Journal of Engineering
journal homepage: www.joe.uobaghdad.edu.iq
Number 1 Volume 26 January 2020

***Chemical, Petroleum and Environmental Engineering***

# Biodegradation of Total Petroleum Hydrocarbon from Al-Daura Refinery Wastewater by Rhizobacteria

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## ABSTRACT

Due to the deliberate disposal of industrial waste, a great amount of petroleum hydrocarbons pollute the soil and aquatic environments. Bioremediation that depends on the microorganisms in the removal of pollutants is more efficient and cost-effective technology. In this study, five rhizobacteria were isolated from *Phragmites australis* roots and exposed to real wastewater from Al-Daura refinery with 70 mg/L total petroleum hydrocarbons (TPH) concentration. The five selected rhizobacteria were examined in a biodegradation test for seven days to remove TPH. The results showed that 80% TPH degradation as the maximum value by *Sphingomonas Paucimobilis* as identified with Vitek® 2 Compact (France).

**Keywords**: petroleum wastewater; biodegradation; *Phragmites australis;* rhizobacteria.

## التحلل الحيوي للهيدروكاربونات النفطية من المياه الملوثة لمصفى الدورة بأستخدام بكتريا الجذور

## الخلاصة

بسبب التخلص المتعمد للنفايات، فإن كمية كبيرة من الهيدروكربونات النفطية تلوث التربة والبيئات المائية. إن المعالجة الحيوية التي تعتمد على الكائنات الدقيقة في إزالة الملوثات هي تقنية أكثر كفاءة وفعالية من حيث التكلفة. في هذه الدراسة ، تم عزل خمسة انواع بكتريا من جذور نبات Phragmites australis بعد تعرضه لمياه مصفى الدورة الملوثة ذات التركيز الإجمالي للهيدروكربونات البترولية 70 مجم / لتر. تم فحص أنواع البكتريا المعزولة في اختبار التحلل الحيوي لمدة سبعة أيام لإزالة TPH.وأظهرت النتيجة انخفاظ 80 TPH ٪ كقيمة قصوى بواسطة *Sphingomonas Paucimobilis* المشخصة بواسطة نظام Vitek® 2 Compact.

**الكلمات الرئيسية:** المياه الملوثة البترولية, التحلل البيولجي, القيصوب, بكتربا الجذور.

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Peer review under the responsibility of University of Baghdad.
https://doi.org/10.31026/j.eng.2020.01.02

Article received: 23/12/2018
Article accepted: 16/2/2019
Article published: 1/1/2020

## 1. INTRODUCTION

The crude oil is the most essential energy source in the industrial countries (**Xaaldi Kalhor, et al., 2017)**. In 2015, 97 million barrels of oil were needed per day in order to meet the energy required **(dos Santos and Maranho, 2018).** During the exploration of oil wells, refining, and transportation of petroleum products, a large amount of crude and refined oil is released into surroundings as a negative approach, **(Yavari, et al., 2015)**. Also, and because of the high cost of treatment, the refinery wastewater is discharged to the rivers and other aquatic bodies without treatment is one of the ways that is occurring in the developing countries **( Nayyef M. Azeez 2016; Musa, et al., 2015)**. The pollution due to crude oil and refinery process has a significant environmental impact on human health and ecosystem quality**(Yavari, et al., 2015; Hussain, et al., 2018).**

There are numerous techniques, such as physical, chemical and biological for treating the petroleum contamination **(Peng et al. 2009)**. Among them, biological methods are more feasible, cost-effective and easy to apply **(dos Santos and Maranho, 2018; Chunli Zheng, et al., 2012).** and offer complete degradation of oil to $CO_2$ and $H_2O$ (**Hussain, et al., 2018)**.

