BioremediationofSoilContaminatedfromPetroleumhydrocarbonsleakedatPetrol Station, Kota

ABSTRACT

Soil contaminatedwithhydrocarbonsofpetroleumanditsproductsneedaverycosteffective processof remediationwhichisknownasbioremediation.Therearenumerousfactorswhich affecttheefficiencyofbioremediationprocesswhichgenerallyincludesmicrobial population, environmental conditionsandoncompositionofhydrocarbonspills.Themainmotiveofpresent workwastofindoutpossiblemethodstoincreasetherateof degradationof hydrocarbonsby bacteriaaerobically (*ex–situ*treatments).In currentwork,application ofbioremediation process weredoneonsandysoilcollectedfrompetrolstations ofKotawhichhas beencontaminatedwith dieseloil,leakedfromundergroundstoragetank.Generalmicrobiologicallaboratory procedures and experimentswere used to evaluate the results of biodegradation of the diesel oil contaminatedsoil.Biostimulation(additionof Tween80surfactantsandphosphorus-nitrogen solutions) and Bioaugmentation by bacterial consortium were used to enhance the biodegradationprocess.Thepresentworkwastofocusonthebiologicalactivitiesandtheir effectonlimitingnutrientsincontrolconditions.Respirometricmethodswereusedtomeasure the efficiency ofbiodegradation process.Thepresentinvestigation resultsshowed thatnatural bioremediation ofdieselcontaminatedsoil canbeachievedbythebiological agents(especially bacteria). It hasbeen observed by respirometricdata indicating that 5% removal of total petroleum hydrocarbons (TPH) in 50 days treatment. Predominantly *Bacillus spp.*,

Staphylococcusspp.andPseudomonasspp.ofbacteriafromsoilwereisolatedandidentifiedat theendoftheexperiment.

Keywords-Bioagumentation,Biostimulation,Bioremediation,Bacterial Consortium, Biodegradation

1. INTRODUCTION

Petrol anddiesel areessentialcompoundsforvehiclesandmotorstorunandrequiredinday to daylife.But manytimesthesecompoundsmaycreateproblemswhenget leakedintosoil. Petroleumhydrocarbonscontaminatethesoilbyaffectingthesoilhealthandlife.Petrol and petroleumproductscandestroy thefertilityofsoilandcanharm theusefulmicrobespresentby decreasingtheirnumber.Leakageofpetrol anddieselinnearbysoilatpetrol pumpsisavery commonproblem.Thiscanbeunnoticeableifleakageisatsmalllevelconcentrations.Butitcan beeasytoseeorsmellifleakageofoil occursatlargescaleconcentrations.Thediscolorationof soilcontaminatedwithpetroleumhydrocarbonsascomparedtonearby areasandpoororless vegetationgrowthshows thelevelof contamination.Thisproblemcanbereduceordegradeby usingenvironmentalfriendlymethods,such asbioremediation.Bioremediationisaprocessby whichmicroorganismsareusedtoreduce ordegradeenvironmentalhazards.Accumulationof toxicchemicalsandwastescreatesenvironmentalhazardsinthenature.Bioremediationisthe way tospeedup theprocessofwastedegradationnaturallyandby thesenaturally occurring microbescanberecycledasfungi,bacteriaand yeastscellsareusedtodegradehazardous pollutantsinthesoil,airandwaterintolesstoxicornon-toxicsubstances.Microorganisms digestcontaminantslikenitrates,carbontetrachlorideandoilintowater,carbondioxideand otherbyproducts andgiveoffttheseproducts.

Biologicalmethodcreatestheconditionstodetoxify thecontaminantsbymicroorganismsto flourishand performtheir metabolicactivities. Microorganismusescontaminantsasenergy sourcetodometabolicactivitiesduringbioremediationprocess.Themainconceptof thisisto givenecessary requirementstomicroorganismsandtopromoteorganismsfordegradation process.Bioremediationmethods canbeappliedonthesamesiteorex-situmeans toincreasethe growth ofmicroflorawhicharelocally presenton thesiteoraddition ofmicrobialconsortium withinthedesiredcharacteristics.Inin-situremediationtreatmentofcontaminatedsoiloccursat

the samelocation where it is found, but in ex-situ remediation contaminated soil treated elsewherefromitsoriginalplaceorexcavationofsampleforthetreatment.

Scientists haveworked a lot to identify the best solution to clean up the hydrocarbon contaminatedsites.Theyhaveresearchedonspecificmicrobeswhichcanhelptoreducethe level ofcontamination.Microorganismsinvolvedinbioremediationare;*Bacillus,Pseudomonas, Flavobacterium, Dechloromonas*etc. Basicallytwo bioremediationapproaches used for the treatmentofcontaminatedsoilarebiostimulationandbioagumentation.

