EFFECT OF VARIOUS HYDROCARBON SOURCES ON THE GROWTH OF MICROBES AND THEIR CHARACTERISATION

Preeti Sing & Dr. Ajit Pandya

Department of Bio-chemistry, C.U Shah Science College, Ashram road, Ahmedabad

Pacific university Udaipur – Ph D student

Abstract:

Decrease in the biodiversity of the bacterial communities was observed during the study on the dynamics of bacterial communities during nutrient – enhanced oil spill Bioremediation. Presence of hydrocarbon boosts the profusion of hydrocarbon – degrading bacteria but decreases microbial diversity due to effect of crude oil on the metabolic activity of mixed microbial population. . In the actual process, 1.0 gm of soil sample was inoculated into 100 mL of Bushnell Haas (BH) broth containing 1.0% (v/v) crude oil as a sole carbon source. The flasks were incubated on a rotary shaker at 120 rpm at 37ºC for 7 days. . ***Certain microbes prefer simple sources like glucose, sucrose, lactose, mannose and other simple sugars, whereas certain microbes can utilize polymers in addition to simple sources.*** *There were many studies which have revealed that a group of microbes are also capable of utilizing tedious compounds like crude oil, PAH, hydrocarbons.*

INTRODUCTIONS

Microbial diversity of the contaminated site is affected by the contamination of hydrocarbons and various microorganisms having competence for degradation of such compounds which are isolated from the contaminated sites. Domination of the microorganisms capable of degrading contaminant is seen on contaminated site. Thus the diversification of the stressed system is less compared to the non stressed system.

Decrease in the biodiversity of the bacterial communities was observed during the study on the dynamics of bacterial communities during nutrient – enhanced oil spill bioremediation. Presence of hydrocarbon boosts the profusion of hydrocarbon – degrading bacteria but decreases microbial diversity due to effect of crude oil on the metabolic activity of mixed microbial population. Pseudomonas, Acinetobacter, Achromobacter, Micrococcus, Alcaligens, Vibrio, Xanthomonas are few well-known bacterial species which are showing capability of hydrocarbon degradation and Aspergillus, Penicillium, Saccharomyces, Fusarium, Trichoderma, Candida are few fungal species taking part in the biodegradation of the hydrocarbon products. (Cemilia 1993; Vivekanandan et al. 1999).

It was also reported in the study by Ridway (1990) that the Pseudomonas, Alcaligens, Nocardia and Micrococcus made up to 89.7 % of the isolated and identified bacteria from a gasoline contaminated aquifer. (Ridway et al. 1990) Napthalene and phenanthrene when present as the sole carbon source, nine out of right isolates are capable of using it. Such species belong to Pseudomonas and Sphingomonas. the Microbial ecological evidences two districts of Gujarat specifies that Pseudomonas was predominant genus amongst the hydrocarbon-degrading bacterial genera. (Patel & Ghosh 2003) Prevalence of 6 genera of bacterial community

Pseudomonas, Flavobacterium, Achromobacter, Bacillus, Alcaligenes and Micrococcus were observed during the analysis of water and sediment samples from Cuban shelf for hydrocarbon degrading bacteria. (Montero et al. 1996) San Diego bay sediments contaminated with different polyaromatic hydrocarbons and hexadecane belonged to various marine genera like Vibrio, Marinobacter.

MATERIALS AND METHODS TO ENRICHMENT MEDIA

Use of enrichment method is one of the most efficient methods for selective isolation of microbes of interest. In this technique media is composed in such a way that it will allow only the growth of only desired microbes. This method is easy as well as highly applicable. (Atlas 1993) Here, in this study a media containing combination of pure salts was incorporated with crude oil as a sole carbon source. Because only crude oil is present in the media as a carbon source so those microbes will be able to growth which ha s ability to degrade the crude oil. In the actual process, 1.0 gm of soil sample was inoculated into 100 mL of Bushnell Haas (BH) broth containing 1.0% (v/v) crude oil as a sole carbon source.

