International Research Journal of Biological Sciences ISSN 2278-3202
Vol. **3(2),** 67-69, February **(2014)** *Int. Res. J. Biological Sci.*

Determination of Crude Oil Degradation Efficiency of Glass Biofilm of Isolated Bacterium and Fungus

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Available online at: www.isca.in, www.isca.me
Received 23rd October 2013, revised 1st December 2013, accepted 30th January 2014

Abstract

The natural ecosystem has greatly affected by the spills of crude and refined oils from various oil refineries. Different physical, chemical and biochemical techniques have been applied to degrade the crude oils spills. One of such technique is use of biofilm of oil degrading microbes. The present work has focused on the use of biofilms as a mean to degrade crude oil due to some of its property which makes its more advantageous then the planktonic cells. In this experiment, microbes having potential to degrade crude oil were isolated and biofilms of these microbes were prepared on glass matrix. These films were utilized for determination of their crude oil degradation efficiency. Not only this, phase contrast microscopic analysis was carried out to determine the biofilm consistency on glass matrix, before and after degradation study. Results of the study have shown that among the isolated microbes, Bacilli has maximum potential to make a firm biofilm with glass as compare to fungal spp. In addition to that, it also has great potential to degrade 90.0% of crude oil within 22hrs.

Keywords: Biofilms, Crude oil degradation, glass matrix.

Introduction

With the increasing in industrial activities, a potential rise in environmental pollution has also been occurred posing a threat to all living organisms including humans[1–4]. Oil spill is one of the major causes of concern, which is actually release of liquid hydrocarbons into the environment. Many oil spills have been reported those have caused extensive damage to life[1,2]. Oil spills generally occurred due to the release of crude oil from tankers, offshore platforms and wells. It may also be due to the spills of refined petroleum products. Small scale spills are generally not hazardous but big spills can affect humans as they posses PAH (polyacylic aromatic hydrocarbon) which can cause cancer and other disease. Marine animals are also affected as oil wets their fur and makes it difficult for them to keep warm. Toxic compounds in the oil kill the eggs and most of the fish die because of no gaseous exchange[2].

Due to above mentioned hazards it is very essential to treat such oil contaminated regions by various techniques. There are physical, chemical and mechanical methods are available for clearing of these spills. Use of floats to trap the oil, skimmer boats to gather oil and absorbent pads to soak up the oil are some physical and mechanical methods. Chemical methods involve coagulating agents to clump it up for easier pickup[2,4]. These methods are very expensive and also have many drawbacks. Therefore, there is a need to establish a different cost effective and eco-friendly method for removal of oil spill. Recent Studies have shown that it is possible to eliminate a wide range of pollutants and waste by using microbes[3,5,6]. This is actually a chemical dissolution of materials by bacteria or other biological means. In general it is known as biodegradation. It has not only been used extensively in clearing up the oil spills but also has been used in degrading heavy metals, industrial dyes and other harmful materials.

Biofilm is a complex aggregation of microorganisms growing on a solid substrate. It is generally found on solid substrates submerged in or exposed to some aqueous solution. Biofilms may consist of bacteria, fungi and achaea living within a matrix of excreted polymeric compounds[7–9]. This matrix protects the cells within it and facilitates communication among them through chemical and physical signals. These biofilms may also have water channels to distribute nutrients and other signaling molecules. This matrix is strong enough that in some cases, biofilms can become fossilized. Biofilms are applicable for degradation of hydrocarbons instead of the free floating bacteria as they have certain advantages. One of the major is the consistency of film which provides continuous source of microbes for degradation of crude oil.

In this work, a thin film of two isolated microbes were prepared on glass matrix and used for degradation of crude oil under laboratory condition. Consistency of microbial film was also determined by phase contrast microscopy.

Material and Methods

Sample collection: Oil contaminated soil as well as crude oil were collected in sterile autoclaved bottles from ONGC oil well, located near Aadraj village, Ahmedabad, gujarat, India. Sample were stored in cold condition and immediately transferred to the laboratory for further experiment.

Isolation of potential oil degrading organisms: BH media with 1.0% crude oil as sole carbon source was used for selective screening of crude oil degrading microorganisms. Flask was incubated for 48 hrs under standard conditions and isolation of microbes was done by streak plate method after serial dilution on the petri plates containing same media. After 48hrs of incubation, colonies having different morphology were isolated and used for the determination of their crude oil degradation capability.

Preparation of Biofilm: Glass slides of 400mm X 150mm X 2mm were used for preparation of biofilms. These slides were first sterilized by autoclaving and then immersed into densely populated active microbial culture for atleast 24 hrs under static condition. After 24 hrs glass slides were washed gently with sterile distilled water to remove unbound cells.

Determination of Crude oil Degradation: For determination of crude oil degradation capacity selected microbes were inoculate in the BH media containing 1.0% crude oil and incubated for 24 hrs. Here, rate of crude oil degradation and microbial growth rate were determined using spectrophotometer.

Determination of biofilm consistency: Phase contrast microscopy was used to determine the consistency of biofilms before and after crude oil degradation.

Results and Discussion

As a result of selective screening, atleast six different microbes were found which have potential for degradation of crude oil. Out of these six strains, two strains were found to be the most efficient. Based on colony morphology, it was found that among the two microbes one belongs to *Bacilli* family and the other belongs to *Aspergillus* family. However, species level identification needs to be done using 16s rRNA sequencing.