The treatment, which depends on the plant and their associated rhizobacteria is one of the most important biological strategies that can be used for removal of pollutants water, because it is more efficient and cost-effective technology **(Liu, et al. 2011; Toyama ,et al. 2011; dos Santos and Maranho, 2018; Cai, et al. 2010)**. The main mechanism in those methods are based on stimulation of rhizobacteria, called rhizoremediation or rhizodegradation **(Gerhardt, et al., 2017; Cai, et al. 2010; Al-Baldawi, et al. 2013; dos Santos and Maranho, 2018)**. Rhizodegradiation is the combination of phytoremediation and bioaugmentation with rhizobacteria (**Hussain, et al. 2018; Pant, et al., 2016)**. In this process, the plant acts indirectly by providing carbon and energy source to rhizobacteria (the bacteria which concentrate on root surface and in the surrounding area to the root). The plant exudes oxygen and nutrient such as organic acids, amino acids, enzymes and sugars to root zone (**Epps 2006; Pant, et al., 2016)**. However, great attention to bacteria that able to degrade petroleum hydrocarbons in water and soil has been received. **Yasseen (2014)** has listed 25 bacterial genera that act as petroleum hydrocarbons degraders, such as Acidovorax, Mycobacterium, Pseudomonas, Rhodococcus, Sphingomonas, Xanthomonas, Achromobacter, Micrococcus and Bacillus. This study aimed to explore plant–rhizobacteria interaction in the rhizosphere zone to improve the biotreatment of petroleum hydrocarbons of refinery wastewater. Rhizobacteria used was isolated from the root of *Phragmites australis* exposed to real wastewater from Al-Daura refinery contains hydrocarbons, and identified by Vitek® 2 Compact systems (bioMérieux, France).

## 2. MATERIALS AND METHOD

### 2.1 Isolation and identification of rhizobacteria

The bacteria strains used in this study were isolated from roots of *Phragmites australis* which was exposed to wastewater containing petroleum hydrocarbons that obtained from Al-Daura refinery, Iraq. Ten grams of *Phragmites australis* root was inserted in 250 mL Erlenmeyer flask containing 100 mL sterilized distilled water and then incubated in a rotary shaker at 30°C with 150 rpm for one hour. Five dilutions were obtained for planting by applying serial dilution method. 1 mL of water from Erlenmeyer flask was mixed with 9 mL sterile saline water (0.85% NaCl) until reaching serial dilution up to $10^{-5}$. 0.1 mL of each dilution ($10^{-2}$, $10^{-3}$, $10^{-4}$ and $10^{-5}$) was pipetted in a petri dish with Tryptic Soya Agar (TSA) and spread with glass cell spreader (hockey stick). The planted Petri dishes were incubated at 30°C overnight **(Al-Baldawi, et al. 2015)**.

A pure culture of more appearance colonies was obtained on TSA Petri dish. The isolated rhizobacteria were coded according to color or shape of the colony (W = white, Y = yellow, T = tree). Then, the selected rhizobacteria were identified using Vitek® 2 Compact systems (bioMérieux, France) after biochemical test of gram stain was examined. Cultures to be examined were regrown from storage on TSA and incubated in 30°C before one day of testing. By a sterile swab, numbers of pure colonies were suspended in 0.3 mL of sterile saline (aqueous 0.45% to 0.50% NaCl, pH 4.5 to 7.0) in a test tube until reach 0.6 NTU which was measured by turbidity meter( **Pincus, 2010)**.

## 2.2 Biodegradation of petroleum hydrocarbon by isolated rhizobacteria

Bushnell Haas medium prepared using refinery wastewater with characteristics as shown in **Table 1.** was inoculated with rhizobacteria to determine the ability of rhizobacteria to degrade petroleum hydrocarbon. The medium was described by **(Anon, 2011).** as follows (g/L): 0.2 $MgSO_4.7H_2O$, 0.02 $CaCl_2.2H_2O$, 1.0 $KH_2PO_4$, 1.0 $K_2HPO_4$, 0.1 $NH_4NO_3$, and 0.05 $FeCl_3$. 20 mL of sterilized Tryptic Soya Broth (TSB) was prepared and inoculum with pure cultures of each rhizobacterium and incubated in a rotary shaker (JSR, Korea) at 30°C with 150 rpm for 24 h **(Płaza, et al. 2008)**. 2 mL of inoculum with 4 McFarland ($1200\times10^6$ CFU/mL) was added to a 100 mL Erlenmeyer flask contained 48 mL of Bushnell Haas medium and kept in a rotary shaker at 30°C and 150 rpm for seven days, and then the biodegradation was tested.

**Table 1.** The main characteristics of Al-Daura refinery wastewater.

| Parameter | Units | Value |
|---|---|---|
| PH | | 7 |
| Turbidity | NTU | 31.3 |
| TSS | mg/L | 142 |
| Phenol | mg/L | 2.5 |
| TPH | mg/L | 70 |
| COD | mg/L | 370 |
| $BOD_5$ | mg/L | 120 |
| ORP | mV | 179 |
| TDS | mg/L | 904 |

## 2.3 Evaluation of TPH Degradation by Rhizobacteria

After seven days of incubation, the remaining TPH was measured using oil content analyzer (Horiba, OCMA-350, USA). 10 mL of liquid medium was extracted by equal volume of Tetrachloromethane ($CCL_4$) and then the solvent layer which contains residual TPH was passed through anhydrous sodium sulfate ($Na_2SO_4$) to remove the remaining water. During extraction the liquid sample was adjusted at pH=2 **(USEPA, 1996).**