The flasks were incubated on a rotary shaker at 120 rpm at 37ºC for 7 days.The incubation period was kept longer for isolation of microbes as there are many microbes which may have longer lag phase under this media. After incubation, an inoculation loop of culture was streaked on Bushnell Haas agar containing 1.0% crude oil and incubated at 37°C until the visible growth is seen. Selective microbial colonies were picked and sub-cultured by streaking onto Bushnell Haas Plates containing crude oil several times to obtain pure cultures. These pure cultures were stored at low temperature for further experiment. (Ajit et al. 2015; Gopinathan et al. 2012)

Result and discussion

Carbon source is the key element for the growth of any microorganism. Each microbes has ability to consume number of carbons sources, however the utilization pattern may vary. Certain microbes prefer simple sources like glucose, sucrose, lactose, mannose and other simple sugars, whereas certain microbes can utilize polymers in addition to simple sources. (Prescott 2002; Atlas 1993; Roy et al. 2007; Onuoha et al. 2011) There were many studies which have revealed that a group of microbes are also capable of utilizing tedious compounds like crude oil, PAH, hydrocarbons. (Prabhakaran et al. 2014; Al-Hadhrami et al. 1996) When microbes are allowed to grow on simple sources they grow exponentially, but in case of complex compound, they tend some time for adaptation. This adaptation is intracellular synthesis of degrading enzymes responsible for degradation of complex compound. This is the reason for longer lag phase in such cases. This phase could be from 6 hrs – 48 hrs depending on the microbes and source of carbon. (Vincent et al. 2011)

From the obtained results it was found that yeast extract is the most efficient carbon source for the growth of all the microbes during the first 24 hrs. Upon extension of incubation period

rapid decrease in the growth was observed. This effect is seen because yeast extract contains many components which are essential for the growth of microbes along with carbon source. These components include certain proteins, peptides and other growth promoters.

These components are readily available in the media resulting into rate of microbial production. In case of glucose growth of microbes was increased gradually with time upto 48hrs for most of the cases. After that reduction is observed in Halomonas daqingensis PS13 and Parapusillimonas granuli PS14. Sucrose has shown steady increase in the growth of microorganisms upto 72 hrs. As compare to other sources, crude oil was found least preferred source for microbial growth. All the microbes were able to grow on crude oil but as compare to other three sources the rate was found very less.

Maximum growth was obtained after the incubation of 72hrs. The reason behind lesser growth is slow degradation of crude oil. This slower degradation results into slower liberation of monomers required for the growth and development of microbes. Maximum degradation of crude oil was achieved by Pseudoxanthomonas Mexicana with optical density of 0.823 at 600nm followed by Halomonas daqingensis, Parapusillimonas granuli and Bacillus cereus. Surprisingly Bacillus species was found least efficient; otherwise in the most of the study it has shown a very potential effect. If only biomass is concern then either glucose or sucrose will be preferred but in this study the main aim was to degrade crude oil hence it was preferred as carbon source and rest on the parameters standardize accordingly.

Table 2 has clearly explained the characteristics of all 4 microbes grown and isolated on the bases of cololny characterization by standard parameters. Numbers of colonies were obtained on the plates after suitable incubation time. Colony morphology of these colonies has suggested that, these microbes may be belonging to families of Bacilli, Rhodococcus, Acinatobater etc. However for confirmation other analysis must be performed. Initially each of the probable microbes was screened for their efficiency of crude oil degradation. However only limited microbes those are potential for crude oil degradation has to be selected for further study. Among the various isolated microbes by enrichment media four potential microbes which are able to degrade crude oil faster with better efficiency was selected for further study. Colony characteristics of the all the four microbes along with their grams nature, spore forming efficiency and motility are noted in table 2.

References:

Ajit Pandya and Preeti Sing, 2015 : OPTIMIZATION OF CARBON (C) SOURCE ON THE GROWTH OF HYDROCARBON DEGRADER AROBES, WJPR- Volume 4, Issue 9, 2182-2185

Al-Hadhrami, M.N., Scott, L. & Fisher, P.J., 1996. Effects of the addition of organic carbon sources on bacterial respiration and n-alkane biodegradation of Omani crude oil. Marine Pollition Bulletine, 32(4), pp.351–357.

Atlas, R., 1993. Handbook of microbiological media, CRC Press.

Cemilia, C., 1993. Biodegradation of polycyclic aromatic hydrocarbons. Current Opinion

in Biotechnology, 4, pp.331–338.

Gopinathan, R., Prakash, M. & Bharathirajan, R., 2012. An experimental study for crude oil biodegradation in contaminated soil. International Jounral of Current Microbiology and Applied Sciences, 1(1), pp.12–16.

