Use of microbial consortium along with biosurfactants in oil sludge treatment

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ABSTRACT

Due to the excessive use of various oils, a large amount of oil sludge or waste water is produced annually which is causing various environmental and health issues. Bioremediation through the use of microbial consortium is an effective method. The use of consortium instead of single specie of bacteria has shown 89-94% improved results. However the microbes are still not able to work effectively due to bioavailability issues. This is mainly because of the low solubility of hydrophobic contaminants and microbes. This problem is overcome by use of biosurfactants which enhance the solubility and emulsification of hydrophobic substrates and microbes. These biosurfactants are produced by various organisms (mainly microbes) which are later isolated and used. Certain new and efficient insitu techniques are used for the application of microbial consortium which includes suspended growth, attached growth and hybrid growth. Waste water treated this way can be reused in the refinery procedures or can be dumped safely.

KEYWORDS: Microbial Consortium, Biosurfactants In Oil Sludge Treatment, Bioremediation Through The Use Of Microbial Consortium

Introduction:

The need for oil as a fuel and lubricant is increasing drastically with advancing technology. There are about 700 oil refineries in the world(2012 census). Water is used in refining and oil drilling processes. Some of this water comes in direct contact with crude oil producing contaminated waste water or sludge. This sludge is dumped in ponds, marshes and open pits, which posses various environmental hazards to superficial & ground water, soil and air. It also causes serious ecological problems. The pollutants in this sludge includes polycyclic aromatic hydrocarbons e.g. benzene, toluene,ethyl benzene^[1], asphaltene, phenol and its derivatives^[2],metal contaminants^[3], some carcinogens and immunotoxicants. Exposure to these contaminants can cause variety of health problems including damage to lungs, respiratory problems. mutations, cancer, nausea, irregular heartbeats, and birth defects. It also effects plants, decreases the fertility of soil and suppresses seed germination^[4].

Various physiochemicalmethods have been devised for the treatment of sludge e.g. oily sludge solidification method, chemical demulsification method. solid -liquid separation method etc. But.the bioremediation (through microbes) is the most cost effective, environment friendly and widely used method. In this paper use and preparation of microbial consortium in the treatment of oily sludge is discussed along with the hindrance in microbial action and the methods to use microbial consortium.

Pretreatment of oil sludge:

Hydrocarbons and other non polar compounds are good source of energy for the microbes but their bioavailability is a limiting factor because of the low solubility, non distribution of uniform spatial microorganisms and pollutants. and retardation of substrate diffusion by soil matrix^[5].

To overcome these obstacles a pretreatment of oil sludge is required. The pretreatment methods include electro coagulation, electro chemical oxidation and other physiochemical methods ^{[5][1] [6]}. These methods are effective (63% COD removal and 92.8% COD removal respectively) but are inconvenient and costly^[1]. However, pretreatment with bio surfactants (or bioemulsifiers) is a widely used method. Biosurfactants are the agents released by certain organisms which enhance the solubility and emulsification of hydrophobic substrates. It reduces the interfacial tension between water and hydrophobic pollutant (removing low solubility and retardation obstacles), allowing microbes to work effectively^[7] ^[8].But, the phenomenon behind this activity of biosurfactants is not known exactly ^[6]. Some scientists believe that the biosurfactants produce certain agents which increase the adhesion of microbes to the hydrocarbons. Table 1^[9] shows the different classifications of biosurfactants and the organisms they are isolated from.

No.	Type of biosurfactant	Name	Bacterial sp.
1. (Glycolipids	Rhamolipids	Nocardioides sp.
		Sophorolipids	Candida sp.
		Trehalose lipids	Rhodococcus sp.
	Lipopeptides & lipoproteins	Fengycin	Bacillus sp.
2.		Arthrofactin	Arthrobacter sp.
		Bile salts	Myroides sp.
		Fatty acids	Mycobacterium sp. Nocardia sp. Candida sp. Cladosporin sp.
3.	Phospholipids		Candida sp. Cladosporni sp.
	&	Phosphotidylth	Rhodococcus sp.
	Fatty acids	amamme	
4.	Polymeric Biosurfactants	Alasan	Acinetobacter sp.
		Bioemulsan	Gordonia sp.
5.	Particulate Biosurfactants	Whole cells	Yarrowia sp.
		Vesicles	Serratia sp.

