October - November 2017; 6(6): 2840-2844

International Journal of Research and Development in Pharmacy & Life Science
An International open access peer reviewed journal
ISSN (P): 2393-932X, ISSN (E): 2278-0238
Journal homepage: http://ijrdpl.com

Original Article

Optimization of Diesel oil degrading Bacterial strains at various culture parameters

T. Sivagamasundari*[1] and N. Jayakumar [2]

[1]Assistant Professor, Department of Microbiology and Biotechnology, N.M.S.S. Vellaichamy Nadar College, Madurai, India
[2]Assistant Professor, Department of Microbiology, Government Arts College, Surandai, India

Keywords: Hydrocarbon, Optimization, Bioremediation, parameters

Article Information:

Received: August 17, 2017;
Revised: September 23, 2017;
Accepted: October 10, 2017

Available online on:
15.10.2017@http://ijrdpl.com

http://dx.doi.org/10.21276/IJRDPL.2278-0238.2017.6(6).2840-2844

ABSTRACT: Environmental pollution with petroleum has been recognized as one of the most serious current problems. Petroleum hydrocarbons are a combination of hydrocarbons obtained from reservoirs of crude petroleum. The petroleum hydrocarbons contain aliphatic hydrocarbons and aromatic hydrocarbons. The most common petroleum hydrocarbons polluting environment are the gasoline, diesel and fuel oils. Numerous microorganisms could use petroleum as only source of carbon as energy for metabolic activities, and these microorganisms are broadly present and distributed in nature. Bioremediation is the use of microorganism metabolism to remove pollutants. Bioremediation is the biological process involving living microorganisms to remove contaminants or pollutants from soil or water. It involves the use of microbes to detoxify and humiliate environmental contaminants. The present study is carried out by optimization of diesel oil degrading microbes from diesel oil polluted sites. *Bacillus sp* and *Enterobacter hormaechei* were isolated according to Bergey's manual and by 16s rRNA gene sequence. Further the optimization was carried out for various culture parameters such as various hydrocarbon concentration, pH, temperature and for best carbon and nitrogen sources. The present study suggests that *Bacillus sp* and *Enterobacter hormaechei* were able to degrade diesel oil at pH 7, 300 C and preliminary hydrocarbon concentration of 1%.

T. Sivagamasundari, Assistant Professor, Department of Microbiology and Biotechnology, N.M.S.S. Vellaichamy Nadar College, Madurai, Tamil Nadu, India. E-mail: sivagamiraj82@gmail.com.
Dr. N. Jayakumar, Assistant Professor, Department of Microbiology, Government Arts College, Surandai, Tamilnadu, India

INTRODUCTION

The biodegradation of petroleum products in the environment depends on physical and chemical factors. The main significance of this is in determining the course and effectiveness of the process is pH. Most heterotrophic bacteria and fungi prefer a pH that is close to neutral. Most aquatic ecosystems are characterized [1] by neutral or slightly alkaline pH whereas the pH of soil can be within a broad range of values (pH 2.5 – 11).

Temperature plays a significant role in controlling the nature and extent of microbial hydrocarbon metabolism (Nedwell, 1999). Temperature affects the rate of biodegradation, as well as physical nature and chemical composition of hydrocarbons [14], Rowland *et al., 2000*). Although microbial activity is generally reduced at low temperatures, many of the components in crude oil and diesel can be degraded by psychrophilic and psychrotrophic microorganisms [14] [5][3][2].

Biodegradation is the process of degrading organic contaminants into simple matters using microorganism [15]. Nowadays, biodegradation become more popular due to its environmental safety, cost effectiveness and high toxic removal efficiency. Oil spills that released into the environment may contaminate soil as well as water resources that results with serious environmental issues. And in that cases biodegradation provides the best solution that results with transforming the toxic chemical into simple non-toxic forms. Hydrocarbon and petroleum products are consider as the major form of energy resources that are originate through biological process (Surridge 2007).

Even, their contamination severely effects the living system include plant, animal and humans because they possess carcinogenic and mutagenic effects [13].

