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# Role of Isolates of Bacillus Species for Biodegradation of Multiple Contaminants

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**Abstract:** Nature is the precious gift for every organism on the earth but, only few species are taking benefits and rest are suffering from scarcity of natural resources because of over exploitation. There exist numbers of hazardous pollutants in environment that are required to eradicate for sustainable use of natural resources. To overcome these pollutants researchers introduced bioremediation with microorganisms. This paper has been prepared by collecting data from various research articles to show numerous applications of bacillus species for sustaining environment. The article is unique from other research studies as it elaborates removal of different pollution causing elements such heavy metals, soil contaminants, removal of dye contaminants from the environment. Although there are large numbers of microbial species to degrade pollutants but according to recent researches, Bacillus is more prominent among all bacterial species. Researchers have proved that Bacillus are safer and cheaper source for conserving environment and reduce toxics from environment. Removal of heavy metals such as cadmium, nickel, copper can be done with the help of Bacillus cereus. In waste water treatment, Bacillus licheniformis and Bacillus acidophilus are also responsible for reducing nitrogenous components like phosphates, nitrites and ammonia.

***Keywords:*** *Bacillus paramycoides, Bacillus subtilis, Bacillus cereus, Bioremediation, Biosurfactants, Dye contaminant, Waste water treatment*

Conflicts of interest: None
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## 1. Introduction

The simplest form of organism is called microorganism. Microorganisms are always being part of researchers' interest due to their various applications, easy availability and highly cheap resources. Bacillus is the group of bacteria that is useful as well as has been known for numerous revolutionary inventions. Bioremediation is defined as involvement of microbes to degrade persisting chemicals and toxic compounds disturbing ecosystem. These microbes has proved that they are powerful tool for biotechnology and environmental technologies by showing their numerous outstanding achievements included pollution control like removal of biofilms, reduction of heavy metals, recovery of silver particles in x ray film and degradation of oil from soil and water. As we know microbes are also very effective in sewage treatment and effluents. For last two decades microbes have shown their best results in dye contamination reductions. Bacillus sp. is also responsible for microbial enzymes production like celluloses, proteases, amylases, lipases, laccases etc. These all microbial enzymes secreting various degradation cellular components that are very much help in numerous companies and served as an important industrial part. Nowadays bioremediation wit bacillus has been increasing day by day. To eradicate toxic chemicals and their persistence ability to protect environment bioremediation is one and only solution (Gadd et al., 2000; Malik et al., 2004; Farhandian et al., 2008).

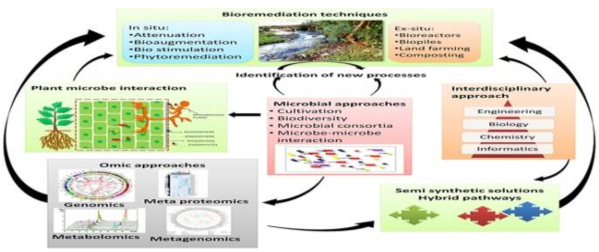


**Figure 1:** Cycle of microbes' interaction with environment

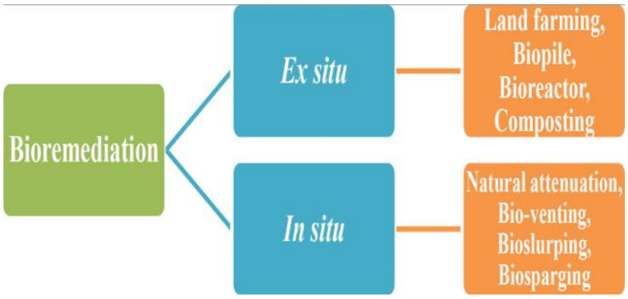


**Figure 2:** Types of bioremediation (Raju et al., 2018)

Bioremediation is the remedy to involve living organisms to protect environment over chemicals. On one hand there is need to explore more the term bioremediation and on other hand there is a difficult observation to recognize suitable strains of bacterium to sustain environment (Guo et al, 2010).

