Global NEST Journal, Vol 20, No 3, pp 504-511

Impact of bioaugmentation of soil with n-hexadecane-degrading bacteria and phosphorus source on the rate of biodegradation in a soil-slurry system

Dehghani M.[1*], Taatizadeh S.B.[1], Samaei M.R.[1], Shamsedini N.[1], Shahsavani S.[1], Derakhshan Z.[2,3,4] and Conti G.O.[5]

[1]Research Center for Health Sciences, Department of Environmental Health, School of Health, Shiraz University of Medical Sciences, Shiraz, Iran

[2]Environmental Science and Technology Research, Center, Department of Environmental Health Engineering, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

[3]Department of Environmental Health, School of Health, Larestan, University of Medical Sciences, Larestan, Iran

[4]Student Research Committee, Shahid Sadoughi University Medical of Sciences, Yazd, Iran

[5]Environmental and Food Hygiene Laboratories (LIAA) of Department of Medical Sciences, Surgical and Advanced Technologies "G.F. Ingrassia", Hygiene and Public Health, University of Catania, Italy

Received: 30/01/2018, Accepted: 18/10/2018, Available online: 31/10/2018

*to whom all correspondence should be addressed: e-mail: mdehghany@sums.ac.ir.

https://doi.org/10.30955/gnj.002607

Abstract

In recent years, n-hexadecane, a component *of diesel petroleum hydrocarbons*, is frequently detected as the contaminant in many soils and water resources. The main aims of this study were focused on the feasibility of using the biodegradation for the removal of n-hexadecane from the soil-slurry system, by assessing the effects of phosphorus sources on biodegradation rate and determining the optimal conditions of the process. This study was carried out using an experimental method at the laboratory scale. The Taguchi method was used to optimize variables and their levels using the Qualitek-4 (w32b) software. We investigated the effects of initial *concentration of n-hexadecane* as the sole source of carbon (1-80 g/kg of soil), the role of phosphorus sources, at the concentration ranges from 10 to 600 g per kg of soil, released by *different bacterial* species (*Acinetobacter radioresistens, Pseudomonas aeruginosa, Bacillus subtilis,* and the bacterial consortium) at the incubation time between 0 and 30 days. The optimum values of the response *variables* were predicted *through signal*-to-*noise ratio* (S/*N*). Results indicated that *Bacillus subtilis* were *more effective* in n-hexadecane degradation *compared* to the others (42%). Optimization process by Taguchi method suggested that the optimal conditions for the removal of n-hexadecane in a soil-slurry system are as follows: the initial n-hexadecane concentration as sole source of carbon in soil, $Na_2HPO_4.12H_2O$ as phosphorus source at the concentration of 300 mg per kg of soil and, finally, level of significance for the study parameters were 74.31, 6.48 and 8.51, respectively. In conclusion, bioaugmentation of soil with n-hexadecane-degrading bacteria providing an *adequate supply* of phosphorus source may enhance the biodegradation rate in a polluted soil.

Keywords: Biodegradation, n-Hexadecane, Phosphorus sources, Taguchi method, *Bacillus subtilis*

1. Introduction

Contamination of soils and water resources with petroleum hydrocarbons is one of the most important environmental issues worldwide (Partovinia A. *et al.*, 2012; Farzadkia M. *et al.*, 2014). Most of these compounds are carcinogenic and persistent in the environment with the high potential to bioaccumulate (Dehghani M. *et al.*, 2014; Sciacca and Oliveri Conti, 2009). The main pollutant of diesel fuel is n-hexadecane (Volke-Sepúlveda T.L. *et al.*, 2003; Dehghani M. *et al.*, 2014).

Bioremediation is a remedial approach treating the contaminated soil with the advantages of simplicity, cost-effective and environmentally friendly technology (Vidali M., 2001; Dehghani M. *et al.*, 2013; Wang S. *et al.*, 2016). But this method has some disadvantages such as long treatment time (Cha D.K. *et al.*, 1997), require supplementary nutrients (Boopathy R., 2000), and need to support with exogenous bacteria to decompose pollutants (Boopathy R., 2000; Zhang C. *et al.*, 2000; Dehghani M. *et al.*, 2007). Biostimulation is also used to remove total petroleum hydrocarbons (TPH) from polluted soil (Alvarez and Illman, 2006). Many studies showed that the addition of phosphorus and nitrogen sources to soil may enhance the biodegradation of hydrocarbons (Graham D. *et al.*, 1999; Dehghani *et al.*, 2013). The application of the phosphorus sources increased the rate of biodegradation up to 8.5% (Adetutu E.M. *et al.*, 2012). The removal of crude oil in sediments at a low ratio of carbon to

phosphorus was 83.13% at the optimal conditions (Mohajeri L. *et al.*, 2010). Another study also demonstrated the positive impact of phosphorus and nitrogen sources on the biodegradation rate of n-hexadecane in soil (Graham D. *et al.*, 1999). However, this process may cause significant changes in the structure of soil microbial communities (Brigmon R.L. *et al.*, 2003; Graham D. *et al.*, 1999).

