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Oil Spill Treatment: Enzymatic Agents, Bio-surfactants, and Toxicity Elimination in Hydrocarbon Clean-up in Aquatic Environments - A Literature Overview

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The following literature review has been produced by the Lawrence Anthony Earth Organization Science and Technology Committee (LAEO STAC) on Oil Spill Response Systems.

In the spirit of Cooperative Ecology and finding a better way forward the LAEO STAC is seeking collaborative partnerships for advancing research in this field. We truely hope that Oil Spill Response Professionals, Oil and Gas Industry members as well as government agency regulators will accept our help and avail themselves of this information as critical to their decision-making process when selecting methods to be used for removing oil and other hazardous chemical spills from our oceans and waters.

LAEO's Optimizing Oil Spill Response Systems Program is a Cooperative Ecology™ Initative.

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I. INTRODUCTION

The prevailing methods of oil spill clean-up applied to open water spills in the U.S. are physical barriers and removal and chemical dispersants. However, when humans complete their clean up activities, the ultimate job of removing hydrocarbons from the environment is carried out by hydrocarbon-consuming bacteria, which respond to spills in large numbers and break down hydrocarbons into more bioavailable compounds. The clean up services provided by these organisms can be facilitated by bioremediation agents, which are better suited to promoting this ecological processes at they they are less toxic than synthetic dispersants, and are naturally biodegradable. This literature review includes peer-reviewed papers published 1991-2016 on the the use of enzymes and biosurfactants in oil spill bioremediation. Topics covered are efficiceny and rate, toxicity, and mode of action.

II. DEFINITIONS

Bioremediation: treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non-toxic substances (US EPA).

Bio stimulation: to enhance the activities of indigenous (autochthonous) pollutant-degrading microorganisms via environmental management, e.g. the addition of nutrients and other growth-limiting factors (Atlas and Bartha 1998).

Enzyme: a substance, typically made of proteins, produced by a living organism that acts as a catalyst to bring about a specific biochemical reaction.

Bio-surfactant: amphiphilic compounds produced on living surfaces, mostly microbial cell surfaces, or excreted extracellularly and contain hydrophobic and hydrophilic moieties that reduce surface tension (ST) and interfacial tensions between individual molecules at the surface and interface, respectively.

- "Biosurfactants (BSs) are “green” amphiphilic molecules produced by microorganisms during biodegradation, increasing the bioavailability of organic pollutants (Antoniou et al 2015)."

III. KEY FINDINGS

1. Enzymatic Agents

- Oil degraders are present throughout marine environments - deep sea, open water, shoreline, etc. (Gao 2015, Ali 2016, Alonso-Gutiérrez et al 2009).

- Extracellular enzyme activity is a key step in degradation and utilization of organic polymers, since only compounds with molecular mass lower than 600 daltons can pass through cell pores. Hydrolytic enzymes disrupt major chemical bonds in the toxic molecules and results in the reduction of their toxicity (Chandrakant 2011).

- The use of enzymes instead of synthetic chemicals or microbes presents the following advantages: (Alcade 2006)

  1). the biotransformation does not generate toxic side products as is often the case with chemical and some microbiological processes, and
  2). the enzymes are digested, in situ, by the indigenous microorganisms after the treatment;
  3). the requirement to enhance bio-availability by the introduction of organic co-solvents or surfactants is much more feasible from an enzymatic point of view than using whole cells; and
  4). the potential to produce enzymes at a higher scale, with enhanced stability and/or activity and at a lower cost by using recombinant-DNA technology.

- Enzymes can degrade many organic pollutants, including – polycyclic aromatic hydrocarbons (PAHs) and other persistent organic pollutants (Ang et al. 2005).

- Enzymes can reduce the presence of aromatics at a higher rate than chemical dispersants (Abdallah 2005).

- OSE2 positively enhances the biodegradation process (Aldrett et al. 1997).

- Bioremediation in arctic waters is attainable and follows the same mode of action, albeit at a slower rate, than in temperate waters (Garrett et al. 2003).

- Bioremediation strategies which can sustain high levels of bacterial diversity rather than the selection of specific taxa may significantly increase the efficiency of hydrocarbon degradation in contaminated marine sediments (Dell'Anno et al. 2012).

2. Bio-surfactants

- Despite the large amount of research on dispersants, there is very little on the use of bio-surfactants as bio-dispersants despite their potential benefits, particularly for enhancing oil biodegradation and solubilization (Mulligan 2004).

- The synthesis and application of microbial biosurfactants have an important practical significance for a range of petroleum pollutants bioremediation. Bio-surfctants can be considered as a key component in the cleanup strategy for petroleum hydrocarbon remediation due to their biodegradability and low toxicity (Matvyeyeva1 et al. 2014).

- Biosurfactants can make hydrocarbon complexes more mobile with potential use in oil recovery, pumping and bioremediation of crude oil contaminant (Bordoloi 2009).

- Bio surfactants were less toxic than the synthetic surfactants to some invertebrate species tested (Silva 2014, Souza 2014).

IV. KEY REFERENCES: ENZYMES IN BIOREMEDIATION

Categorized as 1) Efficacy and Rate*, 2) Toxicity**, 3) Mode of Action***, 4) Supplementary****

Abdallah, R. I., S.Z. Mohamed and F.M. Ahmed **(2005). Effect of Biological and Chemical Dispersants on Oil Spills. *Petroleum Science and Technology,* 23: 463-474

****Ahuja, S.K., G.M. Ferreira and A.R. Moreira **(2004).** Utilization of Enzymes for Environmental Applications. *Critical Reviews in Biotechnology*, 24(2–3): 125–154

*Alcalde, M., M. Ferrer, F. J. Plou and A. Ballesteros **(2006).** Environmental biocatalysis: from remediation with enzymes to novel green processes, *Trends in Biotechnology*, 24(6): 281–287

*Aldrett, S., J.S. Bonner, M.A. Mills, R.L. Autenrieth and F.L. Stephens **(1997).** Microbial Degradation of Crude Oil in Marine Environments Tested in a Flask Experiment. *Water Research,* 31: 2840-2848.

***Ang, E.L., H. Zhao, J. Obbard **(2005).** Recent advances in the bioremediation of persistent organic pollutants via biomolecular engineering. *Enzyme and Microbial Technology,* 37

****Barrios San Martín, Y. **(2011)**. Bioremediation: A Tool for the Management of Oil Pollution in Marine Ecosystems. *Biotecnología Aplicada,* 28: 69-76.

Bidwell, J. R., D.S. Cherry and A.T. Merski **(2003). Toxicity Evaluation of a Commercial Bioremediation Agent Mixed with Crude Oil. *Environmental Toxicology and Chemistry,* 22: 84-91.

*Bio-Aquatic Testing, Inc. **(2009).** Bioremediation Agent Effectiveness Test: Oil Spill Eater II. Carolton, TX

****Cao, B., K. Nagarajan and K. C. Loh **(2009).** Biodegradation of aromatic compounds: current status and opportunities for biomolecular approaches. *Applied Microbiology and Biotechnology*, 85(2): 207–228.

****Cappello, S., D. Russo, S. Santisi, R. Calogero, C. Gertler, F Crisafi, M. De Domenico, and M. Yakimov **(2012).** Presence of Hydrocarbon-Degrading Bacteria in the Gills of Mussel Mytilus Galloprovincialis in a Contaminated Environment: A Mesoscale Simulation Study. *Chemistry and Ecology,* 28: 239-252.

***Chandrakant S.K. and S.S. Rao **(2011).** Role of Microbial Enzymes in the Bioremediation of Pollutants: A Review. *Enzyme Research*, 2011.

***Das, N. and P. Chandran (**2010).** Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. *Research International*, 2011.

****Dell'Anno, A., F. Beolchini, L. Rocchetti, G. M. Luna and R. Danavaro **(2012).** High Bacterial Biodiversity Increases Degradation Performance of Hydrocarbons During Bioremediation of Contaminated Harbor Marine Sediments. *Environmental Pollution,* 167: 85-92.

*Dias, R., L. Ruberto, E. Hernández, S. Vázquez, A. Lo Balbo, M.T. Del Panno and W.P. Mac Cormack **(2012).** Bioremediation of an aged diesel oil-contaminated Antarctic soil: Evaluation of the "on site" biostimulation strategy using different nutrient sources. *International Biodeterioration & Biodegradation,* 75.

****dos Santos, H. F., G.A. Santos Duarte, C.T. da Costa Rachid, R.M. Chaloub, E.N. Calderon, L.F. de Barros Marangoni, A. Bianchini, A.H. Nudi, F.L. do Carmo, J.D. van Elsas, A.S. Rosado, C. Barreira e Castro and R.S. Peixoto **(2015).** Impact of Oil Spills on Coral Reefs Can Be Reduced by Bioremediation Using Probiotic Microbiota. *Scientific Reports,* 5.

***Fan, Chi-Yuan amd S. Krishnamurthy (**1995**). Enzymes for Enhancing Bioremediation of Petroleum-Contaminated Soils. *Air & Waste Manage. Assoc*., 45: 453-460.

***Haritash, A.K. and C.P. Kaushik **(2009)**. Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review. *Journal of Hazardous Materials,* 169: 1–15

***Hoppe, H.G., C. Arnosti and G.F. Herndl **(2002).** Ecological significance of bacterial enzymes in the marine environment. In: Burns, R.G., Dick, R.P. (Eds.), *Enzymes in the Environment,* CRC Press.

****Kleindienst, S., S. Grim, M. Sogin, A. Bracco, M. Crespo-Medina, S.B. Joye **(2016).** Diverse, Rare Microbial Taxa Responded to the Deepwater Horizon Deep-Sea

Hydrocarbon Plume. *Isme Journal,* 10: 400-415.

*Majcherczyk, M., C. Johannes and A. Huttermann **(1998).** Oxidation of polycyclic aromatic hydrocarbons (PAH) by laccase of Trametes versicolor. *Enzyme and Microbial Technology,* 22:335–341.

***Peixoto, R.S., A. B. Vermelho and A. S. Rosado **(2011).** Petroleum-Degrading Enzymes: Bioremediation and New Prospects. *Enzyme Research,* 2011.