Based on the contaminant concentration the biodegradation of petroleum products can be achieved by using native microorganism, bio stimulation or employed by using bio augmentation. The isolation and understanding the behaviour of biodegrading microbes with different parameters is more essential to carry out the biodegradation in an effective way.

MATERIALS AND METHOD

Effect of various concentration of diesel oil on bacterial growth and biodegradation

The midlog bacterial culture (*Bacillus coagulans and Enterobacter hormaechei)* were inoculated mineral salt medium suspended with different concentration of diesel oil (0.5%, 1%, 1.5%, 2.0% and 2.5%). The flasks were incubated at 37°C with pH 7.5 on rotary shaker at 150rpm for 72 hours. Every 12 hours interval the growth was measured at 620 nm.

Effect of pH on bacterial growth and biodegradation

The effect of hydrogen ion concentration on growth and degradation of 1% diesel oil was studied. Mineral salt medium with diesel oil was prepared at different pH (5, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0, 9.5, 10.0) using 1N HCl / 1N NaOH. The flasks were inoculated with bacterial cultures and incubated at 37°C for 24 hours. The growth was measured at 620 nm.

Effect of temperature on bacterial growth and bio-degradation

The influence of different temperatures 15°C, 30°C and 45°C on the growth and degradation of diesel oil by the bacterial isolates at different time intervals was studied using mineral salt medium with 1% diesel oil at pH 7.5. The population and percentage of degradation were determined.

Effect of Various Carbon sources on bacterial growth

Different carbon sources such as Glucose, Sucrose, Maltose and Starch were taken, 1% of these carbon sources were added in the medium with pH 7 and the flasks were incubated at 30^0 C for 7 days. Then the growths of these isolates were determined by using spectrophotometry at 620nm.

Effect of Various Nitrogen sources on bacterial growth

Various nitrogen sources such as Urea, Ammonium Chloride, Sodium nitrate and Peptone were taken, 1% of these nitrogen sources were added in the medium with pH 7 and the flasks were incubated at 30^0 C for 7 days. Then the growths of these isolates were determined by using spectrophotometry at 620nm.

RESULT

Effect of various concentration of diesel oil on bacterial growth and biodegradation

The growth of *Bacillus sp* and Enterobacter *hormaechei* were studied under different concentrations of diesel oil containing medium. The results were tabulated (Table 1 and Table 2). Both organisms were grown well in the medium containing diesel oil.

Table 1: Effect of different concentrations of Diesel oil on the growth of *Bacillus* sp.

Time (Hours)	Diesel Oil Concentration (in %)				
	Optical Density (620 nm)				
	0.5	1	1.5	2	2.5
6	0.1	0.35	0.39	0.45	0.5
12	0.12	0.51	0.61	0.63	0.58
18	0.24	0.63	0.75	0.67	0.8
24	0.35	0.82	0.82	0.79	0.96
30	0.4	0.92	0.9	0.85	1.11
36	0.41	0.98	0.96	0.94	1.15
42	0.45	1	1.1	0.97	1.23
48	0.56	1.3	1.12	1	1.15
54	0.78	1.46	1.16	1.12	1.13
60	0.94	1.5	1.11	1.07	1.08
66	0.9	1.43	1.04	1.01	1.01
72	0.83	1.37	0.95	0.96	0.96
78	0.72	1.29	0.87	0.87	0.89
84	0.64	1.21	0.7	0.79	0.81
90	0.52	1.17	0.64	0.71	0.76
96	0.43	1.01	0.57	0.66	0.69