Also, the microbial consortium has a high capability to remove petroleum hydrocarbons from the oily sludge (Vasudevan N. and P. Rajaram, 2001: Dehghani M. *et al.*, 2014). Many studies showed that *Pseudomonas* bacteria can remove n-hexadecane (Mishra and Singh, 2012), diesel oil, gasoline, kerosene (Wongsa P. *et al.*, 2004), petroleum (Karamalidis A.K. *et al.*, 2010), and alkanes (Belhaj A. *et al.*, 2007) from the environmental media. *Bacillus* is also widely used in bioremediation processes with high capabilities to remove long-chain alkanes such as n-hexadecane (Wang L. *et al.*, 2006). *Bacillus* bacteria was isolated from Riyadh contaminated soils significantly reduce the concentration of n-hexadecane (Abdel-Megeed A. *et al.*, 2010). *Acinetobacter* was able to remove various pollutants (e.g. parathion) in different environmental media (Liu F.y. *et al.*, 2007).

Since Iran is very rich in petroleum reserves, there is highly concern regarding the effect of the petroleum hydrocarbons pollution on public health and the receiving environment. Therefore, the aims of our study were to (i) evaluate efficiency of bioremediation method using different bacterial species to remove n-hexadecane from contaminated soil in a soil-slurry system, (ii) compare the removal efficiency by adding various sources of phosphorus and finally, (iii) determine the optimal conditions so that the standard limit can be achieved by further complementary treatment.

2. Materials and methods

2.1. Bacterial growth

This study was conducted on a laboratory scale using a soil-slurry system in a 50 ml *Erlenmeyer* flask at room temperature (25°C). Studied parameters including initial concentrations of n-hexadecane, different bacterial species, and phosphorus sources at different concentrations were presented in Table 1.

Table 1. The studied parameters for the biodegradation of n-hexadecane

Parameters	Level 1	Level 2	Level 3	Level 4
Initial concentration of n-hexadecane (mg kg^{-1})	1000	10000	40000	80000
Bacterial species	Pseudomonas aeruginosa	Bacillus subtilis	Acinetobacter radioresistens	Bacterial consortium
Phosphorus sources	K_2HPO_4	KH_2PO_4	$Na_2HPO_4.12H_2O$	$HPO_4(NH_4)_2$
Concentration of phosphorus mg kg^{-1} soil)	10	100	300	600

The bacteria used in the current study were isolated from the soil nearby Abadan refineries, in the southern part of Iran (Samaei M.R. *et al.*, 2013). In order to acclimate the bacterial consortium capable of growing in soil containing n-hexadecane as the sole source of carbon, the mineral salt media with supplementary of n-hexadecane were prepared (Wu J. and L.K. Ju, 1998). The medium was amended with 20 µl/L of n-hexadecane after autoclaving. The pH was also adjusted to 7.0. Inoculated cultures were incubated aerobically on a reciprocal shaker (120 rpm) at room temperature. The culture was subcultured on the same medium at a one-week interval. From one-week-old culture, 10 mL was transferred to 50 mL of freshly prepared n-hexadecane medium for the period of 8-10 months to ensure the bacterial consortium have complete adaptation with n-hexadecane and grow rapidly. To determine the rate of microbial growth, optical density (OD) was measured using a spectrophotometer (Dr-5000 HACH, USA) at 600 nm wave length. The samples with OD=1 is *equivalent* to 2.8×10^6-10^7 bacterial cells/ml of suspension (Partovinia A. *et al.*, 2010; Wu J. and L.K. Ju, 1998).

2.2. Chemicals and analytical method

All chemicals were purchased from Merck (Germany). The effective parameters should be optimized to achieve higher efficiency. The Taguchi method of design of experiments was used to optimize variables and their levels by conducting experiments on a real-time basis using the Qualitek-4 (w32b) software. The optimum values of the response *variables* were predicted *using signal*-to-*noise ratio* (S/*N*). The S/*N* ratio was used to measure the effect of the response variables and determine the percent removal of n-hexadecane, according to equation 1. In this equation, the amounts of y_n and n are the measured response and the number of repetition (2 in this case) for each test, respectively (Kniemeyer O. *et al.*, 2007; Dashti N. *et al.*, 2008).

$$\frac{S}{N}=-10\log\frac{(1/Y_1^2+1/Y_2^2+\ldots+1/Y_n)^2}{n} \quad (1)$$

The level of significance is 95% (α=0.05).

2.3. Soil sampling and preparation

Soil samples for the experiments were taken from Shiraz refinery area (22 km northeast of Shiraz, Southern Iran). Soil samples were collected from 0-20 cm of soil depth with a hand-driven soil auger and stored at 4°C until they were used. The soil samples were air-dried and passed through 2 mm sieve and then sterilized at 160°C for 2 hours to be prepared for further microbiological examinations. To remove prior contamination of the soil with n-hexadecane, 30 mL of 99.8% n-hexane was added to the soil and shake for one hour. Then, n-hexane was separated and soil was dried for about 48 hours at 60°C (Dehghani M. *et al.*, 2014). Four hundred grams of soil sample was spiked to the desired amount of n-hexadecane (0.4, 4, 16 and 32 g/100 mg of soil) while mixing. To ensure that the n-hexadecane adsorbed well to the soil, the inoculation of soil with the selected bacterial species was done after 5-7 days of the addition of the compound.

