COMBINATION OF BIOAUGMENTATION AND BIOSTIMULATION AS AN OIL-DRILLING MUD CONTAMINATED SOIL BIOREMEDIATION TREATMENT

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Abstract. The removal of oil-drilling mud contaminated soil generated from oilfields in the Algerian Sahara by bioaugmentation with *Yarrowia lipolytica* and biostimulation with carrot peel waste amendment during 45 days was investigated. Initially, the evaluation of growth and gasoil degrading ability of *Yarrowia lipolytica* in carrot peel waste, and carob kibbles media were compared. Afterwards, the effect of bioaugmentation and organic amendment on oil-drilling mud contaminated soil was studied for 45 days of study period. Total petroleum hydrocarbon (TPH) was measured by distillation using distiller mud. The results indicated that, higher augmentation in growth was observed in carrot peel waste medium and when the concentration of gasoil was increased from 15% to 30%. TPH decreased to 35 ± 1.66% and 30.60 ± 1.50% the first 15 days, 33 ± 2.30% and 26.8 ± 1.66%, respectively at the end of study. TPH rate did not undergo any significant change from its initial value in the control for the entire period of incubation. This study demonstrated the effectiveness of co-application of bioaugmentation with *Yarrowia lipolytica* and biostimulation with carrot peel waste amendment for bioremediation of oil-drilling mud contaminated sites.
Keywords: *bioremediation, carrot peel waste, Yarrowia lipolytica, soil remediation, oil-drilling mud*

Introduction

The mud generated from oil-drilling, causes considerable ecological and health problems like toxicity and carcinogenicity (Singh and Chandra, 2014). About 20% of generated waste drilling mud is treated by thermal treatment, solidification, solvent washing, landfilling of contaminated soils, stabilization and transport which are some of the available techniques that are highly expensive, ineffective, rarely neutral, and environmentally destructive (Lu et al., 2010; Minai-Tehrani et al., 2009; Dadrasnia et al., 2014). While the rest is temporarily deposited in so-called mud pits. Bioremediation of mud pits has proven to be an effective, non-invasive, eco-friendly and clean-up technology (Cerqueira et al., 2014; Silva-Castro et al., 2015). Bioremediation is utilization of specific microorganisms to reduce or transform petroleum products into less toxic forms (Esmaeil and Akbar, 2015). Biostimulation and

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bioaugmentation are bioremediation methods that have been successfully used earlier (Esmaeil and Akbar, 2015). Bioaugmentation involves introducing allochthonous degrading microorganisms to detoxify petroleum contaminated soils (Taccari et al., 2012; Ceci et al., 2019). Biostimulation is based on stimulation of the catabolic activity of degrading microorganisms by adding nutrient-rich organic and inorganic materials to avoid metabolic limitations (Taccari et al., 2012). Bioaugmentation studies using *Yarrowia lipolytica* were reported by Sekova et al. (2015) to degrade hydrocarbons and triglycerides. The yeast *Candida maltosa* was used to degrade phenyl alkanes, which constitute refined gasoline fuel oil, by Awe et al. (2008). In arid areas, addition of nutrients may be necessary to stimulate the growth of biodegrading microbiota in contaminated soils poor in organic matter (Nyman, 1999). Addition of pure nutrients and organic waste material as biostimulants has been well documented, to support microbial activity in the contaminated soils (Decesaro et al., 2016). Compared to pure nutrients, organic waste material supplementation is a cost-effective method (Andreolli et al., 2015). Organic biostimulants, containing enzymes, enhance substantially the growth of microbial activity (Shahi et al., 2016). Besides, biostimulants release biosurfactants that increase the bioavailability of poorly soluble hydrocarbon petroleum compounds (Yi and Crowley, 2007; Yoshitomi and Shann, 2001). In the few last years, few studies have shown the efficiency of co-application of bioaugmentation by yeast with addition of organic amendment for biodegradation of petroleum contaminated soils (Zhang et al., 2011; Qin et al., 2013). Zhang et al. (2011) used bioaugmentation in combination with biostimulation to treat hydrocarbons contaminated soils by incubating *Fusarium* sp. with a mixture of leaves, branches, and biowastes. Qin et al. (2013) used biochar produced from rice straw in bioremediation of hydrocarbons. These authors demonstrated that application of amendment and yeasts simultaneously, gave promising results compared to bioaugmentation only. The aim of this study was to examine the efficiency of this combination strategy for biodegrading of oil-drilling from contaminated soil using carrot peel waste as organic amendment. However, review of the literature shows that there is no information on the application of this organic matter and *Y. lipolytica* for bioremediation of crude oil contaminated soil.

Materials and methods

The oil-drilling mud contaminated soil origin

The oil-drilling mud was collected in the Hassi Messaoud field (Algeria), located at 30° 25.006’ N and 5° 23.637’ E (*Fig. 1*).

Samples were collected at 0-50 cm depth using a stainless steel sampler, placed in appropriate containers thoroughly mixed therein. The oil-drilling mud contaminated soil was sterilized by heating at 180 °C for 2 h in a closed stainless container which prevents loss of volatile fractions, then cooled down overnight before use. The significant characteristics of the oil-drilling mud contaminated soil are listed in *Table 1*.

Physico-chemical characterization of oil-drilling mud contaminated soil

Preparation of the lixiviate

10 g of oil-drilling mud contaminated soil was placed in the beaker containing 100 mL of distilled water. After agitation during 1 h and filtration (Whatman 125 mm), lixiviate obtained was then diluted at 1/100 and used for analysis.

