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Long Haul Bioremediation of Petroleum Contaminated Soil under Varying Moisture Content

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ABSTRACT: *Oil hydrocarbon spills cause defilement of soils, surface springs and groundwater supplies, along these lines negatively affecting the earth. Advances in science and innovation have empowered us to apply the capability of natural assorted variety for contamination decrease which is named as bioremediation. Bioremediation is an inventive innovation for the treatment of wide assortment of contaminants. The current examination was thus centered around treating oil tainted soils using the capability of bioremediation.*
The examination underscores on hydrocarbon debasement during the drawn out bioremediation of oil polluted soil. Dampness substance of the dirt was considered for enhancement, so as to assess its impact on the biodegradation procedure. Biodegradation of Total Petroleum Hydrocarbons (TPH) was inspected for 28 bioreactors with differing dampness content (30% - 90% field limit) and one reactor was taken as control bioreactor. The physico-substance and natural qualities of the dirt were tried on a week after week reason for a time of 23 weeks to decide the TPH debasement rate. It was seen that at dampness substance of 60% field limit, most extreme TPH expulsion of 78.21% was recorded and the debasement rate constants for fast and moderate period of debasement were 0.0250 d-1 and 0.00267 d-1respectively. Since the primary (fast) phase of degradationwas overwhelming, endeavors to upgrade natural movement ought to be coordinated towards the principal period of biodegradation.
Keywords:*LongTermBioremediation,TotalPetroleumHydrocarbons,MoistureContent.*

I. INTRODUCTION

Petroleumhydrocarbonsarewidelyusedinourdailylifeasfuelandchemicalcompounds.Asaresultofthismassiveuse,petroleumhasbecomethemostcommoncontaminantoflargesoilsurfaces,andeventuallyisconsideredasamajorenvironmentalproblem[1].Theincreaseinpublicawarenesstowardstheconservationoftheenvironmenthasledtothedevelopmentofvariousphysicochemicaltechniquesforcleaningupsites.Althoughmostofthephysicochemicalmethodscanbeefficientfortreatingawiderangeofpollutants,theyareextremelyexpensive[2].Consequently,bioremediationhasbecomeavaluablealternativetechnologytomanyphysicochemicalmethodsasitisacosteffectiveandenvironmentalfriendlytreatment[3].Hence,thisresearchworkwasfocusedonstudyingthebiodegradationofhydrocarbonsduringlongtermtreatmentofpetroleumhydrocarboncontaminatedsoils.

1. Literature Review

Bioremediationisaninvaluabletoolboxforwiderapplicationintherealmofenvironmentalprotection.Biologicalagents,mainlymicroorganismsi.e.yeast;fungiorbacteriaareusedtocleanupcontaminatedsoilandwater[4].Bioremediationhasbeensuccessfullyappliedforcleanupofsoil,surfacewater,groundwater,sedimentsandecosystemrestoration.Ithasbeenunequivocallydemonstratedthatanumberofxenobioticscanbecleanedupthroughbioremediation[5]. However,thereareawiderangeoffactorsknowntoreducetheabilityofsoilmicrobestobreakdowncontaminants.Thesefactorsincludenutrients,pH,temperature,moisture,oxygen,soilcharacteristicsandcontaminantbioavailability[6].Optimizingtheseenvironmentalconditionscouldenhancecontaminantsbiodegradationinthesoil[7].Biosurfactantscanbeusedtoimprovecontaminantbioavailabilitytosoilmicrobialdegradersthroughreducingcontaminantviscosityandthusincreasinghydrocarbonsolubility[3].

Overtheyears,lotofstudieshasbeenreportedonpetroleumhydrocarbondegraders[8].But,thereisnocomprehensiveandconclusivereportonthekineticsofbiodegradationofcrudeoil[9].Fewworkshavebeendedicatedtoinvestigatethekineticofsoilbioremediation[10,11,12].Informationonkineticsisextremelyimportantbecauseitcharacterizestheconcentrationofthechemicalremainingatanytimeandpermitspredictionofthelevelslikelytobepresentatsomefuturetime[13].Thus,informationondegradationkineticsandresultingresidualconcentrationsisnecessarytounderstandthebehaviorofpollutantsinsoilsandtoassesstheprospectsofremediation.However,dataonkinetics andresultingresidualconcentrationsfromthedegradationofTPHinlongtermpollutedfieldsoilsarescarce[14,15].Henceforth,thisresearchwasgearedtowardsstudyingthebiodegradationofhydrocarbonsduringthelongtermtreatmentofTPHcontaminatedsoilsandtodescribethetwoconsecutivefirstorderkineticreactions.Itwasalsofocusedontheoptimizationofmoisturecontentrequiredforeffectivebioremediation.