The removal percentage of TPH was determined according to equation (1):

$$\%\text{Biodegradation} = \frac{\text{TPH0} - \text{TPH7}}{\text{TPH0}} \times 100 \qquad (1)$$

where TPH0= total petroleum hydrocarbon at 0 days, and
TPH7= total petroleum hydrocarbon at seven day

The ability of rhizobacteria to degrade n-alkanes ($C_{10}$-$C_{26}$) was evaluated using gas chromatography with a flame ionization detector (GC-FID) (GC-Shimadzu, Japanese company, model 2010) and dichloromethane (DCM) as solvent. The column was programmed to stay for 30 seconds at 100$^{o}$C, and then ramp 10$^{o}$C every minute until reach 330$^{o}$C for 10 minutes.

## 3. RESULTS AND DISCUSSION

### 3.1 Isolation and identification of rhizobacteria

After 24h of growth on TSA, pure cultures of 5 species of rhizobacteria were obtained on TSA medium, as shown in **Fig. 1**.

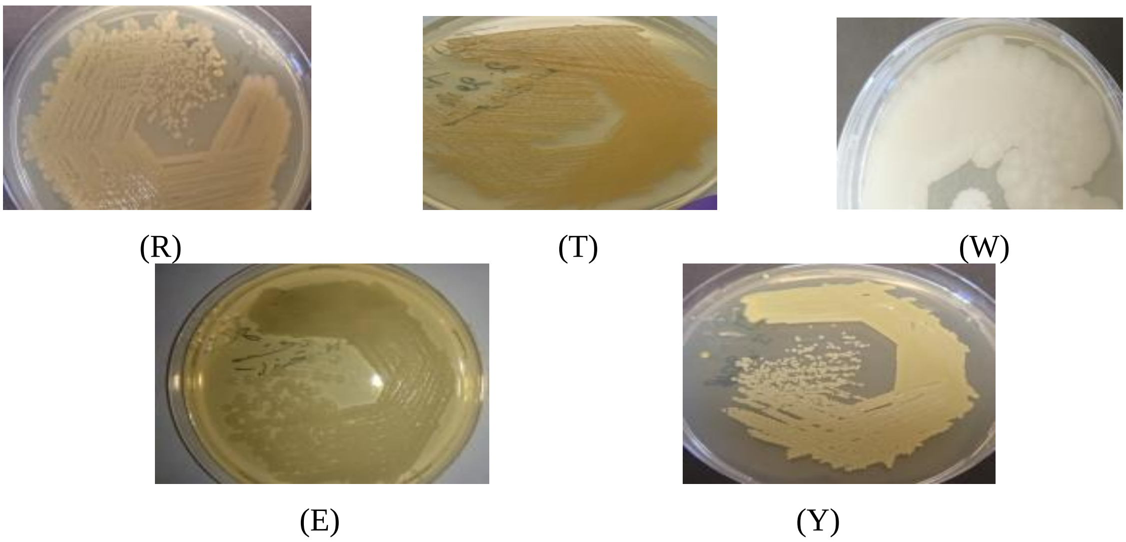



**Figure 1.** Code and pictures of isolated bacteria.

Then, the identification of the five selected rhizobacteria was made using Vitek® 2 Compact systems (bioMérieux, France), the results as shown in **Table 2**.

**Table 2.** Rhizobacteria identification by vitek® 2 compact.

| Code | Bacteria name | Probability | Gram stain |
|---|---|---|---|
| R | Pseudomonas stutzeri | 97% | - |
| T | Sphingomonas paucimobilis | 94% | - |
| Y | Micrococcus luteus | 99% | + |
| W | Staphylococcus aureus | 86% | + |
| E | Staphylococcus lentus | 93% | + |