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Determination of the indicating organic pollution parameters

HACH DR900 Colorimeter was used for the determination of dissolved oxygen, chemical oxygen demand (COD), nitrate, nitrite, phosphorus, sulphates, and ammonia nitrogen content in the oil-drilling mud contaminated soil. Analytical methods were used to determine alkalinity, and chloride by volumetry. Calcium, magnesium were measured by complexometric titration with standard solution of EDTA. Total iron and potassium were measured by an atomic absorption spectrophotometer (PerkinElmer A-Analyst 200).

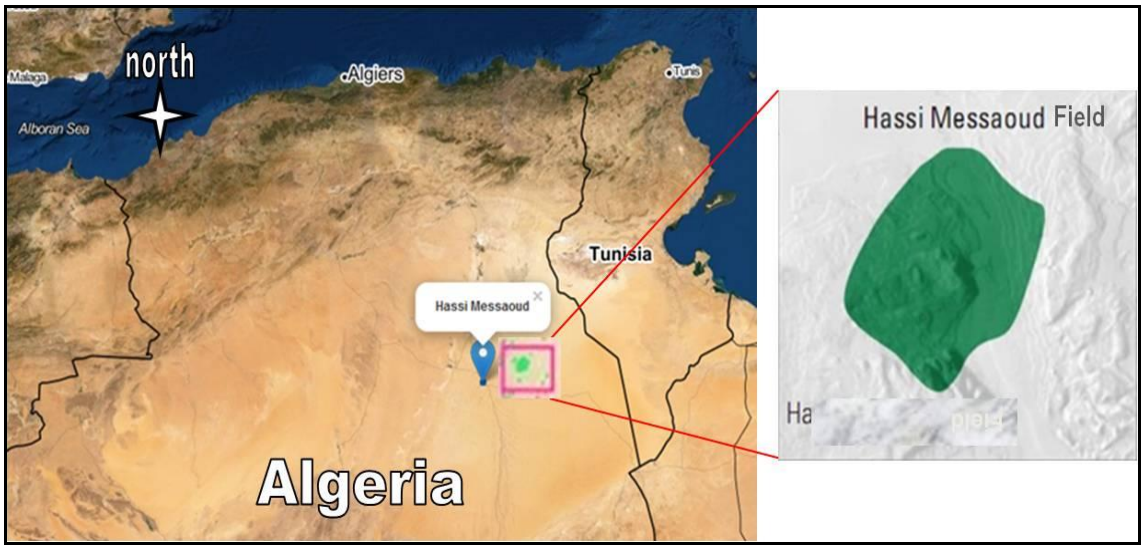

Figure 1. *Location map of the study area*

Biochemical oxygen demand after 5 days (BOD_5)

The Biochemical Oxygen Demand measured for 5 days (BOD_5), was carried out by respirometry (Khodja, 2008).

Evaluation of growth and gasoil degrading ability of Yarrowia lipolytica in carrot peel waste, and carob kibbles media

Carrots were purchased from a local vegetable market and dry pods of carob were obtained from locality of Ighil-Ali (Bejaïa, Algeria). Carrot peels and carob kibbles were macerated separately at a ratio of 1 kg in 2.5 L of distilled water at 85 °C for 45 min with continuous stirring (Acourene and Tama, 2001). After filtration and decantation, media were autoclaved at 120 °C for 20 min and stored at 4 °C before use as biostimulating media.

The yeast *Yarrowia lipolytica* used in this study was isolated and identified earlier in our previous work (published results by Hamoudi-Belarbi et al., 2016). *Y. lipolytica* was grown in 500 mL Erlenmeyer flasks containing 100 mL of Yeast Extract Glucose (YEG broth) prepared using: 10 g/L yeast extract (Himedia, Mumbay, India), 20 g/L glucose (Sigma, Switzerland) in an orbital shaker cabinet maintained at 25 °C under an agitation rate of 80 rpm for 24 h. This 1st culture was then used to inoculate (3% v/v) a carrot peels waste, and carob kibbles media which were incubated at 25 °C for 48 h in an

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orbital shaker (80 rpm). Gasoil (15% or 30%) was added to the 500 mL Erlenmeyer flasks containing 100 mL of sterilized carrot peels waste or carob kibbles media and then incubated at 25 °C in an orbital shaker (80 rpm) for 12 days. At four days regular intervals, turbidity was measured at 600 nm. A volume of 1 mL of each Erlenmeyer flask containing carrot peel waste or carob kibbles media was diluted in 9.9 mL of peptone water solution (Difco, Detroit, USA). After serial dilutions, 1 mL aliquots of suitable dilutions were pour plated in potato dextrose agar medium (Difco). The number of viable cells was counted after 48 h of incubation at room temperature (25 °C).

Bioaugmentation using Yarrowia lipolytica in combination with biostimulation using a selected medium

Experimental design

200 g of sterilized oil-drilling mud in the slurry was used for bioremediation studies and conducted by the following: (i) addition of only yeast (3% v/v); (ii) addition of both the organic amendment and yeast; (iii) sterilized oil-drilling mud in the slurry without yeast and organic amendment (control). Each slurry mixture was placed in circular cell, run in the open air and mixed thoroughly every 3 days to ensure homogenous distribution during 45 days of remediation studies. Each treatment was carried out in duplicate (*Fig. 2*).