The degradation rate of n-hexadecane by bacterial species was measured in 50 ml capped Erlenmeyer flask containing soil samples. Four grams of soil sample was brought to the total volume of 20 ml with n-hexane. The experimental design consisted of 144 flasks with 16 treatment and three replications for each treatment. The bioaugmentation of soil was accomplished with the phosphate buffer containing the selected bacterial species to yield 7.5×10^5 bacterial cell/g soils determined by plating on soil extract agar. A non-inoculated control received only a sterile phosphate buffer containing sodium azide (1%) to prevent any biological activity. A blank was also used without phosphate buffer content. The samples containing various amounts of n-hexadecane at the different phosphorus sources were incubated aerobically on the shaker at room temperature in dark during the incubation period. Soil samples were extracted after 10 days of incubation. The MSB media with no phosphorus content was added to the flask to maintain nutrient for the growth of n-hexadecane degraders every 15 days over a 30 days' period of incubation. Soil samples were extracted at the incubation time of 10, 20 and 30 days and analyzed to determine the remained n-hexadecane concentration at each time intervals. Periodically, pH and dissolved oxygen (DO) and the number of colonies formed unit (CFU) using plate count method were measured.

2.4. Soil analysis

The general physicochemical characteristics of soil were determined. Hydrometer was used to determine soil textures using Guelph method (Nelson and Sommers, 1996). Other soil characteristics such as pH (Thomas G.W., 1996) and organic matter content (Nelson and Sommers, 1996) were determined. The soil texture was silty loam and the amount of sand, silt, and clay were 52.2%, 35.2% and 12.6%, respectively. Soil pH was 7.71. Organic matter content was 17 g/kg of soil. The native soil characteristic at the refinery site was fine-loamy, mixed, thermic, and Typic Calcixerepts.

2.5. Extraction and analytical methods

Liquid-liquid extraction was used to extract n-hexadecane. To extract n-hexadecane from the liquid phase, 10 mL of 99.8% n-hexane was added and shacked for 1 minute at vortex condition. Then the collected organic phase was passed through Teflon filter (0.22 µm in diameter). Then, rinsed with 10 ml of 99.8% n-hexane and stored in a refrigerator prior to GC analysis. The recovery of n-hexadecane with this method of extraction was 95% (Kniemeyer O., 2007). Gas chromatography (GC) coupled with a FID detector was used to measure the amount of the remaining n-hexadecane. The temperatures of detector and injector were set at 250°C and 210°C, respectively. Besides, the column's temperature was maintained at 80°C for 1.5 min and then increased to 125°C for 5 min; afterward, it was increased to 190°C with a rate of 40°C per minute, and, then, it was maintained for 3 min.

3. Results

The removal efficiency of n-hexadecane in a soil-slurry system using *different species* of bacteria within 30 days of incubation was shown in Figure 1. According to data obtained in this study, *Bacillus subtilis* had the maximum n-hexadecane biodegradation rate in a soil-slurry system after 20 days (42%).

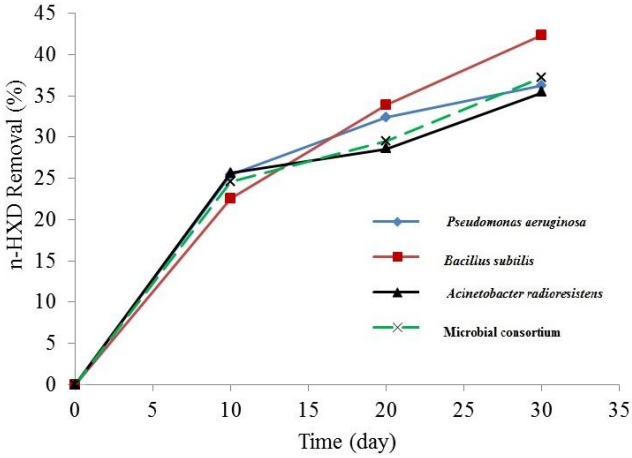

Figure 1. The biodegradation rate of n-hexadecane (n-HXD) in a soil-slurry system *using different* bacterial *species* within 30 days of incubation period

Figures 2 and 3 showed the biodegradation rate of n-hexadecane in a soil-slurry system using different concentrations of n-hexadecane at the various sources of phosphorus at different concentrations. According to these Figures, the maximum biodegradation rate of n-hexadecane occurred with 300 mg/kg of phosphorus source of $Na_2HPO_4.12H_2O$ using 0.1 mg/l of n-hexadecane.

Figure 4 represents the variations of pH during the biodegradation of n-hexadecane in a soil-slurry system at various phosphorus sources *using Bacillus subtilis* within 30 days of incubation. At first, the initial pH was adjusted to 7.0. During 12 to 15 days of incubation period, pH slowly decreased to 6.38. After 30 days of incubation, pH increased up to 7.9. According to Figure 4, the same

variations of pH for different phosphorus sources were observed.