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****Santos, H. F., F.L. Carmo, J. Paes, A.S. Rosado and R.S. Peixoto **(2011).** Bioremediation of Mangroves Impacted by Petroleum. *Water Air and Soil Pollution,* 216: 329-350.

***Sutherland, T.D., I. Horne and K. M. Weir **(2004).** Enzymatic bioremediation: from enzyme discovery to applications. *Clinical and Experimental Pharmacology and Physiology*, 31(11): 817–821.

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**** Zhu, X., A.D. Venosa and M. Suidan **(2004).** Literature review on the use of commercial bioremediation agents for cleanup of oil-cotaminated estuarine environments. EPA/600/R-04/075 July.

V. KEY REFERENCES: BIO-SURFACANTS IN BIOREMEDIATION

*Aparna, A., G. Srinikethan and S. Hedge **(2011).** Effect of addition of biosurfactant produced by Pseudomonas sps. on biodegradation of crude oil. In: 2nd International Proceedings of Chemical, Biological & Environmental Engineering, vol. 6: 71-75, IACSIT Press, Singapore.

*Bao, M., P. Sun, X.Yang, X. Wang, L.Wang, L. Cao, and F. Li **(2014).** Biodegradation of Marine Surface Floating Crude Oil in a Large-Scale Field Simulated Experiment. *Environmental Science-Processes & Impacts,* 16: 1948-1956.

*Bordoloi, N.K. and B.K. Konwar **(2009).** Bacterial biosurfactant in enhancing solubility and metabolism of petroleum hydrocarbons. *Journal of Hazardous Materials*, 170: 495–505.

* Cameotra, S. and P. Singh **(2008).** Bioremediation of oil sludge using crude biosurfactants. *International Biodeterioration & Biodegradation,* 62: 274–280.

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of Marine Sediments Impacted by Petroleum. *Applied Biochemistry and Biotechnology,* 153: 58-66.

* Lai, C., Y.C.Huang, Y. Wei, J. Chang **(2009).** Biosurfactant-enhanced removal of total petroleum hydrocarbons from contaminated soil. *Journal of Hazardous Materials,* 167: 609–614.

*Matvyeyeva, O.L., O.A. Vasylchenko and O. R. Aliieva **(2014).** Microbial Biosurfactants Role in Oil Products Biodegradation. *International Journal of Environmental Bioremediation & Biodegradation*, 2(2): 69-74.

*Montero-Rodríguez, D., R.F.S. Andrade, D.L.R. Ribeiro, D. Rubio-Ribeaux, R.A. Lima, H.W.C. Araújo and G.M. Campos-Takaki **(2015).** Bioremediation of Petroleum Derivative Using Biosurfactant Produced by *Serratia marcescens* UCP/WFCC 1549 in Low-Cost Medium. *Int. Journal of Current Microbiology and App.Sci,* 4(7): 550-562.

* Muligan, C. (**2005).** Environmental applications for biosurfactants. *Environmental Pollution*, 133: 183–198.

* Mohanty, S., J. Jasmine and S. Mukherji **(2013).** Practical Considerations and Challenges Involved in Surfactant Enhanced Bioremediation of Oil. *BioMed Research Intl*, 2013.

** Poremba, K., W. Gunkel, S. Lang and F. Wagner **(1991).** Marine biosurfactants: Toxicity testing with marine microorganisms and comparison with synthetic surfactants. *Z. Naturforsch*, 46: 210–216.

* Rebello, S., A.K. Asok, S. Mundayoor and M.S. Jisha **(2014).** Surfactants: toxicity, remediation and green surfactants. *Environ Chem Letters,* 12: 275–287.

** Silva, F.S., D.G. Almeida, R.D. Rufino, J.M. Luna, V.A. Santos and L.A. Sarubbo **(2014).** Applications of Biosurfactants in the Petroleum Industry and the Remediation of Oil Spills. *Int. Journal of Molecular Sci*., 15: 12523-12542.

*** Souza, E., T.C. Vessoni-Penna, R.P. de Souza Oliveira **(2004).** Biosurfactant-enhanced hydrocarbon bioremediation: An overview. *International Biodeterioration & Biodegradation,* 89: 88-94.

VI. LITERATURE REVIEW ON ENZYMATIC BIOREMEDIATION OF HYDROCARBON POLLUTION IN AQUATIC ENVIRONMENTS

1. Efficiency and rate*

Alcalde, M., M. Ferrer, F. J. Plou and A. Ballesteros **(2006).** Environmental biocatalysis: from remediation with enzymes to novel green processes, *Trends in Biotechnology*, 24(6): 281–287.

- Since Nov. 2002, microbial bioremediation using naturally occurring microbes in combination with mechanical approaches are currently being used as the major mechanism of removing low and high molecular weight, polycyclic aromatic hydrocarbons from the Prestige ship spill on the N. coast of Spain.
- From an environmental point of view, the use of enzymes instead of chemicals or microorganisms undoubtedly presents some advantages:
    - the biotransformation does not generate toxic side products as is often the case with chemical and some microbiological processes, and
    - the enzymes are digested, in situ, by the indigenous microorganisms after the treatment;
    - the requirement to enhance bio-availability by the introduction of organic co-solvents or surfactants is much more feasible from an enzymatic point of view than using whole cells; and
    - the potential to produce enzymes at a higher scale, with enhanced stability and/or activity and at a lower cost by using recombinant-DNA technology.

Aldrett, S., J.S. Bonner, M.A. Mills, R.L. Autenrieth and F.L. Stephens **(1997).** Microbial Degradation of Crude Oil in Marine Environments Tested in a Flask Experiment. *Water Research,* 31: 2840-2848.

- Tested all bioaugmentation and biostimulation products on NCP schedule, including OSE. The different bioremediation agents proved to be significantly better than the control treatment. This experiment showed that the bioremediation agents positively enhanced the biodegradation process. Aromatics were present higher than saturates in both bio and chemical agents.

Bio-Aquatic Testing, Inc. **(2009).** Bioremediation Agent Effectiveness Test: Oil Spill Eater II. Carolton, TX

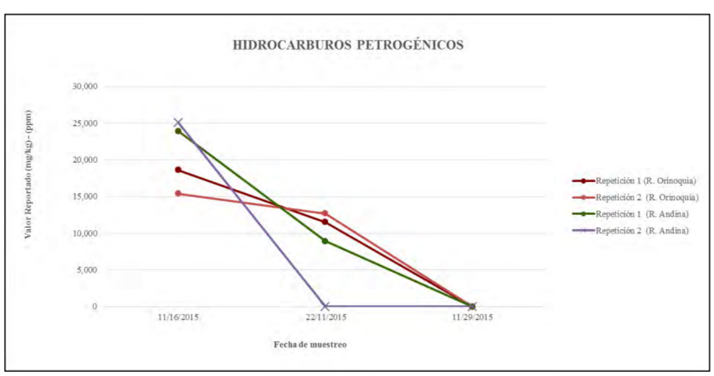
- Tested OSE2, showed to be effective and non-toxic, supports NCP listing approval.

Cravajal, M.T., Y.M.L. Bohorquez and N.G.P. Torres **(2015).** EvaluaciÓn social, ambiental, economica y tecnica de la utilizacion del product Oil Spill Eater – OSEII para la empressa Coltanques S.A.S. Final report to obtain Master Degree in Hydrocarbon Indutry Management, Universidad Vina Del Mar, Escuela de Ingenieria.

- In a plastic container 0.06m3 soil was mixed homogenously with soil with 3 l. of hydrocarbon. CEPSA crude was used. OSEII was added, and diluted with water as by the manufacturer to dilute 50: 1 (gallons water - OSE II) for a total of 4.76 lt x 0.1 l. water = 4.86 lt of solution per container. Three monitoring sites which were tested 1/week for a period of 20 days.
- In all four repetitions, petroleum hydrocarbons were reduced to 0ppm by day 13.

Figura 13

Resultados para Hidrocarburos Petrogénicos.



Fuente: Los Autores

Dias, R., L. Ruberto, E. Hernández, S. Vázquez, A. Lo Balbo, M.T. Del Panno and W.P. MacCormack **(2012).** Bioremediation of an aged diesel oil-contaminated Antarctic soil: Evaluation of the "on site" biostimulation strategy using different nutrient sources. *International Biodeterioration & Biodegradation*, 75.

- Experiment was carried out at Carlini (Jubany) Station (62 140 S, 58 400 W), located on Potter Cove, 25 de Mayo Island (King George Island), South Shetland Islands. Chronically hydrocarboncontaminated soil used in this work had been collected from the concrete pools containing the fuel-storage tanks and was kept for more than two years in metal containers. After manually removal of the large stones the soil was sieved (2 mm mesh) in order to obtain a homogeneous material.
- Commercial product (OSEII) caused the higher reduction (49.4%, $p < 0.05$) of hydrocarbons compared with the control system after 45 d of treatment.
- The continuous increase in HDB observed until the end of the assay in this experimental system also suggests that OSEII maintained the adequate releasing of the inorganic nutrients required by the natural microbiota to grow all along the study.
- OSEII product evidenced the better performance with the minor perturbation of the natural bacterial community structure, constituting a promising alternative for some hydrocarbon contaminated Antarctic soil restoration.

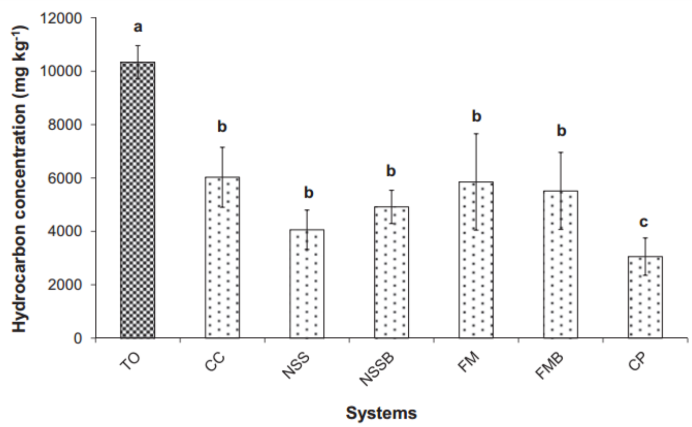


Fig. 2. Total hydrocarbon concentration (THC) in soil at day 1 and at the end of the assay (45 d) in the different microcosms systems. T0: control system at day 1, CC: control system at day 45, NSS: system amended with inorganic salts at day 45, NSSB: system amended with inorganic salts and Brij700 at day 45, FM: system amended with fish meal at day 45, FMB: system amended with fish meal and Brij700 at day 45. Bars represent SD of three independent replicates. Different letters represent significant differences between systems. For description of each microcosm see Table 1.