Microorganisms are the organisms present in the simplest form in nature. Despite large applications of microorganisms and easy availability, still there is more to discover about their eco-friendly approach. A study revealed that microorganisms are not as simple as they found or studied (O'Toole, 2000). Biofilms is also a capability of microorganisms. Biofilms are defined as the combined interactions of various microorganisms at a time on a particular surface (Donlan, 2001). Biofilms are found everywhere like pipelines, food industries, water pipes, medical instruments and many more. Therefore to overcome from these pathogenic bacterial and fungal matrixes is the most challenging task for medical sector. Therefore some species of Bacillus strain secrets cellulases and proteases to degraded these biofilms and reduce their hazardous effects to environment. About 12 species of bacillus are able to act as biosurfactants. Biosurfactants are the components that are responsible for inhibition of bioactive bioilms and their infections. From the studies it has proved that biosurfactants produced by bacillus sp. successfully decreases the functioning and infections spreading with biofilms against P. aerugonisa strain of fungus. (Matthew D et al., 2020)

Bacillus is an important bacterial strain for bioremediation and biotechnological applications. This study also reveals that there were 10 isolated of bacillus strains that were able to degrade all soil contaminants and pathogenic microbes. Among three strains of bacteria in oily sludge of contaminated soil like- Pseudomonas aerugonisa, streptococcus and Bacillus, Bacillus was the acceptable more suitable choice of researchers. They also revealed that bacillus cereus can be recognized as GRAS-generally recognized as safer bacteria (Elenga et al., 2020). Bacillus cereus UCP1615 is a strain of bacterium that is present at low cost and non toxic to environment. These biosurfactant produced by Bacillus cereus are also shows their property as a bioindicator. These biosurfactants are able to reduce toxic hydrophobic contaminants attached to rocks and stones. If these biosurfactants are combined with sorbate they can show their activity against biofilms or a long time (Durval et al., 2021).

## 2. Materials and methods

This paper has been prepared by collecting data from various research articles to show numerous applications of bacillus species for sustaining environment. The Open Access articles were searched in journal databases and search engines. The article is unique from other research studies as it elaborates removal of different pollution causing elements such heavy metals, soil contaminants, removal of dye contaminants from the environment.

## 3. Results and discussion

### 3.1. Removal of soil contaminants

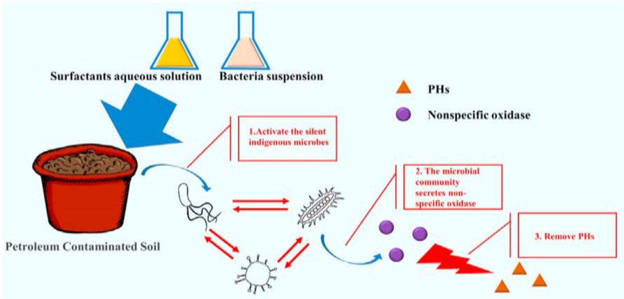


**Figure 3:** Figure represent reduction of soil contaminants from Environment (Rong et al., 2021)

As we know coal and diesel are most prominent challenges to remove from environment globally. So the common practice has been running throughout the ages to discard them into soil as they had extracted from the soil. There is an insertion of new practice with the help of microorganisms to remove soil contamination. Some studies showes that within 30 days hydrocarbon of aged petroleum can be removed from soil by 80% mixing with Bacillus methylotrophicus. This study has proved the bacillus strain if inoculated with soil suspension is able to degrade hydrocarbons of old petroleum and diesel. The strain of Bacillus has a compatible interaction with soil and other living organisms of soil to maintain balanced chain of ecosystem. Microbial enzymes like laccases, oxidases and catalases are responsible for degrading toxic components of hydrocarbons and soil contaminants (Rong et al., 2020).

### 3.2. Waste water treatment by Bacillus

As we know the water treatment is an important a part of our environment and treatment of waste water is an essential process of maintaining water recycling. Large numbers of microbes are involved in waste water treatment like enzymes, inoculums and many more. These all practices are involved to increase water quality. To improving water quality is helpful for aqua system too and the organisms present in the aquaculture. According o the study the sample was isolated from polluted areas have the quantity of cellulolytic and proteolytic bacteria in them. These bacterial strains were to decreases COD, BOD and nitrogenous compounds from the sample that is essential nutrients for aquaculture (Sonia et al., 2015). According to the study among various strains like Nitrosomonas, Nitrobacter and other bacteria, Bacillus subtilis was the suitable to reduce COD, BOD and nitrogenous compounds that is indeed component in ponds for fish and prawns. The usage of bacillus over other bacterium can reduce hazardous effects for fish and prawns and an effective solution of water systems of aquacultures (Reddy et al., 2018). Bacillus licheniformis and Bacillus acidophilus are also responsible for reducing nitrogenous components like phosphates, nitrites and ammonia (Xiang-Hong et al., 2000).