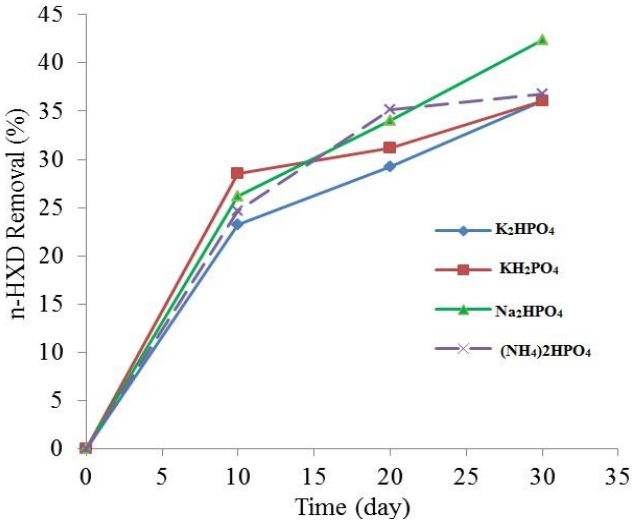

Figure 2. The biodegradation rate of n-hexadecane (n-HXD) in a soil-slurry system at different phosphorus sources within 30 days of incubation period

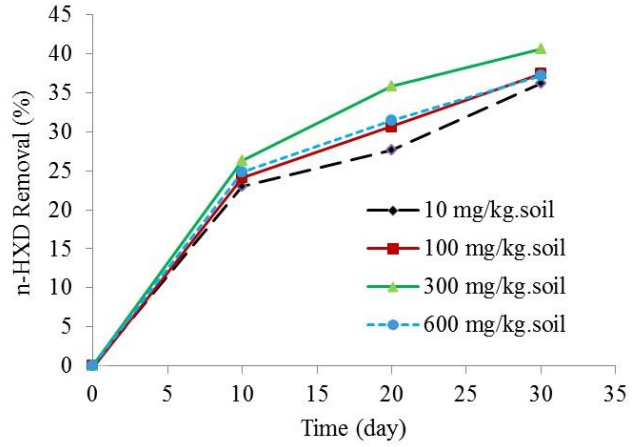

Figure 3. The biodegradation rate of n-hexadecane (n-HXD) in a soil-slurry system at different phosphorus concentrations within 30 days of incubation period

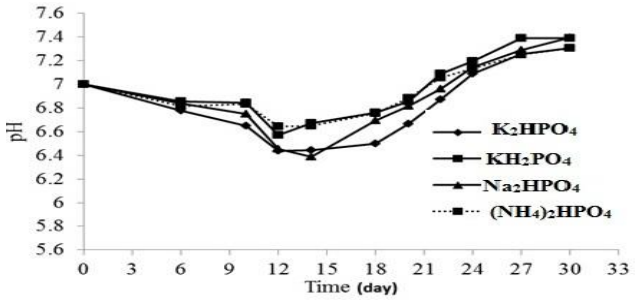

Figure 4. The variation of pH during the biodegradation of n-hexadecane in a soil-slurry system at different phosphorus sources within 30 days of incubation period

Figure 5 shows the fluctuations of pH during the biodegradation of n-hexadecane in a soil-slurry system at different phosphorus concentrations of $Na_2HPO_4.12H_2O$ within 30 days of incubation. The different phosphorus concentrations showed the same variations of pH during the incubation period.

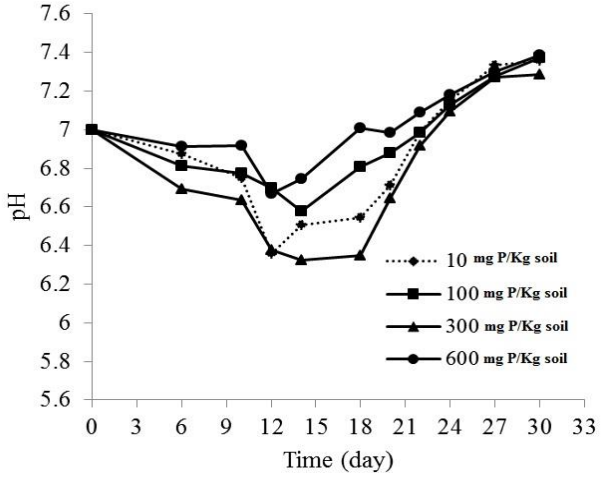

Figure 5. The fluctuations of pH during the biodegradation of n-hexadecane in a soil-slurry system at different phosphorus concentrations of $Na_2HPO_4.12H_2O$ within 30 days of incubation period

Figure 6 shows the effect of initial n-hexadecane concentrations on its biodegradation *using Bacillus subtilis* within 30 days of incubation.

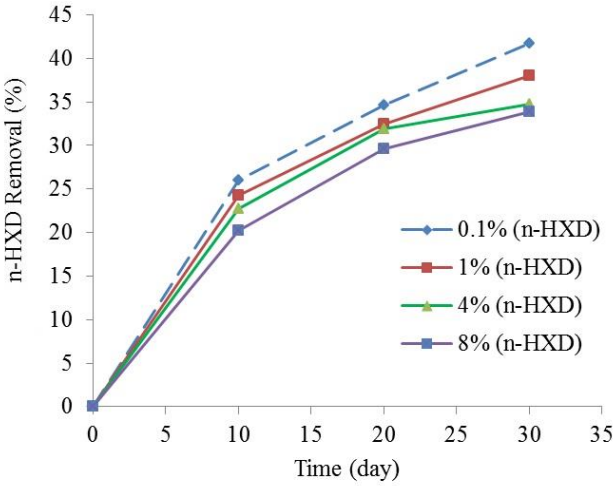

Figure 6. The biodegradation rate of n-hexadecane (n-HXD) in a soil-slurry system at different initial n-hexadecane concentrations *using Bacillus subtilis* within 30 days of incubation period

The variations of dissolved oxygen (DO) in a soil-slurry system were also examined at various phosphorus sources. Initially, the amount of DO declined with relatively steep slopes in the first 6 days of incubation, and then reached almost constant (Figure 7).