Hydrocarboncompoundsareessentialelements forhuman life. Leakage fromunderground storageoiltankscausesseriousproblemsofcontaminationtosoil,soilmicrobesandwater bodies.Oilspillsfromtanksandpipelinescausespollutioninwaterandsoil whichcanbesevere tohumanhealthandcancausecancer.

Harmfuleffects ofhydrocarbons pollution:

- Medicalandhealthissues occurinhumans.
- Soil characteristicslikephysiochemical andbiochemical propertieschangesduetopetrol anddieseloilcontamination.Soilbecomes unfitforvegetationgrowth.
- Growthanddevelopmentof plantsbecomeverylowduetosoilpollution.Duetothis pollution soil's nutritivevaluedegrade.
- Hydrocarbonsshow inhibitoryeffect onmicrobialbiomassand phytotoxiceffect on agriculturalcrops presentinsoil.
- Whenpetroleum products(petrol anddiesel)deeply penetrateintothesoil causes disturbancein thebiogeochemicalcyclesandshowsworseeffecton biotic andabiotic components.

Petroleumhydrocarbonsareseriousconcernfor manycountries,so it becomesvery importanttobringsoilinits originalforminnaturalway(bioremediation).Fromlastfew yearsscientistsworkingonthisproblemandconductingcost effectivetechniquesto removecontaminantsfromthesoilwithoutmuchdamagingtotheenvironment. The currentobjectivesofourworkis toinvestigatetheefficiency ofbioremediationonsoil contaminated frompetrol and diesel oil by respirometricmethod (carbon dioxide

productionbymicrobes)andisolationandidentificationofbacterialconsortiumfromthe contaminatedsoilsample.Aimof thisresearchistofindoutpossiblesolutionforthe inventionof newprocesseswhichreducestimeandeffortsforbioremediation. Bioremediation processisaprocesswhich generallybased on microbialmetabolic activities.The availabletechnologiestoclean upthecontaminatedsoilby thesemethods arealsooneofthemainfactorsofbioremediation.Remediationbybiologicalmethods has severaladvantagesoverchemicalmethods.Theseareasfollows:

Detoxificationof hazardoussubstancesbybiologicalbasedremediationcreateslowor minimaltoxics andnon-transferringfromoneenvironmenttoanother.

Bioremediation arecosteffectivetechniquesto treatcontaminatedsitesascompareto conventionaltreatments.

In comparison toexcavationbasedprocesses,bioremediation areeffectiveandless disruptivetotheenvironment.

2. METHODOLOGY

2.1SAMPLECOLLECTION:

Samplewascollectedfrom petroleumstationatJawaharNagar,Kotawhereunderground dieselleakagefrom thetankhasbeenoccurredinnearbysite.Siteforsamplecollectionwas identifiedbycomparingthediscoloration ofsoilduetohydrocarbon contamination.Soil samplecollectedhavegreatamountofoil andleakedtothegroundwater.Capillaryfringeof depth 1-2 feet was used to collect the sample. Sample thenstored at 4^0 C at normal refrigeratortemperature.

2.2BIOREMEDIATIONONDIESELCONTAMINATEDSOIL:

Thisworkdealswiththeprocessofbioremediation onsoil collectedfrom underground storagetankofpetrolpumpfromJawaharNagar, Kota.

Microbial CO_2production was measured byusing Flask arrangement to biodegrade diesel oil. 500 mLflask arrangement was used for carrying out biodegradation experiment.

Biodegradation processwasenhancedbybiostimulationi.e.addingN andPsolution or tween80surfactantsandbioaugmentationi.e.treatedwithbacterialconsortiuminoculum (whichis known cultureof*Bacillus*species).

Theimpactof threevariables(additionof NandPcontent,tween80surfactantand bacterialconsortium)onpollutantbiodegradationwas presentincollectedsoil sample.

Homogenizationofsoil samplewas donebyblendingandalteredsolutions wereadded.

CorrectionswereperformedofNitrogenbyusing$(NH_4)2.SO_4$(1218mg/200gofsoil) and$PhosphorusbyusingKH_2PO_4$ (195mg/200g)solutions.ThusC:N:Pnutrientratio was adjustedto100:15:1.

Additionoftween80surfactantandbacterialconsortiumi.e.*Bacillus*spp.Culture.

Watercontentofsoilchangedto21.8%byconsideringtheinclusionofamendments. 44.5mL waterwas added.

Incubationwas doneinthedarkfor40days atroomtemperatureandshakedregularly.

