which adds on food scarcity to a new height with the fast growing world population. To combat these crop enemies, pesticides were used which include a wide range of compounds viz. insecticides, fungicides, herbicides, rodenticides, molluscicides, nematicides etc. Use of these pesticides results enhanced economic potential in increased production of food. On the contrary, it causes

serious health implications to man and his environment. Overwhelming evidences are now being found that these chemicals act as a potential risk to human beings and other animates and cause unwanted side effects to the environment and so are considered as serious agricultural pollutants. In India, the first report of poisoning due to pesticides was in Kerala in 1958, where over 100 people died after consuming wheat flour contaminated with Parathion. This prompted the Special Committee on 'Harmful Effects of Pesticides' constituted by the ICAR to focus further on the problem. Thus, approaches to reduce the harmful effect of these pesticide residues become need of the hour to safeguard our environment and human health. Although there are

many physico-chemical strategies to combat the problem, the use of micro-organisms and their consortia direct a new era for bioremediation of these agricultural pollutants which provides a safe, economic and environment friendly approach.

BIOREMEDIATION

Bioremediation may be defined as the use of living organisms, primarily microorganisms, to degrade the environmental contaminants into less toxic forms. It uses naturally occurring bacteria and fungi or plants to detoxify substances hazardous to human health and/or the environment. The micro-organisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Living organisms

through their metabolic reactions transform the contaminant compounds into their less toxic forms. Biodegradation of a compound is often, a result of the actions of multiple organisms. Bioremediation was successfully applied for clean up of soil, surface water, groundwater, sediments and ecosystem restoration.

Principle of bioremediation

The micro-organisms must enzymatically act upon agri-pollutants to transform them into harmless products. For bioremediation to be effective the environmental parameters must be favourable to the micro-organisms permitting their growth and showing their activity to the fullest. So, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate. Bioremediation techniques are typically more economical than traditional methods, and some pollutants can be treated on-site. Most bioremediation systems run under aerobic conditions, but running a system under anaerobic conditions may permit microbial organisms to degrade otherwise recalcitrant molecules.

Factors of bioremediation

The control and optimization of bioremediation process is a complex system of many factors. The Table 1 shows the various factors responsible for bioremediation along with the conditions required:

Table 1. Factors affecting bioremediation

Factors	Conditions required
Microorganisms	Aerobic or anaerobic
Natural biological processes of microorganisms	Catabolism or Anabolism
Environmental factors	Temperature, pH, Oxygen content, Electron acceptor/donor
Nutrients	Carbon, Nitrogen, Oxygen etc.
Soil moisture	25-28% of water-holding capacity
Type of soil	Low clay or silt content

Enzymatic reactions involved in bioremediation

The contaminant present in the environment can become an additional food source for the microorganisms. The contaminant serves two useful purposes for the microbes. First, the contaminant provides a source of carbon for growth of the microbes. Second, the microbes obtain energy by breaking chemical bonds and transferring electrons away from the contaminant. This is known as an oxidation-reduction reaction. The contaminant that loses electrons is oxidized and the chemical that gains the electrons (electron acceptor) is reduced. The energy gained from the electron transfer is used along with the carbon and some electrons to produce more cells. Microbes generally use oxygen as an electron acceptor but nitrate, sulphate, iron, and CO₂ are also commonly used. Other than oxidoreductase enzymes, a large number of enzymes from bacteria, fungi, and plants are involved in the biodegradation of toxic organic pollutants.

Microbial consortia for bioremediation

Fungal and bacterial organisms often share the same micro-habitat and as such interact with each other. In general terms such interactions may be categorized as antagonistic, neutral or beneficial for the microbes. Antagonism may be the production of antibacterial or antifungal compounds or simply the competition for nutrients. Competition for nutrients can lead to great reduction of fungal biomass in the presence of competing bacteria and *vice-versa*, whereas other bacteria may even obtain nutrients by

mycophagy i.e. feeding on living fungi. A neutral interaction is one where there is no positive or negative effect of the presence of the other organism, e.g. where the organisms have different niches. However, what is desirable in a consortium is a beneficial interaction for it to be efficient for degradation of pesticides (Table 2).

More complex contaminants which are not readily degraded by single microorganism can be easily transformed into harmless products by using microbial consortia. The intermediate product from biodegradation of a contaminant, which is not possible for the same organism to degrade it further can be broken down by another microbe present in the consortia. The use of fungal-bacterial consortia is of greater advantage as a more mechanistic type of interaction exists between them, which are the transport of bacteria along the fungal hyphae, termed as the fungal highway. Pollutant-degrading bacteria have limited accessibility to the polluting compound, as well as water and nutrients, in heterogeneous and water-unsaturated environments e.g. soil. The fungal highway may, however, improve this in several ways. Transport of bacteria can occur in the thin water film around the fungal hyphae consequently leading to a more homogenous distribution of these bacteria in the soil. The bioremediation of commonly used pesticides namely chlorpyrifos and methyl parathion can be achieved using developed indigenous microbial consortium (*Acinetobacter* sp., *Pseudomonas* sp., *Enterobacter* sp. and *Photobacterium* sp.). The use of different strains of fungal-bacterial