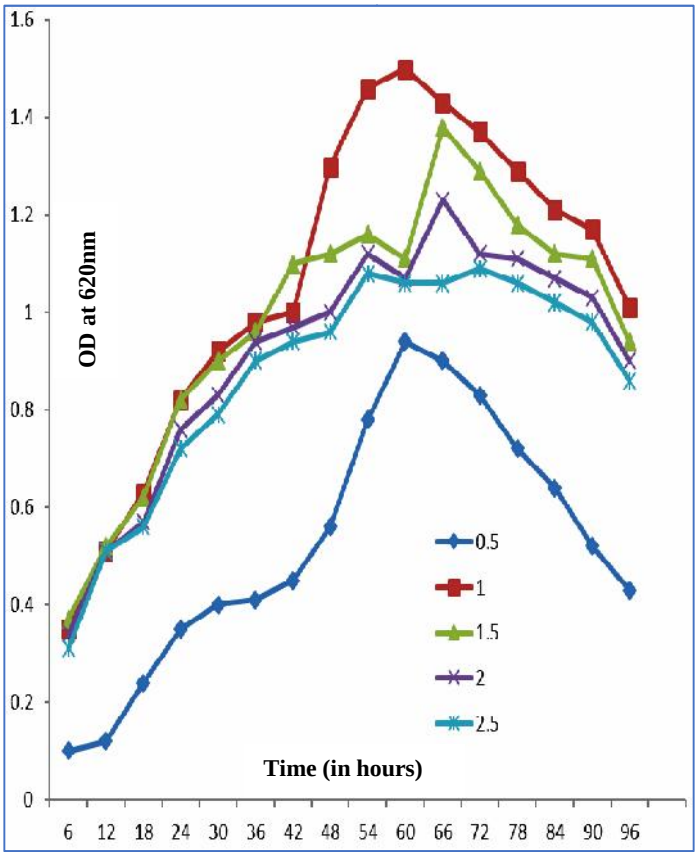

Figure 1: Effect of different concentrations of Diesel oil on the growth of *Bacillus* sp

Table 2: Effect of different concentrations of Diesel oil on the growth of Enterobacter *hormaechei*

Time Hours)	Diesel Oil Concentration (in %)				
	Optical Density (620 nm)				
	0.5	1	1.5	2	2.5
6	0.1	0.35	0.39	0.45	0.5
12	0.12	0.51	0.61	0.63	0.58
18	0.24	0.63	0.75	0.67	0.8
24	0.35	0.82	0.82	0.79	0.96
30	0.4	0.92	0.9	0.85	1.11
36	0.41	0.98	0.96	0.94	1.15
42	0.45	1	1.1	0.97	1.23
48	0.56	1.3	1.12	1	1.15
54	0.78	1.46	1.16	1.12	1.13
60	0.94	1.5	1.11	1.07	1.08
66	0.9	1.43	1.04	1.01	1.01
72	0.83	1.37	0.95	0.96	0.96
78	0.72	1.29	0.87	0.87	0.89
84	0.64	1.21	0.7	0.79	0.81
90	0.52	1.17	0.64	0.71	0.76
96	0.43	1.01	0.57	0.66	0.69

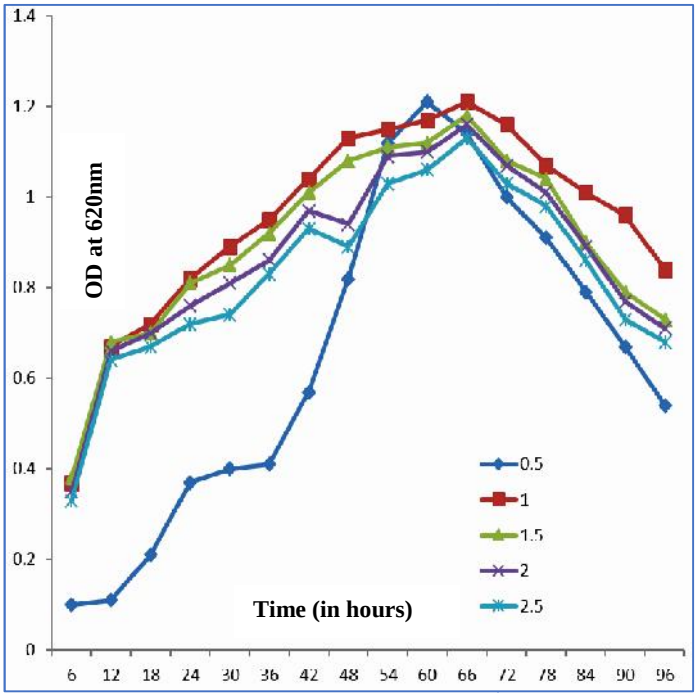

Figure 2: Effect of different concentrations of Diesel oil on the growth of *Enterobacter hormaechei*

Effect of pH on bacterial growth and biodegradation

The growth of *Bacillus sp* and *Enterobacter hormaechei* were studied under different pH (5 to 10). The well growth of these organisms was observed in the medium with the pH of 7.0 (Table 3).

