

Departament de microbiologia. Facultat de biologia.

Universitat de Barcelona

BIODEGRADACIÓ I BIOREMEDIACIÓ DE FUEL DEL *PRESTIGE*

CARACTERITZACIÓ QUÍMICA
I COMUNITATS MICROBIANES IMPLICADES

Memòria de tesi presentada per Núria Jiménez García.

Dirigida per:

Dra. Anna Maria Solanas
Cánovas
Professora titular
Dpt. de Microbiologia
Facultat de Biologia
Universitat de Barcelona

Dr. Josep Maria Bayona
Térmens
Professor d'investigació
Institut de diagnosi
ambiental i estudis de
l'aigua
CSIC

Dr. Joan Albaigés Riera
Professor d'investigació
Institut de diagnosi
ambiental i estudis de
l'aigua
CSIC

Programa de doctorat: "Microbiologia Ambiental i Biotecnologia".

Bienni 2004-2006.

PART IV



Capítol 9

Conclusions

9.1. Processos de degradació del fuel

En l'estudi dels processos de biodegradació i fotooxidació del fuel s'ha observat el següent:

- La biodegradació de les diferents famílies de compostos alifàtics i aromàtics segueix patrons clarament establerts tant in vitro com in situ, independentment de la microbiota present: de manera general disminueix amb la seva massa molecular i, pel que fa als aromàtics, depèn de l'estructura molecular i la posició dels substituents alquílics, i afecta amb preferència els que tenen substituents β .
- La fotooxidació afecta preferentment la fracció aromàtica i dels carbazols. Depèn, també, de l'aromaticitat, del grau d'alquilació i de la conformació de la molècula i s'ha observat certa especificitat isomèrica. Durant el procés es generen compostos més polars que elueixen amb la fracció de les resines.
- Les fraccions de resines i asfaltens no es veuen alterades significativament.
- L'acció conjunta dels processos de biodegradació i fotooxidació incrementa i accelera de manera significativa la degradació del fuel.

9.2. Comunitats microbianes implicades en la degradació del fuel

En relació amb les comunitats microbianes implicades en la degradació del fuel:

- Predominen els α i γ -proteobacteris, presents en tots els experiments, i també els actinobacteris (al camp) i els flavobacteris.
- El gènere *Alcanivorax* s'ha mostrat com un primer colonitzador, de creixement ràpid i que sobresurt respecte els altres microorganismes de la comunitat. La seva absència a l'experiment de bioremediació in situ podria atribuir-se al fet que l'assaig es va dur a terme mesos després del vessament.
- Segons les diferents aproximacions, *Thalassospira*, *Marinobacter*, *Parvibaculum* i *Roseobacter* tindrien papers preponderants en la degradació del fuel, tant pel que fa a la fracció alifàtica com a l'aromàtica. Els gèneres *Lutibacterium*, *Mesorhizobium* i *Flavobacterium* podrien tenir un paper més rellevant en la degradació dels HAP.

A partir d'una mostra de fuel presa al mar, s'ha obtingut un consorci autòcton que presenta una potent capacitat degradadora in vitro. Existeix una molt bona correlació entre la cinètica de degradació de la fracció alifàtica i aromàtica del fuel i l'evolució de les poblacions degradadores de cada fracció.

9.3. Bioremediació del fuel: efecte de l'addició d'un fertilitzant oleofílic

L'addició del fertilitzant oleofílic *S200* incrementa significativament la biodegradació dels *n*-alcans més pesants i els hidrocarburs aromàtics policíclics més alquilats tant in vitro com al camp. Aquest efecte selectiu suggereix que, a més de subministrador de nutrients, l'*S200* actua augmentant la biodisponibilitat dels hidrocarburs més pesants, a causa de la presència d'un tensioactiu a la formulació del producte.

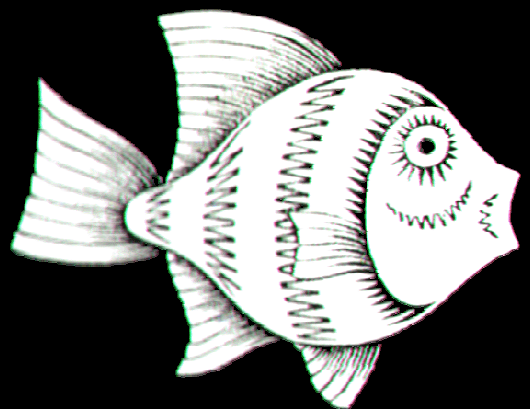
L'experiència pilot de bioremediació duta a terme en el camp, a la costa propera a Santander, ha corroborat els resultats obtinguts in vitro. Donada la naturalesa química del fuel del *Prestige*, amb un alt contingut d'hidrocarburs pesants i alquilats, l'aplicació de l'*S200* ha permès assolir molt bons resultats en la degradació de les fraccions alifàtica i aromàtica.

9.4. Aspectes metodològics

Existeixen diferències considerables a l'hora de caracteritzar les comunitats microbianes, tant pel que fa a l'anàlisi de l'estructura com del nombre de microorganismes, en funció de la metodologia analítica emprada. És per això que és més convenient abordar els estudis emprant diverses metodologies que aporten informacions complementàries.

- En el cas dels recomptes, factors com la font de carboni o la salinitat són d'importància cabdal. Pel que fa a la salinitat, se n'ha detectat una forta influència negativa sobre la població degradadora d'HAP, mentre que la població heteròtrofa i la degradadora d'hidrocarburs alifàtics no es veu afectada.
- En relació amb el substrat, s'ha provat que l'*n*-hexadecà és un substrat restrictiu en l'enumeració i aïllament de microorganismes degradadors d'hidrocarburs alifàtics, de manera que es recomana utilitzar una barreja d'hidrocarburs alifàtics com l'F1 emprada en aquest treball, procedent del fraccionament de un cru de petroli.
- En el cas de l'estructura, s'obtenen perfils molt diferents en funció de la tècnica utilitzada, (llibreria de clons, DGGE, aïllament en placa en diversos medis de cultiu o SIP).