The results of identification show that all rhizobacteria were hydrocarbon-degrading bacteria and that explained by **(Toyama, et al. 2006 )**as due to ability of plant (Phragmites australis) to select and enhance accumulation of contaminants degrading bacteria (TPH) to enhance removal efficiency. Where, Pseudomonas Stutzeri have high ability to degrade crude oil contaminant soil and water **(Kaczorek, et al., 2012; Hassan Shahian, et al., 2012; Biology, et al., 2008; Celik, et al., 2008)**. Sphingomonas paucimobilis has been reported as poly-aromatic hydrocarbons (PAHs) degrading bacteria (**Story, et al. 2004; Haritash and Kaushik, 2009)** and also have the ability to degreed diesel **(Ipung Fitri Purwanti1, et al. 2012; Moliterni, et al. 2012**. **Zhou, et al. 2016)** show that Sphingomonas acts a key role in the degradation of PAH also, (**San Miguel, et al. 2009).** study the ability of Sphingomonas paucimobilis to degrade naphthalene in water and showed that 80% of naphthalene was degraded during three weeks. **(Ojo, 2006).** studied the capability of Micrococcus Luteus with other bacteria as a consortium in the utilization of Petroleum hydrocarbon and the result shown the major effect of the consortium in the biodegradation. Moreover,**( Mustapha, et al. 2015).** isolate Micrococcus Luteus from sub-surface wetland treating refinery wastewater. Also, Micrococcus Luteus able to degrade different organic contaminant such as nitrobenzene (**Zheng, et al. 2009).** aliphatic and aromatic hydrocarbons **Benedek, et al. 2010; Toledo, et al. 2006**, and Pesticide**( Kanjilal, et al., 2015**. **Musa, et al. 2015** isolate Staphylococcus aureus from petroleum refinery wastewater and **Length, 2010).** revealed that Staphylococcus aureus is the best in degradation of kerosene. (**Moliterni, et al. 2012)** isolated Staphylococcus Lentus and other bacteria from oil refinery soil to study biodegradation of diesel by them, and then the result shows 80% of diesel was degraded after 40h.

### 3.2 Biodegradation of Petroleum Hydrocarbon by Selected Rhizobacteria

The five selected rhizobacteria were tested for biodegradation of TPH in refinery wastewater during seven days. The degradation of TPH was ranged between 68.6 to 80% as shown in **Fig. 2**. The higher TPH removal was for *Sphingomonas Paucimobilis* while the lower TPH removal was for *Pseudomonas stutzeri*.

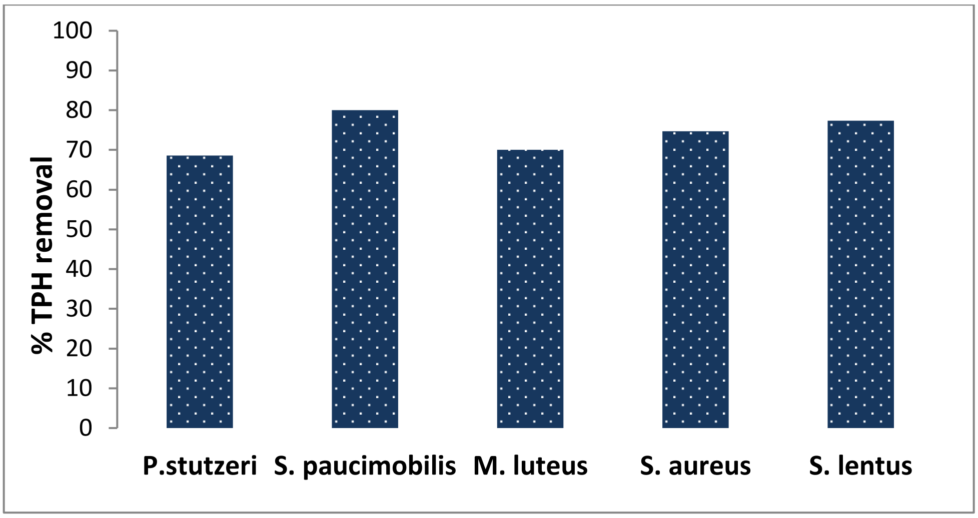
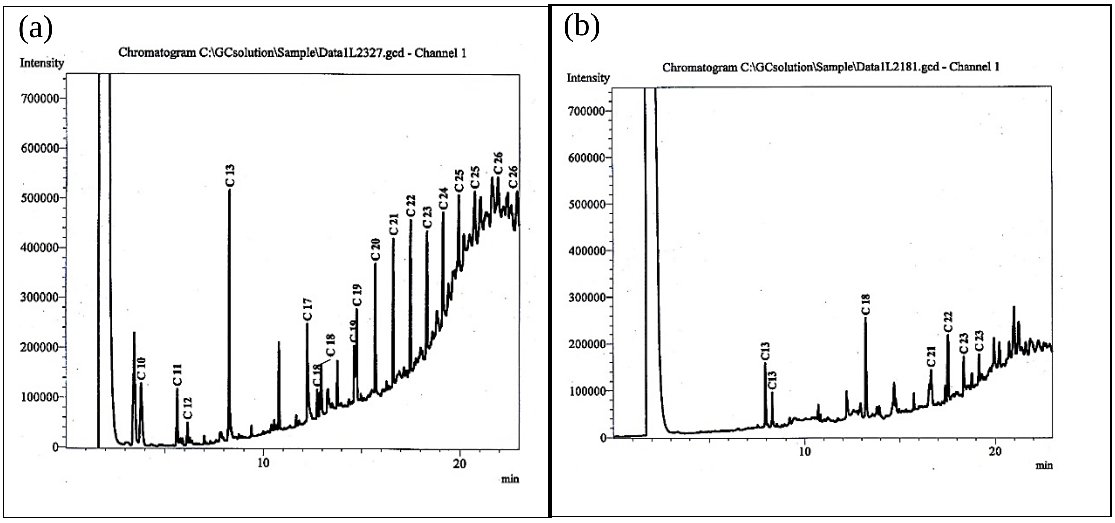