Montero, J., Perez, G. & Rodriguez, F., 1996. Biodiversity of hydrocarbon-degrading marine bacteria isolated in tropical sea water. European Marine Microbiology Symposium, pp.11–15.

Onuoha, S.C. et al., 2011. Biodegradation Potentials of Hydrocarbon Degraders from Waste-lubricating Oil-spilled Soils in Ebonyi State , Nigeria. International Journal of Agriculture and Biology, 13(4), pp.586–590.

Patel, A.C. & Ghosh, S.K., 2003. Spatial and temporal diversity in petroleum degrading bacterial population. Asian J. of Microbiol. Biotechnol. and Environ. Sci.,, 3, pp.65–68.

Prabhakaran, P. et al., 2014. Bioremediation of Crude Oil in Synthetic Mineral Salts Medium Enriched With Aerobic Bacterial Consortium. International Jounral of Innovative Research in Science and Engineering and Technology, 3(2), pp.9236–9242.

Ridway, H.F. et al., 1990. Identification and catabolic activity of well- derived gasoline-degrading bacteria from a contaminated aquifer. Environmental Microbiology, 56, pp.3565–3567.

Roy, R. et al., 2007. Degradation of polyaromatic hydrocarbons by mixed culture isolated from oil contaminated soil — A bioprocess engineering study. Indian Journal of Biotechnology, 6(January), pp.107–113.

Vivekanandan, G., Vasudevan, N. & Laxmanperumalswami, P., 1999. Degradation of crude oil by bacterial consortium. Pollution Research, 18(3), pp.245–249.

Vincent, A.O. et al., 2011. Microbial Degradation and its Kinetics on Crude Oil Polluted Soil. Research Journal of Chemical Sciences, 1(6), pp.8–14.

Result Table			
Effect of various carbon sources on the growth of microbes			
Carbon Source	**24 hrs**	**48 hrs**	**72 hrs**
Bacillus cereus PS11			
Glucose	0.440±0.018	1.320±0.078	2.143±0.103
Sucrose	0.216±0.022	1.166±0.066	1.781±0.089
Yeast extract	1.568±0.051	1.353±0.075	0.825±0.066
Crude oil	0.344±0.022	1.325±0.016	0.393±0.016
Pseudoxanthomonas mexicana PS12			
Glucose	0.529±0.041	1.224±0.077	1.925±0.121
Sucrose	0.370±0.023	1.333±0.092	1.542±0.079
Yeast extract	1.316±0.067	1.560±0.106	1.523±0.088
Crude oil	0.357±0.021	0.377±0.025	0.823±0.054
Halomonas daqingensis PS13			
Glucose	.763±0.051	1.862±0.102	1.739±0.078
Sucrose	0.359±0.024	1.236±0.087	2.179±0.121
Yeast extract	1.510±0.084	1.209±0.068	0.924±0.086

Crude oil	0.373±0.022	0.452±0.032	0.754±0.054
Parapusillimonas granuli PS14			
Glucose	0.822±0.041	1.971±0.078	1.964±0.079
Sucrose	0.298±0.012	0.532±0.032	1.720±0.025
Yeast extract	1.281±0.069	1.915±0.089	0.762±0.034
Crude oil	0.272±0.009	0.320±0.012	0.549±0.021

Table 2 Colony characteristic of microbes

	Microorganism 1	**Microorganism 2**
Size	Medium	Medium
Shape	Round	Circular
Margin	Wavy	Entire
Elevation	Convex	Slightly Raised
Texture	Rough	Smooth
Opacity	Opaque	Translucent
Pigmentation	No Pigmentation	Yellowish
Gram's Staining	Gram Positive	Gram Negative
Spore Forming Efficiency	Spore Forming	Non Spore Forming
Motility	Motile	Motil

	Microorganism 3	**Microorganism 4**
Size	Medium	Medium
Shape	Circular	Circular
Margin	Entire	Wrinkled
Elevation	Flat	Raised
Texture	Smooth	Smooth
Opacity	Translucent	Opaque
Pigmentation	Yellowish	Light Yellow
Gram's Staining	Gram Negative	Gram Negative
Spore Forming Efficiency	Non Spore Forming	Non Spore Forming
Motility	Motile	Motile