REFERÈNCIES



- Ábalos A, Viñas M, Sabaté J, Manresa MA, Solanas AM. 2004. Enhanced biodegradation of Casablanca crude oil by a microbial consortium in presence of a rhamnolipid produced by *Pseudomonas aeruginosa* AT10. *Biodegradation* 15:249-260.
- Albaigés J, Albrecht P. 1979. Fingerprinting marine pollutant hydrocarbons by computerized gas chromatography-mass spectrometry. *Int J Environ Anal Chem* 6:71-190.
- Albaigés J, Bayona JM. 2003. El Fuel. A: Rey S. La Huella del fuel. Ensayos sobre el *Prestige*. A Coruña: Fundación Santiago Rey Fernández-Latorre. pp. 80-103.
- Alexander M. 1994. Biodegradation and Bioremediation. London: Academic Press.
- Alonso-Gutiérrez J, Costa MM, Figueras A, Albaigés J, Viñas M, Solanas AM, Novoa B. 2008. Alcanivorax strain detected among the cultured bacterial community from sediments affected by the Prestige oil-spill. *Mar Ecol Prog Ser* 362:25-36.
- Alonso-Gutiérrez J, Figueras A, Albaigés J, Jiménez N, Viñas M, Solanas AM, Novoa B. 2009. Bacterial communities from the shoreline environments ("Costa da Morte" (NW Spain)) affected by the Prestige Oil-spill. *Appl Environ Microbiol* 11:3407-3418.
- Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 25:3389-3402. [citad el 06 d'octubre de 2008] Disponible a URL:<http://www.ncbi.nlm.nih.gov/BLAST/>
- Alzaga R, Montuori P, Ortiz L, Bayona JM, Albaigés J. 2004. Fast solid-phase extraction-gas chromatography-mass spectrometry procedure for oil fingerprinting Application to the *Prestige* oil spill. *J Chromatogr A* 1025:133-138.
- Amann RI, Binder BJ, Olson RJ, Chisholm SW, Devereux R, Stahl DA. 1990. Combination of 16S rRNA-targeted oligonucleotide probes with flow cytometry for analyzing mixed microbial populations. *Appl Environ Microbiol* 56:1919-1925.
- Amann RI, Ludwig W, Schleifer K-H. 1995. Phylogenetic identification and in situ detection of individual microbial cells without cultivation. *Microbiol Rev* 59:143-169.
- Arey JS, Nelson RK, Plata, DL, Reddy CM. 2007. Disentangling Oil Weathering Using GCxGC. *Environ Sci Technol* 41:5738-5746.
- Atlas RM, Bartha R. 1992. Hydrocarbon biodegradation and oil spill bioremediation. A: Marshall KC ed. *Advances in Microbial Ecology* 12:287-338.
- Atlas RM. 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiol Rev* 45:180-209.
- Atlas RM. 1995. Petroleum biodegradation and oil spill bioremediation. *Mar Pollut Bull* 31:178-182.
- Austin B, Calomiris JJ, Walker JD, Colwell RR. 1977. Numerical taxonomy and ecology of petroleum-degrading bacteria. *Appl Environ Microbiol* 34:60-68.
- Bayona JM, Albaigés J, Solanas AM, Parés R, Garrigues P, Ewald M. 1986. Selective aerobic degradation of methyl-substituted polycyclic aromatic hydrocarbons in petroleum by pure microbial cultures. *Int J Environ Anal Chem* 23:289-303.
- BBSRC 2004. [citad el 06 de març de 2005]. Disponible a URL:<http://www.bbsrc.ac.uk>
- Beal R, Betts WB. 2000. Role of rhamnolipid biosurfactants in the uptake and mineralization of hexadecane in *Pseudomonas aeruginosa*. *J Appl Microbiol* 89:158-168.
- Bedoya JL, Alberti AP. 2004. Distribución e impacto de la marea negra del buque "Prestige" en la costa noroccidental gallega. *Xeografía: revista de xeografía, territorio e medio ambiente* 4: 117-140.
- Bennett B, Chen M, Brincat D, Gelin FJP, Larter SR. 2002. Fractionation of benzocarbazoles between source rocks and petroleum. *Org Geochem* 33:545-559.
- Bharati S, Patience R, Mills N, Hanesand T. 1997. A new North Sea oil-based standard for latroscan analysis. *Org Geochem* 26:49-57.

- Boehm PD, Douglas GS, Burns WA, Mankiewicz PJ, Page DS, Bence AE. 1997. Application of petroleum hydrocarbon chemical fingerprinting allocation techniques after the Exxon Valdez oil spill. *Mar Pollut Bull* 34:599-613.
- Boonchan S, Britz ML, Stanley GA. 1998. Surfactant-enhanced biodegradation of high molecular weight polycyclic aromatic hydrocarbons by *Stenotrophomonas maltophilia*. *Biotechnol Bioeng* 59:482-494.
- Boström CE, Gerde P, Hanberg A, Jernström B, Johansson C, Kyrklund T, Rannug A, Törnqvist M, Victorin K, Westerholm R. 2002. Cancer risk assessment, indicators, and guidelines for polycyclic aromatic hydrocarbons in the ambient air. *Environ Health Perspect* 110: 451-488.
- Boschker HTS, Nold SC, Wellsbury P, Bos D, de Graaf W, Pel R, Parkes RJ, Cappenberg TE. 1998. Direct linking of microbial populations to specific biogeochemical processes by ¹³C-labelling of biomarkers. *Nature* 392:801-804.
- Boukir A, Aries E, Guiliano M, Asia L, Doumenq P, Mille G. 2001. Subfractionation, characterization and photooxidation of crude oil resins. *Chemosphere* 43:279-286.
- Bragg JR, Prince RC, Harner EJ, Atlas RM. 1994. Effectiveness of bioremediation for the Exxon Valdez oil spill. *Nature* 368:413-418.
- Brakstad OG, Lodeng AGG. Microbial diversity during biodegradation of crude oil in seawater from the North Sea. *Microb Ecol* 49:94-103.
- Bruns A, Rohde M, Berthe-Corti L. 2001. *Muricauda ruestringensis* gen. nov., sp. nov., a facultatively anaerobic, appendaged bacterium from German North Sea intertidal sediment. *Int J Syst Evol Microbiol* 51:1997-2006
- Budzinski H, Raymond N, Nadalig T, Gilewicz M, Garrigues P, Bertrand JC, Caumette P. 1998. Aerobic biodegradation of alkylated aromatic hydrocarbons by a bacterial community. *Org Geochem* 28:337-348.
- Bulot J. 2003. A Costa da Morte. A: Rey S. La Huella del Fuel. Ensayos sobre el Prestige. A Coruña: Fundación Santiago Rey Fernández-Latorre pp. 80-103.
- Bustin SA. 2000. Absolute quantification of mRNA using real-time reverse transcription polymerase chain reaction assays. *J Molec Endocr* 15:169-193.
- Caldwell ME, Garrett RM, Prince RC, Suflita JM. Anaerobic biodegradation of long-chain n-alkanes under sulfate-reducing conditions. *Environ Sci Technol* 1998; 32:2191-2195.
- Cedre. 2002. Foto del Prestige durante el naufragio. [citat el 06 d'octubre de 2008]. Disponible a URL:<http://www.cedre.fr/es/accidentes/prestige/sauvetage.php>
- Cedre. 2003. Determinación del producto. [citat el 06 d'octubre de 2008]. Disponible a URL:<http://www.cedre.fr/es/accidentes/prestige/produit.php>
- Cerniglia CE. 1992. Biodegradation of polycyclic aromatic hydrocarbons. *Biodegradation* 3:351-368.
- Cerniglia CE. 1984. Microbial transformation of aromatic hydrocarbon. A: Atlas RM ed. Petroleum Microbiology. New York: Macmillan Publishing Company pp. 99-128.
- Chaerun SK, Tazaki K, Asada R, Kogure K. 2004. Bioremediation of coastal areas 5 years after the *Nakhodka* oil spill in the Sea of Japan: isolation and characterization of hydrocarbon-degrading bacteria. *Environ Int* 30:911-922.
- Chang Y-C, Stephen JR, Richter AP, Venosa AD, Brüggeman J, MacNaughton SJ, Kowalchuk GA, Haines JR, Kline E, White DC. 2000. Phylogenetic analysis of aerobic freshwater and marine enrichment cultures efficient in hydrocarbon degradation: effect of profiling method. *J Microbiol Methods* 40:19-31.
- Chung WK, King GM. 2001. Isolation, characterization, and polyaromatic hydrocarbon potential of aerobic bacteria from marine macrofaunal burrow sediments and description of *Lutibacterium anuloederans* gen. nov., sp. nov., and *Cycloclasticus spirillensus* sp. nov. *Appl Environ Microbiol* 67:5585-5592.