Majcherczyk, M., C. Johannes and A. Huttermann (**1998).** Oxidation of polycyclic aromatic hydrocarbons (PAH) by laccase of Trametes versicolor. *Enzyme and Microbial Technology*, 22: 335–341.

- Laccase of Trametes versicolor was able to oxidize in vitro most of the 14 polycyclic aromatic hydrocarbons (PAH) tested.
- Acenaphthylene was removed by 37% followed by anthracene and benzo[a]pyrene which were oxidized by 18 and 19%, respectively.
- Lower but significant oxidation of about 10% was found for eight additional PAH: acenaphthene, fluoranthene, pyrene, benzo[a]anthracene, chrysene, benzo[b]fluoranthene, benzo[k]fluoranthene, and perylene. Naphthalene, fluorene, and phenanthrene were recovered unchanged after incubation for 72 h with laccase. Addition of 1-hydroxybenzotriazole (HBT) to the reaction mixture increased oxidation of PAH: acenaphthylene, acenaphthene, fluorene, anthracene, benzo[a]pyrene, and perylene were almost completely removed from the reaction mixture. Oxidation of pyrene and benzo[a]anthracene increased from 8 and 6% without a mediator to 48 and 53% in the presence of HBT. Other PAH were not significantly influenced by the addition of this mediator. PAH-quinones as oxidation products were formed from all PAH to different extents.

Ruggaber, T.P and J.W. Talley **(2006).** Enhancing Bioremediation with Enzymatic Processes: A Review. *Practice periodical of hazardous, toxic, and radioactive waste management,* April 2006.

- Enzymes are defined as substances that alter a reaction's rate and/or a reaction's activation energy without being present in the reaction products (Uhlig 1998). They are naturally produced by nearly every known organism in order to aid processes such as digestion, metabolism and cell synthesis (Madigan et al. 2003). Some microbes may require unique conditions difficult to optimize in the field. These factors can limit the overall success of bioremediation.
- Enzymes are able to act in a large range of environmental conditions and remain active even if these conditions quickly change (Ahuja et al. 2004; Gianfreda and Rao 2004).
- A very pragmatic benefit of enzymatic treatment is that the enzymes themselves are biodegradable proteins, meaning that the enzymes that are not recovered will degrade in the environment after they are no longer needed. Unlike other remediation methods, there is no buildup of biomass or chemicals that must be removed (Ahuja 2004).
- Some enzymes have been shown to be effective in degrading PAHs, phenolic substances, and other hydrocarbon associated pollutants such as chlorophenols, pyrene, anthracene, phenanthrene, flourene, and others.

2. Toxicity**

Abdallah, R. I., S.Z. Mohamed and F.M. Ahmed **(2005).** Effect of Biological and Chemical Dispersants on Oil Spills. *Petroleum Science and Technology,* 23: 463-474.

- The efficiency of four dispersants and enzymes on crude oil decomposition were studied according to the (IP–AS/84) standard method at 20C. This method is used to estimate the quantities of crude oil and other petroleum products that could be dispersed into seawater. Gas chromatography was also used for the identification and characterization of saturated fractions. One key finding is that that increasing the efficiency of dispersant will decrease the content of saturates in the undispersed oil and consequently increase the aromatic content. These results are in agreement with the conclusion given by some authors (Shepard and Hyzy, 1992; Amer, 1994). Aromatics remained in higher proportion with chemical dispersant over enzymatic (Table 5). NOTE: "Aromatics, which are based on a 6-carbon ring, tend to be the molecular compounds in oil that are the most toxic to marine life. (NOAA, http://response.restoration.noaa.gov/about/media/chemistry-oil-spill.html).
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Table 5. Hydrocarbon component of undispersed crude oil.

Sample	Asphaltene wt %	Resin wt %	Oil wt %	Oil: Saturates wt%	Oil: Aromatics wt%
Crude oil	3.47	11.95	84.58	68.31	31.67
Crude + enzyme (2 min)	7.0	18.8	74.2	65.34	34.64
Crude + enzyme (4 min)	6.83	19.2	73.9	62.67	36.22
Crude + dispersant (1)	6.3	16.5	77.2	67.19	32.68
Crude + dispersant (2)	7.34	24.62	68.04	60.01	39.96
Crude + dispersant (3)	8.32	25.92	65.76	58.84	41.45
Crude + dispersant (4)	9.49	31.01	59.5	54.18	45.8

Bidwell, J. R., D.S. Cherry, and A.T. Merski, **(2003).** Toxicity Evaluation of a Commercial Bioremediation Agent Mixed with Crude Oil. *Environmental Toxicology and Chemistry,* 22: 84-91.

- Tested undeclared NCP schedule approved bioremediation agent. Good example for how this testing is implemented and reported.

3. Mode of action***

Al-Awadhi, H., R.H. Al-Hasan and S.S. Radwan **(2002).** Comparison of the Potential of Coastal Materials Loaded with Bacteria for Bioremediating Oily Sea Water in Batch Culture. *Microbiological Research,* 157: 331-336.

- Sand hosts more bacteria than open water, looks at effects of fertilization with KNO3 (increased efficacy).

Ali, N., N. Dashti, S. Salamah, N. Sorkhoh, H. Al-Awadhi and S. Radwan **(2016).** Dynamics of Bacterial Populations During Bench-Scale Bioremediation of Oily Seawater and Desert Soil Bioaugmented with Coastal Microbial Mats. *Microbial Biotechnology,* 9: 157-171.

- Added microbes with coastal mats, but they were poorly adapted to seawater. The fact that individual autochthonous isolates consumed between one fifth and one third of oil implies that the total (seawater) microbial consortia must be quite effective in cleaning suitable oily habitats.

Alonso-Gutierrez, J., A. Figueras, J.Albaiges, N. Jimenez, M. Vinas, Anna M. Solanas and B. Novoa. **(2009).** Bacterial Communities from Shoreline Environments (Costa da Morte, Northwestern Spain) Affected by the Prestige Oil Spill. *Applied and Environmental Microbiology,* June 2009: 3407–3418.

- Many oil degraders present in shoreline sediments.

Ang, E.L., H. Zhao and J. Obbard. (**2005).** Recent advances in the bioremediation of persistent organic pollutants via biomolecular engineering. *Enzyme and Microbial Technology*, 37

- Enzymes can degrade many organic pollutants, including – polycyclic aromatic hydrocarbons (PAHs) and other persistent organic pollutants. Reviews types and mode of actions.

Atlas, R.M. **(1995).** Petroleum Biodegradation and Oil Spill Bioremediation. *Marine Pollution Bulletin,*31: 178-182.

- Nutrient addition helps bioremediation by 3-5 times, states enzymes of indigenous microbes not usually limiting factor, but has no direct evidence of this fact

Chandrakant, S.K. and S.S. Rao **(2011).** Role of Microbial Enzymes in the Bioremediation of Pollutants: A Review. *Enzyme Research,* 2011.

- The pollution of soil and water by industrial chemicals and petroleum hydrocarbons is a serious problem of the modern world. Due to their extensive use, they are found as environmental contaminants in numerous aquatic and terrestrial ecosystems.
- The use of bioremediation technologies for removing these contaminants provides a safe and economic alternative to commonly used physical-chemical treatment. Bacterial activity is the major process involved in the hydrolysis of organic pollutants.
- Extracellular enzyme activity is a key step in degradation and utilization of organic polymers, since only compounds with molecular mass lower than 600 daltons can pass through cell pores. Hydrolytic enzymes disrupt major chemical bonds in the toxic molecules and results in the reduction of their toxicity

Das, N. and P. Chandran **(2010).** Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview. *Research International*, 2011

- The degradation of petroleum hydrocarbons can be mediated by specific enzyme systems.

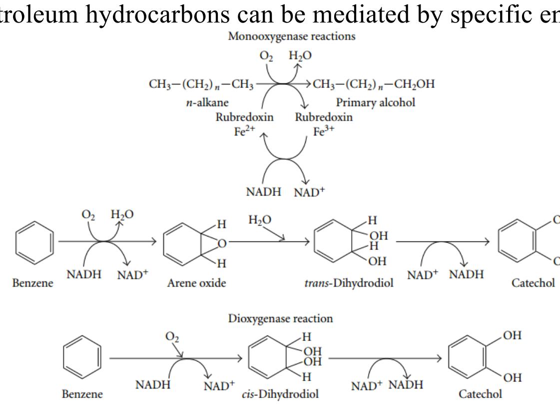

Figure 3: Enzymatic reactions involved in the processes of hydrocarbons degradation.

Fan, C.Y. and S. Krishnamurthy **(1995).** Enzymes for Enhancing Bioremediation of Petroleum-Contaminated Soils. Air and Waste Management Assoc.. 45: 453-460.

- A great review of hydrocarbon structure and enzyme action

Gao, X., W. Gao, Z. Cui, B. Han, P. Yang, C. Sun and L. Zheng **(2015).** Biodiversity and degradation potential of oil-degrading bacteria isolated from deep-sea sediments of South Mid-Atlantic Ridge. *Marine Pollution Bulletin,* 97: 373–380.

- Eleven oil degraders were collected from deep-sea mid Atlantic ridge from *Alcanivorax, Bacillus, Dietzia, Erythrobacter, Marinobacter, Nitratireductor, and Oceanicola*. Results indicated that the intrinsic biodegradation capacity of oil contaminants by indigenous microbial communities exists in South MAR sediments.

Haritash, A.K. and C.P. Kaushik **(2009).** Biodegradation aspects of Polycyclic Aromatic Hydrocarbons (PAHs): A review. *Journal of Hazardous Materials*, 169: 1–15.