II. MATERIALS AND METHODS

Thefreshsoilwasexcavatedfromanopenfieldnear CivilEngineeringDepartment,JBCampus,Bangalore,atadepthof50cmfromthegroundsurface.Itwasthenairdried,pulverizedandsievedthrough4.75mm.Thesoilpassedthrough4.75mmandretainedon75micronwastakenfortheexperimentalwork.Thefreshsoilwasanalyzedforthevariousphysicochemicalandbiologicalcharacteristicsinordertoascertainitssuitabilityforbioremediationprocess(Table1).Thewasteoil(oroilysludge)wascollectedfromVRLLogisticslocatedatadistanceof4kmsfromJBCampus.Thefreshsoilwasmixedwithwasteoilandacclimatizedsoilintheratio10:2:1 (i.e.4kg offreshsoil:800gmof wasteoil:400gmof acclimatizedsoil).BiosurfactantrhamnolipidproducedinEnvironmentalEngineeringlaboratory,CivilEngineeringDepartment,JBCampus,Bangalorewasaddedtothebioreactorsin1:4ratio(i.e.1gmofbiosurfactantper4gmofsoil)toincreasethecontaminantbioavailabilitytosoilmicrobes.Thesoilwasthenmixeduniformlyandfilledinto PVCreactorsupto75%workingvolumeandremaining25%wasfreeboardforefficientdegradationofcontaminatedsoil.

28bioreactorsalongwithonecontrolbioreactorwereusedforsinglebatchexperiments.Theenvironmentalparametersaffectingbioremediationprocessweremaintainedatoptimumconditionsinallthebioreactorsthroughoutthestudyperiod.ThepHwasmaintainedwithinarangeof6.58.5,temperatureat20ºCto30ºCandC:N:Pratioat100:10:1[16].Thebioreactorswere maintainedatadifferentmoisturecontentof30%,40%,50%,60%,70%,80%and90%fieldcapacityandwererepresentedasM1,M2,M3,M4,M5,M6andM7respectively.Thesesevenbioreactors(withdifferentmoisturecontent)hadfourreplicateseach(numberofbioreactorsforthestudywasselectedbasedonstatisticalanalysis).OnebioreactorwaskeptasControlReactor(MC).Thecontrolreactorhadnoalterationsdonetoitsmoisturecontentthroughoutthestudyperiod.

Thebioreactorsweremonitoredregularlyandwereanalyzedonaweeklybasisforvariousphysicochemicalandbiologicalcharacteristicsforaperiodof23weeks.TheweeklyreductioninTPHconcentrationsinthebioreactorswasevaluatedtooptimizethemoisturecontentrequiredforefficientbioremediationandtounderstandthedegradationkinetics.

III. RESULTS AND DISCUSSION

3.1 Physico-chemicalandbiologicalcharacteristicsoffreshsoilandsimulatedsoil

TheinitialphysicochemicalandbiologicalcharacteristicsofthefreshsoilandsimulatedsoilareshowninTable1.Theresultsofthevariousphysicochemicalandbiologicalcharacteristicsofthesimulatedsoilinthebioreactorsafterseven andtwentythreeweeksoftreatment(i.e.afterIandIIstage)istabulatedinTable2andTable3respectively.