- Clark RC, MacLeod WD. 1997. Inputs, transport mechanisms and observed concentrations of petroleum in the marine environment. A Malins DC ed. *Effects of Petroleum on Arctic and Subarctic Marine Environments and Organisms*. London: Academic Press pp. 91-223.
- Clegg H, Wilkes H, Santamaria-Orozco D, Horsfield B. 1998. Influence of maturity on carbazole and benzocarbazole distributions in crude oils and source rocks from the Sonda de Campeche, Gulf of Mexico. *Org Geochem* 29:183-194.
- Cole JR, Chai B, Farris, RJ, Wang Q, Kulam SA, McGarrell DM, Garrity GM, Tiedje JM. 2005. The Ribosomal Database Project (RDP-II): sequences and tools for high-throughput rRNA analysis. *Nucleic Acids Res* 33: D294-D296. doi: 10.1093/nar/gki038. [Citat el 06 d'octubre de 2008] Disponible a URL:<http://rdp.cme.msu.edu/html/>
- Colthup NB, Daly LH, Wiberley SE. 1975. *Introduction to Infrared and Raman Spectroscopy*, second ed. New York: Academic Press.
- CONCAWE. 1981. A field guide to coastal oil spill control and cleanup techniques. Report No. 9/81, concawe, Den Haag, Netherlands.
- Connan J. 1984. *Biodegradation of crude oils in reservoirs*. London: Academic Press.
- Coulon F, McKew BA, Osborn AM, McGenity TJ, Timmis KN. 2007. Effects of temperature and biostimulation on oil-degrading microbial communities in temperate estuarine waters. *Environ Microbiol* 9:177-186.
- Cui Z, Lai Q, Dong C, Shao Z. 2008. Biodiversity of polycyclic aromatic hydrocarbon-degrading bacteria from deep sea sediments of the Middle Atlantic Ridge. *Environ Microbiol* 10:2138-2149.
- Cunico RL, Sheu EY, Mullins OC. 2004. Molecular weight measurement of UG8 asphaltene using APCI mass spectroscopy. *Petrol Sci Technol* 22:787-798.
- Daling PS, Faksness L-G, Hansen AB, Stout SA. 2002. Improved methodology for oil spill fingerprinting. *Environ Forensics* 3:263-278.
- Das P, Mukherjee S, Sen R. 2008. Improved bioavailability and biodegradation of a model polyaromatic hydrocarbon by a surfactant producing bacterium of marine origin. *Chemosphere* 72:1229-1234.
- De Zwart JMM, Nelisse PN, Kuenen JG. 1996. Isolation and characterisation of *Methylophaga sulfidovorans* sp. nov.: an obligately methylotrophic, aerobic, dimethylsulphide oxidising bacterium from a microbial mat. *FEMS Microbiol Ecol* 20:261-270.
- DeLong EF, Wickham GS, Pace NR. 1989. Phylogenetic stains: ribosomal RNA-based probes for the identification of single cells. *Science* 243: 1360-1363.
- Díez S, Jover E, Bayona JM, Albaigés J. 2007. Prestige oil spill III: fate of a heavy oil in the marine environment. *Environ Sci Technol* 41:3075-3082.
- Díez S, Sabaté J, Viñas M, Bayona JM, Solanas AM, Albaigés J. 2005. The Prestige oil spill. I. Biodegradation of a heavy fuel oil under simulated conditions. *Environ Toxicol Chem* 24:2203-2217.
- dos Santos VAPM, Yakimov MM, Timmis KN, Golyshin PN. 2008. Genomic insights into oil biodegradation in marine systems. A: Díaz E ed. *Microbial Biodegradation Genomics and Molecular Biology*. Norfolk, UK: Caister Academy Press. pp. 269-296.
- Dong W, Eichhorn P, Radajewski S, Schleheck D, Denger K, Knepper TP, Murrell JC, Cook AM. 2004. *Parvibaculum lavamentivorans* converts linear alkylbenzenesulphonate surfactant to sulphophenyl-carboxylates, α,β -unsaturated sulphophenylcarboxylates and sulphophenyldicarboxylates, which are degraded in communities. *J Appl Microbiol* 96: 630-640.
- Douglas GS, Bence AE, Prince RC, McMillen SJ, Butler EL. 1996. Environmental stability of selected petroleum hydrocarbon source and weathering ratios. *Environ Sci Technol* 30:2332-2339.