- Enzymes involved in the degradation of PAHs are oxygenase, dehydrogenase and lignolytic enzymes. Fungal lignolytic enzymes are lignin peroxidase, laccase, and manganese peroxidase. They are extracellular and catalyze radical formation by oxidation to destabilize bonds in a molecule. The biodegradation of PAHs has been observed under both aerobic and anaerobic conditions and the rate can be enhanced by physical/chemical pretreatment of contaminated soil.
- Addition of biosurfactant-producing bacteria and light oils can increase the bioavailability of PAHs and metabolic potential of the bacterial community.

Hoppe, H.G., C. Arnosti and G.F. Herndl **(2002).** Ecological significance of bacterial enzymes in the marine environment. In: Burns, R.G., Dick, R.P. (Eds.), Enzymes in the Environment. CRC Press.

- Excellent description of natural enzyme processes in marine environment.
- Enzymes break up organic matter and feed free living bacteria.

Peixoto, R.S., A. B. Vermelho and A. S. Rosado **(2011).** Petroleum-Degrading Enzymes: Bioremediation and New Prospects. *Enzyme research,* 2011.

- Aromatic hydrocarbons, such as benzene, toluene, xylene, and naphthalene, can be degraded in aerobic conditions. The degradation of these compounds usually serves as an initial step in the formation of catechol or a structurally related compound. Once formed, catechol can be degraded, resulting in compounds that can be introduced into the citric acid cycle. Also these compounds can be completely degraded to CO_2. The catechol dioxygenase class of bacterial iron-containing enzymes is an example of an enzyme class involved in the degradation of aerobic aromatic hydrocarbons.
- Despite the fact that petroleum degradation under aerobic conditions occurs faster than under anaerobic conditions, it is important to note that anaerobic degradation is also essential to the bioremediation process because in several cases the environmental conditions can include limitations of the oxygen availability, such as in mangroves, aquifers, and sludge digesters.

Perelo, L. **(2010).** Review: In situ and bioremediation of organic pollutants in aquatic sediments. *Journal of Hazardous Materials,* 177: 81–89.

- Targets of phytoremediation include PCBs, PAHs, nitroaromatics, and linear halogenated hydrocarbons. The mechanisms of phytoremediation include biophysical and biochemical processes like adsorption, transport and translocation, as well as transformation and mineralization by plant enzymes. Plants have been shown to be able to degrade halogenated compounds like TCE by oxidative degradation pathways, including plant specific dehalogenases. Dehalogenase activity was observed to be maintained after the plants death. Enzymes can become bound to the organic matrix of the sediment as plants die, they decay and they are buried in the sediment, thus contributing to the dehalogenase activity observed in organic-rich sediments.

Shacker, M. (**2014).** Nitrogen and Phosphorus: Unyielding Destruction in the Gulf. *International Pollution Issues,* December 2014.

- High levels of nitrogen and phosphorous in the Gulf of Mexico. They are stratified due to low density of freshwater and so higher concentration at surface.

Sutherland, T.D., I. Horne and K.M. Weir **(2004).** Enzymatic bioremediation: from enzyme discovery to applications. *Clinical and Experimental Pharmacology and Physiology*, 31(11): 817–821.

- Enzymes applicable to pesticide remediation – degrade toxins rapidly.

Trombly, J. **(1995).** Engineering enzymes for better bioremediation. *Environmental Science & Technology*, *29*(12): 560–564.

- Enzymes are generally the active agents behind biochemical transformations that take place through bioremediation.
- The transformation takes place as the enzyme encounters its substrate (the target pollutant) and splits the substrate into component parts or removes part of the molecule. This process occurs very rapidly, leaving the enzyme unaltered and ready to deal with further molecules of substrate. Enzymes are classified broadly as hydrolytic, oxidizing, or reducing, depending on the type of reaction they control.

Venosa, A. D and X.Q. Zhu **(2003).** Biodegradation of Crude Oil Contaminating Marine Shorelines and Freshwater Wetlands. *Spill Science & Technology Bulletin,* 8: 163-178

- ONLY looks at nutrient addition for biostimulation. In theory, approximately 150 mg of nitrogen and 30 mg of phosphorus are utilized in the conversion of 1 g of hydrocarbon to cell materials (Rosenberg & Ron, 1996). When a major oil spill occurs in marine and freshwater environments, the supply of carbon is dramatically increased and the availability of nitrogen and phosphorus generally becomes the limiting factor for oil degradation (Atlas, 1984; Leahy & Colwell, 1990). In marine environments, nutrient limitation is generally correlated to the low background levels of nitrogen and phosphorus in seawater (Floodgate, 1984).

4. Supplementary information****

Ahuja, S.K., G.M. Ferreira and A.R. Moreira **(2004).** Utilization of Enzymes for Environmental Applications. *Critical Reviews in Biotechnology*, 24(2–3):125–154

- Enzymes are natural catalysts, which are universally found in all living organisms. They may either be used for building more complex molecules from simple ones or for selective breakdown of a mixture of larger molecules. Just one microorganism can contain over 1,000 different enzymes (Novo Nordisk, 1995).
- Enzymes are powerful tools that help sustain a clean environment in several ways. They are utilized for environmental purposes in a number of industries including agro-food, oil, animal feed, detergent, pulp and paper, textile, leather, petroleum, and specialty chemical and biochemical industry. Enzymes also help to maintain an unpolluted environment through their use in waste management.

Arulazhagan, P. and N. Vasudevan **(2009).** Role of a Moderately Halophilic Bacterial Consortium in the Biodegradation of Polyaromatic Hydrocarbons. *Marine Pollution Bulletin,* 58: 256-262.

- Polycyclic aromatic hydrocarbons are ubiquitous pollutants in the environment, and most high molecular weight PAHs cause mutagenic, teratogenic and potentially carcinogenic effects. While several strains of bactiera have been identified that degrade PAHs, the present study is focused on the degradation of PAHs in a marine environment by a moderately halophilic bacterial consortium. The bacterial consortium was isolated from a mixture of marine water samples collected from seven different sites in Chennai, India. The low molecular weight (LMW) PAHs phenanthrene and fluorine, and the high molecular weight (HMW) PAHs pyrene and benzo(e)pyrene were selected for the degradation study.
- The consortium metabolized both LMW and HMW PAHs. The consortium was also able to degrade PAHs present in crude oil-contaminated saline wastewater. The bacterial consortium was able to degrade 80% of HMW PAHs and 100% of LMW PAHs in the saline wastewater. The strains present in the consortium were identified *as Ochrobactrum sp*., *Enterobacter cloacae* and *Stenotrophomonas maltophilia*. This study reveals that these bacteria have the potential to degrade different PAHs in saline wastewater.

Arulazhagan, P., N. Vasudevan, I.T. Yeom **(2010).** Biodegradation of Polycyclic Aromatic Hydrocarbon by a Halotolerant Bacterial Consortium Isolated from Marine Environment. *International Journal of Environmental Science and Technology,* 7: 639-652.

- The biodegradability of polycyclic aromatic hydrocarbons such as naphthalene, fluorene, anthracene and phenanthrene by a halotolerant bacterial consortium isolated from marine environment was investigated. The polycyclic aromatic hydrocarbons degrading bacterial consortium was enriched from mixture saline water samples collected from Chennai (Port of Chennai, salt pan), India.
- The consortium potently degraded polycyclic aromatic hydrocarbons (> 95%) at 30g/L of NaCl concentration in 4 days. The consortium was able to degrade 39 to 45% of different polycyclic hydrocarbons at 60 g/L NaCl concentration.

Atlas R.M., and R. Bartha **(1998)** Microbial Ecology: Fundamentals and Applications, 4th edn Canada: Benjamin/Cummings Publishing.

- Provides a definition for bio-stimulation.

Barrios San Martín, Y. **(2011**) Bioremediation: A Tool for the Management of Oil Pollution in Marine Ecosystems. *Biotecnología Aplicada,* 28: 69-76.

- The first patent for a biological agent to be used in remediation processes was granted in 1974, protecting an oil-degrading strain of *Pseudomonas putida*. By 1991 there were already more than 70 oildegrading microbial genera in literature; a figure that almost doubled in the two successive decades. These microorganisms belong to at least 11 different prokaryotic divisions.
- Microorganisms (mainly bacteria and fungi) can degrade a vast array of structurally dissimilar environmental pollutants. Recalcitrant contaminants, such as the polychlorinated biphenyls produced by oil refineries, polycyclic aromatic hydrocarbons, resins and asphaltenes as well as complex mixtures containing them, can be mineralized into carbon dioxide by several ligninolytic fungi and bacteria.

Bragg, J. R., R.C. Prince, E.J. Harner, R.M. Atlas **(1994).** Effectiveness of Bioremediation for the Exxon-Valdez Oil-Spill. *Nature,* 368: 413-418.

- Discusses successful bioremediation post Exxon-Valdez.

Cao, B., K. Nagarajan and K.C. Loh **(2009).** Biodegradation of aromatic compounds: current status and opportunities for biomolecular approaches. *Applied Microbiology and Biotechnology*, 85(2): 207–228.

- Biodegradation can achieve complete and cost effective elimination of aromatic pollutants through harnessing diverse microbial metabolic processes. Aromatics biodegradation plays an important role in environmental cleanup and has been extensively studied since the inception of biodegradation.