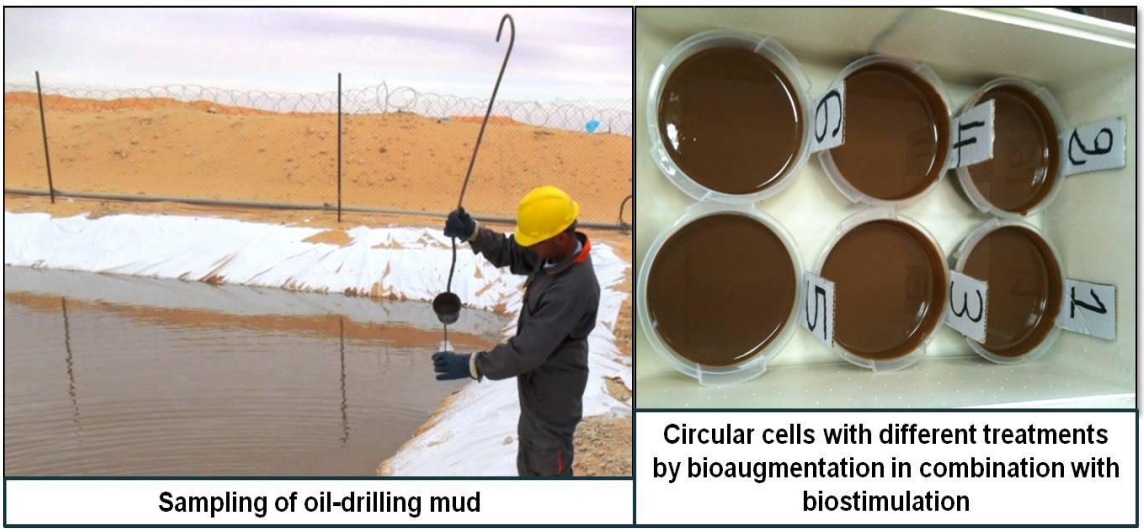

Figure 2. *Experimental design of oil-drilling mud remediation under laboratory scale*

Physico-chemical characterization

Temperature, pH and residual moisture measurements

The temperature and pH were determined using thermometer (MRC 201, France) and, pH meter (Accumet AE150 instrument; Fisher Scientific, France), respectively. The residual moisture (RM) of oil-drilling mud contaminated soil samples was determined (in duplicate) by the difference in weight before and after drying in a vacuum oven at 105 °C for 3 h in the presence of P_2O_5.

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Total petroleum hydrocarbon (TPH) measurement by distillation

TPH percentage of oil-drilling mud samples was measured by distillation using Fann distiller and by determination of the percentage of water/oil in the mud. 20 mL of each soil sample is placed in a distiller, then heated up to 800 °C. The vapors of water and oil are then condensed back into liquid form and collected (distillate). After about 30 to 60 min of decantation, the volumes of water and oil are read directly. After distillation, the remaining mass of mud is weighed. The percentages of water and oil are directly determined. Two replications were conducted for all measurements.

Microbiological analysis

Two replicate samples from each oil-drilling mud (i) with yeast only; (ii) with both the organic amendment and yeast; (iii) without yeast and organic amendment (control) were withdrawn at the end of the second, fourth and sixth week of study for the enumeration of yeast. A volume of 1 mL of each sample was diluted in 9.9 mL of peptone water solution (Difco, Detroit, USA). After serial dilutions, 1 mL of aliquots from suitable dilutions was pour-plated in potato dextrose agar (PDA) medium (Difco). The PDA agar was acidified to pH 3.5 using 100 g/L of tartaric acid solution.

Statistical analysis

Data were analyzed by Data Analysis Tool pack of Microsoft Office Excel 2007 (Microsoft, New York, NY, USA).

Results and discussion

Physicochemical properties of oil-drilling mud contaminated soil

Table 1 lists the physico-chemical properties of oil-drilling mud contaminated soil. The temperature of oil-drilling mud was approximately 21.8 °C ± 0.28; the effect of temperature on the physical nature and chemical composition of the oil, rate of hydrocarbon metabolism by microorganisms and composition of the microbial community, influences oil biodegradation (Atlas, 1981). Moisture content of oil-drilling mud was 51.0 ± 2.12%; according to Kumari et al. (2016), moisture is a limiting factor during biodegradation. As shown in the table, dissolved oxygen is low (7.6 mg/mL). Indeed, a high chemical oxygen demand (COD) (1362 mg O_2/L), that is a measure of the oxygen required to degrade the organic matter present in oil-drilling mud, is an indicator of strong pollution at the site studied (Damo and Icka, 2015). Total organic carbon (TOC) is considered as the most relevant parameter for quantifying organic pollution in soil (Wang et al., 2013). The highest value observed in this study (17.94%) confirmed that the soil studied was polluted by hydrocarbons present in the oil-drilling mud contaminated soil. pH value of oil-drilling contaminated soil used in this study was basic (8.5 ± 0.26). In arid areas, soils tend to be alkaline (Ma et al., 2015), mainly due to the dry environmental conditions. According to Khodja (2008), alkalinity of oil-drilling contaminated soil is maintained between 9.5 and 10.5 in order to prevent corrosion and control the solubility of calcium and magnesium salts. Oil-drilling contaminated soil was mineralized with predominance of ions such as Cl^-, Ca^{+2}, SO_4^{-2}, and Mg^{+2}. However, nitrites (HNO_2^-), nitrates ($N\text{-}HNO_3^-$), phosphorus (PO_4^{-3}), and Fe^{+2} were negligible. According to Abd-Alla et al. (2014), alkaline soils are characterized by poor

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availability of phosphorus and micronutrient transition metals. Analysis of carrot peel waste showed that it contains 25.10 ± 1.20 mg/L phosphorus, and 1.4 mg/L nitrogen (Hamoudi-Belarbi et al., 2018). Therefore this waste can compensate for the nutrient deficiencies in the oil-drilling mud contaminated soil.