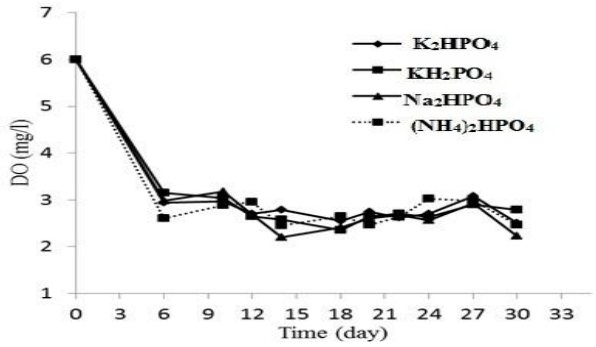

Figure 7. The variations of DO during the biodegradation of n-hexadecane in a soil-slurry system at different phosphorus sources *using Bacillus subtilis* within 30 days of incubation period

Figure 8 shows the effects of n-hexadecane biodegradation on optical density (OD) within 30 days of incubation period. At first, the bacterial growth rate was very fast in the first 10 days of incubation considering all the sources of phosphorus at each concentration were consumed very fast. Then the rate was considerably reduced after 18 days and reached a plateau.

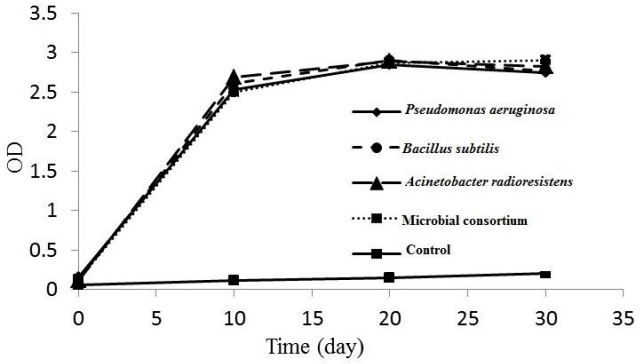

Figure 8. The effects of n-hexadecane (n-HXD) biodegradation on optical density (OD) within 30 days of incubation period

4. Discussion

4.1. Effects of different bacterial species *on n-hexadecane biodegradation*

The feasibility of the biodegradation of n-hexadecane as the sole source of carbon was studied by *different bacterial* species including *Acinetobacter radioresistens*, Pseudomonas aeruginosa, Bacillus *subtilis,* and the bacterial consortium. According to Figure 1, the maximum biodegradation rate of n-hexadecane was occurred by Bacillus subtilis in a soil-slurry system at 30 days of incubation. The biodegradation rate of n-hexadecane by Bacillus subtilis was more than the two other bacterial species and the bacterial consortium. The biodegradation rate of n-hexadecane in liquid culture by the different bacterial species showed an initial sharp increasing slope in the first 10 days of incubation and then reaching constant with a relative slower rate due to the lack of n-hexadecane and phosphorus as carbon and nutrient sources, respectively.

Seti *et al.* demonstrated that 86.4% of hexadecane was removed by *Pseudomonas s.p.p* with an initial concentration of 12g/l over a period of 31 days. In addition, Dashti *et al.* (2008) revealed that *Micrococcus* and *Pseudomonas* as the predominant bacteria for removing of n-hexadecane from all soil samples (Dashti N. *et al.*, 2008). The isolated bacterial consortium degraded hydrocarbons in contaminated soil by petroleum, crude oil, and motor oil (Ghazali, F.M. *et al.*, 2004). Another study showed that *Pseudomonas aeruginosa* was more effective in removing crude oil than *Bacillus* (Das, K. and A.K. Mukherjee, 2007). Hui *et al.* (2010) isolated n-hexadecane degrading *Enterobacteria* from soil. The isolated microbial consortium removed 75% of petroleum hydrocarbons in 270 days of incubation (Chalneau C.-H. *et al.*, 1995). The results of the current study showed that the maximum removal of n-hexadecane by *Bacillus subtilis* and bacterial consortium were 42% and 36%, respectively.

4.2. Effect of initial n-hexadecane concentration on the biodegradation rate

At first, 5 days of the incubation time period, the degradation rates for the initial concentration of 1 to 80 g/kg soil were almost the same (Figure 6). After that the biodegradation rate was lower for the higher initial concentration. At 30 days of incubation period, the maximum removal of n-hexadecane (42%) was for the lowest initial concentration of n-hexadecane (1 g/kg soil). Our data completed Velky *et al.* (2006) study that investigated lower initial concentrations of n-hexadecane (18-717 mg/kg of soil). They showed that the removal efficiency increased at higher initial concentrations of n-hexadecane.

