2011 International Conference on Environmental, Biomedical and Biotechnology
IPCBEE vol.16 (2011) © (2011)IACSIT Press, Singapoore

Optimization of growth condition for hydrocarbon degrading bacteria isolated from oily sludge in order to achieve the most efficient conditions in environmental bioremediation

Hossein Motamedi[1], Amin Afifi[1], Hadi Leilavi[1], Bahram Alizadeh[2] and Mostafa Amopour Bahnamiry[1]

1- Department of Biology, Faculty of Science, Shahid Chamran University, Ahvaz, Iran

2- Department of Geology, Faculty of Geological Science, Shahid Chamran University, Ahvaz, Iran

Abstract. Petroleum refineries around the world have generated the solid wastes during the refining process and stocking of crude oil. Oily sludge leads to destructive effects in the environment. This investigation concentrated in degrading oily compounds from oily sludge in order to environmental bioremediation. Eight bacterial strains able to grow on the oily sludge as sole source of carbon and energy isolated from oily sludge and soil-contaminated with oily sludge in Abadan refinery. Two bacterial strains were adopted as efficient strains and were named AOR1 and AOR2. Influence of different nitrogen and phosphorous sources on the growth of these two bacterial strains were investigated by measuring the growth rate through serial dilution and colony counting in T.S.A medium. Sodium nitrate and disodium hydrogen phosphate were found to be the best nitrogen and phosphorous sources for the AOR1 strain. Ammonium chloride and diammonium hydrogen phosphate were found to be the best nitrogen and phosphorous sources for the AOR2 strain. It can be concluded that these isolates have potential for environmental pollution bioremediation and supplying these nitrogen and phosphor resources to their medium can accelerate this process.

Key words: oily sludge, biodegradation, environmental bioremediation, optimization

1. Introduction

Crude oil is an oily mixture with different content. During oil extraction and through petroleum based industries including oil refineries, tremendous amount of oily sludge is released to the environment, which pollutes both terrestrial as well as aquatic ecosystems(3). The higher concentration of these pollutants is likely to affect soil texture (1). Contamination with refinery sludge creates a distinct type of ecological habitat that invariably changes the physico-chemical properties of soil and leading to affect germination of seeds and growth and development of plant lives. This oily sludge may completely eliminate the vegetation of a place hampering soil aeration, destroying rhizospheric system including microbial populations. This brings a serious and disastrous changes in composition of flora leading to lost this habitat (2). In situ bioremediation using indigenous microorganisms is by far the most widely used. Indigenous bacteria in the soil can degrade a wide range of target constituents of the oily sludge, but their population and efficiency are affected when any toxic contaminant is present at high concentrations. The reintroduction after enrichment of indigenous microorganisms isolated from a contaminated site helps to overcome this problem, as microorganisms can degrade the constituents and have a higher tolerance to toxicity(4). In the present investigation, therefore, an attempt was made to optimize growth conditions of hydrocarbon degrading bacteria isolated from oily sludge in order to achieve the best efficient conditions in environmental bioremediation.

2. Material and Methods

In this investigation optimization of growth condition were examined for two bacterial strains isolated from oily sludge and soil-contaminated with oily sludge. These bacteria based on biochemical and morphological tests belonging to the genus Pseudomonas and Klebsiella, respectively, were named AOR1 and AOR2. Some characteristics of these two strains is as follows:

Strain of AOR1 through biochemical and morphological tests identified as gram negative bacilli, catalase positive, oxidase positive, urease positive and SIM negative. Strain of AOR2 through biochemical and morphological tests identified as gram negative bacilli, catalase positive, oxidase negative, urease positive and SIM negative.

2.1. Drawing growth curves

Growth curves were depicted by serial dilution method. In this method an inoculums of desired bacteria was prepared in the zero time and was inoculated to the mineral base medium containing oily sludge as the sole carbon and energy source. Then, 1ml values was removed from the mineral base medium and after preparing serial dilution were cultured on the Tryptic-soy agar medium. Numbers of colonies were counted in each culture. This colony counting was repeated every 12 hours and bacterial growth curves were drawn after 6 days.

2.2. Optimization of growth condition for isolated strains

Influence of different nitrogen and phosphorous sources on the growth of isolated bacteria were investigated in the oily sludge.

2.3.1. Optimization of nitrogen source

Influence of sodium nitrate, ammonium chloride, ammonium nitrate and urea on the growth of two bacterial strains in mineral base medium was investigated by measuring the growth rate by serial dilution in exponential phase.