## 3.3. Treatment of heavy metals by Bacillus

Reduction of heavy metals from the environment is the most challenging task for researchers. As we know that there are large numbers of heavy metals present in the environment like cadmium, copper, lead and many more. So there is need for reduce these contaminants from soil and water. According to the study microbes isolated from a particular contaminated site the microbe was belong to the bacillus species and has greater ability to reduce copper, cadmium and lead at a time about 75%, 84% and 50%. For removal of heavy metals like cadmium, lead and copper can be removed from the soil and water with the help of bacillus species. (Guo et al, 2010).

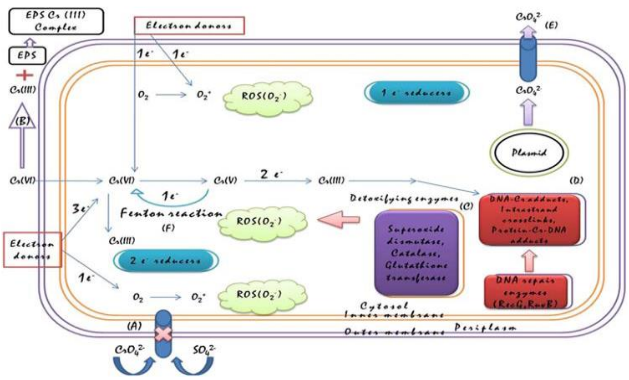

**Figure 4:** Representing reduction of heavy metals by microbes (Pattnaik et al., 2021)

The appropriate term called a biotic precipitation or biosorption for complete removal of heavy metals from the environment (Guo et al, 2010).

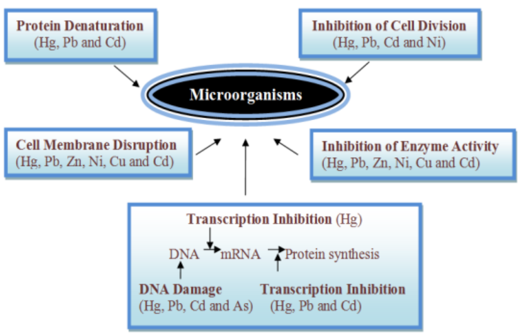


**Figure 5:** Degradation of heavy metals by microorganisms (Ahmed et al., 2014)

According to the research bacillus cereus had fund from the contaminated sites and also capability of bioremediation of heavy metals (Pugazhendhi et al., 2018). Cooper heavy particles were also found to be reduced with Bacillus cereus strain (Migocka, 2015).One study also revealed that the microbes are able to degrade heavy metals like zinc, mercury and are also have ability to show many antibiotic drug resistant ability and tolerance (Naik et al. 2012). Bacillus strains are able to show biomonitoring by reducing toxic effects of heavy metals like zinc, copper, lead, iron and mercury (Kumari et al., 2021). This study reveals that bacillus strains are capable for growing at unfavorable conditions and can be utilized for long term bioremediation (Pattnaik et al., 2022).

## 3.4. Coal contaminants

There is lack of proper management of old coal mining and lands to recover after remains of soil contaminants and utilization of that particular area. With the help of microbes that are able to reduce these coal contaminants and enhance soil quality for further usage. After bioremediation of soil of ex-coal mining can be utilize as road and new landscapes. But these all can be done a technique called Microbial induced calcite precipitation (MICP) to improve the soil quality. This study has revealed the capability of Bacillus subtilis after mixing with coal. The coal contaminants were degrading with increasing time period. It was observed that treated soil were involved MICP 15times more than untreated soil. The main quality of bacillus strain was one that it can be reused after the treatment with soil contaminants. One more thing were concluded from their study that bacillus subtilis spores can remain as it is more than 6 years and it can survive in nutrient-poor soil and medium (Indriani et al., 2021).