Table 1. *Physicochemical properties of oil-drilling mud contaminated soil*

Parameters	Values
Temperature	21.7
Residual moisture %	51.0 ± 2.12
pH	8.5 ± 0.26
COT (%)	17.94
$N\text{-}HNO_3^-$	0.152
HNO_2^-	1.3
PO_4^{-3}	0.23
COD (mg/L)	1362
BOD_5 (mg/L)	56.5
Dissolved O_2 (mg/L)	7.60
CO_3^-	-
HCO_3^-	51.85
SO_4^{2-}	80
Cl^-	4627.13
Ca^{++}	841.68
Mg^{++}	60.8
Na^+	1454.4
K^+	28.67
Fe^{++}	21.45

Evaluation of growth and gasoil degrading ability of Yarrowia lipolytica in carrot peel waste, and carob kibbles media

Figure 3 shows the growth of *Yarrowia lipolytica* after incubation using biostimulating media (carrot peel waste, and carob kibbles) with adding gasoil at 15%, and 30% concentration as sole source of carbon, in comparison with a control without adding gasoil, during 12 days. The nature of biostimulating media and concentration of gasoil affected the growth of *Y. lipolytica*. Higher augmentation in growth was observed in carrot peel waste medium and when the concentration of gasoil was increased from 15% to 30%, indicating that phosphorus and nitrogen nutrients present in carrot peel waste played a major role in growing the cultures. As an example, at 4 days of incubation at 15% concentration, log (CFU/mL) was 11.93 (*Fig. 3A* and *B*). At 8 days of incubation, log (CFU/mL) decreased to 9.00 for 15% and 7.97 for 30%. After 8 days of incubation, log (CFU/mL) increased to reach 11.27 for all 15% and 30% concentration at 12 days of incubation. Carob kibbles are widely used as biostimulating medium during bioremediation of petroleum (Hamoudi-Belarbi et al., 2017, 2018). Carob kibbles did not provide a good biostimulating medium to *Y. lipolytica*, with log (CFU/mL) of 10.38 for 15% and 30% concentration simultaneously at 4 days of incubation. Log (CFU/mL) increased then decreased to 11.16, and 10.41 for 15% and 30%, respectively at 8 days of incubation. A drastic decreasing was observed at 12 days

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of incubation with log [CFU/mL] of 8.34 for 15% and 30% concentration simultaneously. This better growth using carrot peel waste than carob kibbles can be explained by the fact that phosphorus and nitrogen nutrients present in carrot peel waste played a major role in growing the cultures of *Y. lipolytica*. Vidali (2001) demonstrated that the additional nutrient nitrogen and phosphorus contained in the carrot peel waste stimulated microbial growth and led to synthesized enzymes required to degrade hydrocarbon compounds. On the other hand, carrots are known to release linoleic acid, which can increase the bioavailability of poorly soluble hydrocarbon compounds (Yi and Crowley, 2007; Yoshitomi and Shann, 2001; Kosaric, 2001).

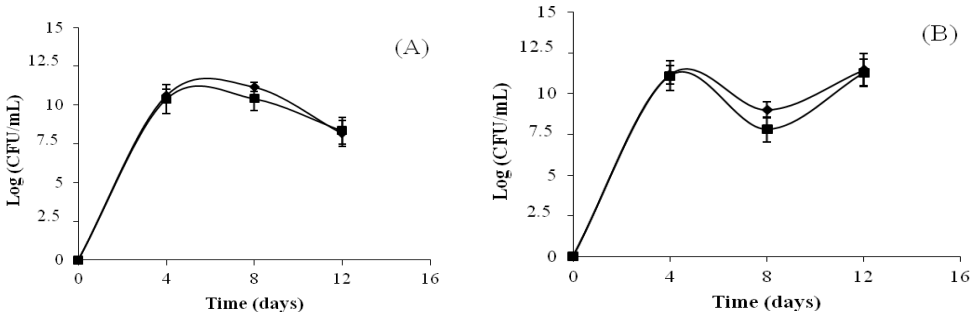

Figure 3. *Growth and gasoil degradation by Yarrowia lipolytica during 12 days at concentrations (♦) 15% and (■) 30% v/v. Y. lipolytica was grown in (A) carob kibbles medium; (B) carrot peel waste medium. Error bars represent the standard error of replicates*

Effect of bioaugmentation and organic amendment on oil-drilling mud contaminated soil

Figure 4A, B, and *C* illustrate variation of temperature, moisture content and pH of oil-drilling mud contaminated soil using bioaugmentation by *Y. lipolytica* and biostimulation with carrot peel waste for one week intervals and during 45 days of study period. *Figure 4A* shows the evolution of temperature as a function of time throughout the study. The effect of temperature on the chemical composition and physical nature of the oil, metabolism of hydrocarbon by microorganisms, influences oil-petroleum biodegradation (Atlas, 1981). At the beginning of the study, the temperature of oil-drilling mud with both the organic amendment and yeast, and without yeast and organic amendment (control) was approximately 21.8 °C ± 0.28.