Table 1 Major groups of anaerobic bacteria in aromatic biodegradation

Pollutants	Bacteria	Comments	References
Benzene	*Geobacter* spp. *Desulfobacterium* spp.	Oxidize benzene in Fe(II)-reducing conditions Mineralize benzene into CO_2 in 5 days	(Coates et al. 2001; Rooney-Varga et al. 1999)
Toluene	*G. metallireducens* *Azoarcus* spp. *Thauera* spp.	First pure culture for toluene oxidation Facultative toluene-oxidizing nitrate-reducers	(Chakraborty and Coates 2004; Lovley et al. 1989)
Ethylbenzene	*Thauera*-related	Denitrifying bacteria completely mineralize methylbenzene	(Ball et al. 1996; Rabus and Widdel 1995)
Xylene	*D. acetonicum*-related *Desulfosarcina variabilis*-related	Mineralizes *o*- and *m*-xylene	(Harms et al. 1999; Hess et al. 1997; Rabus and Widdel 1995)
PAHs	*Acidovorax* *Bordetella* *Pseudomonas* *Sphingomonas* *Variovorax*	Complete degradation for naphthalene and partial for three to five ring PAHs	(Eriksson et al. 2003; Rockne et al. 2000)
	P. stutzeri *Vibrio pelagius*-related	Mineralizes 7–20% naphthalene	
PCBs	*Desulfitobacterium dehalogenans*	Dehalogenates flanking Cl of OH-PCBs	(Wiegel et al. 1999)
PCP	*Desulfitobacterium frappieri*	90–99% PCP removal forming 3-CP	(Tartakovsky et al. 1999; Beaudet et al. 1998; Bouchard et al. 1996; Shelton and Tiedje 1984)
	Desulfitobacterium halogenans *Desulfitobacterium chlororespirans* *Desulfomonile tiedje*	Dechlorinates at *o*- and *m*-position	

Coulon, F., B.A. McKew, A.M. Osborn, T.J. McGenity, K.N. Timmis **(2007).** Effects of Temperature and Biostimulation on Oil-Degrading Microbial Communities in Temperate Estuarine Waters. *Environmental Microbiology,* 9: 177-186.

- Relatively rapid degradation was found at $4^{O}C$, the lowest temp. tested; and it was temperature rather than nutriet addition that most influenced the community. A phylogenetic analysis of oil-degrading microcosms showed hydrocarbonclastic organisms like Thalassolituus and Cycloclasticus, as wellas oil degrader Roseobacter, were present at both $4^{O}C$ and $20^{O}C$, demonstrating thermos-versatiity of such organisms.

Cappello, S., D. Russo, S. Santisi, R. Calogero, C. Gertler, F. Crisafi, M. De Domenico and M. Yakimov **(2012)**. Presence of Hydrocarbon-Degrading Bacteria in the Gills of Mussel *Mytilus Galloprovincialis* in a Contaminated Environment: A Mesoscale Simulation Study. *Chemistry and Ecology,* 28: 239-252.

- The data obtained show that the presence of hydrocarbons affected the abundance of bacteria inside the gills of specimens and determines selection for specific (hydrocarbon-degrading) bacteria (i.e. Alcanivorax sp. and Marinobacter sp.). However, is not yet clear whether the presence of such genera of bacteria inside the mussel is due to symbiosis or as a result of filtration.

Dombrowski, N. J.A. Donaho, T. Gutierrez, K.W. Seitz, A.P. Teske and B.J. Baker **(2016).** Reconstructing metabolic pathways of hydrocarbon-degrading bacteria from the Deepwater Horizon oil spill. *Nature Microbiology*, 2016.

- The repertoire of polycyclic aromatic hydrocarbon use varied among different bacterial taxa and the combined capabilities of the microbial community exceeded those of its individual components, indicating that the degradation of complex hydrocarbon mixtures requires the non-redundant capabilities of a complex oil-degrading community.
- Alternatively, a coordinated community activity might be required to completely degrade polycyclic hydrocarbons during the DWH spill.

El-Zahab, B., L. Meza, T. Cutright and P. Wang **(2004).** Enzymatic degradation of trichloroethylene using enzyme extracts isolated from a bacterial consortium. *Applied Biochemistry,* 117(3).

- These observations indicated that both the extracellular and the mixed extracts contained enzymes that were effective for trichloroethylene degradation.

Dashti, N., N. Ali, M. Eliyas, M. Khanafer, N.Sorkhoh and S. Radwan **(2015).** Most Hydrocarbonoclastic Bacteria in the Total Environment Are Diazotrophic, Which Highlights Their Value in the Bioremediation of Hydrocarbon Contaminants. *Microbes and Environments,* 30: 70-75.

- Concluded that most hydrocarbonoclastic bacteria are diazotrophic (able to fix atmospheric nitrogen gas into a more usable form such as ammonia and so able to grow without external sources of fixed nitrogen) which allows for their wide distribution in the total environment. Therefore, these bacteria are useful for the cost-effective, environmentally friendly bioremediation of hydrocarbon contaminants.

Dell'Anno, A., F. Beolchini, L. Rocchetti, G. Luna, and R. Danavaro **(2012).** High Bacterial Biodiversity Increases Degradation Performance of Hydrocarbons During Bioremediation of Contaminated Harbor Marine Sediments. *Environmental Pollution,* 167: 85-92.

- In both aerobic and anaerobic conditions, biodegradation efficiencies of hydrocarbons were significantly and positively related with bacterial richness and evenness.
- Overall results presented here suggest that bioremediation strategies, which can sustain high levels of bacterial diversity rather than the selection of specific taxa, may significantly increase the efficiency of hydrocarbon degradation in contaminated marine sediments.

dos Santos, H. F., G.A. Santos Duarte, C.T. da Costa Rachid, R.M. Chaloub, E.N. Calderon, L.F. de Barros Marangoni, A. Bianchini, A. Nudi, F.L. do Carmo, J.D. van Elsas, A.S. Rosado, C. Barreira e Castro and R.S. Peixoto **(2015).** Impact of Oil Spills on Coral Reefs Can Be Reduced by Bioremediation Using Probiotic Microbiota. *Scientific Reports,* 5.

- As expected, oil (WSF) impacted *M. harttii* health in varying (negative) ways, and they also affected the *M. harttii* microbiome. Remarkably, the bioremediation strategy, which was based on a single dosage of a consortium composed of 10 oleophilic morphotypes selected on a specific growth medium already improved M. harttii health. It also significantly accelerated the degradation of petroleum hydrocarbons.

Garrett, R. M., S.J. Rothenburger, and R.C. Prince **(2003).** Biodegradation of Fuel Oil under Laboratory and Arctic Marine Conditions. *Spill Science & Technology Bulletin,* 8: 297-302.

- Low temperatures clearly slow biodegradation, but the physical progress seems to follow that seen at warmer temperatures.

Kleindienst, S., S. Grim, M. Sogin, A. Bracco, M. Crespo-Medina and S.B. Joye **(2016)**. Diverse, Rare Microbial Taxa Responded to the Deepwater Horizon Deep-Sea Hydrocarbon Plume. *Isme Journal,* 10: 400-415.

- Major hydrocarbon degraders, adapted to the slow-diffusive natural hydrocarbon seepage in the Gulf of Mexico, appeared unable to cope with the conditions encountered during the DWH spill or were outcompeted. In contrast, diverse, rare taxa increased rapidly in abundance, underscoring the importance of specialized subpopulations and potential ecotypes during massive deep-sea oil discharges and perhaps other large-scale perturbations.

Nichols, D., J. Bowman, K. Sanderson, C. Nichols, T. Lewis, T. McMeekin and P.D. Nichols **(1999).** Developments with Antarctic Microorganisms: Culture Collections, Bioactivity Screening, Taxonomy, Pufa Production and Cold-Adapted Enzymes. *Current Opinion in Biotechnology,* 10: 240-246.

- Cold-adapted enzymes are produced by organisms existing in permanently cold habitats located in polar zones, at high altitudes or in the deep sea and in sea ice, seawater, lake water, sediments, snow and permafrost.

Resource Analysts, Inc. Personal cumminication with S. Pedigo, June, 11 1990

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P.O. Number: 2473E

Date Received: 05/25/90 (1415)

Lab Number: 21,986

Date Reported: 06/11/90

Attached please find test results for acid/base/neutral extractable organic compounds.

Field Identification: OSE BATCH 9522 Matrix: Water
Laboratory Number: 21986-1

Parameter	Concentration	Date Analyzed	Method/Ref.
Arsenic, total (mg/L)	<0.01	05/29/90	7060/1
Cadmium, total (mg/L)	<0.005	05/29/90	3010,6010/1
Chromium, total (mg/L)	<0.01	05/29/90	3010,6010/1
Copper, total (mg/L)	0.04	05/29/90	3010,6010/1
Mercury, total (mg/L)	<0.0003	05/30/90	7470/1
Nickel, total (mg/L)	<0.03	05/29/90	3010,6010/1
Lead, total (mg/L)	<0.005	05/29/90	3020,7421/1
Zinc, total (mg/L)	0.06	05/29/90	3010,6010/1

References: 1) EPA SW 846, 3RD Edition

Santos, H. F., F.L. Carmo, J. Paes, A.S. Rosado and R.S. Peixoto **(2011)**. Bioremediation of Mangroves Impacted by Petroleum. *Water Air and Soil Pollution,* 216: 329-350.

- In some environments, such as mangroves, bioremediation may be the most appropriate approach for cleanup.

Scott, C., S. E. Lewis and R. Milla **(2010).** A free-enzyme catalyst for the bioremediation of environmental atrazine contamination. *Journal of Environmental Management*, 91(10): 2075–2078.

- We propose an alternative strategy using a free-enzyme bioremediant, which is unconstrained by the issues surrounding the use of live organisms. Here we report an initial field trial with an enzyme-based product, demonstrating that the technology is technically capable of remediating water bodies contaminated with the most common triazine herbicide, atrazine

Tanokura, M., T. Miyakawa, L. Guan and F. Hou **(2015).** Structural analysis of enzymes used for bioindustry and bioremediation. *Bioscience, Biotechnology, and Biochemistry*, *79*(9): 1391–1401.

- This review partly summarizes research into the structural biology of enzymes used for bioindustry and bioremediation

Walsh. C. **2002.** Enabling the Chemistry of Life. *Nature*, 409 (11).

- One of the most active areas of applied enzymology in the past two decades has been the study of enzymes capable of bioremediation: the breakdown of organic and inorganic pollutants. There are now substantial databases of enzymes and the bioremedial transformations they catalyse, which inlude the breakdown of aromatic and heteroaromatic pollutants by oxidative, reductive and hydrolytic transformations.

Zhu, X., A.D. Venosa and M. Suidan **(2004).** Literature review on the use of commercial bioremediation agents for cleanup of oil-cotaminated estuarine environments. EPA/600/R-04/075 July

- This report states that Zwick et al. 1997 is the only peer-reviewed paper available on a NCP-Schedule-listed bioremediation agent OSEII, other than numerous publications on Inipol EAP 22. *This is no longer correct and needs to be updated*.