**Figure 6:** Types of Soil Contaminants

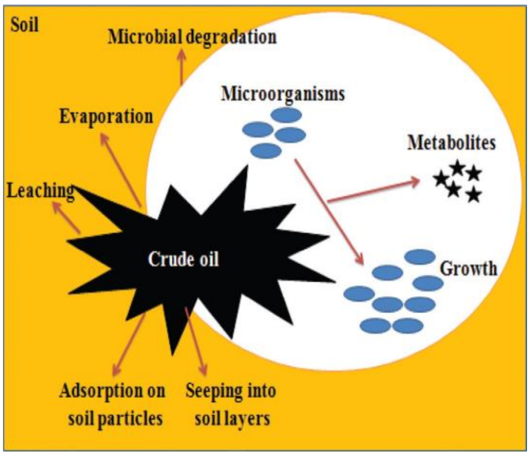


**Figure 7:** Represents oil spillages in soil (Raju et al., 2018)

It has proved that Bacillus subtilis can remove hydrocarbons from soil and water ( Das and Mukherjee 2007) Bacillus strain can capable of reduce oil and rude petroleum from soil wit in 20 days about 60% (Varjani and Upasani 2016). Chromatographic studies revealed that light and heavy aromatic hydrocarbon chains are also to degrade with Bacillus subtilis. (Safdari et al., 2020).

## 3.5. Bacillus as Biosurfactants

Oil and petroleum is the major part of our lifestyle involves its large production as well transportation from several places (Nriagu, 2011). These transportations and productions involved greater chances of spills and damaged. Most of the time water bodies like oceans and soil go-downs are being part of loss of their flora fauna. The major components of these oil spills are hydrocarbons and their derivatives.

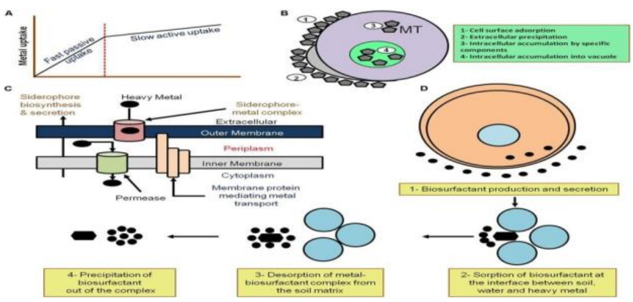

**Figure 8:** Figure represents mechanism of microbes as Biosurfatants

The large number of microbes involved to remove these contaminants like bacteria, fungi and sometimes yeast too (Varjani, 2017). Despite there are some chemical or synthetic surfactants such cethylmethyl ammonium bromide and sodium lauryl sulphate for these oil cleanup process but they are highly toxic and non biodegradable (lima et al., 2011). Biosurfactants are highly surface active, stress and starvation resistant and have several applications involved antibiotic resistance and eco-friendly in nature (Naeem and Qazi, 2019) (Prakash et al., 2021).

## 3.6. Bacillus for degradation of dye contaminants

According to the survey China is known as world's largest country for producing dyes and textile at a same time. About 60% of dye has been produced by China every year. Dye is a deniable chemical because it is compulsory for not only textile industry but for food, plastic and many other industries for providing different vibrant colors and their shades. Even in the pharmaceutical industries dye plays an important role (Piaskowaski et al., 2018). Untreated dye polluted water can cause serious effects to aqua system if not treated before discarded (Ren et al., 2013). Discarding these dye without treating is not a solution for environment in fact dyes found to be carcinogenic in nature. These carcinogenic dyes every year discarded in water bodies without treatment about 10-20%. These untreated dyes lead to death of aquatic lives. However, treatment of polluted dyes are short term, highly cost effective and insufficient (Vikrant et al., 2018). There are about 10,000 of synthetic dyes present at commercial level as their important of the process. There are availability of dyes such as anionic dyes, cationic dyes, azo dyes and many more. In this study Bacillus amyloliquiferous was isolated from the dye contaminated sample and were treated with dyes like- cromasive brilliant blue, congo red and sarraine. About 95% of reduction of dyes were taken place and made the sample colorless (Liu et al., 2020).

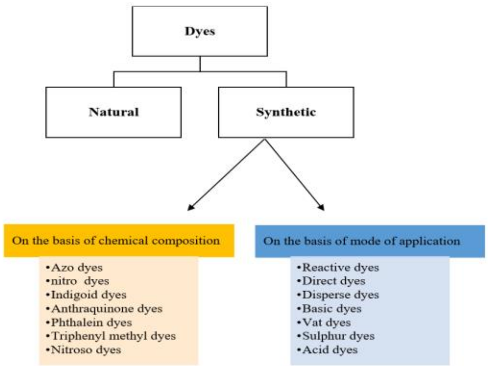


**Figure 9**: Figure represents classification of synthetic textile dyes (Verma et al., 2022)

With carcinogenic and teratogenic cancer properties in dye contaminants, it is very crucial process to protect aquatic life as well environmental hazards. Treatment of dyes effluents can be helpful to recycle water for agriculture practices (Verma et al., 2022).