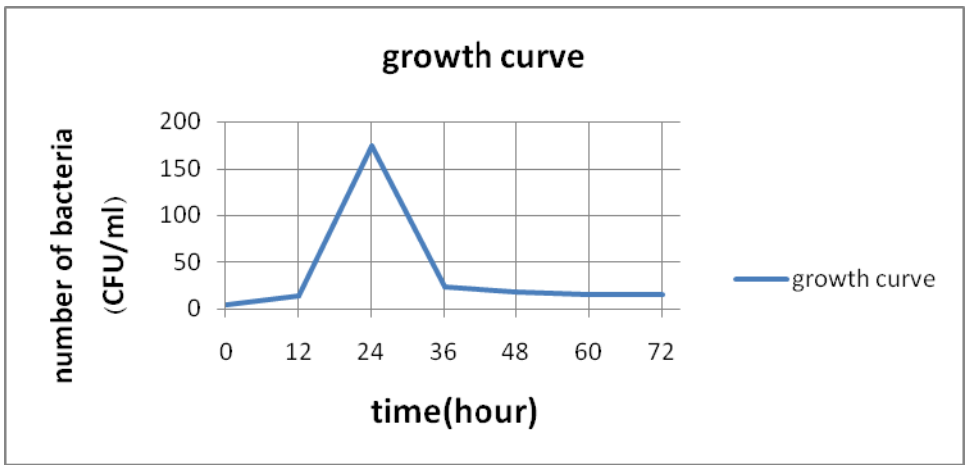
2.3.2 Optimization of phosphorous source

Influence of dipotasium hydrogen phosphate, disodium hydrogen phosphate and diammonium hydrogen phosphate on the growth of two bacterial strains in mineral base medium were investigated by measuring the growth rate by serial dilution in exponential phase.

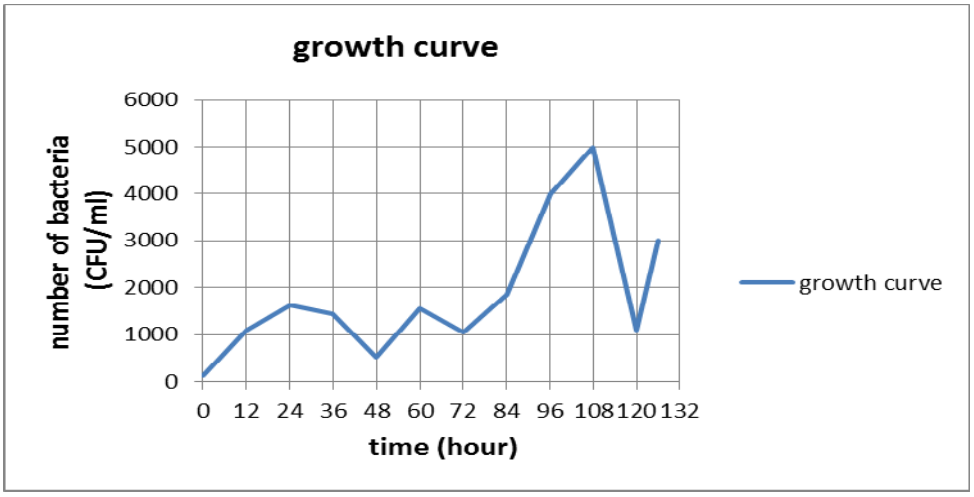
3. Results and Discussion

As can be seen from the growth curve, AOR2 strain grow much faster than AOR1 strain and more quickly reaches to stationary phase (24h). Therefore, in the biological bioremediation processes that aim to achieve faster results in less time it can be useful.

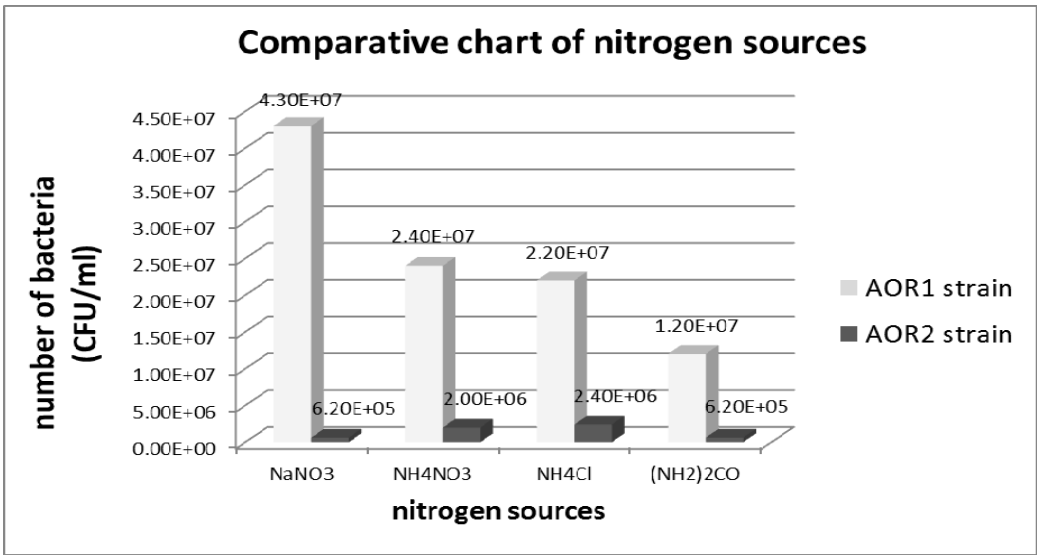
Diagrams 3 and 4 indicate optimization results of nitrogen and phosphate sources. Based on the results, ammonium chloride and sodium nitrate cause highest growth rate in the mineral base medium (Chart 4). In examination of optimum Sources of phosphate for growth in the mineral base medium containing oily sludge as the sole carbon and energy source by AOR1 and AOR2 strain it was determined that the ammonium hydrogen phosphate and sodium hydrogen phosphate cause highest growth rate in the mineral base medium (see Figure 3). Hydrocarbon degrading bacteria in oily sludge through the decomposition of these compounds reduce the waste volumes and are able to reduce their toxicity. The bacteria can also through the production of Biosurfactant and conversion of heavy oil compounds to lighter compounds and use them as the substrate have also important economically. In addition, due to existing toxic and carcinogenic compounds in oily sludge, decomposition of these compounds by the bacteria neutralized this destructive effects.