Table1:Initialphysico-chemicalandbiologicalcharacteristicsoffreshsoilandsimulatedsoil

Parameters	**Unit**	**FreshSoilConcentrations**	**SimulatedSoilConcentrations**
TypeofSoil	---	Sandy	Sandy
Porosity	%	37	---
Texture		WellGraded	WellGraded
Co-efficientofUniformity Cu	---	6.5	---
Co-efficientofCurvature Cz	---	1.10	---
pH	---	6.9	7.49
Temperature	^{o}C	28	26.9
MoistureContent	%	3.8	4.14
TotalOrganicCarbon	mg/gmofsoil	54.6	78.2
TPH	mg/kgofsoil	0	128000
Nitrogen	mg/gmofsoil	2.62	6.28
Phosphorous	mg/gmofsoil	0.24	0.64
MicrobialCount	CFU/gmofsoil	$47x10^{5}$	$19x10^{6}$

Table2:Physico-chemicalandbiologicalcharacteristicsofsimulatedsoilafter7weeksoftreatment(StageI)

Bioreactors / **Parameters**	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**MC**
pH	7.15	7.21	7.24	7.42	7.41	7.47	7.28	7.44
Temperature(^{o}C)	24.5	25.1	24.5	24.5	25.3	25.1	24.5	25.1
TOC(mg/gm)	37.55	36.18	34.06	32.55	34.43	39.82	38.20	39.90
TPH(mg/kg)	91050	67991	53012	37620	42216	61110	83001	102923
Nitrogen(mg/gm)	3.50	3.42	3.28	2.90	3.13	3.65	3.76	3.40
Phosphorus(mg/gm)	0.35	0.35	0.31	0.27	0.29	0.35	0.34	0.35
MicrobialCount(CFU/ gm)×10^{6}	17.4	26.1	46.4	48.2	43	30.1	17.1	15

*Concentrationsdepictedaretheaveragevaluesobtainedbyconsideringallfourreplicatesofeachbioreactor.

Table3:Physico-chemicalandbiologicalcharacteristicsofsimulatedsoilafter23weeksoftreatment(StageII)

Bioreactors / **Parameters**	**M1**	**M2**	**M3**	**M4**	**M5**	**M6**	**M7**	**MC**
pH	7.42	7.20	7.34	7.40	7.28	7.40	7.47	7.12
Temperature(^{o}C)	28.6	28.6	28.6	28.6	28.6	28.6	28.6	28.7
TOC(mg/gm)	18.33	15.99	15.43	13.23	14.02	17.11	17.80	22.01
TPH(mg/kg)	84203	60268	44869	27883	32864	53120	75500	99206
Nitrogen(mg/gm)	1.61	1.50	1.38	1.13	1.28	1.53	1.81	2.77
Phosphorus(mg/gm)	0.12	0.11	0.10	0.08	0.09	0.10	0.12	0.20
MicrobialCount(CFU/ gm)×10^{6}	7.2	14.2	21.3	29.2	20.4	13	8.2	7

*Concentrationsdepictedaretheaveragevaluesobtainedbyconsideringallfourreplicatesofeachbioreactor.

3.2 Analysis of Data and Interpretation

3.2.1 pH:TheinitialpHofthesimulatedcontaminatedsoilforallthebioreactorswas7.49.ThepHvaluesfluctuatedinaverysmallrangeinallbioreactors.ThefinalpHofallthebioreactorswaswithinthepHrangeof6.5to8.0whichisconsideredasoptimumvalueforoildegradation.[16,17].

3.2.2 Temperature:Thetemperaturerangedfrom20.7°Cto28.9°Cinallbioreactorsduringthestudy.Thetemperature variationsdidnotfollowadefinitepatternwithtime.However,thetemperaturefellwithintheoptimumrangerequiredforeffectivebioremediationprocess[16,18].Thisfacilitatedoptimalgrowthofmicrobialpopulationswhichinturnwasresponsibleforbiodegradationofpetroleumproducts.

3.2.3 MoistureContent:Themoisturecontentwerevariedlike30%,40%,50%,60%,70%,80%and90%offieldcapacity(incrementsof10%)forthebioreactorsM1,M2,M3,M4,M5,M6andM7respectivelyandweremaintainedassuchthroughoutthestudy.Themoisturecontentofthecontrolreactor(MC)wasnotmaintainedthroughoutthestudyperiod,i.e.allotherparametersotherthanmoisturecontentwasmonitoredforthecontrolrectorduringthestudyperiod.