At the beginning of study, the temperature of crude oil unamended and amended soil was approximately 21.8 °C ± 0.34. An increase in soil temperature was obtained for all samples after 15 days followed by gradual decreasing then stabilisation at 21.3 °C ± 1.5 for unamended soil control at the end of the study, while temperature values of amended samples continue their progression. After 15 days of treatment, an increase in oil-drilling mud temperature was obtained for all samples, followed by gradual decreasing then stabilization at 21.3 °C ± 0.42 for control at the end of the study, while temperature values continue their progression followed by gradual decreasing then stabilization at 22.85 °C ± 0.42 for oil-drilling mud with yeast only at the end of the study, while temperature values of amended samples continue their progression. These results may be due to the intensity of the metabolic activity of *Y. lipolytica* present in carrot peel waste

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amended oil-drilling mud soil. According to Insam et al. (2010), during microbial activities, fragmentation of complexes molecules liberates energy; some of this energy is used for anabolism and the rest is dissipated as heating. At the laboratory scale, the metabolism of hydrocarbons occurs in the temperature range from 4 to 30 °C (Aislabie et al., 2006).

Moisture content of the oil-drilling mud with both the organic amendment and yeast ranged between 51.0 ± 2.12% and 56.50 ± 2.32% at the end of the treatment while that of oil-drilling mud amended with yeast only ranged from 51.0 ± 2.12% and 53.11 ± 2.63% at the end of treatment, respectively (*Fig. 4B*). However, the moisture content of control stabilized at 51.01 ± 1.11% at the end of treatment. According to Kumari et al. (2016), moisture is a limiting factor during biodegradation; further more during microbial catabolism, energy and water are produced.

pH value of all oil-drilling mud contaminated soil with yeast only, with both the organic amendment-yeast, without yeast and organic amendment (control) was basic at the beginning of study (8.5 ± 0.26) (*Fig. 4C*). pH value of organic amended samples with yeast decreased gradually then increased to reach, at the end of study, 8.08 ± 0.10 for carrot peel waste amended oil-drilling mud and yeast and 7.61 ± 0.11 for yeast only. According to Vero et al. (2019), solubilization and formation of ammonia by organic nitrogen ammonification by *Y. lipolytica* results in increased pH. It has also been reported that alkaline condition enhances hydrocarbon degradation in contaminated soil (Morgan and Atlas, 1989).

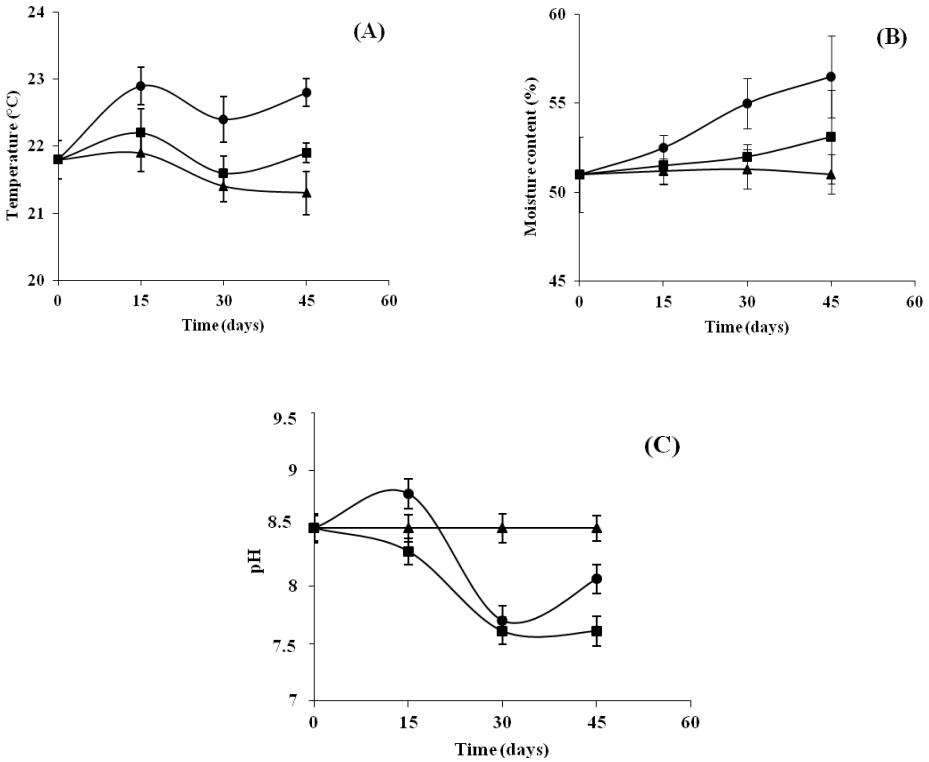

Figure 4. *Evolution of temperature (A), moisture (B), and pH (C) during 45 days of treatment. Mud + Carrot + Yeast (•); Mud + Yeast (■); Mud (▲). Error bars represent the standard error of replicates*