Effect of temperature on bacterial growth and bio-degradation

The growth of *Bacillus sp* and *Enterobacter hormaechei* were studied under different temperature (15, 30 and 45°C).

The optimum growths of these organisms were observed in the medium that incubated with the temperature of 30°C (Table 4).

Table 3: Effect of pH on the growth of *Bacillus* sp and *Enterobacter hormaechei*

pH	Optical Density (620 nm)	
	Bacillus sp	*Enterobacter hormaechei*
5.0	0.5	0.7
5.5	1.1	1.3
6.0	1.2	1.5
6.5	1.5	1.8
7.0	1.8	2.0
7.5	1.4	1.7
8.0	1.2	1.4
8.5	1.0	1.2
9.0	0.62	0.61
9.5	0.56	0.53
10.0	0.37	0.40

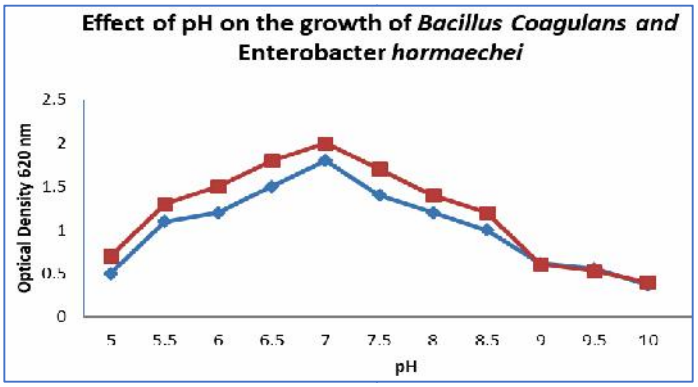

Figure 3: Effect of pH on the growth of *Bacillus* sp and *Enterobacter hormaechei*

Table 4: Effect of Temperature on the growth of *Bacillus* sp and *Enterobacter hormaechei*

Temperature (°C)	OPTICAL DENSITY (620 nm)	
	Bacillus sp	*Enterobacter hormaechei*
15	1.0	1.2
30	2.5	2.7
45	1.8	1.6

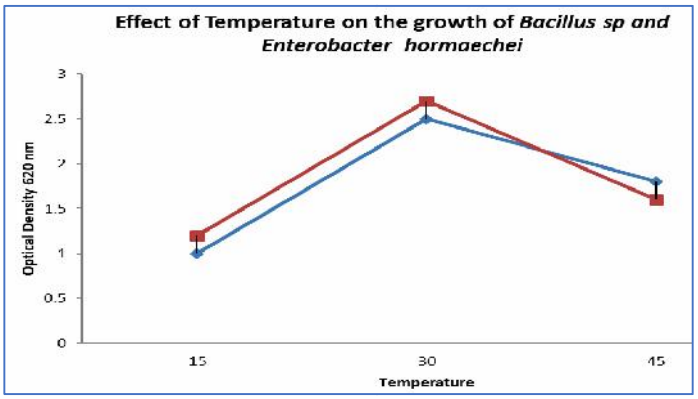

Figure 4: Effect of Temperature on the growth of *Bacillus* sp and *Enterobacter hormaechei.*

Effect of various Carbon sources on bacterial growth

Different carbon sources such as Glucose, Sucrose, Maltose and Starch were used to study the bacterial growth. Excellent growths of the organisms were observed in Sucrose (Table 5).

Effect of Various Nitrogen sources on bacterial growth

Various nitrogen sources such as Urea, Ammonium Chloride, Sodium nitrate and Peptone were used to study the bacterial growth. Peptone optimally supports the growth of the bacterial strains (Table 6).