**Figure 2.** Removal Percentage of TPH by the five selected rhizobacteria monoculture.

The changes in concentration of TPH with carbon number range C10-C26 showed using GC-FID. **Fig. 3** shows gas chromatography of petroleum hydrocarbon extracted at 0 days and seven days of the biodegradation study of Pseudomonas stutzeri, Sphingomonas Paucimobilis, Micrococcus luteus, Staphylococcus aureus, and Staphylococcus Lentus.



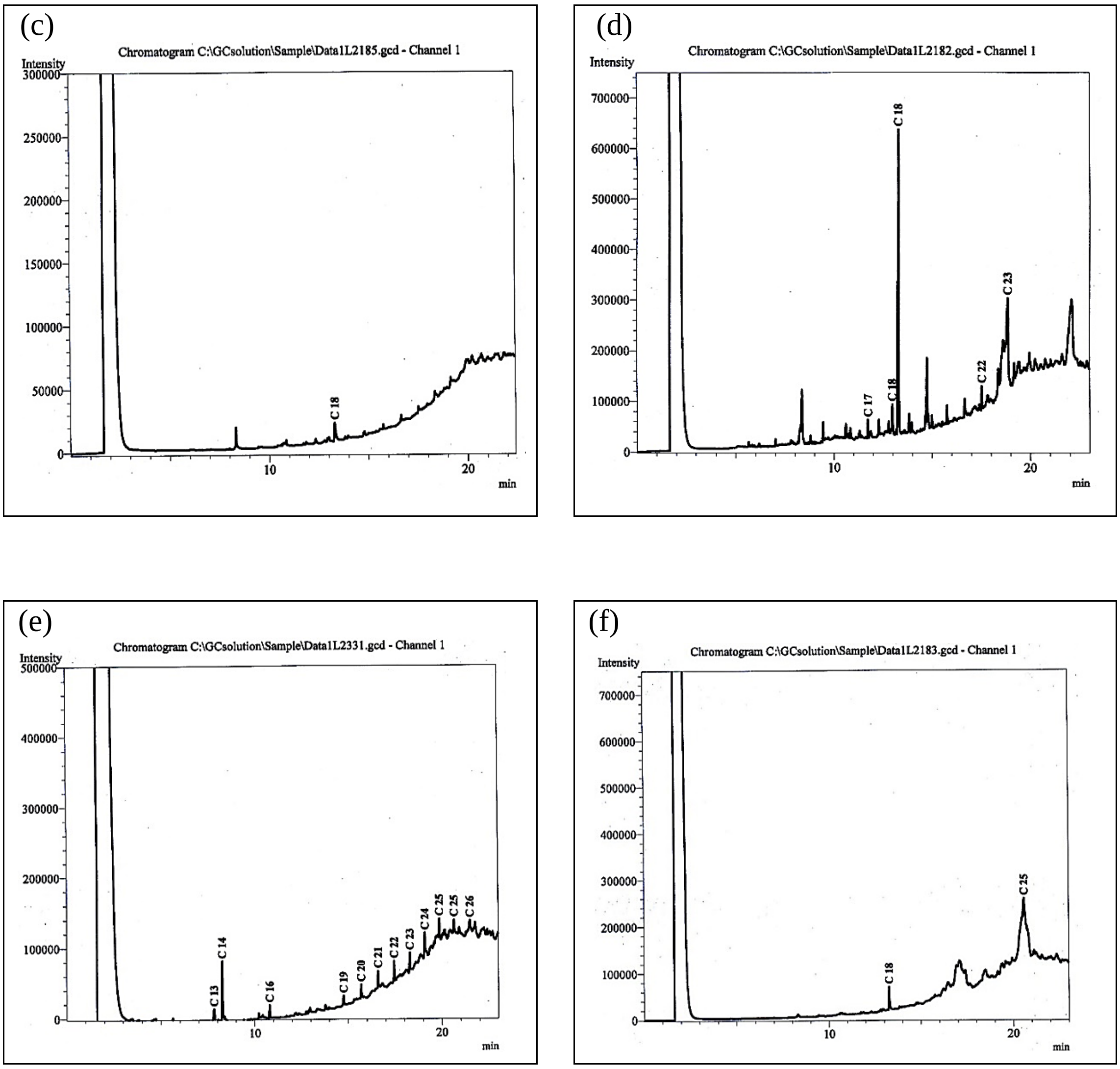



**Figure 3** Biodegradation of TPH: (a) Control (0 day), (b) Pseudomonas stutzeri- 7 days, (c) Sphingomonas paucimobilis- 7 days, (d) Micrococcus luteus- 7s day, (e) Staphylococcus aureus- 7s day, and f) Staphylococcus lentus- 7s day.

The results of GC analysis after biodegradation show that each isolated rhizobacteria have the ability to degrade specific n-alkanes more than others. Where, *Pseudomonas stutzeri* shows high ability to degrade $C_{10}$-$C_{12}$, $C_{17}$, $C_{19}$-$C_{20}$, and $C_{24}$-$C_{26}$. *Sphingomonas paucimobilis* show high efficiency to degrade all carbon range stander $C_{10}$-$C_{26}$. *Micrococcus Luteus* show high efficiency to degrade $C_{10}$-$C_{13}$, $C_{19}$-$C_{21}$, and $C_{24}$-$C_{26}$. While *Staphylococcus aureus* could degrade all carbon stander range spatially $C_{10}$-$C_{12}$, and $C_{17}$-$C_{18}$. Also, *Staphylococcus lentus* can efficiently degrade $C_{10}$-$C_{17}$, $C_{19}$-$C_{4}$, and $C_{26}$.

## 4. CONCLUSIONS

Five colonies were isolated from the root of Phragmites australis after being exposed to 70 mg/L TPH. The selected rhizobacteria were classified by morphology and biochemical tests, then identification using Vitek® 2 compacts as Pseudomonas stutzeri, Sphingomonas paucimobilis, Micrococcus luteus, Staphylococcus aureus and

Staphylococcus lentus. The biodegradability of petroleum hydrocarbons using isolated rhizobacteria was tested by monoculture. The maximum removal of TPH was 80% and achieved by Sphingomonas paucimobilis. The results indicate that the rhizodegredation of an organic contaminant in water by rhizobacteria is excellent technology.