- Douglas GS, Owens EH, Hardenstine J, Prince RC. 2002. The OSSA II pipeline oil spill: The character and weathering of the spilled oil. *Spill Sci Technol Bull* 7:135-148.
- Dutta TK, Harayama S. 2001. Biodegradation of n-alkylcycloalkanes and n-alkylbenzenes via new pathways in *Alcanivorax* sp. strain MBIC 4326. *Appl Environ Microbiol* 67:1970-1974.
- Elmendorf DL, Haith CE, Douglas GS, Prince RC. 1994. Relative rates of biodegradation of substituted polycyclic aromatic hydrocarbons. A: Hinchee RE, Leeson A, Semprini L, Ong SK eds. *Bioremediation of Chlorinated and Polycyclic Aromatic Hydrocarbon Compounds*. Boca Raton, FL: Lewis Publishers pp. 188-202.
- Environment Canada. 2008. Tanker Spills database. [citat el 06 d'octubre de 2008]. Disponible a URL:<http://www.etc-cte.ec.gc.ca/databases/TankerSpills/Default.aspx>
- ESA. 2002. Prestige Tanker Disaster in Galicia (Spain): ASAR Wide Swath Acquisition. [citat el 06 d'octubre de 2008]. Disponible a URL:http://envisat.esa.int/asar_oil_spill/
- Euzéby JP. 2008. List of prokaryotic names with standing in nomenclature. [citat el 06 d'octubre de 2008]. Disponible a URL:<http://www.bacterio.cict.fr/number.html>
- EVOSTC. 2008 [citat el 06 d'octubre de 2008]. Disponible a URL:<http://www.evostc.state.ak.us>
- Ezra S, Feinstein S, Pelly I, Bauman D, Miloslavski I. 2000. Weathering of fuel oil spilt on the east Mediterranean coast, Ashdod, Israel. *Org Geochem* 31:1733-1741.
- Faksness L-G, Daling PS, Hansen AB. 2002. Round Robin study-oil spill identification. *Environ Forensics* 3:279-291.
- Fernández-Álvarez P, Vila J, Garrido JM, Grifoll M, Lema JM. 2006. Trials of bioremediation on a beach affected by the heavy oil spill of the Prestige". *J Hazard Mater* 137:1523-1531.
- Fernández-Martínez J, Pujalte MJ, García-Martínez J, Mata M, Garay E, Rodríguez-Valera F. 2003. Description of *Alcanivorax venustensis* sp. nov. and reclassification of *Fundibacter jadensis* DSM 12178^T (Bruns and Berthe-Corti 1999) as *Alcanivorax jadensis* comb. nov., members of the emended genus *Alcanivorax*. *Int J Syst Evol Microbiol* 53:331-338.
- Fingas MF, Fieldhouse B, Lane J, Mullin JV. 2000. Studies of water-in-oil emulsions: Long-term stability, oil properties, and emulsions formed at sea. *A Proceedings of the Twenty-third Arctic and Marine Oil Spill Program Technical Seminar*. Environment Canada. pp. 145-160.
- Fischer SG, Lerman LS. 1979. Length-independent separation of DNA restriction fragments in two-dimensional gel electrophoresis. *Cell* 16:191-200.
- Fleming JT, Sanseverino J, Sayler GS. 1993. Quantitative relationship between naphthalene catabolic gene frequency and expression in predicting PAH degradation in soils at town gas manufacturing sites. *Environ Sci Technol* 27:1068-1074.
- Floodgate G. 1984. The fate of petroleum in marine ecosystems. A:Atlas RM, ed. *Petroleum Microbiology*. New York:Macmillan Publishing Company pp. 355-398.
- Forsyth JV, Tsao YM, Blem RD. 1995. Bioremediation:when is augmentation needed? A: Hinchee RE. et al. eds. *Bioaugmentation for Site Remediation*. Columbus, OH: Battelle Press. pp. 1-14.
- French-McCay DP. 2004. Oil spill impact modeling: development and validation. *Environ Toxicol Chem* 23: 2441-2456.
- Gallego JR, González-Rojas E, Peláez AI, Sánchez J, García-Martínez MJ, Ortiz JE, Torres T, Llamas JF. 2006. Natural attenuation and bioremediation of *Prestige* fuel oil along the Atlantic coast of Galicia (Spain). *Org Geochem* 37:1869-1884.
- García L, Viada C, Moreno-Opo R, Alcalde A, González F. 2003. Impacto de la marea negra del *Prestige* sobre las aves marinas. Madrid:SEO/BirdLife.
- Garrett RM, Pickering IJ, Haith CE, Prince RC. 1998. Photooxidation of crude oils. *Environ Sci Technol* 32:3719-3723.
- Gauthier MJ, Lafay B, Christen R, Fernandez L, Acquaviva M, Bonin P, Bertrand J-C. 1992. *Marinobacter hydrocarbonoclasticus* gen. nov., sp. nov., a New, Extremely Halotolerant, Hydrocarbon-Degrading Marine Bacterium. *Int J Syst Bacteriol* 42: 568-576.

- Gentili AR, Cubitto MA, Ferrero M & Rodriguez MS. 2006. Bioremediation of crude oil polluted seawater by a hydrocarbondegrading bacterial strain immobilized on chitin and chitosan flakes. *Int Biodeter Biodegr* 57: 222-228.
- Gibb SW, Hatton AD. 2004. The occurrence and distribution of trimethylamine-N-oxide in Antarctic coastal waters. *Mar Chem* 91: 65-75.
- Gogotov IN, Khodakov RS. 2008. Surfactant production by the *Rhodococcus erythropolis* sH-5 bacterium grown on various carbon sources. *Appl Biochem Microbiol* 44:207-212.
- Goindi HK, Saini VS, Verma PS Adhikari DK. 2002. Dibenzothiophene desulfurization in hydrocarbon environment by *Staphylococcus* sp. resting cells. *Biotech Lett* 24:779-781.
- Goldstein RM, Mallory LM, Alexander M. 1985. Reasons for possible failure of inoculation to enhance biodegradation. *Appl Environ Microbiol* 50:977-983.
- Gray ND, Head IM. 2001. Linking genetic identity and function in communities of uncultured bacteria. *Environ Microbiol* 3:481-492.
- Griest WH, Tomkins BA, Epler JL, Rao TK. 1979. *Characterization of multialkylated polycyclic aromatic hydrocarbons in energy-related materials*. A: Jones PW i Leber P eds. Carcinogenesis, Vol. 4. New York: Raven-Press. pp.395-409.
- Grifoll M, Bayona JM, Solanas AM. 1992. Isolation and characterization of a fluorene-degrading bacterium: Identification of ring oxidation products and ring fission products. *Appl Environ Microbiol* 58:2910-2917.
- Guiesse B, Viklund G. 2005. Sequential UV-biological degradation of polycyclic aromatic hydrocarbons in two-phases partitioning bioreactors. *Chemosphere* 59:369-376
- Guo CL, Zhou HW, Wong YS, Tam NFY. 2005. Isolation of PAH-degrading bacteria from mangrove sediments and their biodegradation potential. *Mar Pollut Bull* 51:1054-1061.
- Hall TA. 1999. A user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl Acids Symp Ser* 41:95-98.
- Handelsman J. 2005. Metagenomics or megagenomics? *Nat Rev Microbiol* 3:457-458.
- Hara A, Syutsubo K, Harayama S. 2003. *Alcanivorax* which prevails in oil-contaminated seawater exhibits broad substrate specificity for alkane degradation. *Environ Microbiol* 5:746-753
- Harayama S, Kishira H, Kasai Y, Shutsubo K. 1999. Petroleum biodegradation in marine environments. *J Mol Microbiol Biotechnol* 1:63-70.
- Head IM, Jones DM, Röling WFM. 2006. Marine microorganisms make a meal of oil. *Nat Rev Microbiol* 4:173-182.
- Head IM, Saunders JR, Pickup RW. 1998. Microbial evolution, diversity and ecology: a decade of ribosomal RNA analysis of uncultivated microorganisms. *Microb Ecol* 35:1-21.
- Head IM, Swannell PJ. 1999. Bioremediation of petroleum hydrocarbon contaminants in marine habitats. *Curr Opin Biotechnol* 10:234-239.
- Heid CA, Stevens J, Livak KJ, Williams PM. Real time quantitative PCR. 1996. *Genome Res* 6:986-994.
- Heitkamp MA, Freeman JP, Miller DW, Cerniglia CE. 1988. Pyrene degradation by a *Mycobacterium* sp.: identification of ring oxidation and ring fission products. *Appl Environ Microbiol* 54:2556-2565.
- Hibbs DE, Gulliver JS, Voller VR, Chen Y-H. 1999. An aqueous concentration model for riverine spills. *J Hazard Mater* 64:37-53.
- Hostettler FD, Kvenvolden KA. Geochemical changes in crude-oil spilled from the Exxon-Valdez supertanker into Prince-William-Sound, Alaska. *Org Geochem* 1994; 21:927-936.
- Hughey CA, Rodgers RP, Marshall AG, Qian K, Robbins WK. 2002. Identification of acidic NSO compounds in crude oils of different geochemical origins by negative ion electrospray Fourier transform ion cyclotron resonance mass spectrometry. *Org Geochem* 33:743-759.