CO_2producedwascapturedin100mLKOHsolution(0.2N)inthesidearmoftheFlask arrangement.Measurementofbiodegradationefficiency wasdoneby respirometricmethods (productionofmicrobial CO_2)bytitration.

Periodicallywithdrawn ofKOH solution was done byusing syringe after 15 days. ResidualKOH(10mL)wasusedtotitratetheamountof CO_2absorbed;1mL phenolphthaleinindicatior(giving pinkcolour)wasaddedafterandtitrateditwith HCL standardsolution(0.1N).

Samemethodwas followedandrepeatedafter40days.

DeterminationofTotalhydrocarbonContent:

Initial hydrocarbon contentin thesoil samplewasmeasuredusingsilicacruciblewhich washeateduptoredhot.Afterthiscruciblewasremovedfromtheburnerandallowedto coolatroomtemperature.Nowweightwasrecorded.Samplekeptinovenat100^0Cfor

1hourtoremoveitmoisture.Placethesampleinthecrucibleandweightwas takenagain. Cruciblewasheatedagain.Duetoheatingof thecrucible(becomesredhot) oilfumes wereproducedandlaternofumeswereproduced,thisleadstocompleteevaporation of organicmatterfromthesample.Aftercompleteevaporationcrucibleweretakenoutfrom theburnerandweightedagainaftercoolingatroomtemperature.Thedifferencebetween previousweightandlaterweightwascalculatedandthiswastheTotal Petroleum Hydrocarbon(TPH).

2.3MICROBIALISOLATION:

Isolationofmicroorganismswasdoneby general microbiological laboratory methods.Serial dilutionsupto10^{-6} werepreparedindistilledwaterfromcontaminatedsoilsample.General bacteriologicalmediai.e.nutrientagarwaspreparedforinoculation.Soil samplesfrom different dilutions wereinoculatedonnutrientagarplates andincubatedfor24-48hours at37^0C.

2.4 MICROBIALIDENTIFICATION:

Cellsgrownonnutrientagarplateswereusedforidentificationby Gramstainingandendospore stainingtechniques.Biochemical testbyCarbohydratetest,Indoleproductiontest,Catalasetest, Oxidasetest,Ureasetest,CitrateutilizationtestandNitratereductiontestwereperformedforthe identificationofmicrobes.

3.OBSERVATIONS

3.1DeterminationbytitrationofCo₂absorbedbyKOH:

After20and40days ofobservationshowedthathydrocarbonpresentwas convertedintoCO^2 whichreacts withKOHsolution.Whentitratedwith0.1NHClwegotthefollowingreadings:

Titration days	**VolumeofKOH(mL)**	**Usedvolumeof HCl(mL)**		
		A	B	C
20	10	2.3	2.0	2.0

40	10	6.9	6.6	6.6

3.2Bacterialisolation:

Bacterialcoloniesonagarplateswereobservedfornumbers andcolonialdistribution which showthefollowingobservation:

S.No.	SampleDilutions	No.ofColonies
1	10^{-3}	248
2	10^{-4}	213
3	10^{-5}	159
4	10^{-6}	131

3.3 Bacterialidentification:

24 hoursold cultures grownwere used for staining and for morphological studies further biochemicaltests wereperformedwhichshows thefollowingobservations:

Name	Typeof Bacteria	Shape	Appearance	Carbo-hydrate test	Indole Test	Catal-ase test	Oxi-dase test	Urease test	Citrate test	Nitrate reduction Test	Spore Forming Bacteria
A	Gram -ve	Rod	Brownish Pigment	(-ve)	-	+	+	-	+	+	-
B	Gram +ve	Rod	White	(-ve)	-	-	+	-	-	-	-
C	Gram	Rod	Translu-	(+ve)	-	-	-	-	+	-	-

	+ve		cent								
D	Gram +ve	Rod	Yellow	(-ve)	-	-	-	-	-	+	-
E	Gram +ve	Rod	White	(-ve)	-	-	+	-	-	+	+
F	Gram +ve	Cocci	White	(+ve)	-	+	-	-	-	+	-

4. RESULTS

Attheendoftheworkwegotthefollowingresults:

4.4 Results ofTitration:

CalculationofTotalDegradedHydrocarbon:

After20days: TakeninitialKOHsolution0.1N=10mL

Titratedwith0.1HCl

VolumeofHClwereconsumed =2.3mL

TotalusedKOHinCO_2 =2.5mL

2.5*0.1N =0.1N*2.5mL CO_2(mol.wt. 44)