Zwick, T.C., E.A. Foote, A.J. Pollack, L.J. Boone, B.C. Alleman, R.E. Hoeppel, and L. Bowling **(1997).** Effects of nutrient addition during bioventing of fuel contaminated soils in an arid environment. *In-Situ and On-Site Bioremediation*, 1: 403-409.

- Although this field trial suggested that irrigation and OSE II addition might have enhanced microbial activities in the deeper soils at the site, the report was inconclusive in regards to direct evidence that hydrocarbons were degraded.

VII. LITERATURE REVIEW ON BIO-SURFCTANT BIOREMEDIATION OF HYDROCARBON POLLUTION IN AQUATIC ENVIRONMENTS

1. Efficiency and rate*

Aparna, A., G. Srinikethan and S. Hedge **(2011).** Effect of addition of biosurfactant produced by *Pseudomonas sps.* on biodegradation of crude oil. In: 2nd International Proceedings of Chemical, Biological & Environmental Engineering, 6: 71-75. IACSIT Press, Singapore

- A good surfactant can lower surface tension of water from 72 to 35 mN/m. The initial surface tension of the supernatant of *Pseudomonas sps.* was 70 mN/m, which reduced to 27 mN/m respectively. There was a positive correlation between the reduction of surface tension and population of microorganism. This shows that biosurfactant can increase the bioavailability of crude oil and biodegradation process. Similar results were obtained by Banat et al., 1991.

Bao, M., P. Sun, X, Yang, X. Wang, L. Wang, L. Cao, and F. Li **(2014).** Biodegradation of Marine Surface Floating Crude Oil in a Large-Scale Field Simulated Experiment. *Environmental Science-Processes and Impacts,* 16: 1948-1956.

- Biodegradation of marine surface floating crude oil with hydrocarbon degrading bacteria, rhamnolipid biosurfactants, and nutrients was carried out by a large-scale field simulated experiment in this paper. After a 103 day experiment, for n-alkanes, the maximum biodegradation rate reached 71% and the results showed hydrocarbon degrading bacteria, rhamnolipid biosurfactants, and nutrients have a comprehensive effect. It also showed that rhamnolipid biosurfactants could shorten the biodegradation time through an emulsifying function; the nutrients could greatly increase the biodegradation rate by promoting production of hydrocarbon-degrading bacteria.

Bordoloi, N.K. and B.K. Konwar **(2009).** Bacterial biosurfactant in enhancing solubility and metabolism of petroleum hydrocarbons. *Journal of Hazardous Materials,* 170: 495–505.

- Biosurfactant can make hydrocarbon complexes more mobile with the potential use in oil recovery, pumping of crude oil and in bioremediation of crude oil contaminant. In the investigation, bacterial isolates capable of utilizing poly-cyclic aromatic hydrocarbons like phenanthrene, pyrene and fluorene were used. A gradual decrease of the supplemented hydrocarbons in the culture medium was observed with corresponding increase in bacterial biomass and protein.
- Biosurfactants were found to be lipopeptide and protein–starch–lipid complex in nature and they could reduce the surface tension of pure water (72 mN m−1) to 35 mN m−1. The critical micelle concentration (CMC) was also lower than the chemical surfactant sodium dodecyl sulphate (SDS).

Cameotra, S. and P. Singh. **(2008).** Bioremediation of oil sludge using crude biosurfactants. *International Biodeterioration & Biodegradation,* 62: 274–280.

- A microbial consortium consisting of two isolates of *Pseudomonas aeruginosa* and one of *rhodococcal* from soil contaminated with oily sludge was able to degrade 90% of hydrocarbons in 6 weeks in liquid culture. The ability of the consortium to degrade sludge hydrocarbons was tested in two separate field trials. In addition, the effect of two additives (a nutrient mixture and a crude biosurfactant preparation) on the efficiency of the process was also assessed.
- The biosurfactant used was produced by a consortium member and was identified as being a mixture of 11 rhamnolipid congeners. The consortium degraded 91% of the hydrocarbon content of soil contaminated with 1% (v/v) Ratnagiri, India, crude oil sludge in 5 weeks. Separate use of any one additive along with the consortium brought about a 91–95% depletion of the hydrocarbon content in 4 weeks, with the crude biosurfactant preparation being a more effective enhancer of degradation.
- However, more than 98% hydrocarbon depletion was obtained when both additives were added together with the consortium. Similar results were observed with soil contaminated with sludge from IOCL, Faridabad, India. The data substantiate the use of a crude biosurfactant preparation for hydrocarbon remediation

Cappello, S. A. Crisari, M. Hassanshahian, M. Genovese, S. Santisi and M. Yakimov **(2012).** Effect of a Bioemulsificant Exopolysaccharide (Eps2003) on Abundance and Vitality of Marine Bacteria. *Water Air and Soil Pollution,* 223: 3903-3909.

- It is evident that the EPS2003, with respect to commercial products, favors the growth of marine microbial population. Data obtained evidence that thanks to its biodegradability and its low level of toxicity, the EPS2003, in connection with the bacterial community, is revealed optimal for use in the participations of recovery of environment polluted from oil.

Cappello, S., M. Genovese, R. Denaro, S. Santisi, A. Volta, M. Bonsignore, G. Mancini, L. Giuliano, L. Genovese, and M. Yakimov **(2014).** Quick Stimulation of Alcanivorax Sp by Bioemulsificant Eps2003 on Microcosm Oil Spill Simulation. *Brazilian Journal of Microbiology,* 45: 1317-1323.

- Data obtained indicated that bioemulsificant addition stimulated an increase of total bacterial abundance and, in particular, selection of bacteria related to Alcanivorax genus; confirming that EPS2003 could be used for the dispersion of oil slicks and could stimulate the selection of marine hydrocarbon degraders thus increasing bioremediation process.

Chandankere, R., J.Yao , M. Cai, K. Masakorala, A.K. Jain and M. Choi **(2014).** Properties and characterization of biosurfactant in crude oil biodegradation by bacterium Bacillus methylotrophicus. *Fuel*, 122: 140–148

- An effective biosurfactant-producer and hydrocarbon degrading bacterial strain, *Bacillus methylotrophicus* USTBa was isolated from hydrocarbon contaminated aqueous medium using crude oil as sole source of carbon. The produced biosurfactant had the ability to decrease the surface tension of water from 72 to 28 mN/m, with the critical micelle concentration (CMC) of 35 mg/L. The biosurfactant exhibited 90% emulsification activity (EI) on crude oil.

Dang, N.P., B. Landfaldb and N.P. Willassen **(2016).** Biological surface-active compounds from marine bacteria. *Environmental Technology,* 37(9): 1151–1158

- Surface-active compounds (SACs) are widely used in different industries as well as in many daily consumption products. However, with the increasing concern for their environmental acceptability, attention has turned towards biological SACs which are biodegradable, less toxic and more environmentally friendly.
- In this work, 176 marine hydrocarbon-degrading bacterial isolates from petroleum-contaminated sites along the Norwegian coastline were isolated and screened for their capacity to produce biological SACs. Among them, 18 isolates were capable of reducing the surface tension of the culture medium by at least 20 mN m−1 and/or capable of maintaining more than 40% of the emulsion volume after 24 h when growing on glucose or kerosene as carbon and energy source. These isolates were members of the genera Pseudomonas, Pseudoalteromonas, Rhodococcus, Catenovulum, Cobetia, Glaciecola, Serratia, Marinomonas and Psychromonas. Two isolates, Rhodococcus sp. LF-13 and Rhodococcus sp. LF-22, reduced surface tension of culture medium by more than 40 mN m−1 when growing on kerosene, n-hexadecane or rapeseed oil. The biosurfactants were produced by resting cells of the two Rhodococcus strains suggesting the biosynthesis of the biosurfactants was not necessarily associated with their growth on hydrocarbons
- The extracted biosurfactant from Rhodococcus sp. strain LF-22 was able to enhance the biodegradation rate of n-hexadecane at 13°C

da Silva, A. C.; F. de Oliveira, D.S. Bernardes and F.P. de Franca **(2009).** Bioremediation of Marine Sediments Impacted by Petroleum. *Applied Biochemistry and Biotechnology,* 153: 58-66.

- In this paper, the enhancement of oil-contaminated soil bioremediation by the use of biostimulation techniques was verified. Experimental design tools were used to study the effects of commercial fertilizer and biosurfactant on biodegradation of the Heat of Formation (HOF) by indigenous microbiota. A variable screening procedure showed that the oil biodegradation depended primarily on the biosurfactant concentration, while the use of 25 to 45 g kg−1 of fertilizer had a slight effect on oil biodegradation. One can conclude that the experimental design tool led to an increase in the contaminants biodegradation—expressed as HOF biodegradation—from 20% to 30% at 30 days of bioprocess.
- Under the studied biostimulation conditions, the indigenous microbial population in the mid-tide zone of Guanabara Bay was able to degrade 100% of the n-alkanes between C15 and C30 and reduce 65% of HOF, at the end of 60 days of process. The intrinsic bioremediation tests showed a lower level of biodegradation, varying from 85% to 100% of the n-alkanes between C15 and C30 at the end of the 60 days of experimentation.

El-Sheshtawy, H. S., N.M. Khalil, W. Ahmed and R. Abdallah **(2014)**. Monitoring of Oil Pollution at Gemsa Bay and Bioremediation Capacity of Bacterial Isolates with Biosurfactants and Nanoparticles. *Marine Pollution Bulletin,* 87: 191-200.

- In this study, two crude oil-degrading bacterial strains were isolated from the Gemsa Bay, in the Red Sea, Egypt. There is a direct relationship between both the emulsification activity (E24) and the decrease in surface tension with increasing the growth rate on hydrocarbons. The presence of biosurfactant with different types of nanoparticles helps the bacterial isolates to consume the iso-paraffins more than n-paraffins. This result seems to be newly and valuable biodegradation trend. The complete degradation of some different membered rings of polyaromatics and the percentage biodegradation of other polyaromatics increased in microcosms containing two different types of nanoparticles with biosurfactant at 7 days. Thus, these bacterial isolates have a potential to be applied in the bioremediation of petroleum contaminated sites using biosurfactant and specific concentration of Fe_2O_3 and $Zn_5(OH)_8Cl_2$ nanoparticles.