**5. REFERENCES:**


- Al-Baldawi, Israa Abdulwahab, Siti Rozaimah Sheikh Abdullah, Nurina Anuar, Fatihah Suja, and Idris Mushrifah. 2015. Phytodegradation of Total Petroleum Hydrocarbon (TPH) in Diesel-Contaminated Water Using Scirpus Grossus. *Ecological Engineering* 74. Elsevier B.V.:463–73.
- Al-Baldawi, Israa Abdulwahab, Siti Rozaimah Sheikh Abdullah, Fatihah Suja', Nurina Anuar, and Mushrifah Idris. 2013. Phytotoxicity Test of Scirpus Grossus on Diesel-Contaminated Water Using a Subsurface Flow System. *Ecological Engineering* 54. Elsevier B.V.:49–56.
- Benedek, T, I Máthé, A Táncsics, S Lányi, and K Márialigeti. 2010. Investigation of Hydrocarbon-Degrading Microbial Communities of Petroleum Hydrocarbon Contaminated Soils in Harghita County, Romania. *Bulletin Ştiinţific Al Universităţii de Nord Baia Mare* XXIV (2):15–24.
- Cai, Zhang, Qixing Zhou, Shengwei Peng, and Kenan Li. 2010. Promoted Biodegradation and Microbiological Effects of Petroleum Hydrocarbons by Impatiens Balsamina L. with Strong Endurance. *Journal of Hazardous Materials* 183 (1–3):731–37.
- Celik, Gokcen Yuvali, Belma Aslim, and Yavuz Beyatli. 2008. Enhanced Crude Oil Biodegradation and Rhamnolipid Production by Pseudomonas Stutzeri Strain G11 in the Presence of Tween-80 and Triton X-100. *Journal of Environmental Biology* 29 (6):867–70.
- Chunli Zheng, Ling Zhao, Xiaobai Zhou, Zhimin Fu, and An Li. 2012. Treatment Technologies for Organic Wastewater. *World's Largest Science, Technology & Medicine Open Access Book Publisher*.
- Epps, Amanda Van. 2006. Phytoremediation of Petroleum Hydrocarbons. *Environmental Protection Agency, US*, no. August.
- Gerhardt, Karen E., Perry D. Gerwing, and Bruce M. Greenberg. 2017. Opinion: Taking Phytoremediation from Proven Technology to Accepted Practice. *Plant Science* 256. Elsevier Ireland Ltd:170–85.
- Haritash, A. K., and C. P. Kaushik. 2009. Biodegradation Aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A Review. *Journal of Hazardous Materials* 169 (1–3):1–15.
- Hassanshahian, Mehdi, Giti Emtiazi, and Simone Cappello. 2012. Isolation and Characterization of Crude-Oil-Degrading Bacteria from the Persian Gulf and the Caspian Sea. *Marine Pollution Bulletin* 64 (1). Elsevier Ltd:7–12.
- Hussain, Imran, Markus Puschenreiter, Soja Gerhard, Philipp Schöftner, Sohail Yousaf, Aijie Wang, Jabir Hussain Syed, and Thomas G. Reichenauer. 2018. Rhizoremediation of Petroleum Hydrocarbon-Contaminated Soils: Improvement Opportunities and Field Applications. *Environmental and Experimental Botany* 147. Elsevier B.V.:202–19.
- Ipung Fitri Purwanti1, Siti Rozaimah Sheikh Abdullah2, Hassan Basri1, Muhammad Mukhlisin1, Mushrifah Idris3, and Mohd Talib Latif4. 2012. Identification of Diesel-Tolerant Rhizobacteria of Scirpus Mucronatus. *African Journal of Microbiology*

*Research* 6 (10):2395–2402.

- Kaczorek, Ewa, Teofil Jesionowski, and Anna Giec. 2012. Cell Surface Properties of Pseudomonas Stutzeri in the Process of Diesel Oil Biodegradation, 857–62.