Table 5: Effect of various Carbon sources on the growth of *Bacillus* sp and *Enterobacter hormaechei*

CARBON SOURCES	*Bacillus* sp	*Enterobacter hormaechei*
GLUCOSE	0.11	0.13
SUCROSE	0.15	0.18
MALTOSE	0.09	0.1
STARCH	0.07	0.08

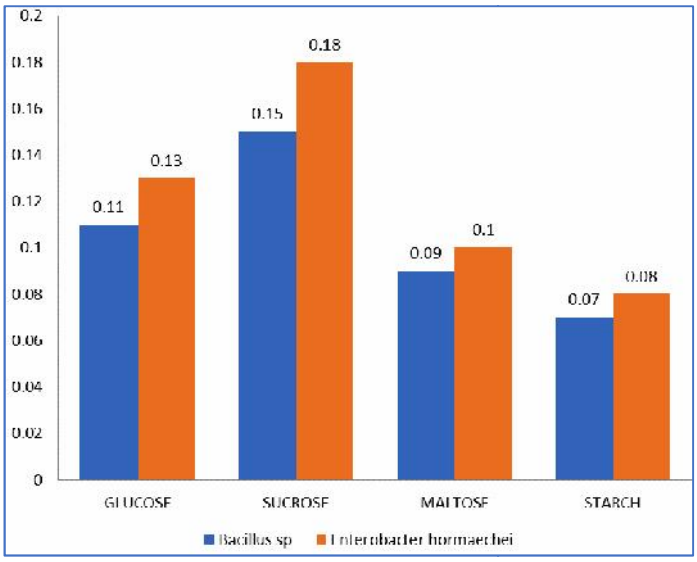

Figure 5: Effect of various Carbon sources on the growth of *Bacillus* sp and *Enterobacter hormaechei*

Table 6: Effect of various Nitrogen sources on the growth of *Bacillus* sp and *Enterobacter hormaechei*

NITROGEN SOURCES	*Bacillus* sp	*Enterobacter hormaechei*
UREA	0.02	0.03
AMMONIUM CHLORIDE	0.01	0.02
SODIUM NITRATE	0.07	0.06
PEPTONE	0.18	0.2

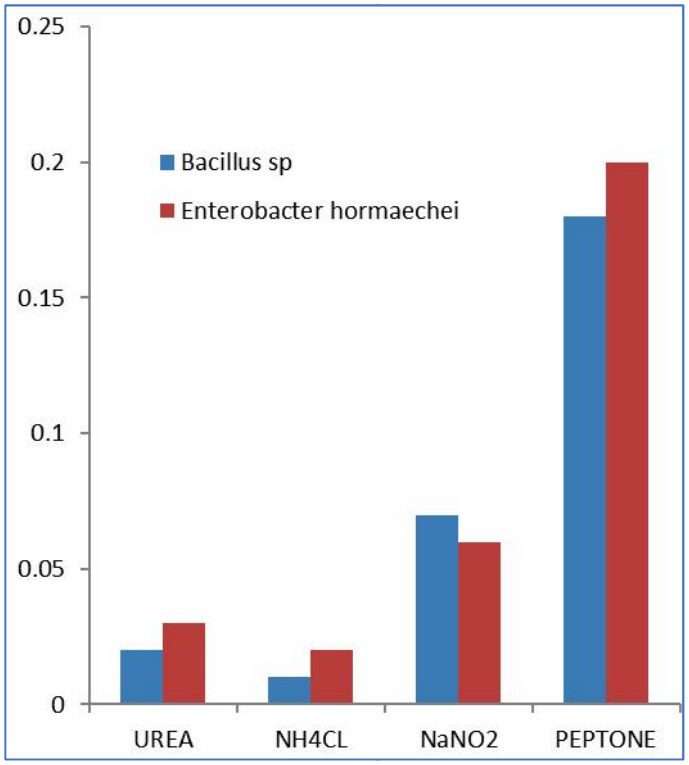

Figure 6: Effect of various Nitrogen sources on the growth of *Bacillus* sp and *Enterobacter hormaechei*