4.3. Effects of phosphorus sources on n-hexadecane biodegradation

Theoretically, nutrient *supplementation may* enhance *bacterial growth rates* for successful biodegradation. The addition of *phosphorus* sources increases the biodegradation rate. The bacterial growth rate significantly related to the *type and the amount* of nutrient *added* (Graham D. *et al.*, 1999).

Adequate nutrients for bacterial metabolic activity must be provided for proper degradation. Therefore, the biodegradation rate of n-hexadecane can be limited by the bioavailability of nutrient. *Supplementing soils* with the *necessary nutrients* are *needed* to *provide* sufficient biodegradation of the contaminant.

Initially, there was no phosphorus in the studied soil. Therefore, the activity of the degrading *bacteria* is highly influenced by enrichment of soil with *phosphorus* sources. The choice of phosphorus sources is an important issue because not all sources are easily accessible for the bacterial growth *added* (Graham D. *et al.*, 1999). The *solubility* of the various inorganic *phosphorus* compounds directly affects the accessibility of the bacterial growth. In addition, the addition of proper amount of phosphorus is also very important to enhance the bioavailability. Mohajeri *et al.* (2010) showed that increasing nitrogen to 2 g/kg soil led to an increase in bioremediation. However, further increase can significantly reduce the bioremediation (Mohajeri L. *et al.*, 2010). The removal of

n-hexadecane in soil was mainly influenced by the addition of appropriate phosphorus and nitrogen sources *at right doses*.

According to Figure 2, the maximum n-hexadecane biodegradation was observed for the addition of $Na_2HPO_4.12H_2O$ as phosphorus source at 300 mgp/kg soil. The appropriate phosphorus sources for the biodegradation of n-hexadecane mostly depend on the soil type and the nutrient contents of the soil (Tania Volke-Sepúlveda *et al*., 2006).

4.4. The variation of pH during n-hexadecane biodegradation

The rate of n-hexadecane degradation decreased quickly when the initial concentration of n-hexadecane increased from 1 to 80 g/kg of soil. Data showed that sample with the highest initial concentration of n-hexadecane (80 g/kg soil) had the lowest microbial activity.

Bacillus subtilis in a soil-slurry system exhibited the maximum removal efficiency with the highest influence on its biological activity. The pH is one of the important parameters affecting cells' metabolism and enzymes function (Dehghani M. *et al.*, 2013). So, pH variations during the incubation period are indicative of biological reactions. Any changes in chemical properties of hydrocarbons may cause pH variation during biodegradation process. Moreover, microbial activity is mainly influenced by pH variations (Tang *et al.*, 2012). According to our data (Figures 4-5), pH variations were only observed in the inoculated samples while pH in the control samples was almost constant during the period. In first 10 days of incubation, high amount of n-hexadecane was removed and pH reduction rate showed an initial sharp decreasing slope. However, pH value increased with relative slower rate between the time intervals of 20-30 day. The maximum pH variation due to higher microbial activity was at the phosphorus concentration of 300 mg phosphorus/kg of soil (Figure 3).

4.5. The fluctuations of DO during n-hexadecane biodegradation

According to Figure 7, DO reduction rate showed an initial sharp decreasing slope during 10 days of incubation (decrease from 7±2 to about 3±0.5 mg/l) and then DO value was almost constant from 20-30 days of incubation (average 3.2±0.5 mg/l). Since high amount of n-hexadecane was removed during 10 days of incubation, significant amount of DO was also consumed due to bacterial growth. In the control samples (without bacteria inoculation), DO was almost constant. Our results were consistent with Michelson *et al.* showed that the variations of DO during the bioremediation process could be used as the indicator of biological activity (Michaelsen M. *et al.*, 1999).

4.6. The effects of n-hexadecane biodegradation on OD

Data obtained in this study showed that there is no significant relationship between number of CFU and n-hexadecane removal rate ($p>0.05$). Tang *et al.* (2012) also reported that there was no significant relationship between number of CFU and the removal rate of total petroleum hydrocarbons.

However, the variation of OD during the first ten days of the incubation period showed sharp increasing slope (Figure 8). Then, the rate became slow and finally constant. The quickly increasing of OD was related to the immediate bacterial growth due to presence of n-hexadecane as the only carbon source. After that, OD variations became constant due to the lack of carbon sources. Therefore, it can be assumed that there is a relationship between OD and the rate of microbial growth and n-hexadecane removal rate. Moreover, the variation of OD for control samples (without bacteria inoculation) was not significant. Therefore, it can be assumed that there is a relationship between OD and the rate of microbial growth and n-hexadecane removal rate.

5. Conclusions

In conclusion, process optimization by Taguchi method suggests that the optimal conditions for the removal of n-hexadecane in a soil-slurry system are as follows: the n-hexadecane concentration of 1 g/kg of soil, appropriate phosphorus source ($Na_2HPO_4.12H_2O$), phosphorus concentration of 300 mg/kg of soil, bacteria species (*Bacillus subtilis*) and level of significance for the study parameters were 74.31, 6.48, 8.51, and 3.0, respectively.