**Table 1:** Role of Bacillus in industries to decolorize different textile dyes (Bhatia et al., 2017)

| S.N. | Textile Dyes | Bacteria |
|---|---|---|
| 1. | Acid red 128 | *Bacillus endophyticus* VITABR13 |
| 2. | Crystal violet | *Bacillus subtilis* |
| 3. | Provisional pink | *Bacillus sp.* |

| | | |
|---|---|---|
| 4. | Congo red | *Bacillus pumillus* |
| 5. | Dri marane Red | *Bacillus sp.SG2* |
| 6. | Methyl Red and Navy Blue | *Bacillus sp.* |

From the study it was proved that bacillus species involved B. subtilis, B. cereus and Bacillus mycoides were main isolates for color removal practices in the studies. These species of bacillus are also important for molecular and biochemical studies. According to these studies bacillus sp is more effective and eco-friendly bacterium than others (Aydin et al., 2021).

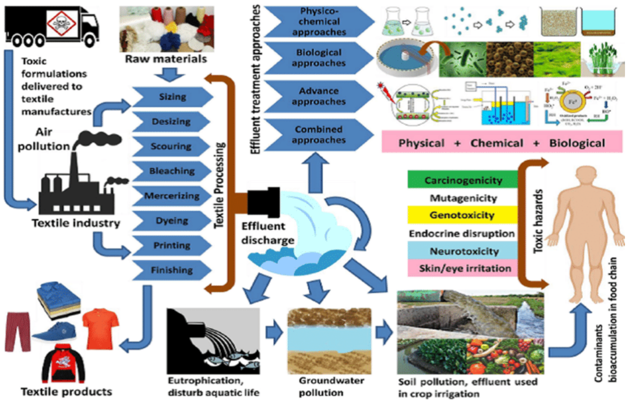


**Figure 10**: Textile dyes and its types with their impacts on environment and health hazards to humans

In this study author has concluded that the bacterial sample was taken from textile effluents and treated Orange -10 Azo dye. After some period of time they had observed that the complete remove of color and degradation of toxic compounds taken place. Therefore Bacillus subtilis is responsible for removal of Orange-10 azo dyes (Hema et al., 2020).

### 3.7. Silver recovery

There is an another important metal in the world known as silver that is required in numerous application like jewelries, x-ray, silver wares, photo graphic and many other electronic gadgets and appliances. Due to large number of applications of silver in various objects it is consider as a vital metal. Silver is required in making of images via x-ray photographic process but it has some left over after the completion of process. It was indeed required to reuse and recover the silver particles. Nowadays it is possible to recover and reuse silver particles after the completion of process of x-ray photography. Enzyme Proteases produced by bacillus is able to recover these silver particles. Alkaline proteases by Bacillus are successfully recovers the silver particles in just 30 minutes and its activity is increases with decreasing its incubation time. During this process gelatin also plays an important role by making gelatin hydrolysate. According to the study in first 30 minutes proteases were utilized more in number and as on time goes on increases silver particles are recovering directly proportional to incubation time. Silver recovery is also a major part of environment protection and bacillus are eligible strain to protect environment. (Abdalall et al., 2016).

## 4. Conclusion

From the study it was concluded that Bacillus are very crucial strain for sustaining environment. There are large applications of bacillus with their different make it more prominent in researcher's interest. Not only research but it is highly needful bacterium for bioremediation. In the water bodies there are release of dye waste from industries takes place that can lead toxic ailment and even death for aquatic life. To overcome this issue dyes like Acid red, crystal violet, provisional pink, congo red and methyl red , presence of bacteria like B. subtillus, B. aureus, B. cereus, B. endophytics, B.pumilus, B. mycoides and many more. Bacillus subtilis having larger capability to degrade coal contaminants after coal mining. Removal of heavy metals such as cadmium, nickel, copper can be reduce with help of Bacillus cereus. In waste water treatment, Bacillus licheniformis and Bacillus acidophilus are also responsible for reducing nitrogenous components like phosphates, nitrites and ammonia.

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