Upon observation in phase contrast microscope at low power and high power, a fine definite arrangement of *Bacilli* was observed. A layer of exopolysaccharide was found present in the surrounding of each cell which plays a vital role formation of biofilm. Not only this, it is also responsible for a strong and sticky binding among the cells and with matrix[10–13]. In case of fungi, filamentous structure was observed without any specific pattern of arrangement. This irregular arrangement is because of presence of fungal mycelia. No doubt these mycelia increase the surface area but cannot increase the rate of crude oil degradation directly[7,10].

In the biodegradation assay, flasks inoculated with *Bacilli* biofilms were able to degrade more than 90.0% of crude oil within 22 hrs. However, for the initial 6 hrs no such activity or microbial growth were observed. This is because of longer lag phase and lower number of microbes in the culture media to degrade crude oil. After 6 hrs, the degradation started at an exponential way and reached maximum at 14 hrs. Similar pattern of growth for the fungus biofilms was observed but rate of degradation was found lesser than *Bacilli* biofilm. It took 24 hrs for 70.0% degradation of crude oil.

Phase contrast microscopic observation has revealed that after 24hrs of degradation of crude oil *Bacilli* biofilm remain intact as compare to fungus (figure 1 and 2). In fungus biofilm, clumps of fungus were found present in floating condition in the crude oil containing media indicating the loose binding with the glass (figure 3 and 4). These clumps are because of loose binding of fungus with glass matrix resulted into breakage of biofilm[7,10].

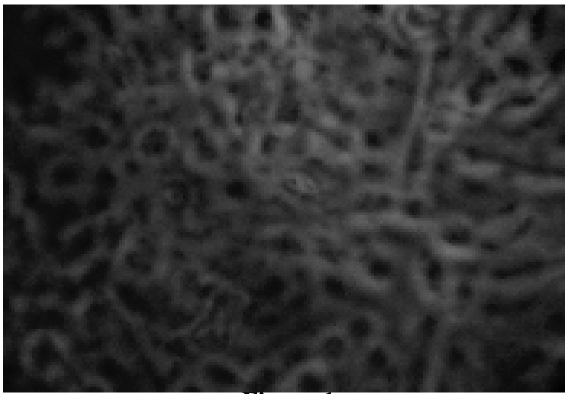

Figure 1
***Bacilli* biofilm before degradation**

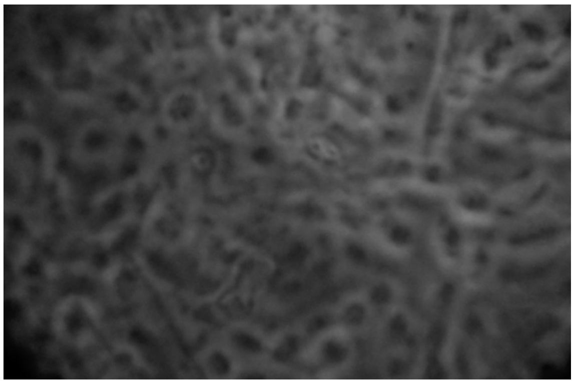

Figure 2
***Bacilli* biofilm after degradation**

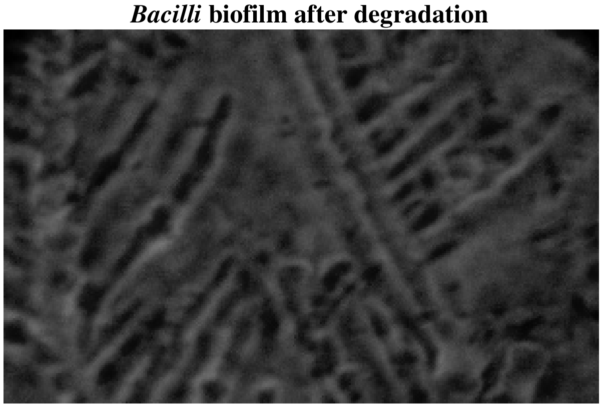

Figure 3
Fungus biofilm before degradation

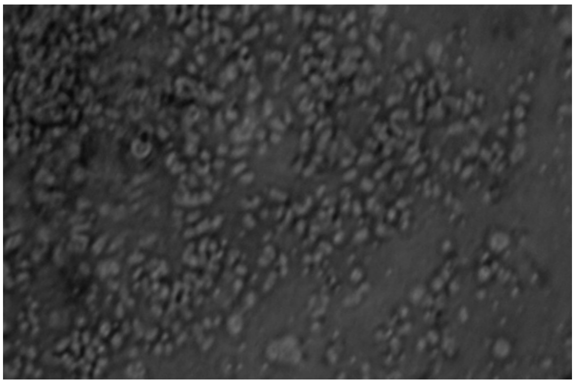

Figure 4
Fungus biofilm after degradation

Conclusion

From overall study, it was found that *Bacilli* can be a potential microorganism for glass biofilm production for degradation of crude oil. However, variety of other matrixes could also be used for the same purpose but one need to standardize the methods. In addition to that, cross linking and binding of microbes with the aid of other chemical or physical agents with the matrix can improve the biofilm stability and its efficiency.

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