Table 2. Microorganisms producing different enzymes and pesticides degraded

Microorganisms	Enzymes produced	Pesticides degraded
<i>Pseudomonas</i> sp. <i>Klebsiella</i> sp.	Hydrolytic enzymes	Atrazine
<i>Pseudomonas</i> sp. <i>Alcaligenes</i> sp.	Oxygenase, Hydroxylase, Hydrolase, Isomerase	2,4-D
White rot fungi <i>Trichoderma</i> sp.	Cellulase	Phenylamide, Phenylurea, Triazine, Dicarbimide, Chlorinated and Organophosphorus compounds
<i>Pseudomonas diminuta</i> <i>Flavobacterium</i> sp.	Phosphotriesterase	Organophosphorus pesticides

consortia viz. bacteria (*Sphingomonas* sp. SRS 2, *Variovorax* sp. SRS 16, and *Arthrobacter globiformis* D 47) and fungi (*Mortierella* sp. LEJ 702 and LEJ 703) may be useful for the increased remediation of agricultural pollutants such as Phenylurea herbicide diuron [N-(3,4-dichlorophenyl)-N,Ndimethyl-urea]. *Enterobacter* sp., *Stenotrophomonas* sp., *Sphingomonas* sp., *Paracoccus* sp., *Bacillus pumilus* and *Bacillus laterosporus* degraded chlorpyrifos to its hydrolysis product 3,5,6-Trichloro-2-Pyridinol (TCP), which is a persistent metabolite and serious contaminant. But these microbes are unable to further degrade TCP into

non-toxic form. So inclusion of a new fungal strain *Cladosporium cladosporioides* Hu-01a in the consortia showed the ability to degrade TCP.

SUMMARY

Bioremediation is a natural process which involves in complete destruction of pollutants and can be achieved without using any harmful chemicals. The choice of the bioremediation strategy should be made on the basis of type of pesticide, environmental matrix and the organisms present in the ecosystem. Since the organism is the only eligible factor, the knowledge

about features, advantages or disadvantages of organisms could be a decisive factor on bioremediation proficiency. To get all the benefits from this strategy it is necessary to carefully select the most adequate microorganism and the enzyme, and to have it well-characterized.

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Success story

Organic ginger cultivation

Shri Nim Tshering Lepcha, the President of Lower Nandok of Nandok-Naitam GPU, East Sikkim district, is an innovative farmer engaging himself in different farm activities like off-season vegetable cultivation in low-cost houses, large cardamom cultivation, fishery, backyard poultry, dairy etc. to earn additional income. These ventures however, were not very much profitable with the amount of labour involvement. There was a lot of interest among the farmers, of the village to start the production of organic ginger in the region but the technological inputs and knowledge of cultivation was lacking. ShriLepcha was in a dilemma in growing this crop for economic viability and in fact, he was having the problem in procurement of quality planting materials for further multiplication, proper processing and organized marketing.

Technology/process intervened: The scientists ofKVK, East Sikkim visited the village and the organic production of ginger technology was imparted through skill based trainings and demonstrations at farmers' field. At first, he was provided with 200 kilograms of good quality ginger rhizomes (cv. Bhaisey) as seed material during the year 2013-14. ShriLepcha successfully adopted the improved ginger cultivation technology under organic condition.

Impacts

The crop is ready for harvest after 8.5-9 months of sowing with an average yield of 137.13 q/ha and average net return of Rs. 2,84,905/ha per year resulting B:C ratio of the crop to the tune of 3.28.

Most of the farmers of Nandok village now are engaged in growing ginger which has become an important economic activity of the farmers of this area. The other fellow farmers in the village are motivated by the quality planting material based production technology of ginger under organic condition for its high return. Among the growers, Mr.PhurbaLepcha of Upper Nandok is best known in the field of ginger production. He has wide practical knowledge and better experience in the field of ginger cultivation. It has also been reported that he has been able to produce 6500 kg of ginger crop out of 200 kg rhizome seed for two consecutive years. The ginger seeds so produced by Mr.PhurbaLepcha have a high demand in the local area for seed production. Thus, it became a necessary story in ginger cultivation, which encouraged other farmers of the region to opt for cultivation as high value cash crop ginger.

Effect of the technology /process :The achievements of technological interventions of ginger cultivation with regard to the results from 2013-14 to 2017-18 is presented below:

Year	Area under cultivation (ha)	Yield/ha (q/ha)	Gross expenditure (q/ha)	Gross Return (q/ha)	Net Return (q/ha)	B:C ratio
2013-14	0.10	129.5	1,28,250.00	3,88,500.00	2,60,250.00	3.0
2014-15	0.25	132.2	1,25,100.00	3,96,600.00	2,71,500.00	3.2
2015-16	0.45	135.0	1,24,650.00	4,05,000.00	2,80,350.00	3.2
2016-17	0.50	140.8	1,22,420.00	4,22,400.00	2,99,980.00	3.4
2017-18	1.00	144.2	1,20,155.00	4,32,600.00	3,12,445.00	3.6
Average		137.13	1,24,115.00	4,09,020.00	2,84,905.00	3.28

mandarin intercrop. This case study of ginger production technology has led to the adoption of this technology amongst many farmers, farm women and rural youth of Nandok and has also spread to nearby villages Thanzing, Upper Khamdong, Yangthang, Thanka and Lingtam villages of East Sikkim covering an area of 50 ha for sole crop of ginger.

The fresh ginger rhizomes are being sold to the traders/retailers and/or to the local merchants. Marketing agencies are managed by the private and government traders with the few co-operative societies that handle this product.

The technology demonstrated in an area of 0.10 ha in the field of ShriNimTsheringLepcha at Lower Nandok during 2013-14 has now extended to an area of 1.0 ha. Presently, more than 8 ha land is under ginger cultivation in Nandok village of East District. The productive potential of the technology was tapped by majority of the maize growers and they have started ginger cultivation purely as sole crop, a suitable alternative to