High levels of soil contamination with n-hexadecane had inhibitory effects on the biodegradation rate. Excessive phosphorus concentrations decreased the removal of n-hexadecane. Bacteria species was also the major parameters affecting the biodegradation rate. The maximum biodegradation rate was related to *Bacillus subtilis*. However, there were no significant differences between bacterial consortium and three bacteria species regarding the removal efficiency. It is very important to note that the biodegradation of n-hexadecane in the controlled laboratory conditions was accomplished by biostimulation through phosphorus addition and without the interaction of environmental factors. Physicochemical conditions of soils (temperature, pH, soil texture and organic matter content) and competition with native microorganisms may quickly decrease the population of the inoculums and limit biodegradation capacity. To determine the feasibility of the biodegradability in a real situation, *the* biostimulation technique should be applied to *real contaminated* soils with n-hexadecane.

Acknowledgements

This article was extracted from the thesis written by Mr. Seyed Behnam Taatizadeh, MSc student of Environmental Health Engineering. The authors would like to thank the Deputy of Research and Technology of Shiraz University of Medical Sciences for their financial support of this project # 91-6334.

Authors' contributions

The overall implementation of this study including design, experiments, data analysis, and manuscript preparation were the results of the corresponding author's efforts. All authors have made extensive contribution into the review and

finalization of this manuscript. All authors have read and approved the final manuscript.

Conflict of interest

The authors declare that they have no competing interests.

References

Abdel-Megeed A., Al-Harbi N. and Al-Deyab S. (2010). Hexadecane degradation by bacterial strains isolated from contaminated soils, *African Journal of Biotechnology*, **9**, 7487-7494.

Adetutu E.M., Ball A.S., Weber J., Aleer S., Dandie C.E. and Juhasz A.L. (2012). Impact of bacterial and fungal processes on 14C-hexadecane mineralisation in weathered hydrocarbon contaminated soil, *Science of the Total Environment*, **414**, 585-591.

Belhaj A., Desnoues N. and Elmerich C. (2002). Alkane biodegradation in Pseudomonas aeruginosa strains isolated from a polluted zone, identification of alkB and alkB-related genes, *Research in Microbiology*, **153**, 339-344.

Brigmona R. (2002). Bioremediation of compounds hazardous to health Biotransformations: bioremediation technology for health and environmental protection, **36**, 1-28.

Das K. and Mukherjee A.K. (2007). Crude petroleum-oil biodegradation efficiency of Bacillus subtilis and Pseudomonas aeruginosa strains isolated from a petroleum-oil contaminated soil from North-East, *India Bioresource Technology*, **98**, 1339-1345.

Das N. and Chandran P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: an overview, *Biotechnology Research International*, **2011**, 1-13.

Dashti N., Al-Awadhi H., Khanafer M., Abdelghany S. and Radwan S. (2008). Potential of hexadecane-utilizing soil-microorganisms for growth on hexadecanol, hexadecanal and hexadecanoic acid as sole sources of carbon and energy, *Chemosphere*, **70**, 475-479.

Dehghani M., Nasseri S., Ahmadi M., Samaei M.R. and Anushiravani A. (2014). Removal of penicillin G from aqueous phase by Fe^{+3}-TiO_2/UV-A process, *Journal of Environmental Health Science and Engineering*, **12**, 56.

Dehghani M., Nasseri S., Amin S., Naddafee K., Taghavi M., Yunesian M. and Maleky N. (2007). Isolation and identification of atrazine-degrading bacteria from corn field soil in Fars province of Iran, *Pakistan Journal of Biological Sciences*, **10**, 84-89.

Dehghani M., Nasseri S., Amin S. and Zamanian Z. (2010). Assessment of atrazine distribution in Shiraz soils, south of Iran Pakistan journal of biological sciences, *Pakistan Journal of Biological Sciences*, **13**, 66-72.

Dehghani M., Nasseri S. and Zamanian Z. (2013). Biodegradation of alachlor in liquid and soil cultures under variable carbon and nitrogen sources by bacterial consortium isolated from corn field soil, *Iranian Journal of Environmental Health Science & Engineering*, **10**, 21.

Derakhshan Z., Ehrampoush M.H., Mahvi A.H., Dehghani M., Faramarzian M. and Eslami H. (2019). A comparative study of hybrid membrane photobioreactor and membrane photobioreactor for simultaneous biological removal of atrazine and CNP from wastewater, A performance analysis and modeling, *Chemical Engineering Journal*, **355**, 428-438.

Derakhshan Z., Ehrampoush M.H., Mahvi A.H., Dehghani M., Faramarzian M., Ghaneian M.T., Mokhtari M., Ebrahimi A.A. and Fallahzadeh H., (2018a). Evaluation of a moving bed biofilm reactor for simultaneous atrazine, carbon and nutrients removal from aquatic environments: Modeling and optimization, *Journal of Industrial and Engineering Chemistry*, **67**, 219-230. doi:https://doi.org/10.1016/j.jiec.2018.06.032

Derakhshan Z., Mahvi A.H., Ghaneian M.T., Mazloomi S.M., Faramarzian M., Dehghani M., Fallahzadeh H., Yousefinejad S., Berizi E. and Ehrampoush M.H., (2018b). Simultaneous removal of atrazine and organic matter from wastewater using anaerobic moving bed biofilm reactor: A performance analysis, *Journal of Environmental Management*, **209**, 515-524.