=2.2gin1000mL

=2.2*2.5/1000gCO_2in2.5mL

Totaldegradedhydrocarbon =0.0055gCO_2in2.5mL

After40days: TakeninitialKOHsolution0.1N=10mL

Titratedwith0.1HCl

VolumeofHClwereconsumed =6.9mL

TotalusedKOHinCO_2 =3.5mL

3.5*0.1N =0.1N*3.5mL CO_2(mol.wt. 44)

=2.2gin1000mL

=2.2*3.5/1000gCO_2in3.5mL

Totaldegradedhydrocarbon =0.0077gCO_2in3.5mL

CALCULATIONOF BIODEGRADATIONEFFICIENCY:

After20days: Thebiodegradationefficiencycanbeexpressedas:

. BE%= (totalbiodegradedcarbon/initial soilorganiccarboncontent).100

BE%= (0.0055/0.1559). 100

BE%= 3.527%

After40days: Thebiodegradationefficiencycanbeexpressedas:

BE%= (totalbiodegradedcarbon/initial soilorganiccarboncontent).100

BE%=(0.0077/0.1559).100

BE%= 4.939%

4.2BACTERIAISOLATION:

Formulaforcalculatingnumberofbacterial coloniesgrownonnutrientagarplates:

Dilution10^{-3}: No.ofcells/mL.=248/1*10^{-3}

Dilution10^{-4}: No.ofcells/mL.=213/1*10^{-4}

Dilution10^{-4}: No.ofcells/mL.= 159/1*10^{-5}

Dilution10^{-4}: No.ofcells/mL.= 131/1*10^{-6}

4.3BACTERIAIDENTIFICATION:

Microbesgrownondifferentialmediawereidentifiedandvariousspeciesofbacteriaidentified bystainingandbiochemical tests.*Staphylococcus*spp.wereidentifiedaswhitishcolor,gram positivecoccusshaped,catalasepositivebacteria.*Bacillus*spp.wasidentifiedasgram positive, rodshapedandnitratepositive.*Pseudomonas*spp.wasidentifiedasgramnegative,brownish, rodshapedandcitratepositivebacteria.

5.DISCUSSION

Refinedproductsofcrudeoil areknownaspetroleum productswhichincludedieselfuels, keroseneetc.crudeoiland theseproductsarecomposed ofpetroleumhydrocarbons. Their characteristicsdependontheamountofcarbonpresentintheirmolecularstructure.Problems canbeincreasebymany ofthemifpresentinsoil.Petroleum productsmay causeseverehealth risktohumansiftransportedfrom soil towaterresourcesastheseproductsextremelymobile. Toluene,Benzene,XyleneandEthylbenzenearesomeof examplesof petroleumhydrocarbons whichcausecancer.Contaminatedsoil alsoreleasesthefumesofpetroleum hydrocarbonsinthe atmospherewhichgenerallygetinhaledbythehumans.Someofthepetroleumhydrocarbonsare carcinogenicatverylowlevels.Mostof thepetroleumhydrocarbonsarebrokendownintoless harmfulsmallerproductsinhumanbody.Inhalationoffumesof petroleumhydrocarbonsor ingestionofcontaminatedsoilcreates healthissues toadults andchildren.Therefore reducingthe levelofpetroleumhydrocarbonsfromsoilisaneedtoberemovedonalargescale.Theresults of ourworkwillhelptoreduceorremovethelevelof contaminationoccursduetopetroleum hydrocarbonsinsoilofpetrolpumpsbywhichwecan try toimprovethefertilityandqualityof soil.Thisisverylittleeffortanddoneonasmallareabutifmajorconcernoccurstowards this problemwecanachieveabiggoal.

6.CONCLUSION

Satisfactoryresultsgotforthestrategiesusedforbioremediationtoincreasethebiodegradation ofdiesel oilcontaminatedsoilfrom petrol pump.Whenall theamendmentswereadded the efficiencyintermsofmineralization showedthedoublingprocess ofbiodegradationafterduring 40daysoftreatmentwithremovalofTotal PetroleumHydrocarbon(TPH).Themainlimiting factorwas thenutrientshortage.Bacteriahelpfulinbioremediationprocesswere*Pseudomonas* spp.,*Bacillus*spp.and*Staphylococcus*spp.Bioremediationprocessmainlydepends ontheeffect ofbioaugmentationwithbacterial species.

COMPETINGINTERESTS

Authors havedeclaredthatnocompetinginterests exist.

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Bioremediationpotentialofhydrocarbondegradingbacteria: isolation, characterization,andassessment

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BioremediationofPetroleumhydrocarbonby using Pseudomonas*speciesisolatedfrom Petroleum contaminatedsoil*

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