DISCUSSION

The current study engaged bacteria isolated from diesel contaminated soil for the biodegradation of diesel oil. Culture parameters for well-organized degradation of diesel oil by a bacterial isolate were optimized. It consumes diesel oil as the only source of carbon and degrades it to maximum level. Haritash and Kaushik demonstrated that microorganisms are the main degraders of hydrocarbons. There are different abiotic features which were optimized for maximum degradation of diesel oil. In this paper, isolation of pure cultures and effect of a various factors on hydrocarbon degradation, such as various hydrocarbon concentration, pH, temperature, different carbon and nitrogen sources, were reported. Effect of preliminary hydrocarbon concentration for growth of *Bacillus* sp and *Enterobacter hormaechei* with diesel oil was calculated. Preliminary hydrocarbon concentrations of 0.5 to 2.5% were used for this degradation studies in which MSM containing 1% of diesel oil explained highest growth of both the organisms. According to Luo *et al.*, [11], diesel oil offered a healthier carbon source for the growth of bacteria. Result of pH for growth of *Bacillus* sp and *Enterobacter hormaechei* with diesel oil was evaluated. Both organisms confirmed utmost growth in pH 7. The growth of the bacterial species was reduced while decreasing or increasing the pH. Hence it is understood that neutral pH is essential for optimum growth of bacteria, acidic and basic conditions did not support the growth of these organisms. According to Whang *et al.*, [11], microbial growth and diesel biodegradation was found to be at a pH 7.2, while decreasing or increasing the pH reduced the degradation efficiency considerably. According to Luo *et al.*, [8] at pH level of 7 *Pseudomonas* sp strain F4 demonstrated efficient diesel oil degradation potential.

Hence, the optimization of pH is very essential for the improved growth of bacteria and for selection of effective bioremediation approach. Sathish Kumar *et al.*, reported that the optimum pH for the degradation of diesel oil by individual bacterial strains and an assorted bacterial consortium was initiate to be 7. Effect of temperature for growth of *Bacillus* sp and *Enterobacter hormaechei* with diesel oil were evaluated. The temperature is an essential feature that affects the diesel degradation potential by bacteria. The present study analyzed the optimum temperature for the degradation of diesel oil and it was create maximum at 30°C. The minimum growth was experimental at 15°C and 45°C. Maintenance of temperature is as essential as pH which powerfully influences bacterial growth. The growths of both the organisms were directly proportional to diesel oil degradation, since the medium with diesel oil as the individual source of carbon. The degradation efficiency decreased significantly with the raise of temperature. Mnif *et al.*, found that 30°C was the optimum situation for the degradation of diesel by *Bacillus subtilis* SPB1. Responsibility of carbon and nitrogen sources for growth of *Bacillus* sp and *Enterobacter hormaechei* with diesel oil was evaluated. A variety of carbon sources such as Glucose, Sucrose, Maltose and Starch (1%) were added to the MSM medium holding 1% diesel oil as nutrient addition for diesel oil degradation, amongst them Sucrose initiate to enhance diesel oil degradation. Then variety of Nitrogen sources such as Urea, Ammonium Chloride, Sodium nitrate and Peptone (1%) were added to the MSM medium holding 1% diesel oil as nutrient addition for diesel oil degradation, amongst them Peptone is used to improve diesel oil degradation. Prathima Devi *et al.*, report that addition of external nutrients improving the degradation of crude petroleum sludge. Carbon sources were not utilized by the bacterial species at previous phase and there was minimum growth in the occurrence of carbon sources.

CONCLUSION

Clean out of petroleum hydrocarbons in the environment is an existing problem. An improved understanding of the method of biodegradation has a high environmental importance that depends on the native microorganisms to change or mineralize the organic contaminants. Microbial degradation carries out the removal of spilled oil from the environment by various physical and chemical methods. In this paper focused on the degradation of diesel oil by a bacterial species isolated from diesel contaminated site. The attainments of oil bioremediation depend on the capability to optimize a range of physical, chemical and biological conditions in the polluted environment. Here, *Bacillus* sp and *Enterobacter hormaechei* were able to degrade diesel oil at pH 7, 30^{o}C and preliminary hydrocarbon concentration of 1%.