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Figure 5 shows the degradation of total petroleum hydrocarbons (TPH) of the oil-drilling mud contaminated soil using the various bioremediation strategies and during 45 days. At the beginning of study, initial concentration of TPH was about 38 ± 1.4% (corresponding to 3.60 g/kg) for all samples (*Fig. 5*). When oil-drilling contaminated soil was incubated with only *Y. lipolytica* (6 mL, 3×10^7 CFU/mL) and both *Y. lipolytica* (6 mL, 3×10^7 CFU/mL) and carrot peel waste amendment (4 mL), TPH decreased to 35 ± 1.66% and 30.60 ± 1.50% the first 15 days, 33 ± 2.30% and 26.8 ± 1.66% respectively at the end of study. TPH rate did not undergo any significant change from its initial value in the control for all the period of incubation. These results indicate that combining application of *Y. lipolytica* with carrot peel waste has a positive effect on TPH biodegradation in oil-drilling contaminated soil. Without organic amendment, *Y. lipolytica* activity was slow due to low organic matter content in the oil-drilling mud contaminated soil. This low TPH removal was attributed to nutrition deficiency in the soil. Mancera-López et al. (2008) found that *Rhizopus* sp., *Penicillium funiculosum* and *Aspergillus sydowii* fungi removed, respectively, 36%, 30% and 17% more polycyclic aromatic hydrocarbon (PAH) from crude oil in comparison with biostimulation alone. Besides, Ataikiru et al. (2018) demonstrated that yeasts have great potentials to degrade hydrocarbons, and their use is one of the cheaper solutions to remediation in comparison to highly expensive physical and chemical techniques.

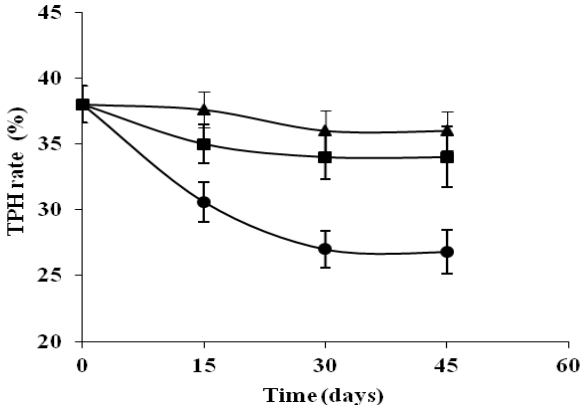

Figure 5. *The rate of TPH during 45 days of treatment. Mud + Carrot + Yeast (•); Mud + Yeast (■); Mud (▲). Error bars represent the standard error of replicates*

The growth and evolution of *Y. lipolytica* cells in the oil-drilling mud contaminated soil, as function of incubation time, is represented in *Figure 6*. At the end of the second week, the log (CFU/g) reached a value of 7.47 ± 0.53 for both *Y. lipolytica* and carrot peel waste amendment and 7.50 ± 0.77 for yeast only. At 30 days of incubation, log (CFU/g) of oil-drilling mud contaminated soil reached a value of 8.20 ± 0.55 for both *Y. lipolytica* and carrot peel waste amendment and 7.60 ± 0.51 for yeast only. At 45 days of incubation, log (CFU/g) increased to reach a value of 8.90 ± 0.54 for both *Y. lipolytica* and carrot peel waste amendment and 7.80 ± 0.60 for yeast only. The log (CFU/g) of oil-drilling mud soil (control) did not change from its initial value. Without

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organic amendment, the activity of *Y. lipolytica* was slow due to the low organic matter content in the oil-drilling mud contaminated soil. Li et al. (2015) and Borowik et al. (2017) had also reported increase of biomass on bioaugmentation with microorganisms and organic amendment. Presence of considerable quantities of P in carrot peel waste (25.10 ± 1.20 mg/L), which is a necessary nutrient for microbial biodegradative activities (Hamoudi-Belarbi et al., 2018). In addition, production of linoleic acid biosurfactant by carrot increases the solubility and availability of hydrocarbons to biodegrading microorganisms (Marinescu et al., 2017) and contributes to the biodegradation of petroleum hydrocarbons. Desert soils have been previously shown to be nutrients-limited (Al-Saleh and Hassan, 2016).

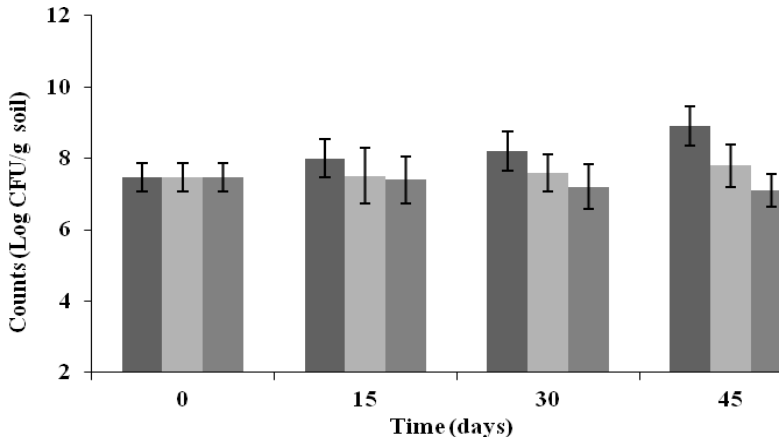

Figure 6. *Evolution of cell count during 45 days of treatment. Mud+ Carrot + Yeast (■); Mud + Yeast (■); Mud (■). Error bars represent the standard error of replicates*

Conclusion

This study demonstrated that combination of bioaugmentation by the isolate *Yarrowia lipolytica* and biostimulation with carrot peel waste as an organic amendment is a good strategy for bioremediation of oil-drilling mud contaminated soil. The TPH degradation in the oil-drilling mud contaminated soil was enhanced by biostimulation with nutrients present in the carrot peel waste and bioaugmentation by *Y. lipolytica* in comparison to *Y. lipolytica* only and control. Carrot peel waste, containing high amounts of phosphorus, enhanced bioremediation of oil-drilling mud contaminated soil by increasing activities of yeast *Y. lipolytica*. Besides, linoleic acid produced by carrot peel waste, increased the solubility and availability of hydrocarbons to biodegrading yeast. For future research, the selection of other bioproducts as biostimulants in combination with consortium of yeasts and bacteria for bioremediation should be recommended. It will be interesting to select the efficient combination for the better results.