- Kanjilal, Tiyasha, Chiranjib Bhattacharjee, and Siddhartha Datta. 2015. Bio-Degradation of Acetamiprid from Wetland Wastewater Using Indigenous Micrococcus Luteus Strain SC 1204: Optimization, Evaluation of Kinetic Parameter and Toxicity. *Journal of Water Process Engineering* 6. Elsevier Ltd:21–31.
- Laboratories, HiMedia. 2011. Bushnell Haas Broth. *Technical Data*.
- Length, Full. 2010. Degradative Activity of Bacteria Isolated from Hydrocarbon-Polluted Site in Ilaje, Ondo State, Nigeria 4 (23):2484–91.
- Liu, Xiaoyan, Zhenzhen Wang, Xinying Zhang, Jun Wang, Gang Xu, Zhengnan Cao, Chenglin Zhong, and Pengcheng Su. 2011. Degradation of Diesel-Originated Pollutants in Wetlands by Scirpus Triqueter and Microorganisms. *Ecotoxicology and Environmental Safety* 74 (7). Elsevier:1967–72.
- Moliterni, E, R. G. Jiménez-Tusset, M. Villar Rayo, L Rodriguez, F. J. Fernández, and J. Villaseñor. 2012. Kinetics of Biodegradation of Diesel Fuel by Enriched Microbial Consortia from Polluted Soils. *International Journal of Environmental Science and Technology*.
- Musa, N M, S Abdulsalam, and A D I Suleiman. 2015. Bioremediation of Petroleum Refinery Wastewater Effluent via Augmented Native Microbes. *Journal of Emerging Trends in Engineering and Applied Sciences* 6 (1):1–6.
- Mustapha, Hassana Ibrahim, J. J.A. van Bruggen, and P. N.L. Lens. 2015. Vertical Subsurface Flow Constructed Wetlands for Polishing Secondary Kaduna Refinery Wastewater in Nigeria. *Ecological Engineering* 84. Elsevier B.V.:588–95.
- Ojo, O A. 2006. Petroleum-Hydrocarbon Utilization by Native Bacterial Population from a Wastewater Canal Southwest Nigeria 5 (February):333–37.
- Pant, Richa, Piyush Pandey, and Rhitu Kotoky. 2016. Rhizosphere Mediated Biodegradation of 1,4-Dichlorobenzene by Plant Growth Promoting Rhizobacteria of Jatropha Curcas. *Ecological Engineering* 94. Elsevier B.V.:50–56.
- Peng, Shengwei, Qixing Zhou, Zhang Cai, and Zhineng Zhang. 2009. Phytoremediation of Petroleum Contaminated Soils by Mirabilis Jalapa L. in a Greenhouse Plot Experiment. *Journal of Hazardous Materials* 168 (2–3):1490–96.
- Pincus, David H. 2010, Microbial Identification Using the BioMérieux VITEK® 2 System. *Encyclopedia of Rapid Microbiological Methods*, 1–32.
- Płaza, Grazyna A., Kamlesh Jangid, Krystyna Łukasik, Grzegorz Nałęcz-Jawecki, Christopher J. Berry, and Robin L. Brigmon. 2008. Reduction of Petroleum Hydrocarbons and Toxicity in Refinery Wastewater by Bioremediation. *Bulletin of Environmental Contamination and Toxicology* 81 (4):329–33.
- San Miguel, V., C. Peinado, F. Catalina, and C. Abrusci. 2009. Bioremediation of Naphthalene in Water by Sphingomonas Paucimobilis Using New Biodegradable Surfactants Based on Poly (ε-Caprolactone). *International Biodeterioration and Biodegradation* 63 (2). Elsevier Ltd:217–23.
- Santos, Jéssica Janzen dos, and Leila Teresinha Maranho, 2018. Rhizospheric Microorganisms as a Solution for the Recovery of Soils Contaminated by Petroleum: A Review. *Journal of Environmental Management* 210:104–13.
- Story, S. P., E. L. Kline, T. A. Hughes, M. B. Riley, and S. S. Hayasaka. 2004. Degradation of Aromatic Hydrocarbons by Sphingomonas Paucimobilis Strain EPA505. *Archives of Environmental Contamination and Toxicology* 47 (2):168–76.