REFERENCES

[1] Bossert I & Bartha R, 1984, The fate of petroleum in soil ecosystems in Petroleum Microbiology, *edited by R M Atlas. Macmillan, New York. Pp. 435 – 473*.

[2] Baraniecki C A *et al*, 2002, Characterization of Sphingomonas sp. Ant17, an aromatic hydrocarbon-degrading bacterium isolated from Antarctic soil. *Microb Ecol. 42, 958 – 963*.

[3] Delille D & Delille B, 2000, field observations on the variability of crude oil impact in indigenous hydrocarbon degrading bacteria from sub-Antarctic intertidal sediments. *Mar Environ Res, 49, 403 – 417.*

[4] Leahy J G & Colwell R R, 1990. Microbial degradation of hydrocarbons in the environment. *Microbiol Rev, 54, 305 – 315.*

[5] Margestin R & Schinner F, 1998. Low temperature bioremediation of a wastewater contaminated with anionic surfactants and fuel oil. *Appl Microbiol Biotechnol, 49, 482 486.*

[6] Haritash AK, Kaushik CP. Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): a review. *J Hazard Mater. 2009; 169:1–15. doi: 10.1016/j.jhazmat.2009.03.137*.

[7] Whang L-M, Liu P-WG, Ma C-C, Cheng S-S. Application of rhamnolipid and surfactin for enhanced diesel biodegradation-Effects of pH and ammonium addition. *J Hazard Mater. 2009; 164:1045–1050.*

[8] Luo Q, Zhang J-G, Shen X-R, Sui X, Fan Z-Q. Characterization of a novel diesel oil-degrading pseudomonas sp. strain F4. *Fresenius Environ Bullet. 2013; 22:689–697.*

[9] Sathishkumar M, Binupriya AR, Baik S-H, Yun S-E. Biodegradation of crude oil by individual bacterial strains and a mixed bacterial consortium isolated from hydrocarbon contaminated areas. *Clean-Soil Air Water. 2008; 36:92–96.*

[10] Mnif I, Sahnoun R, Ellouze-Chaabouni S, Ghribi D. Evaluation of *B. subtilis* SPB1 biosurfactants' potency for diesel-contaminated soil washing: optimization of oil desorption using Taguchi design. *Environ Sci Pollut Res. 2014; 21:851–861.*

[11] Luo Q, Xian-Rong S, Jian-Guo Z, Zheng-Qiu F, Ying H. Isolation, identification and biodegradation ability of diesel oil degrading *Pseudomonas* sp. strain C7 from bilge water. *Afr. J Microbiol. 2012; 6:1033–1040.*

[12] Prathima Devi M, Venkateswar Reddy M, Juwarkar A, Nageswara Sarma P, Venkata Mohan SR. Effect of co-culture and nutrients supplementation on bioremediation of crude petroleum sludge. *Clean-Soil Air Water. 2011; 39:900–907.*

[13] Vasudevan, N. and Rajaram, P. (2001) Bioremediation of Oil Sludge-Contaminated Soil. *Environment International, 26, 409-411.*

[14] Whyte, L. G., L. Bourbonnie`re, and C. W. Greer. 1997. Biodegradation of petroleum hydrocarbons by psychotropic *Pseudomonas* strains possessing both alkane (alk) and naphthalene (nah) catabolic pathways. *Appl. Environ. Microbiol. 63:3719–3723.*

[15] Alexander M (1994), Biodegradation and Bioremediation, *San diego: Academic press, pp. 43 – 48.*

How to cite this article:

Sivagamasundari T. and Jayakumar N. Optimization of Diesel oil degrading Bacterial strains at various culture parameters. *Int. J. Res. Dev. Pharm. L. Sci.* 2017; 6(6): 2840-2844. Doi: 10.13040/IJRDPL.2278-0238.6(6).2840-2844.