The addition of Biosurfactants improves the efficiency of microbial activity for example At 20 °C, rhamnolipids (11.2 mg/L) increased the removal efficiency of crude oil from 17.7% (in the absence of rhamnolipids) to 63%. At 25 °C, the removal efficiency of crude oil was over 80% with the presence of rhamnolipids compared with 22.3% in the absence of rhamnolipids.^[10]

Microbial Agents for Oil Sludge Treatment:

The indigenous microbes can degrade the contaminant to large extent but if the concentration of these pollutants is higher than it becomes difficult for the microbes to work efficiently and effectively. So, these microbes have to be supplemented with nutrients e.g. Nitrogen, Potassium and Sulphur^[7].

Moreover, it is difficult for a single type of microorganism to degrade the wide range of hydrocarbons and non polar compounds. This was a hindrance in the process of bioremediation so scientists started using microbial consortium ^[2]. Some of the microbes known to degrade polycyclic aromatic hydrocarbons include Pseudomonas sp (P. aeruginosa, P. fluorescence and P. putida) [7] [2], Acinetobacterbaumannii strains [11] cornvbacteriumsp. micrococcus sp. flavobacteriumsp^[12] etc. Some Agrobacterium sp, rhizobia, leguminosarum and bv. Trifolli are used to remove metallic contamination ^[3].

To prepare consortium soil samples are collected from different areas (Oil rich soil). The different bacterial strains are isolated using standard serial dilution procedure. The isolated strains were further characterized on the basis of their substrate specificity and gram character. They are maintained on nutrient agar slants at 4°C and with 50% glycerol at - 20 °C for future use^[12]. The isolated strains are then individually inoculated by single streaking on selective media (i.e. with oily substrate) and checked for a zone of clearing around each bacterial isolate. The strains showing the positive results are subjected to gram staining to check morphology. To prepare successful microbial consortium, bacterial cultures must be compatible with each other in order to simultaneously produce all these enzymes required for the degradation.After the successful degradation of oil substrates in lab trials by the bacterial consortia large scale trials were also set up in closed container. The consortium needs a carrier material for the safe transfer of microbes e.g. corn cob.

It is possible to assess the performance of a waste water treatment plant by measuring the BOD of the inflow and the outflow. The use of consortium has shown to cause 60% reduction in BOD level of waste water as compared to inflow ^[13] and in another research where Baumannii strains were used 89 to 94% removal of total petroleum hydrocarbons was observed depending upon the consortium used. ^[4]

Methods of Application of microbes:

Basically the role of microbes in oil sludge treatment is to convert the complex hydrocarbons into simple compounds like water, carbondioxide and methane. After the pretreatment phase the sludge is treated with microbial consortium. Typically There are three methods for the application of microbial consortium: suspended, attached and hybrid ^[1].

Suspended growth: Aerobic microbes are used in this form of remediation. The microbes are present in the form of suspension i.e. they float freely in the reactor. It is of 5 types i.e. CSTR (continuous stirred tank reactor), SBR (sequence batch reaction), plug flow, complete mix and membrane bioreaction. The basic mechanism behind all these processes is the same which is demonstrated in the figure 2 below.

This method is efficient but the waste water has to be re-purified from the consortium of microbes. So, this method is replaced by attached and hybrid methods.

Attached growth: In this method the effective microbe is immobilized on an inert surface (e.g. rocks, slag or plastic). This

material is then placed in the bioreactors. When these immobilized bacteria come in contact with the sludge (hydrocarbon substrate) enzymes are released which produces a bio film. These biofilm act as sieve that filters the sludge as it passes through. (This filtration is done by converting the complex compounds into simpler ones)

Hybrid growth: It is a combination of attached growth and suspended growth method. Fixed bio filters are present in the bioreactors accompanying suspended microorganisms and carrier material. These biofilters are made up of polyurethane. As the activated sludge (sludge with microorganisms and carrier material) passes through the urethane filters, the microbes in the sludge immobilize themselves on the surface of the filters. Urethane filters also filter sludge from micro granules. The process has been termed Activated Sludge Biofilm Waste Water Treatment System (ASBWTS)^[12].

Conclusion:

Oil is the need of this era but the refining procedures of oil produce a large amount of sludge or waste water. This oil sludge is responsible for causing environmental pollution leading to health problems. Treating this with microbial consortium has proven to be an effective remedy. Microbial consortium used is a combination of hydrocarbon degrading microbes which work together to secrete enzymes that convert the complex hydrocarbons into simpler ones. Generally used microbes for treating oil sludge include Pseudomonas sp,

Acinobactersp, Micrococcus sp, cornybacteriumsp and flavobacter sp.

