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Minor Research Project

On

Degradation of Organic Pollutants From Ash of Thermal Power Plants of Parli By Bacteria

By

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Summary of Minor Research Project:

Title: Degradation of Organic Pollutants from Ash of Thermal Power Plants of Parli by Bacteria.

Summary:

Increase population, industrialization and urbanization are responsible for environmental contamination. The fly ash emitted by power plan a byproduct from burning coal for electricity carries into surrounding environment 100 times more radiation than nuclear power plants producing same amount of energy.

In september-2010, report by physicians for social responsibility and earth justice, states,"…..coal ash commonly contains some of world deadliest toxic metals: arsenic lead, mercury, cadmium, chromium and selenium. These and other toxicants in coal ash can cause cancer and neurological damage in humans.'' Sixteen polycyclic aromatic hydrocarbons are classified as priority pollutants by U.S. Environmental Protection Agency.

They are produced during fossil fuel combustion, waste incineration, or as byproducts of industrial processes, such as coal gasification, production of aluminum/iron/steel and petroleum refining, component of wood preservatives, smoke houses and wood stoves .

Bioremediation is an invaluable tool box for wider application in the realm of environmental protection. These technologies have been attractive alternative to cleanup technologies due to relatively low capital costs and their inherently aesthetic nature. Carbon demand management is priority task of any country due to industrial pollution.Fly ash pollution is major problem of world due to coal combustion.

Electricity production is integral part of all country by using coal combustion for its daily life activity. So, there is need to develop most efficient technique to mineralization of toxic pollutant into ecofriendly product. Microorganisms are more efficient to degrade xenobiotic, polycyclic aromatic hydrocarbons and heavy metals as compared to animals and plants.

Bioremediation is most emergent remedy to mineralization of xenobiotic carcinogenic pollutant by of microorganisms Insitu bioremediation of pollutants microorganism is most applicable, simple, inexpensive technique can apply through- out the world under environmental conditions.

Due to industrialization, modernization and globalization day by day there is increase pollution level of environment. Due to excess pollution level in environment any country may get suffered from economic loss due to carbon demand. This study is helpful to identification of pollutants its type, level in soil. It should provide information about microflora of contaminated soil. It should isolate efficient bacteria which can degrade carcinogenic pollutants.

This study is helpful to give technique to cleanup environment. This study is significant to prepare probes in molecular biology. This study is helpful to design of biosensor which is helpful in bioinformatics. This study may give protection to society from diseases which are originated due to the pollutants. It should control increase in temperature of environment.

Bioremediation of pollutants is easy, simplest inexpensive technology without any by product which is not harmful to nature. Bioremediation can convert waste to best product. This is zero waste technique by using indigenous microflora of site for the removal of pollutants from any part of world. Microorganisms are most efficient to degrade high molecular weight recalcitrant and water insoluble carcinogenic pollutants. This technique can be used for treatment of contaminated air, water, soil sewage, industrial, and hospital, agricultural and domestic waste.

Any nation can use this technique for CDM; this work is helpful to avoid global warming. It is most efficient simplest environment clean up technique for any pollutants from any part of world biosphere.

Polycyclic aromatic hydrocarbons are ubiquitous compounds in the environment and they are known as suspected to be carcinogenic .The study of their fate in nature is therefore a matter of great environmental concern. Persistence of PAHs in the environment is linked to their hydrophobic character and low water solubility. Furthermore, they often get adsorbed to soil or sediments. As a consequence, they are poorly available for degrading microorganisms. One possible way of enhancing their bioavailability for their easy biodegradation is the addition of biological or chemical surface active agents.

Bioremediation is an emergent technology intended to achieve the remediation of soil via biologically mediated transformation of pollutants. It includes augmentation with various pollutant degrading organisms (bioaugmentation) or the provision of amendments to exploit the existing natural degradative capacity of the soil indigenous microflora (biostimulation).

Gas chromatography coupled with mass spectrophotometery 4 peak out of 19 reports shown that the these peaks were of 4-Oxalocorotonic acid, 2-Hydroxy-2 hydro-chrome

carboxylic acid, 3,4-dihydroxy,3-4-dihydrophenanthrene and 7-methoxy-8-hydroxy fluoranthene. These were polycyclic aromatic hydrocarbons organic pollutants observed in ash of thermal power plants of parli vaidyanath.