Lai, C., Y.C.Huang, Y. Wei, J. Chang **(2009).** Biosurfactant-enhanced removal of total petroleum hydrocarbons from contaminated soil. *Journal of Hazardous Materials*, 167: 609–614

- A screening method was developed to evaluate the oil removal capability of biosurfactants for oilcontaminated soils collected from a heavy oil-polluted site. The ability of removing total petroleum hydrocarbon (TPH) from soil by two biosurfactants was identified and compared with that of synthetic surfactants. The results show that biosurfactants exhibited much higher TPH removal efficiency than the synthetic ones examined.

Matvyeyeva,O.L., O.A. Vasylchenko, O. R. Aliieva **(2014).** Microbial Biosurfactants Role in Oil Products Biodegradation. *International Journal of Environmental Bioremediation & Biodegradation,* 2(2): 69-74

- The synthesis of microbial biosurfactants and their application have an important practical significance for a range of petroleum pollutants bioremediation. It may be concluded that microbial degradation can be considered as a key component in the cleanup strategy for petroleum hydrocarbon remediation due to their biodegradability and low toxicity.
- One more promising result was achieved by Obayori et al. who investigated the biodegradative properties of biosurfactant produced by Pseudomonas sp. LP1 strain on crude oil and diesel. They reported 92.34% degradation of crude oil and 95.29% removal of diesel oil. Biodegradative properties of biosurfactant producing Brevibacterium sp. PDM-3 strain were tested by Reddy et al. They reported that this strain could degrade 93.92% of the phenanthrene and also had ability to degrade other polyaromatic hydrocarbons such as anthracene and fluorene. Kang et al. used sophorolipid in studies on biodegradation of aliphatic and aromatic hydrocarbons and Iranian light, crude oil under laboratory conditions. Addition of this biosurfactant to soil increased also biodegradation of tested hydrocarbons with the rate of degradation ranging from 85% to 97% of the total amount of hydrocarbons.

Table 1. Classification of biosurfactants and their use in remediation of hydrocarbon contaminated sites

Class of Biosurfact	Microorganism	Application	Ref.
Rhamnolipids	*Pseudomonas aeruginosa, Pseudomonas* sp.	Enhancement of the degradation and dispersion of different classes of hydrocarbons; emulsification of hydrocarbons and vegetable oils	[31,32,33]
Trehalolipids	*Mycobacterium tuberculosis, Rhodococcus erythropolis, Arthrobacter* sp., *Nocardia* sp., *Corynebacterium* sp.	Enhancement of the bioavailability of hydrocarbons	[34]
Sophorolipids	*Torulopsis bombicola, Torulopsis petrophilum, Torulopsis apicola*	Recovery of hydrocarbons from dregs and muds; enhancement of oil recovery	[32,35,36]
Trehalolipids	*Mycobacterium tuberculosis, Rhodococcus erythropolis, Arthrobacter* sp., *Nocardia* sp., *Corynebacterium* sp.	Enhancement of the bioavailability of hydrocarbons	[34]
Corynomycolic acid	*Corynebacterium lepus*	Enhancement of bitumen recovery	[37]
Surfactin	*Bacillus subtilis*	Enhancement of the biodegradation of hydrocarbons	[38]
Lichenysin	*Bacillus licheniformis*	Enhancement of oil recovery	[39]
Emulsan	*Acinetobacter calcoaceticus* RAG-1	Stabilization of the hydrocarbon-in-water emulsions	[40]
Alasan	*Acinetobacter radioresistens* KA-53		[41]
Liposan	*Candida lipolytica*	Stabilization of hydrocarbon-in-water emulsions	[42]
Mannoprotein	*Saccharomyces cerevisiae*		[43]

Montero-Rodríguez, D., R.F.S. Andrade, D.L.R. Ribeiro, D. Rubio-Ribeaux, R.A. Lima, H.W.C. Araújo and G.M. Campos-Takaki **(2015).** Bioremediation of Petroleum Derivative Using Biosurfactant Produced by *Serratia marcescens* UCP/WFCC 1549 in Low-Cost Medium. *Int. Journal of Current Microbiology and App.Sci,* 4(7): 550-562.

- The crude biosurfactant showed ability to emulsify petroleum derivatives (EI24 > 60% of diesel, engine oil and burned engine oil), as well as be stable in a wide range of pH, temperature and salinity. Also, the crude biosurfactant exhibited excellent properties to dispersing engine oil in water (78%) and to removing burned engine oil in beach sand and mangrove sediment (88.27% and 73.70%, respectively). These results demonstrated the high potential of biosurfactant produced by S. marcescens as sustainable for application in bioremediation processes of hydrophobic pollutants derivated of petroleum.

Muligan, C. **(2004).** Environmental applications for biosurfactants. *Environmental Pollution*, 133: 183–198.

- Shafeeq et al. (1989) Showed that biosurfactants were produced during biodegradation of a hydrocarbon mixture by *P. aeruginosa*. Chhatre et al. (1996) showed that four bacterial isolates from crude oil were able to degrade 70% of the Gulf and Bombay High Crude Oil. At 25 C and a salinity of 35&, a solution of 2% rhamnolipids diluted in saline water and applied at a dispersant to oil ratio (DOR) of 1:2, immediately dispersed 65% of a crude oil. Co-addition of 60% ethanol and 32% octanol with 8% rhamnolipids applied at a DOR of 1:8 improved dispersion to 82%.
- Despite the large amount of research on dispersants, there is very little on the use of biosurfactants as biodispersants despite their potential benefits, particularly for enhancing oil biodegradation and solubilization.

Mohanty, S., J. Jasmine and S. Mukherji**. (2013**). Practical Considerations and Challenges Involved in Surfactant Enhanced Bioremediation of Oil. *BioMed Research Intl*, 2013.

- Chemically, the hydrophilic head group of biosurfactants may consist of a carbohydrate, peptide, amino acid, phosphate, carboxylic acid, or alcohol while the hydrophobic tail may consist of fatty acids, hydroxy fatty acids, or α-alkyl-β-hydroxy fatty acids.
- For total petroleum hydrocarbons (TPHs) associated with soil, biosurfactants such as rhamnolipids and surfactin have been found to remove TPH at higher rates compared to the synthetic surfactants. Biosurfactants are preferable compared to synthetic surfactants as they are easily biodegradable.
- The introduction of surfactants into oil contaminated soil and aquatic environments may add to pollution through the accumulation of petroleum hydrocarbon degradation intermediates and partial biotransformation products of the surfactants. This may pose a threat to aquatic and terrestrial plants and animals. These ecotoxicological implications need to be considered for successful application of SEB. Cationic and nonionic surfactants have much higher sorption on soil and sediment than anionic surfactants.

Nikolopoulou, M., P. Eickenbusch, N. Pasadakis, D. Venieri, and N. Kalogerakis **(2013).** Microcosm Evaluation of Autochthonous Bioaugmentation to Combat Marine Oil Spills. *New Biotechnology,* 30: 734-742.

- Chemical analysis (gas chromatography–mass spectrometry) of petroleum hydrocarbons confirmed the results of previous work demonstrating that the biodegradation processes were enhanced by the addition of lipophilic fertilizers (uric acid and lecithin) in combination with biosurfactants (rhamnolipids), resulting in increased removal of petroleum hydrocarbons as well as reduction of the lag phase within 15 days of treatment.
- Considering this outcome and examining the results, the use of biostimulation additives in combination with naturally pre-adapted hydrocarbon-degrading consortia (bioaugmentation) has proved to be an effective treatment and is a promising strategy that could be applied specifically when an oil spill approaches near a shore line and an immediate hydrocarbon degradation effort is needed.

Rebello, S., A.K. Asok, S. Mundayoor and M. S. Jisha **(2014).** Surfactants: toxicity, remediation and green surfactants. *Environ Chem Letters*, 12: 275–287.

- Biosurfactants are biological compounds with high surfaceactive properties (Georgia and Poe 1931), produced by microorganisms, plants, animals and humans (Christofi and Ivshina 2002). They are produced on microbial cells surfaces or excreted extracellularly and contain both hydrophilic and hydrophobic moieties. They have several advantages over the chemical surfactants, such as lower toxicity higher biodegradability (Zajic et al. 1977), better environmental compatibility (Georgiou et al. 1992), higher foaming ability (Razafindralambo et al. 1996), high selectivity and specific activity at extreme temperatures, pH and salinity (Velikonja and Kosaric 1993) and the ability to be synthesized from renewable feed stock (Desai and Banat 1997).
- In general, biosurfactants are more effective and efficient, and their CMC is about 10–40 times lower than that of chemical surfactants, i.e., less surfactant is necessary to get a maximum decrease in surface tension (Desai and Banat 1997), and biosurfactants also have higher EC50 than synthetic surfactants (Poremba et al. 1991). Biosurfactants constitute an interesting alternative to the commercial chemical surfactants.

2. Toxicity**

Edwards, K., JE. Lepo and M.A. Lewis **(2003).** Toxicity comparison of biosurfactants and synthetic surfactants used in oil spill remediation to two estuarine species. *Marine Pollution Bulletin*, 46 (10): 1309–1316.

- Five major classes of biosurfactants are: (1) glycolipids, (2) phospholipids and fatty acids, (3) lipopeptide/lipoproteins, (4) polymeric surfactants, and (5) particulate surfactants (Parra et al., 1989; Desai and Desai, 1993; Nabholz et al., 1993).
- In this study, PES-61 (synthetic surfactant) and Emulsan (biosurfactant) were consistently the least toxic surfactants, Triton X-100 (synthetic surfactant) the most toxic. Based primarily on changes in weight after 7 d exposure to many of the surfactants, *M. bahia* appeared to be more sensitive than *M. beryllina*. There was no consistent indication that a particular class of surfactants, synthetic or biologically produced, was more toxic than the other. The toxicities of the biosurfactants, in general terms, were intermediate to those for the synthetic surfactants. Lang and Wagner (1993) and Poremba et al. (1991a,b), among others, have reported that biosurfactants are generally less toxic than synthetic surfactants based on the responses of other test species.