These bacterial species are isolated from oil rich soil samples and then checked for efficiency and their compatibility to work with other microbes. These species are then shifted to a carrier material and applied in the treatment of sludge. However, the hydrocarbons in the sludge are not readily available for microbial activity because of solubility problems. Biosurfactants resolve this problem by increasing the solubility and adhesion rate of hydrocarbons to microbes. So, sludge has to be treated with biosurfactants before microbial consortium is applied. The insitu application techniques of microbes include suspended growth in which microbial consortium float freely in the bioreactor, attached growth in which the microbial consortium is first immobilized on an inert surface and then inserted in the bioreactor, and hybrid growth which is a combination of both suspended and attached growth methods.

So, using advanced technologies, biosurfactant and consortium we can clean our environment from hydrocarbon contaminated waste water.

References:

S Ishak, A malakahmad and MH Isa. Refinery of waste water biological treatment, Journal of scientific & industrial researchVol 71 April 2012 pg 251-256

Ojumu, T.V, Bello O.O, Sonibare J.A and Solomon B.O. Evaluation of microbial systems for bioremediation of petroleum refinery effluents in Nigeria, African Journal of Biotechnology Vol. 4 (1), pp. 31-35, January 2005

SP McGrath, AM Chaudri, K E Giller, Long term effects of metals in sewage sludge on soils, microorganisms and plants, <u>Journal of</u> <u>Industrial Microbiology</u>.

A.K Mandal, PM Sarma, C P Jeyasleelan, V A Chanashettar, Beena Singh, Banwari L and J Datta. Large scale bioremediation of petroleum hydrocarbon contaminate waste at Indian Oil refineries International Journal of life sciences and PharmaResearchVol 2 Issue 4 (2012)

S Venkata Mohan. TakuroKisa. TakeruOhkuma. Robert A. Kanaly, Yoshihisa Bioremediation Shimizu for treatment of PAHtechnologies contaminated soil and strategies to enhance process efficiency, **Reviews** in Environmental science & Biotechnology, Vol 5 Issue 4 pp 347-374

Hong-zi Zhang, Xu-wei Long, Ru-yiSha, Guoliang Zhang, and Qin Meng, Biotreatment of oily wastewater by rhamnolipids in aerated active sludge system

K.S.M. Rahman, G. Street, R. Lord, G. Kane, T.J. Rahman, R. Marchant, and I.M.Banat. Bioremediation of petroleum sludge using bacterial consortium with biosurfactant,Environmental bioremediation technologies (2007).

C. Calvo, M. Manzanera, G.A. Silva-Castro, I. Uad, J. González-López. Application of bioemulsifiers in soil oil bioremediation processes. Future prospects, science of the total environment (2009)

I.E. Klosowska, K. medrzycka, E. Karpenko, Biosurfactants_biodegradability, toxicity, efficence in comparison with synthetic surfactants. (unpublished) FadiGebara, Activated sludge biofilm wate water treatment system, Water Research, <u>Volume 33, Issue 1</u>, January 1999, Pages 230–238

S Mishra, J Jyot, R C Kuhad and B Lal. Evaluation of Innoculum addition to stimulate In-Situ Bioremediation of oilysludge-contaminated soil, Applied and environmental Microbiology 2001

K.P.Y. Fong &H.M.Tan, Isolation of microbial consortium for activated sludge for the biological treatment of food waste, World Journal of microbiology & Biotechnology.

Calvo, F.L.Toledo, J. Gonzalez-Lopez, Surfactant activity of a nepthalene degrading bacillus pumilus strain isolated from oil sludge C, Journal of biotechnology.

V. Echeverria, G. Monsalve and H. vidales (2002) Continuous Treatment of oily sludge at Colombian refineries, CT&F cienciatecnologia y futuroVol 2 (2002). S Mishra, J Jyot, R C Kuhad and B Lal. Evaluation of Innoculum addition to stimulate In-Situ Bioremediation of oilysludge-contaminated soil, Applied and environmental Microbiology 2001 Wanj J, Shi Han Chang and Qian Yi, Waste Water Treatment in a hybrid biological reactor: effect of organic loading rates. Process Biochemistry, Vol 36, Issue 34, Nov 2000.