3.2.4 TotalOrganicCarbon:TOCinthesimulatedcontaminatedsoilwasinitially78.2mg/gmofsoilandwasfinallyreducedto13.23mg/gmofsoilinbioreactorM4(60%MoistureContent)owingtoamaximumcarbonutilizationof83.08%bythemicroorganisms.Maximumcarbonutilizationwasobservedinthebioreactor(M4),whichalsohadamaximumbacterial countof91.5x10^{6}CFU/gmofsoil.

3.2.5 NutrientConcentration:C:N:Pratioof100:10:1isconsideredoptimalforbioremediation[18].Thenutrientswhenintheoptimalrangeallowmicrobestocreatenecessaryenzymestobreakdownthecontaminants.Hence,bioreactorswere supplementedwithUreaasasourceofNitrogenandSuperPhosphateasthesourceofPhosphorustobringthenutrientconcentrationstotherequiredlevels.

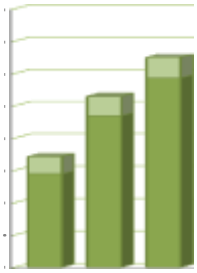
3.2.6 Microbial Activity: Microorganisms play a major role in bioremediation and their absolute numbers can determine the overall degradative ability [19]. The results of bacterial counts showed that the profiles of all the bioreactors followed a typical microbial growth pattern. The microbialcounts varied from 19 x 106 to 91.5 x 106

CFU/gm of soil in the third week and decreased to 48.2 x 106 CFU/gm of soil in the seventh week and further down to 29.2 x 106 CFU/gm of soil at the end of23 week sinbioreactorM4(60%MoistureContent).Thus,increaseinbacterialcounts had a profound influence on therate of TPHreduction.

3.2.7 TotalPetroleumHydrocarbons:TheconcentrationsofTPHinallthebioreactorstakenforthestudyhadadecreasingtrendwithincreasingbioremediationtimewhichistypicalofanydegradationprocess.ThepercentageofTPHreductionrangedbetweenaminimumof22.49%inbioreactorMCtoamaximumof78.21%inthebioreactorM4during23weeksoftreatment.Sinceallotherenvironmentalconditionswerekeptsameinallthebioreactors,themoisturecontentof60%offieldcapacityinreactorM4seemsadvantageousfortheindigenousmicroorganismstogrowandtherebycausemaximumdegradationofTPH.Table4andFigure1showpercentagereductionofTPHinthebioreactors.

Table4: TPHReductionintheBioreactors

Bioreactors	InitialTPH (mg/Kg)	FinalTPH(mg/Kg)		Reduction(%)		
		IStage(7Weeks)	IIStage(23Weeks)	IStage	IIStage	Total
M1=30%	128000	91050	84203	28.86	5.36	34.21
M2=40%	128000	67991	60268	46.88	6.03	52.91
M3=50%	128000	53012	44869	58.58	6.36	64.94
M4=60%	128000	37620	27883	70.60	7.61	78.21
M5=70%	128000	42216	32864	67.01	7.31	74.32
M6=80%	128000	61110	53120	52.25	6.25	58.50
M7=90%	128000	83001	75500	35.15	6.86	41.01
MC=Control	128000	105923	99206	17.24	5.25	22.49



3.3KineticsofBiodegradation

KineticanalysisperformedontheTPHdegradationrevealedadegradationpatterncharacterized bytwoconsecutivefirstorderreactions(biphasicprocess)inmostoftheexperimentalsettings.Thedegradationprocesswascharacterizedbyaperiodoffastdecrease(StageI)inthehydrocarbonconcentrationsduringthefirstsevenweeksfollowed byaperiodofsloweractivity(Stage2)inthesubsequentweeksoftreatment.

After sevenweeks oftreatmentthe TPHconcentrationsweredegraded down to91050, 67991,53012, 37620,42216,61110 and83001mg/kgofsoilwhichresultedin28.86,46.88,58.58, 70.60,67.01,52.25and35.15percentTPHreductioninbioreactorsM1toM7respectively(Table4).Aftertheinitialrapiddegradationphase,thebiodegradationrateslowlydecreasedinthelatterweeksoftreatment.ThefinalconcentrationsofTPHinthesimulatedcontaminatedsoilattheendoftreatmentperiod,i.e.twentythreeweeksoftreatmentwere84203,60268,44869,27883,32864,53120and75500mg/kgofsoilwhichresultedin34.21,52.91,64.94,78.21,74.32,58.50and41.01percentTPHreductioninbioreactorsM1toM7respectively(Table4).