Table 4
Toxicities of biosurfactants and synthetic surfactants to *M. bahia* and *M. beryllina* after 7 d exposure

Surfactant	Response parameter[a]	Calculations					
		M. bahia			*M. beryllina*		
		FEC[b]	NOEC	LOEC	FEC	NOEC	LOEC
Biosurfactants							
BioEM	S	16.8	13.0	21.6	15.5	12.0	20.0
	G	5.8	4.3	7.8	–[c]	20.0	>20.0[d]
	F	3.3	2.6	4.3	–		
Emulsan	S	–	200	>200	232.4	180.0	300.0
	G	–	200	>200	–	300	>500
	F	154.9	120.0	200.0	–	–	–
PES-51	S	10.1	7.8	13.0	21.7	16.8	28.0
	G	10.1	7.8	13.0	–	16.8	>16.8
	F	16.8	13.0	21.6			
Synthetic surfactants							
PES-61	S	–	1000	>1000	464.8	360.0	600.0
	G	–	1000	>1000	–	1000	>1000
	F	–	1000	>1000			
Corexit 9500	S	4.2	3.2	5.4	77.5	10.0	100.0
	G	4.2	3.2	5.4	–	100	>100
	F	15.5	12.0	20.0	–	–	–
Triton X-100	S	2.8	2.2	3.6	2.3	1.8	3.0
	G	1.7	0.8	3.6	–	3.0	>3.0
	F	1.0	0.8	1.3			

Effect and no effect concentrations (mg/l) represent nominal concentrations. Fecundity not determined for toxicity tests conducted with *M. beryllina*.
[a] Effects represent survival (S), growth (G), and fecundity (F).
[b] First effect concentration (FEC)—Geometric mean of NOEC and LOEC.
[c] Not calculable.

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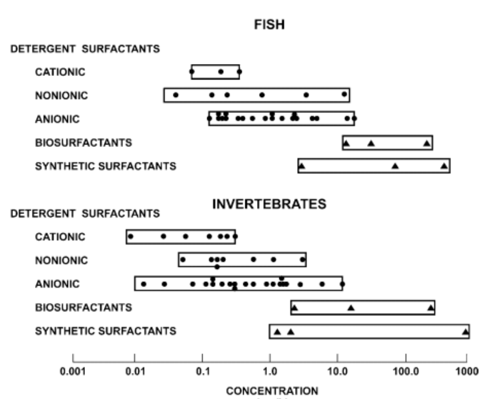

Fig. 3. Comparison of the first effect concentrations of the six surfactants used in this study to those previously reported for detergent surfactants. Data adapted from Lewis (1991, 1992).

Elliot, R., N. Singhal and S. Swift **(2010).** Surfactants and Bacterial Bioremediation of Polycyclic Aromatic Hydrocarbon Contaminated Soil—Unlocking the Targets. *Critical Reviews in Environmental Science and Technology*, 41(1): 78-124

- Removal of pollutants and remediation of sites contaminated with elevated levels of PAHs are of great importance due to the proven toxicity and inherent human health risks associated with these mutagenic or carcinogenic compounds (Bostrom et al., 2002; Makkar & Rockne, 2003). Though the toxicity of PAH molecules is structurally dependent, there are many examples of high toxicity, and several PAH molecules have been linked to or proven to be carcinogenic; for example, phenanthrene, benzo(a)pyrene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, dibenz(a,h)anthracene, and indeno(123-c,d)pyrene (see Fig. 1) (Boffetta et al., 1997; Mastrangelo et al., 1996; Samanta, Singh, & Jain, 2002; Sram and Binkova, 2000). This group of chemicals can also have detrimental effects on many ecosystems due to toxicity and bioaccumulation within food chains. PAHs are highly lipid soluble and thus are readily absorbed from the gastrointestinal tracts of mammals. Although these molecules can be found in a wide variety of tissues, they have a marked tendency for localization in adipose tissues (Cerniglia, 1984; Samanta et al.) and are often coupled with a slow natural degradation process and as a result persist in the ecosystem.
- Surfactants, as the name implies, are surface-acting agents and are a class of natural and synthetic chemicals that promote the wetting, solubilisation, and emulsification of various types of organic and inorganic contaminants. Although this category of molecules is very diverse, collectively they are described as amphiphilic molecules consisting of a hydrophobic and hydrophilic domain. Due to the amphiphilic properties, surfactants often behave by partitioning at the interface of phases with different degrees of polarity and hydrogen bonding, such as between air and water or oil and water boundaries. Biosurfactants include classes of molecular structures that are quite unlike synthetic surfactants, ranging in chemical structures from polymeric and particulate surfactants and simple fatty acids to complex glycolipids, lipopeptides and lipoproteins, lipopolysaccharides, and phospholipids.
- Concerns have been raised about the large-scale use of synthetic surfactants to flush groundwater for remedial purposes. Of particular concern is the varying degree of toxicity that these molecules can exhibit toward humans and the local ecology (Mulligan et al., 2001). Surfactants can also be resistant to biodegradation and may lead to increased toxicant pollution. The addition of surfactants to soils can also form highly viscous emulsions, which are sometimes difficult to remove (Boving & Brusseau, 2000). In fact, many synthetic surfactants have been shown to inhibit PAH degrading microorganisms and, in several instances where they are applied in excess of the CMC, can cause complete inhibition of PAH degradation (Churchill et al., 1995; Laha & Luthy, 1991, 1992; Tsomides, Hughes, Thomas, & Ward, 1995).

Poremba, K., W.Gunkel, S. Lang, F. Wagner **(1991).** Marine biosurfactants, Toxicity testing with marine microorganisms and comparison with synthetic surfactants. *Z. Naturforsch*, 46: 210–216.

- Eight synthetic and nine biogenetic surfactants were tested on their toxicity. Because of their possible application as oil dispersants against oil slicks on sea. the test organisms used were marine microorganisms (mixed and pure cultures of bacteria, microalgae, and protozoa). Bacterial growth was hardly effected or stimulated, whilst that o f algae and flagellates was reduced. All substances tested were biodegradaded in sea water. The bioluminescence o f Photobacter phosphoreum (M icrotox test) was the most sensitive test system used.
- A ranking shows that most biogenetic surfactants were less toxic than synthetic surfactants. No toxicity could be detected with the glucose-lipid produced by the marine bacterium.

Silva, F.S., D.G. Almeida, R.D. Rufino, J.M. Luna, V.A. Santos and L.A. Sarubbo **(2014).** Applications of Biosurfactants in the Petroleum Industry and the Remediation of Oil Spills. *Int. Journal of Molecular Sci.*, 15: 12523-12542.

- The remediation of contaminated sites can be achieved by physicochemical or biological methods. Conventional physicochemical methods can rapidly remove the majority of spilled oil, but, in most cases, removal simply transfers contaminants from one environmental medium to another and can even produce toxic byproducts. Moreover, crude oil cannot be completely cleaned up with physicochemical methods. Thus, more attention is being given to biological alternatives.
- Biosurfactants play an important role in remediation processes due to their efficacy as dispersion and remediation agents as well as their environmentally friendly characteristics, such as low toxicity and high biodegradability.

- Sobrinho et al. tested a biosurfactant produced by Candida sphaerica for the removal of motor oil from soil and seawater and found removal rates of 75% and 92% from clay and silty soil, respectively; in tests carried out with seawater, the biosurfactant exhibited an oil spreading efficiency of 75%, demonstrating its potential for application as an adjuvant in biotechnological processes of environmental decontamination. Edwards et al., in a comparison of toxicity of three synthetic surfactants and three microbial surfactants, concluded that the biosurfactants were less toxic than the synthetic surfactants to some invertebrate species. This paper includes toxicity information for a number of biosurfactants.

3. Mode of Action***

Eliora Z.R. and E. Rosenberg **(2002).** Biosurfactants and oil bioremediation. *Current Opinion in Biotechnology*, 13: 249–252.

- Oil pollution is an environmental problem of increasing importance. Hydrocarbon-degrading microorganisms, adapted to grow and thrive in oil-containing environments, have an important role in the biological treatment of this pollution. One of the limiting factors in this process is the bioavailability of many fractions of the oil. The hydrocarbon-degrading microorganisms produce biosurfactants of diverse chemical nature and molecular size. These surface-active materials increase the surface area of hydrophobic water-insoluble substrates and increase their bioavailability, thereby enhancing the growth of bacteria and the rate of bioremediation.

Souza, E., T.C. Vessoni-Penna, R.P. de Souza Oliveira **(2014).** Biosurfactant-enhanced hydrocarbon bioremediation: An overview. *International Biodeterioration and Biodegradation*, 89: 88-94.

- Biosurfactants present many advantages in comparison with synthetic surfactants, such as high biodegradability, low toxicity, biocompatibility, biodigestibility (which allows their application in cosmetic and pharmaceutical products and as food additives), possibility to be produced from low-cost sources and industrial waste, use in bioremediation of oil-affected sites, biodegradation and detoxification of industrial effluents, in addition to efficacy in extreme temperature, pH and salinity conditions (Pirôllo, 2006).

Enzymatic Bioremediation Field Use Research Consortium

2017 Workshop

The Lawrence Anthony Earth Organization's (LAEO) involvement in oil spill response research and, in particular, Bioremediation Enzyme Additive (EA) agents as a prospective alternative process to chemical dispersants is a long-term commitment as part of our Clean Waters Program. We engage in the examination and validation of alternative technologies.

Catalyzed by the DWH disaster, the LAEO Science and Tech Advisory Committee have been committed to oil spill response research and systems improvement advocacy for nearly 6 years. We have experience with and vetted numerous biological agents and hazardous chemical spill clean up processes over that period. We collaborate with all stakeholders and notably oil spill response professionals from deep water drilling engineers, geo-hazard pros, rig operations experts, marine biologists, chemical analysts to academia, consulting groups and NGOs. We are looking to build even stronger partnerships in order to effect adoption of alternative technologies for the removal of HC and other chemical pollutants from fresh water and ocean ecosystems.