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DOI: http://dx.doi.org/10.15666/aeer/…..

REFERENCES

[1] Abd-Alla, M. H., El-Enany, A. W. E., Nafady, N. A., Khalaf, D. M., Morsy, F. M. (2014): Synergistic interaction of *Rhizobium leguminosarum* bv. *viciae* and *arbuscular* mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. – Microbiological Research 169(1): 49-58.

[2] Acourene, S., Tama, M. (2001): Utilisation des dattes de faible valeur marchande (rebuts de Deglet-Nour, Tinissine et Tantboucht) comme substrat pour la fabrication de la levure boulangère. – Revue des Energies Renouvelable 1-10. http://www.cder.dz/download/bio_1.pdf.

[3] Aislabie, J., Saul, D. J., Foght, J. M. (2006): Bioremediation of hydrocarbon-contaminated polar soils. – Extremophiles 10: 171-179.

[4] Al-Saleh, E., Hassan, A. (2016): Enhanced crude oil biodegradation in soil via biostimulation. – International Journal of Phytoremediation 18(8): 822-831.

[5] Andreolli, M., Lampis, S., Brignoli, P., Vallini, G. (2015): Bioaugmentation and biostimulation as strategies for the bioremediation of a burned woodland soil contaminated by toxic hydrocarbons: a comparative study. – Journal of Environmental Management 153: 121-131.

[6] Ataikiru, T. L., Okerentugba, P. O., Iheanacho, C. C. (2018): Bioremediation of bonny light crude oil polluted soil by bioaugmentation using yeast isolates (*Candida adriatica* ZIM 2468 and *Candida taoyuanica* MYA-4700). – International Journal of Environmental Research and Public Health 5(4): 52-61.

[7] Atlas, R. M. (1981): Microbial degradation of petroleum hydrocarbons: an environmental perspective. – Microbiological Reviews 45(1): 180-209.

[8] Awe, S., Mikolasch, A., Hammer, E., Schauer, F. (2008): Degradation of phenylalkanes and characterization of aromatic intermediates acting as growth inhibiting substances in hydrocarbon utilizing yeast *Candida maltosa*. – International Biodeterioration and Biodegradation 62(4): 408-414.

[9] Borowik, A., Wyszkowska, J., Oszust, K. (2017): Functional diversity of fungal communities in soil contaminated with diesel oil. – Frontiers in Microbiology 8: 1862.

[10] Ceci, A., Pinzari, F., Russo, F., Persiani, A. M., Gadd, G. M. (2019): Roles of saprotrophic fungi in biodegradation or transformation of organic and inorganic pollutants in co-contaminated sites. – Applied Microbiology and Biotechnology 103(1): 53-68.

[11] Cerqueira, V. S., Maria do Carmo, R. P., Camargo, F. A., Bento, F. M. (2014): Comparison of bioremediation strategies for soil impacted with petrochemical oily sludge. – International Biodeterioration and Biodegradation 95: 338-345.

[12] Dadrasnia, A., Salmah, I., Emenike, C. U., Shahsavari, N. (2015): Remediation of oil contaminated media Using organic material supplementation. – Petroleum Science and Technology 33: 1030-1037.

[13] Damo, R., Icka, P. (2015): Environmental impact assessment generated by albanian petroleum industry into Gjanica rIVeR. – Romanian Biotechnological Letters 20(1): 10151.

[14] Decesaro, A., Rampel, A., Machado, T. S., Thomé, A., Reddy, K., Margarites, A. C., Colla, L. M. (2016): Bioremediation of soil contaminated with diesel and biodiesel fuel using biostimulation with microalgae biomass. – Journal of Environmental Engineering 143(4): 04016091.

[15] Esmaeil, A. S., Akbar, A. (2015): Occurrence of *Pseudomonas aeruginosa* in Kuwait soil. – Chemosphere 120: 100-107.

[16] Hamoudi-Belarbi, L., Nouri, L. H., Belkacemi, K. (2016): Effectiveness of convective drying to conserve indigenous yeasts with high volatile profile isolated from algerian fermented raw bovine milk (Rayeb). – Food Sciences and Technology 36(3): 476-484.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/…..

[17] Hamoudi-Belarbi, L., Bendifallah, L., Hamoudi, S., Belkacemi, K. (2017): Biostimulation of Microbial Community by Carob (*Ceratonia siliqua*) to Degrade Total Petroleum Hydrocarbon (TPH) in Contaminated Soil. – In: Kallel, A., Ksibi, M., Dhia, H. B., Khélifi, N. (eds.) Euro-Mediterranean Conference for Environmental Integration. Springer, Cham.

[18] Hamoudi-Belarbi, L., Hamoudi, S., Belkacemi, K., Nouri, L. H., Bendifallah, L., Khodja, M. (2018): Bioremediation of polluted soil sites with crude oil hydrocarbons using carrot peel waste. – Environments 5(11): 124.

[19] Insam, H., Franke-Whittle, I., Goberna, M. (2010): Microbes in Aerobic and Anaerobic Waste Treatment. – In: Insam, H., Franke-Whittle, I., Goberna, M. (eds.) Microbes at Work. Springer, Berlin.

[20] Khodja, M. (2008): Drilling fluid: performance study and environmental considerations. – PhD Thesis, Institut National Polytechnique, Toulouse, France.