Polycyclic aromatic hydrocarbons forms homogenous solution in acetone and acetone evaporates immediately. Five of the tested polycyclic aromatic hydrocarbons maximally solublized in acetone were observed. So acetone was used as solvent for further research work for solubilization of anthracene and fluoranthene it.

Enrichment of Polycyclic aromatic aromatic hydrocarbons degrading microorganisms were carried out by providing anthracene and fluoranthene as a carbon and energy source separatelyin basal salt medium. Growth of microorganisms were indicated by change in colour of medium. Degradation of anthracene and fluoranthene were seen by change in colour of growth medium containing PAHs by microorganisms present in ash contaminated soil samples collected from thermal power plants parli vaidaynath Dist. Beed. The results indicated that ash contaminated soil microflora shown PAHs utilization capability.

From anthracene enrichment, 24 bacterial isolates and 05 fungal isolates were isolated by spray plate technique. Form fluoranthene enrichment, 27 bacterial isolate and 05 fungal isolates were isolated by spray plate technique.

After enrichement of ash contaminated soil samples. The bacterial consortia were screened for its efficiency for PAHs degradation shown very versatile nature.

Eleven isolates from anthracene enrichment shown zone of clearance out of 24 with anthracene and 05 isolates with all of the tested Polycyclic aromatic aromatic hydrocarbons. These 11 isolates were specific efficient and 05 isolates were general efficient.

Nine isolates from fluoranthene enrichment shown zone of clearance out of 27 isolates with fluoranthene and 05 isolates with all of the tested Polycyclic aromatic aromatic hydrocarbons. These 09 isolates were specific efficient and 05 isolates were general efficient.

A Pure culture of bacterial isolates shown rapid degradation and having larger diameter of zone of clearance with anthracene and fluoranthene. The screened efficient bacterial

isolate tentatively named as P-1. Selected bactrial isolate p-1 was used for further research work as a most efficient anthracene and fluoranthene degraders.

Utilization of PAHs were indicated by change in color of growth medium from colourless to yellow orange. The growth of growing bacteria was confirmed by biomass and decreasing concentration of acetone extracted residual hydrocarbons and Gram negative rod shaped bacteria. The degradation was also confirmed by the identification of catechol, salicylic acid, carbon dioxide production and reduces in surface tension.

After the growth was confirmed qualitatively, strain was subjected to increasing concentration from 0.2 to 2 mg/10ml of Basal Salt medium containing of each anthracene and fluoranthene separately. The bacterial isolate P-1 under study was capable of utilizing tested PAHs up to concentration of 2 mg/ml of growth medium. It was also found that the optimal concentration were 1mg/ml of anthracene and fluoranthene.

In order to find out the optimum inoculum needed for maximal growth by utilizing available PAHs by *Achromobacter xylosoxidans*. A trend of increase in growth in terms of optical density at 600 nm was noticed with increase in inoculums size from 0.2 to 1.0 ml/10 ml of growth medium. However, beyond 1.2 to 2ml/10 ml inoculums size of growth medium, the rate of increase in degradation was not very significant.

The culture was incubated from 22 to 37^0C temperature for period of 7 days in presence of 0.1% of each anthracene and fluoranthene as a carbon source in Basal Salt medium having p^H 7.0. maximum growth was observed at 28^0C temperature. Observation indicating that average environmental temperature is optimal for growth of *Achromobacter xylosoxidans*.

Achromobacter xylosoxidans can grow in the p^H range of 5.5. to 8.5 in presence of PAHs. It was also confirmed that p^H 7.0 to 7.5 were optimal p^H for growth of *Achromobacter xylosoxidans* in presence of PAHs.

The *Achromobacter xylosoxidans* culture was incubated in the presence of 0.1% of each anthracene and fluoranthene in Basal Salt liquid medium of p^H 7.2 at 28^0 C for period from 3 to 21 days. There was increase in growth as incubation period was increased up to 21 days.

When the bacterial culture was tested for utilization of 0.1% of each anthracene and fluoranthene in Basal Salt liquid medium. It was found that under shaking and dark conditions that isolate was capable of utilizing tested PAHs in maximal amount as compared to on shaker in light and static condition in dark.