Ebrahimi A., Hashemi H., Eslami H., Fallahzadeh R.A., Khosravi R., Askari R. and Ghahramani E. (2018). Kinetics of biogas production and chemical oxygen demand removal from compost leachate in an anaerobic migrating blanket reactor, *Journal of Environmental Management*, **206**, 707-714.

Ghazali F.M., Rahman R.N.Z.A., Salleh A.B. and Basri M. (2004). Biodegradation of hydrocarbons in soil by microbial consortium, *International Biodeterioration and Biodegradation*, **54**, 61-67.

Graham D., Smith V., Cleland D. and Law K. (1999). Effects of nitrogen and phosphorus supply on hexadecane biodegradation in soil systems, *Water, Air, & Soil Pollution*, **111**, 1-18.

Hua X., Wu Z., Zhang H., Lu D., Wang M., Liu Y. and Liu Z. (2010). Degradation of hexadecane by Enterobacter cloacae strain TU that secretes an exopolysaccharide as a bioemulsifier, *Chemosphere*, **80**, 951-956.

Karamalidis A., Evangelou A., Karabika E., Koukkou A., Drainas C. and Voudrias E. (2010). Laboratory scale bioremediation of petroleum-contaminated soil by indigenous microorganisms and added Pseudomonas aeruginosa strain, Spet, *Bioresource Technology*, **101**, 6545-6552.

Kniemeyer O., Musat F., Sievert S.M., Knittel K., Wilkes H., Blumenberg M., Michaelis W., Classen A., Bolm C. and Joye S.B. (2007). Anaerobic oxidation of short-chain hydrocarbons by marine sulphate-reducing bacteria, *Nature*, **449**, 898.

Liu F., Hong M.-z., Liu D.-m., Li Y.-w., Shou P.-s., Hai Y. and Shi G.-q. (2007). Biodegradation of methyl parathion by Acinetobacter radioresistens USTB-04, *Journal of Environmental Sciences*, **19**, 1257-1260.

Michaelsen M., Hulsch R., Höpner T. and Berthe-Corti L. (1992). Hexadecane mineralization in oxygen-controlled sediment-seawater cultivations with autochthonous microorganisms, *Applied and Environmental Microbiology*, **58**, 3072-3077.

Mishra S. and Singh S. (2012). Microbial degradation of n-hexadecane in mineral salt medium as mediated by degradative enzymes, *Bioresource Technology*, **111**, 148-154.

Mohajeri L., Aziz H.A., Isa M.H. and Zahed M.A. (2010). A statistical experiment design approach for optimizing biodegradation of weathered crude oil in coastal sediments, *Bioresource Technology*, **101**, 893-900.

Nekouei F. and Nekouei S. (2017). Enhanced enzymatic and ex situ biodegradation of petroleum hydrocarbons in solutions using Alcanivorax borkumensis enzymes in the presence of nitrogen and phosphorus co-doped reduced graphene oxide

as a bacterial growth enhancer, *Journal of Materials Chemistry A*, **5**, 24462-24471.

Partovinia A., Naeimpoor F. and Hejazi P. (2010). Carbon content reduction in a model reluctant clayey soil, slurry phase n-hexadecane bioremediation, *Journal of Hazardous Materials*, **181**, 133-139.

Samaei M.R., Mortazavi S.B., Bakhshi B. and Jafari A.J. (2013). Isolation, genetic identification, and degradation characteristics of n-Hexadecane degrading bacteria from tropical areas in Iran, *Fresenius Environmental Bulletin*, **22**, 1304-1312.

Shahi A., Aydin S., Ince B. and Ince O. (2016). Reconstruction of bacterial community structure and variation for enhanced petroleum hydrocarbons degradation through biostimulation of oil contaminated soil, *Chemical Engineering Journal*, **306**, 60-66.

Tang J., Lu X., Sun Q. and Zhu W. (2012). Aging effect of petroleum hydrocarbons in soil under different attenuation conditions, *Agriculture, Ecosystems & Environment*, **149**, 109-117.

Volke-Sepulveda T., Gutiérrez-Rojas M. and Favela-Torres E. (2003). Biodegradation of hexadecane in liquid and solid-state fermentations by Aspergillus niger, *Bioresource Technology*, **87**, 81-86.

Volke-Sepulveda T., Gutiérrez-Rojas M. and Favela-Torres E. (2006). Biodegradation of high concentrations of hexadecane by Aspergillus niger in a solid-state system: Kinetic analysis, *Bioresource Technology*, **97**, 1583-1591.

Wang L., Tang Y., Wang S., Liu R.-L., Liu M.-Z., Zhang Y., Liang F.-L. and Feng L., (2006). Isolation and characterization of a novel thermophilic Bacillus strain degrading long-chain n-alkanes, *Extremophiles*, **10**, 347-356.

Wang S., Guo S., Li F., Yang X., Teng F. and Wang J. (2016). Effect of alternating bioremediation and electrokinetics on the remediation of n-hexadecane-contaminated soil, *Scientific Reports*, **6**, 23833.

Wongsa P., Tanaka M., Ueno A., Hasanuzzaman M., Yumoto I. and Okuyama H. (2004). Isolation and characterization of novel strains of Pseudomonas aeruginosa and Serratia marcescens possessing high efficiency to degrade gasoline, kerosene, diesel oil, and lubricating oil, *Current Microbiology*, **49**, 415-422.