[21] Kosaric, N. (2001): Biosurfactants and their application for soil bioremediation. – Food Technology and Biotechnology 39(4): 295-304.

[22] Kumari, B., Singh, S. N., Singh, D. P. (2016): Induced degradation of crude oil mediated by microbial augmentation and bulking agents. – International Journal of Environmental Science and Technology 13(4): 1029-1042.

[23] Li, H., Tan, L., Ning, S., He, M. (2015): Reactor performance and microbial community dynamics during aerobic degradation and detoxification of Acid Red B with activated sludge bioaugmented by a yeast *Candida tropicalis* TL-F1 in MBR. – International Biodeterioration and Biodegradation 104: 149-156.

[24] Lu, M., Zhang, Z., Sun, S., Wei, X., Wang, Q., Su, Y. (2010): The use of goosegrass (*Eleusine indica*) to remediate soil contaminated with petroleum. – Water, Air, and Soil Pollution 209: 181-189.

[25] Ma, H., Yang, H., Lü, X., Pan, Y., Wu, H., Liang, Z., Ooi, M. K. (2015): Does high pH give a reliable assessment of the effect of alkaline soil on seed germination? A case study with *Leymus chinensis* (Poaceae). – Plant Soil 394(1-2): 35-43.

[26] Mancera-López, M. E., Esparza-García, F., Chávez-Gómez, B., Rodríguez-Vázquez, R., Saucedo-Castaneda, G., Barrera-Cortés, J. (2008): Bioremediation of an aged hydrocarbon-contaminated soil by a combined system of biostimulation–bioaugmentation with filamentous fungi. – International Biodeterioration and Biodegradation 61(2): 151-160.

[27] Marinescu, M., Lacatusu, A., Gament, E., Plopeanu, G., Carabulea, V., Marinescu, M. (2017): A review of biological methods to remediate crude oil polluted soil. – Annals of the University of Craiova-Agriculture, Montanology, Cadastre Series 46(1): 35-340.

[28] Minai-Tehrani, D., Minoui, S., Herfatmanesh, A. (2009): Effect of salinity on biodegradation of polycyclic aromatic hydrocarbons (PAHs) of heavy crude oil in soil. – Bulletin of Environmental Contamination and Toxicology 82: 179-184.

[29] Morgan, P., Atlas, R. M. (1989): Hydrocarbon degradation in soils and methods for soil biotreatment. – Critical Reviews in Biotechnology 8(4): 305-333.

[30] Nyman, J. A. (1999): Effect of crude oil and chemical additives on metabolic activity of mixed microbial populations in fresh marsh soils. – Microbial Ecology 37(2): 152-162.

[31] Qin, G., Gong, D., Fan, M. Y. (2013): Bioremediation of petroleum-contaminated soil by biostimulation amended with biochar. – International Biodeterioration and Biodegradation 85: 150-155.

[32] Sekova, V. Y., Isakova, E. P., Deryabina, Y. I. (2015): Biotechnological applications of the extremophilic yeast *Yarrowia lipolytica*. – Applied Biochemistry and Microbiology 51(3): 278-291.

[33] Shahi, A., Aydin, S., Ince, B., 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.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/…..

[34] Silva-Castro, G. A., Uad, I., Rodríguez-Calvo, A., González-López, J., Calvo, C. (2015): Response of autochthonous microbiota of diesel polluted soils to land-farming treatments. – Environmental Research 137: 49-58.
[35] Singh, K., Chandra, S. (2014): Treatment of petroleum hydrocarbon polluted environment through bioremediation: a review. – Pakistan Journal of Biological Sciences 17(1): 1-8.
[36] Taccari, M., Milanovic, V., Comitini, F., Casucci, C., Ciani, M. (2012): Effects of biostimulation and bioaugmentation on diesel removal and bacterial community. – International Biodeterioration and Biodegradation 66(1): 39-46.
[37] Vero, S., Garmendia, G., Martínez-Silveira, A., Cavello, I., Wisniewski, M. (2019): Yeast Activities Involved in Carbon and Nitrogen Cycles in Antarctica. – In: Castro-Sowinski, S. (ed.) The Ecological Role of Micro-organisms in the Antarctic Environment. Springer, Cham.
[38] Vidali, M. (2001): Bioremediation. An overview. – Pure and Applied Chemistry 73(7): 1163-1172.
[39] Wang, M., Liu, X., Pan, B., Zhang, S. (2013): Photodegradation of acid orange 7 in a UV/acetylacetone process. – Chemosphere 93(11): 2877-2882.
[40] Yi, H., Crowley, D. E. (2007): Biostimulation of PAH degradation with plants containing high concentrations of linoleic acid. – Environmental Science and Technology 41(12): 4382-4388.
[41] Yoshitomi, K. J., Shann, J. R. (2001): Corn (*Zea mays* L.) root exudates and their impact on 14C-pyrene mineralization. – Soil Biology and Biochemistry 33(12-13): 1769-1776.
[42] Zhang, Y., Zhu, Y. G., Houot, S., Qiao, M., Nunan, N., Garnier, P. (2011): Remediation of polycyclic aromatic hydrocarbon (PAH) contaminated soil through composting with fresh organic wastes. – Environmental Science and Pollution Research 18(9): 1574-1584.

http://www.aloki.hu ● ISSN 1589 1623 (Print) ● ISSN 1785 0037 (Online)
DOI: http://dx.doi.org/10.15666/aeer/…..