- Huu N, Denner EBM, Ha DTC, Wanner G, Stan-Lotter H. 1999. *Marinobacter aquaeolei* sp. nov., a halophilic bacterium isolated from a Vietnamese oilproducing well. *Int J Syst Bacteriol* 49:367-375.
- Ilori MO, Amobi CJ, Odocha AC. 2005. Factors affecting the production of oil degrading *Aeromonas* sp. Isolated from a typical environment. *Chemosphere* 61:985-992.
- Inoue K, Habe H, Yamane H, Omori T, Nojiri H. 2005. Diversity of carbazole-degrading bacteria having the *car* gene cluster: Isolation of a novel gram-positive carbazole-degrading bacterium. *FEMS Microbiol Lett* 245:145-153.
- Iwabuchi N, Sunairi M, Urai M, Itoh C, Anzai H, Nakajima M, Harayama S. 2002. Extracellular polysaccharides of *Rhodococcus rhodochrous* S-2 stimulate the degradation of aromatic components in crude oil by indigenous marine bacteria. *Appl Environ Microbiol* 68: 2337-2343.
- ITOPF. 2008. Statistics. [citat el 06 d'octubre de 2008]. Disponible a URL:<http://www.itopf.com/information-services/data-and-statistics/statistics/>
- Janvier M, Grimont PAD. 1995. The genus *Methylophaga*, a new line of descent within phylogenetic branch γ of proteobacteria. *Res Microbiol* 146:543-550.
- Jobson AM, Cook FD, Westlake DWS. 1974. Effect of amendments on the microbial utilization of oil applied to soil. *Appl Microbiol* 27:166-171.
- Johnsen AR, Karlson U. 2004. Evaluation of bacterial strategies to promote the bioavailability of polycyclic aromatic hydrocarbons (PAHs). *App Microbiol Biot* 63:452-459.
- Johnsen AR, Wick LY, Harms H. 2005. Principles of microbial PAH-degradation in soil. *Environ Pollut* 133:71-84.
- Jover E, Adahchour M, Bayona JM, Vreuls RJJ, Brinkman UAT. 2005. Characterization of lipids in complex samples using comprehensive two- dimensional gas chromatography with time-of-flight mass spectrometry. *J Chromatogr A* 1086:2-11.
- Juhasz AL, Naidu R. 2000. Bioremediation of high molecular weight polycyclic aromatic hydrocarbons:a review of the microbial degradation of benzo[a]pyrene. *Int Biodet Biodeg* 45:57-88.
- Kalyuzhnaya MG, Lapidus A, Ivanova N, Copeland AC, McHardy AC, Szeto E, Salamov A, Grigoriev IV, Suciú D, Levine SR, Markowitz VM, Rigoutsos I, Tringe SG, Bruce DC, Richardson PM, Lindstrom ME, Chistoserdova L. 2008. High-resolution metagenomics targets specific functional types in complex microbial communities. *Nat Biotechnol* 26:1029-1034.
- Kaplan CW, Kitts CL. 2004. Bacterial succession in a petroleum land treatment unit. *Appl Environ Microbiol* 70:1777-1786.
- Karlsen DA, Larter SR. 1991. Analysis of petroleum fractions by TLC-FID: applications to petroleum reservoir description. *Org Geochem* 17:603-607.
- Karpenko EV, Vil'danova-Martishin RI, Shcheglova NS, Pirog TP, Voloshina IN. 2006. The Prospects of Using Bacteria of the Genus *Rhodococcus* and Microbial Surfactants for the Degradation of Oil Pollutants. *Appl Biochem Microbiol* 42:156-159.
- Karrick NL. 1977. Alteration in petroleum resulting from physical-chemical and microbiological factors. A: Malins DC ed. Effects of petroleum on Arctic and Subarctic environments and organisms. A: Nature and fate of petroleum vol I. New York:Academic Press, Inc. pp. 225-299.
- Kasai Y, Kishira H, Sasaki T, Syutsubo K, Watanabe K, Harayama S. 2002. Predominant growth of *Alcanivorax* strains in oil-contaminated and nutrient-supplemented sea water. *Environ Microbiol* 4:141-147.
- Kasai Y, Kishira H, Syutsubo K, Harayama S. 2001. Molecular detection of marine bacterial populations on beaches contaminated by the *Nakhodka* tanker oil-spill accident. *Environ Microbiol* 3:246-255.