- Toledo, F L, C Ã Calvo, B Rodelas, and J Gonza. 2006. Selection and Identification of Bacteria Isolated from Waste Crude Oil with Polycyclic Aromatic Hydrocarbons Removal Capacities 29:244–52.
- Toyama, Tadashi, Tetsuya Furukawa, Noritaka Maeda, Daisuke Inoue, Kazunari Sei, Kazuhiro Mori, Shintaro Kikuchi, and Michihiko Ike. 2011. Accelerated Biodegradation of Pyrene and Benzo[a]Pyrene in the Phragmites Australis Rhizosphere by Bacteria-Root Exudate Interactions. *Water Research* 45 (4). Elsevier Ltd:1629–38.
- Toyama, Tadashi, Ning Yu, Hirohide Kumada, Kazunari Sei, Michihiko Ike, and Masanori Fujita. 2006. Accelerated Aromatic Compounds Degradation in Aquatic Environment by Use of Interaction between Spirodela Polyrrhiza and Bacteria in Its Rhizosphere. *Journal of Bioscience and Bioengineering* 101 (4):346–53.
- USEPA. 1996. EPA Method 3510C. *Epa*, no. December:1–8.
- Xaaldi Kalhor, Aadel, Ali Movafeghi, Adel Dabbagh Mohammadi-Nassab, Ehsan Abedi, and Ahmad Bahrami. 2017. Potential of the Green Alga Chlorella Vulgaris for Biodegradation of Crude Oil Hydrocarbons. *Marine Pollution Bulletin* 123 (1–2). Elsevier:286–90.
- Yasseen, B. T. 2014, Phytoremediation of Industrial Wastewater from Oil and Gas Fields Using Native Plants : The Research Perspectives in the State of Qatar. *Central European Journal of Experimental Biology* 3 (4):6–23.
- Yavari, Sara, Amirhossein Malakahmad, and Nasiman B. Sapari. 2015. A Review on Phytoremediation of Crude Oil Spills. *Water, Air, and Soil Pollution* 226 (8).
- Zheng, Chunli, Baocheng Qu, Jing Wang, Jiti Zhou, Jing Wang, and Hong Lu. 2009. Isolation and Characterization of a Novel Nitrobenzene-Degrading Bacterium with High Salinity Tolerance: Micrococcus Luteus. *Journal of Hazardous Materials* 165 (1–3):1152–58.
- Zhou, Lisha, Hui Li, Ying Zhang, Siqin Han, and Hui Xu. 2016. Sphingomonas from Petroleum-Contaminated Soils in Shenfu, China and Their PAHs Degradation Abilities. *Brazilian Journal of Microbiology* 47 (2). Sociedade Brasileira de Microbiologia:271–78.