The *Achromobacter xylosoxidans* indicated that xylulose was readily utilized as a carbon source over utilization of PAHs. But glucose does not affects the growth and degradation of PAHs considerably. The arabinose mannitol, raffinose and rhamnose shows adverse effect on growth and degradation of PAHs. At the same time it was observed that the growth of bacteria is not directly related to the degradation efficiency of PAHs. Another possible effect of carbon sources was that it could enhance microbial activity including biosorption and biostimulation for utilization of PAHs.

In our studies, the various organic and inorganic nitrogn sources were used, yeast extract was the best nitrogen source for efficient degradation of PAHs. This may be owing to the metabolism of yeast extract, which is considered essential for the regeneration of NADH. It was found that 0.1% (w/v) concentration of yeast extract, peptone, soybean meal, calcium nitrate, potassium nitrate and Ammonium nitrate shown enhanced growth and PAHs degradation.

Achromobacter xylosoxidans treated with ethyl methyl sulphonate shown increased efficiency of degradation of PAHs as compared to ultra violet light exposed bacterial isolate P-1 and non ethyl methyl sulphonate treated *Achromobacter xylosoxidans* culture.

Achromobacter xylosoxidans relveaved that as incubation period increased, carbon dioxide production also increased. On third day of incubation period, tested PAHs shown minimal amount of carbon dioxide production.

High performance liquid chromatogram of anthracene degraded by *Achromobacter xylosoxidans* shown the capability of anthracene degradation. It was also noticed that *Achromobacter xylosoxidans shown* complete transformation of anthracene showing 9 peaks of various compounds having different retation time and percentage area in 14 days as compared to undegraded anthracene sample.

High performance liquid chromatogram of fluoranthene degraded by *Achromobacter xylosoxidans shown* the capability of fluoranthene degradation. It was also noticed that *Achromobacter xylosoxidans* shown complete transformation of fluoranthene showing 9 peaks of various compudnds having defferent retation time and perecentage area in 14 days as compared to undegraded fluoranthene sample.

Gas chromatography coupled with mass spectrophotometer peak reports shown that the these peaks were of Pthalic acid, protocatechuic acid and Trans-9, 10-2Hydroxy 2 hydro-anthracene. These were intermetabolites products of anthracene by *Achromobacter xylosoxidans in* 14 days.

Thus GC/MS analysis clearly confirmed that *Achromobacter xylosoxidans* degrades anthracene by pathway proposed for anthracene degradation by Habe and Omori, (2003).

Gas chromatography coupled with mass spectrophotometer peak reports shown that the these peaks were of Pthalic acid and 7,8 Dihydroxy fluoranthene. These were intermetabolites products of fluoranthene by *Achromobacter xylosoxidans* in 14 days.

Thus GC/MS analysis clearly confirmed that *Achromobacter xylosoxidans* degrades fluoranthene by pathway proposed for fluoranthene degradation by Juhasz, (1997) and 1-hydroxy-2-naphthoic acid by Habe and Omori, (2003).

Bacterial isolate P-1 was identified by characterization of morphological, physiological and biochemical characteristics as per Bergey's manual determinative bacteriology. It was identified upto genus level as *Achromobacter* which was formerly known as *Alcaligenes.*

It was also identified by 16SrRNA sequencing using NCBI nucleotide database for BLAST analysis. 16SrRNA sequences of bacterial isolated P-1 shown 718 base pair similarity and 99.190 percentage relatedness to *Achromobacter xylosoxidans.*

Conclusions:

The key outcomes of minor research project were clearly revealed that:

1. *Achromobacter xylosoxidans* having ability of degradation of polycyclic aromatic hydrocarbons efficiently under natural environmental conditions.

2. The *Achromobacter xylosoxidans* can be used for remediation of ash pollutants in environment which having hazardous effects on human health.

3. The *Achromobacter xylosoxidans* can used for treatment of industrial waste effluents for degradation of xenobiotics.

4. The *Achromobacter xylosoxidans* can be used for degradation of hospitals waste recalcitrant materials .

5. The *Achromobacter xylosoxidans* can be used as a biosurfactant, fungicide and biofertilizer.

6. The *Achromobacter xylosoxidans* can be used for management of e-waste.

7. *Achromobacter xylosoxidans* can be used for remediation of high molecular weight hydrophobic recalcitrant pollutants of carcinogenic and mutagenic nature from various sources in the biosphere.