- Kästner M, Breuer-Jammali M, Mahro B. 1998. Impact of Inoculation Protocols, Salinity, and pH on the Degradation of Polycyclic Aromatic Hydrocarbons (PAHs) and Survival of PAH-Degrading Bacteria Introduced into Soil. *Appl Environ Microbiol* 64:359-362.
- Kim J-S, Crowley DE. 2007. Microbial Diversity in Natural Asphalts of the Rancho La Brea Tar Pits. *Appl Environ Microbiol* 73:4579-4591.
- Kim S, Stanford LA, Rodgers RP, Marshall AG, Walters CC, Qian K, Wenger LM, Mankiewicz P. 2005. Microbial alteration of the acidic and neutral polar NSO compounds revealed by Fourier transform ion cyclotron resonance mass spectrometry *Org Geochem* 36:1117-1134.
- Kodama Y, Stiknowati LI, Ueki A, Ueki K, Watanabe K. 2008. *Thalassospira tepidiphila* sp. nov., a polycyclic aromatic hydrocarbon-degrading bacterium isolated from seawater. *Int J Syst Evol Microbiol* 58:711-715;
- Kreuzer-Martin HW. 2007. Stable Isotope Probing: linking functional activity to specific members of microbial communities. *Soil Sci Soc Am J* 71:611-619.
- Lacotte DJ, Mille G, Acquaviva M, Bertrand J-C. 1995. In vitro biodegradation of Arabian Light 250 by a marine mixed culture using fertilizers as Nitrogen and Phosphorous sources. *Chemosphere* 31:4351-4358.
- LaDousse A, Tramier B. 1991. Results of 12 years of research in spilled oil bioremediation: inipol EAP 22. A *Proceedings of the 1991 International Oil Spill Conference*. Washington DC: American Petroleum Institute.
- Lane DJ. Sequencing. 1991. A Stackebrandt E, Goodfellow M eds. Nucleic acid techniques in bacterial systematics. Chichester: John Wiley & Sons pp. 115-175.
- Larson RA, Bott, TL, Hunt LL, Rogenmuser K. 1979. Photo-oxidation products of fuel oil and their antimicrobial activity. *Environ Sci Technol* 13:965-969.
- Larson RA, Hunt LL, Blankenship DW. 1977. Formation of toxic products from a #2 fuel oil by photo-oxidation. *Environ Sci Technol* 11:492-496.
- Lattuati A, Metzger P, Acquaviva Mb, Bertrand J-C, Largeau C. 2002. *n*-Alkane degradation by *Marinobacter hydrocarbonoclasticus* strain SP 17: long chain β -hydroxy acids as indicators of bacterial activity . *Org Geochem* 33:37-45.
- LaVoie EJ, Bedenko V, Hirota N, Hecht SS, Hoffmann D. 1979. *Comparison of the mutagenicity, tumor initiating activity and complete carcinogenicity of polynuclear aromatic hydrocarbons* A Jones PW i Leber P eds. Carcinogenesis, Vol 4. New York: Raven Press. pp.705-721.
- Lee DH, Zo YG, Kim SJ. Nonradioactive method to study genetic profiles of natural bacterial communities by PCR single-strand-conformation polymorphism. *Appl Environ Microbiol* 1996; 63:3112-3120.
- Lee K, Levy EM. 1989. Enhancement of the natural biode-gradation of condensate and crude oil on beaches of Atlantic Canada. A *Proceedings of 1989 International Oil Spill Conference*. Washington DC: American Petroleum Institute. pp. 479-486.
- Lee K, Merlin FX. 2003. Bioremediation of oil on shoreline environments: development of techniques and guidelines. *Pure Appl Chem* 71:161-171.
- Lee K, Tremblay GH, Gauthier J, Cobanli SE, Griffin M. 1997. Bioaugmentation and biostimulation: a paradox between laboratory and field results. A *Proceedings of 1997 International Oil Spill Conference*. Washington DC: American Petroleum Institute. pp. 697-705.
- Li M, Huanxin Y, Stasiuk LD, Fowler MG, Larter SR. 1997. Effect of maturity and petroleum expulsion on pyrrolic nitrogen compound yields and distributions in Duvernay Formation petroleum source rocks in Central Alberta, Canada. *Org Geochem* 16:731-744.
- Liesack W, Weyland H, Stackebrandt E. 1991. Potential risks of gene amplification by PCR as determined by 16S rDNA analysis of a mixed-culture of strict barophilic bacteria. *Microb Ecol* 21:1891-198.

- Lin C-W, Lin H-C, Lai C-Y. 2007. MTBE biodegradation and degrader microbial community dynamics in MTBE, BTEX, and heavy metal-contaminated water. *Int Biodet Biodeg* 59:97-102.
- Liu W-T, Marsh TL, Cheng H, Forney LJ. 1997. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA. *Appl Environ Microbiol* 63:4516-4522.
- Lueders T, Manefield M, Friedrich MW. 2004. Enhanced sensitivity of DNA- and rRNA-based stable isotope probing by fractionation and quantitative analysis of isopycnic centrifugation gradients. *Environ Microbiol* 6:73-78.
- MacNaughton SJ, Stephen JR, Venosa AD, Davis GA, Chang YJ, White DC. 1999. Microbial population changes during bioremediation of an experimental oil spill. *Appl Environ Microbiol* 65:3566-3574.
- Mahajan MC, Phale PS, Vaidyanathan CS. 1994. Evidence for the involvement of multiple pathways in the biodegradation of 1- and 2-methylnaphthalene by *Pseudomonas putida* CSV86. *Arch Microbiol* 161:425-33.
- Maki H, Sasaki T, Harayama S. 2001. Photo-oxidation of biodegraded crude oil and toxicity of the photo-oxidized products. *Chemosphere* 44:1145-1151.
- Maki H, Utsumi M, Koshikawa H, Hiwatari T, Kohata K, Uchiyama H, Suzuki M, Noguchi T, Yamasaki T, Furuki, M, Watanabe M. 2003. Intrinsic biodegradation of heavy oil from Nakhodka and the effect of exogenous fertilization at a coastal area of the Sea of Japan. *Water Air Soil Pollut* 145:123-138.
- Mallick S, Dutta TK. 2008. Kinetics of phenanthrene degradation by *Staphylococcus* sp. strain PN/Y involving 2-hydroxy-1-naphthoic acid in a novel metabolic pathway. *Proc Biochem* 43:1004-1008.
- Manefield M, Whiteley AS, Griffiths RI, Bailey MJ. 2002. RNA stable isotope probing, a novel means of linking microbial community function to phylogeny. *Appl Environ Microbiol* 68:5367-5373.
- Manz W, Amann R, Ludwig W, Vancanneyt M, Schleifer K-H. 1996. Application of a suite of 16S rRNA-specific oligonucleotide probes designed to investigate bacteria of the phylum cytophaga-flavobacter-bacteroides in the natural environment. *Microbiol* 142:1097-1106.
- Manz W, Amann R, Ludwig W, Wagner M, Schleifer K-H. 1992. Phylogenetic oligodeoxynucleotide probes for the major subclasses of Proteobacteria: problems and solutions. *Syst Appl Microbiol* 15:593-600.
- Marcial Gomes NC, Borges LR, Paranhos R, Pinto FN, Leda CS, Mendonça-Hagler, Smalla K. 2008. Exploring the diversity of bacterial communities in sediments of urban mangrove forests. *FEMS Microbiol Ecol* 66:96-109.
- Margesin R, Labbé D, Schinner F, Greer CW, Whyte LG. 2003. Characterization of Hydrocarbon-Degrading Microbial Populations in Contaminated and Pristine Alpine Soils. *Appl Environ Microbiol* 69:3085-3092.
- Margesin R, Hämmerle M, Tscherko D. 2007. Microbial activity and community composition during bioremediation of diesel-oil-contaminated soil: effects of hydrocarbon concentration, fertilizers, and incubation time. *Microb Ecol* 53: 259-269.
- Maruyama A, Ishiwata H, Kitamura K, Sunamara M, Fujita T, Matsuo M, Higashihara T. 2003. Dynamics of microbial populations and strong selection for *Cycloclasticus pugetii* following the Nakhodka oil spill. *Microb Ecol* 46:442-453.
- McKew BA, Coulon F, Osborn AM, Timmis KN, McGenity TJ. 2007a. Determining the identity and roles of oil-metabolising marine bacteria from the Thames Estuary, UK. *Environ Microbiol* 9:165-176.
- McKew BA, Coulon F, Yakimov MM, Denaro R, Genovese M, Smith CJ, Osborn AM, Timmis KN, McGenity TJ. 2007b. Efficacy of intervention strategies for bioremediation of crude oil in