The initialfast degradationphaseismediatedby bacterialutilizationof bioavailable

compoundsandisgovernedbyenzymekinetics.Itwasalsobenefitedbyadequatenutrientspresentintheinitialweeks.Inco ntrast,slowphasemaybegovernedbytherateofpetroleumdissolutionfromsoilparticles.

3.4DegradationRateConstant(k)

Thebiodegradationofhydrocarbonsincontaminatedsoilisassumedtofollowthefirstorderdegradation,assucht hefirstorderdegradationrateforvariousenvironmentalconditionsarecalculatedasfollowsusingfirstorderdegradationki neticequation.Thedegradationrateconstantsobtainedforthebioreactorsaretabulatedintheabulated5.

Table5:DegradationrateconstantsforthebioreactorsM1toM7

Bioreactors	**Degradationrateconstant(k)d^{-1}**	
	IStage(k1)	**IIStage(k2)**
M1=30%MoistureContent	0.0069	0.00070
M2=40%MoistureContent	0.0129	0.00108
M3=50%MoistureContent	0.0180	0.00149
M4=60%MoistureContent	0.0250	0.00267
M5=70%MoistureContent	0.0226	0.00223
M6=80%MoistureContent	0.0151	0.00125
M7=90%MoistureContent	0.0088	0.00084

Theresultsclearlyreflecttwodistinctphasesofbiodegradation.Thek1constantswereresponsibleforthefirststag eoffastdegradationandk2wasresponsibleforsecondstagedegradation.Itisthusconcludedthattheextentofresidualconce ntrationinthesoilwasdeterminedbythebiodegradationefficiencyduringthefirststageoftreatmentwhenthebiologicalpr ocessesdominated.Duringthefollowingperiod,abioticprocessesleadingtoreducedbioavailabilityofTPHwerelimitingt hebiodegradationrate.

IV. CONCLUSIONS

Moisturecontentofthepetroleumcontaminatedsoilhadprofoundinfluenceonbioremediation,sincebioremedi ationefficiencyvarieswithdifferentmoisturecontent.Thestudyrevealedthatpercentagereductionof TPHconcentrationi nbioreactorshaving30%,40%and50%moisturecontentwas34.21%,52.91%and64.94%respectively.Maximumdegra dationof78.21%wasobservedfor60%moisturecontent.Thereafterthepercentagereductionof TPHgraduallydecreasedfor70%,80%and90%moisturecontentas74.32%,58.50%and58.50%respectively.Thecontro lbioreactorMCshowedaTPHreductionof22.49%.Thereforeitisconcludedthattheoptimalconditionsforbetterdegradat ionofTPHismoisturecontentof60%fieldcapacityundertheC:N:Pratioof100:10:1.

Twodistinctphasesofbiodegradationwereobservedduringthelongtermtreatmentoftheabulatedcontaminatedsoil .Thereby,thereweretwodegradationrateconstants(k1andk2)obtainedforthestudyperiodof23weeks.Thek1constantsw ereresponsibleforthefirststageoffastdegradationandk2wasresponsibleforsecondstagedegradation.Thedegradationra teconstantsoftherapidphase(k1)rangedfrom0.0069to0.0250,whereas,fortheslowdegradationphasethedegradationrat e(k2)rangedfrom0.00070to0.00267forbioreactorsM1toM7.Itisthusconcludedthattheextentofresidualconcentrationi nthesoilwasdeterminedbythebiodegradationefficiencyduringthefirststage(sevenweeks)oftreatmentwhenthebiologi calprocessesdominated.Duringthesecondstage,abioticprocessesleadingtoreducedbioavailabilityofTPHwerelimitin gthebiodegradationrate.Therefore,asthefirstfewweeksoftreatmentdetermineitsefficiency,effortstoenhancethebiolog icalactivityshouldbedirectedtothatperiod.

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