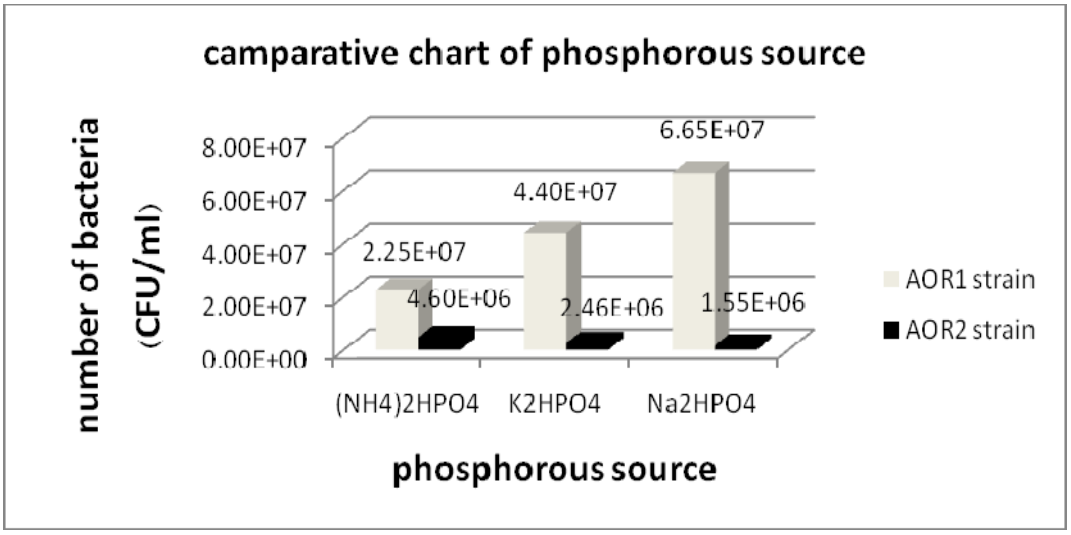

growth curve
number of bacteria (CFU/ml)
200
150
100
50
0
0 12 24 36 48 60 72
time(hour)
growth curve

AOR2


growth curve
number of bacteria (CFU/ml)
6000
5000
4000
3000
2000
1000
0
0 12 24 36 48 60 72 84 96 108 120 132
time (hour)
growth curve

AOR1


Comparative chart of nitrogen sources
number of bacteria (CFU/ml)
4.50E+07
4.00E+07
3.50E+07
3.00E+07
2.50E+07
2.00E+07
1.50E+07
1.00E+07
5.00E+06
0.00E+00
4.30E+07
6.20E+05
2.40E+07
2.00E+06
2.20E+07
2.40E+06
1.20E+07
6.20E+05
NaNO3
NH4NO3
NH4Cl
(NH2)2CO
nitrogen sources
AOR1 strain
AOR2 strain



4. Acknowledgements

The authors wish to thanks for the Vice Chancellor of Research in Shahid Chamran University, Ahvaz, Iran.

5. References

[1] Alexander, M. (1981) Biodegradation of chemicals of environmental concern. *Science,* 211**,** 132.

[2] Baruah, D. & S. K. Sarma (1996) Effects of crude oil pollution on species composition, biomass and production of a herbaceous community in the Lakwa oil fields of Assam (India). *International Journal of Ecology and Environmental Sciences,* 22**,** 95-100.

[3] Bossert, I. & R. Bartha. 1984. The fate of petroleum in the soil ecosystems. In *Petroleum Microbiology,* ed. A. R.M., 435-473. New York: Macmillan.

[4] Mishra, S., J. Jyot, R. C. Kuhad & B. Lal (2001) Evaluation of inoculum addition to stimulate in situ bioremediation of oily-sludge-contaminated soil. *Applied and Environmental Microbiology,* 67**,** 1675.