- marine systems and effects on indigenous hydrocarbonoclastyc bacteria. *Environ Microbiol* 9:1562-1571.
- Medina-Bellver JI, Marín P, Delgado A, Rodríguez-Sánchez A, Reyes E, Ramos JL, Marqués S. 2005. Evidence for *in situ* crude oil biodegradation after the *Prestige* oil spill. *Environ Microbiol* 7:773-779.
- Minai-Tehrani D, Minoui S, Herfatmanesh A. Effect of Salinity on Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) of Heavy Crude Oil in Soil. 2009. *Bull Environ Con Tox* 82:179-184.
- Mondello L, Tranchida PQ, Dugo P, Dugo G. 2008. Comprehensive two-dimensional gas chromatography-mass spectrometry: A review. *Mass Spectrom Rev* 27:101-124.
- Morgan P, Watkinson RJ. 1994. Biodegradation of components of petroleum. A: Ratlege C ed. Biochemistry of microbial degradation. Dordrecht: Kluwer Academic Publishers pp. 1-31.
- Moter A, Göbel UB. 2000. Fluorescence in situ hybridization (FISH) for direct visualization of microorganisms. *J Microbiol Methods* 41:85-112.
- Muñoz D, Guiuliano M, Doumenq P, Jacquot F, Scherrer P, Mille G. 1997. Long term evolution of petroleum biomarkers in mangrove soil (Guadeloupe). *Mar Pol Bull* 34:868-874.
- Muyzer GE, DeWaal ED, Uitterlinden AG. 1993. Profiling of complex microbial populations by denaturing gradient gel electrophoresis analysis of polymerase chain reaction-amplified genes coding for 16S rRNA. *Appl Environ Microbiol* 59:695-700.
- Nakamura S, Sakamoto Y, Ishiyama M, Tanaka D, Kunii K, Kubo K, Sato C. 2007. Characterization of two oil-degrading bacterial groups in the Nakhodka oil spill. *Int Biodet Biodeg* 60:202-207.
- NAS. 2003. Oil in the Sea III: Inputs, fates, and effects. Washington DC: The National Academies Press.
- Nedashkovskaya OI, Kim SB, Han SK, Lysenko AM, Rohde M, Zhukova NV, Falsen E, Frolova GM, Mikhailov VV, Bae KS. 2003. *Mesonia algae* gen. nov., sp. nov., a novel marine bacterium of the family Flavobacteriaceae isolated from the green alga *Acrosiphonia sonderi* (Kütz) Kornm. *Int J Syst Evol Microb* 53:1967-1971
- Neef A. 1997. *Anwendung der in situ Einzelzell-Identifizierung von Bakterien zur Populationsanalyse in komplexen mikrobiellen Biozönosen*. Tesi doctoral. Technische Universität München.
- Neufeld JD, Vohra J, Dumont MG, Lueders T, Manefield M, Friedrich MW Murrell JC. 2007a. DNA stable-isotope probing. *Nat Protocols* 2:860-866.
- Neufeld JD, Schäfer H, Cox MJ, Boden R, McDonald IR, Murrell C. 2007b. Stable-isotope probing implicates *Methylophaga* spp and novel Gammaproteobacteria in marine methanol and methylamine metabolism. *ISME J* 1:480-491.
- Ní Chadhain S, Norman RS, Pesce KV, Kukor JJ, Zylstra GJ. 2006. Microbial dioxygenase gene population shifts during polycyclic aromatic hydrocarbon biodegradation. *Appl Environ Microbiol* 72:4078-4087.
- NOAA. 1992. Oil Spill Case Histories 1967-1991. Report HMRAD 92-11. NOAA. Hazardous Materials Response and Assessment Division, Seattle.
- Noordman, W.H., Janssen, D.B. 2002. Rhamnolipid stimulates uptake of hydrophobic compounds by *Pseudomonas aeruginosa*. *Appl Environ Microbiol* 68:4502-4508.
- Ogino A, Koshikawa H, Nakahara T, Uchiyama H. 2001. Succession of microbial communities during a biostimulation process as evaluated by DGGE and clone library analyses. *J Appl Microbiol* 91:625-635.
- Outdot J, Merlin FX, Pinvidic P. 1998. Weathering rates of oil components in a bioremediation experiment in estuarine sediments. *Mar Environ Res* 45:113-125.
- Outdot J. 2000. Biodégradabilité du fuel de l'Erika. *CR Acad Sci Paris Life Sci* 323:945-950.

- Pastor D, Sanchez J, Porte C, Albaigés J. 2001. The Aegean Sea oil spill in the Galicia Coast (NW Spain). I. Distribution and fate of the crude oil and combustion products in subtidal sediments. *Mar Pol Bull* 42:895-904.
- Payne JR, Phillips CR. 1985. Photo-chemistry of petroleum in water. *Environ Sci Technol* 19:569-579.
- Pedros-Alió C. 2006. Marine microbial diversity: can it be determined? *Trends Microbiol* 14:257-263.
- Peters KE, Moldowan JM. 1993. The Biomarker Guide, Interpreting Molecular Fossils in Petroleum and Ancient Sediments. Englewood Cliffs, NJ, USA: Prentice Hall.
- Peters KE, Moldowan J, McCaffrey MA, Fago FJ. 1996. Selective biodegradation of extended hopanes to 25-norhopanes in petroleum reservoirs. Insights from molecular mechanics. *Org Geochem* 1996; 24, 765-783.
- Pirnik MP, Atlas RM, Bartha R. 1974. Hydrocarbon metabolism by *Brevibacterium erythrogenes*. Normal and branched alkanes. *J Bacteriol* 119:868-78.
- Poston TM, Bean RM, Kalkwarf, DR, Thomas BL, Clark, ML, Killand BW. 1988. Photo-oxidation products of smoke generator fuel (SGF) no. 2 fog oil and toxicity to *Hyalloella azteca*. *Environ Toxicol Chem* 7:753-762.
- Powell SM, Bowman JP, Snape I, Stark J. 1993. Microbial community variation in pristine and polluted near shore antarctic sediments. *FEMS Microbiol Ecol* 45:135-145
- Prince RC, Bragg JR. 1997. Shoreline bioremediation following the Exxon Valdez oil spill in Alaska. *Bioremediation J* 1:97-104.
- Prince RC, Elmendorf DL, Lute JR, Hsu CS, Haith CE, Senius JD, Dechert GJ, Douglas GS, Butler EL. 1994. 17 α (H),218(H)-Hopane as a conserved internal marker for estimating the biodegradation of crude-oil. *Environ Sci Technol* 28:142-145.
- Prince RC, Garrett RM, Bare RE, Grossman MJ, Townsend T, Suflita JM, Lee K, Owens EH, Serty GA, Braddock JF, Lindstrom JE, Lessard RR. 2003. The roles of photooxidation and biodegradation in long-term weathering of crude and heavy fuel oils. *Spill Sci Technol Bull* 8:145-156.
- Pritchard PH, Costa CF. 1991. EPA's Alaska oil spill bioremediation project. *Environ Sci Technol* 25:372-379.
- Quatrini P, Scaglione G, de Pasquale C, Riela S, Puglia AM. 2008. Isolation of Gram-positive n-alkane degraders from a hydrocarbon-contaminated Mediterranean shoreline. *J Appl Microbiol* 104:251-259.
- Radajewski S, Ineson P, Parekh NR, Murrell JC. 2000. Stable-isotope probing as a tool in microbial ecology. *Nature* 103:646-649.
- Rahman PKSM, Gakpe E. 2008. Production, characterisation and applications of biosurfactants-review. *Biotechnology* 7:360-370.
- Riesenfeld CS, Schloss PD, Handelsman J. 2004. Metagenomics: Genomic Analysis of Microbial Communities. *Annu Rev Genet* 38:525-552.
- Rivas R, Sánchez-Márquez S, Mateos PF, Martínez-Molina E, Velázquez E. 2005. *Marteella mediterranea* gen. nov., sp. nov., a novel α -proteobacterium isolated from a subterranean saline lake. *Int J Syst Evol Microbiol* 55:955-959.
- Rodgers RP, Marshall AG. 2007. Advanced characterization of petroleum-derived materials by fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS). A Mullins OC, Sheu EY, Hammami A, Marshall AG, eds. *Asphaltenes, Heavy Oils and Petroleomics*. New York: Springer pp. 63-93.
- Rodríguez S. 2007. Ciencia y Prestige. La investigación desarrollada a raíz del accidente del Prestige. Vigo: Oficina Técnica de Vertidos Marinos.

- Röling WFM, Milner MG, Jones DM, Fratepietro F, Swannell RJP, Daniel F, Head IM. 2004. Bacterial community dynamics and hydrocarbon degradation during a field-scale evaluation of bioremediation on a mudflat beach contaminated with buried oil. *Appl Environ Microbiol* 70:2603-2613.
- Röling WFM, Milner MG, Jones DM, Lee K, Daniel F, Swannell RJP, Head IM. 2002. Robust hydrocarbon degradation and dynamics of bacterial communities during nutrient-enhanced oil spill bioremediation. *Appl Environ Microbiol* 68:5537-5548.
- Ron EZ, Rosenberg E. 2002. Biosurfactants and oil bioremediation. *Curr Opin Biotechnol* 13: 249-252.
- Rontani JF, Bosser-Joulak F, Rambeloarisoa E, Bertrand JC, Giusti G, Faure R. 1985. Analytical study of ASTHART crude oil: asphaltenes biodegradation. *Chemosphere* 14:1413-1422.
- Tjessem K, Aaberg A. 1983. Photochemical transformation and degradation of petroleum residue in the marine environment. *Chemosphere* 12:1373-1394.
- Rontani JF, Gilewicz MJ, Michotey VD, Zheng TL, Bonin PC, Bertrand JC. 1997. Aerobic and anaerobic metabolism of 6,10,14-trimethylpentadecan-2-one by a denitrifying bacterium isolated from marine sediments. *Appl Environ Microbiol* 63:636-643.
- Roux KH. Optimization and troubleshooting in PCR. *Genome Res* 1995; 4:S185-S194
- Rowland S, Donkin P, Smith E, Wraige E. 2001. Aromatic hydrocarbon “humps” in the marine environment: unrecognized toxins? *Environ Sci Technol* 35:2640-2644.
- Rowland SJ, Alexander R, Kagi RI, Jones DM, Douglas AG. 1986. Microbial-degradation of aromatic components of crude oils - a comparison of laboratory and field observations. *Org Geochem* 9:153-161.
- Sabaté J, Viñas M, Bayona JM, Solanas AM. 2003. Isolation and taxonomic and catabolic characterization of a 3,6-dimethylphenanthrene-utilizing strain of *Sphingomonas* sp. *Can J Microbiol* 49:120-129.
- Sabaté J, Viñas M, Solanas AM. 2004. Laboratory-scale bioremediation experiments on hydrocarbon-contaminated soils. *Int Biodet Biodeg* 54:19-25
- Schaeffer TL, Cantwell, SG Brown JL, Watt DS, Fall RR. 1979. Microbial growth on hydrocarbons: terminal branching inhibits biodegradation. *Appl Environ Microbiol* 38:742-746.
- Schaub TM, Hendrickson CL, Quinn JP, Rodgers RP, Marshall AG. 2005. Instrumentation and Method for Ultrahigh Resolution Field Desorption Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry of Nonpolar Species. *Anal Chem* 77:1317-1324.
- Schleheck D, Tindall BJ, Rosselló-Mora R, Cook AM. 2004. *Parvibaculum lavamentivorans* gen. nov., sp. nov., a novel heterotroph that initiates catabolism of linear alkylbenzenesulfonate. *Int J Syst Evol Microbiol* 54:1489-1497.
- Schwermer CU, Lavik G, Abed RMM, Dunsmore B, Ferdelman TG, Stoodley P, Gieseke A, de Beer D. 2008. Impact of Nitrate on the Structure and Function of Bacterial Biofilm Communities in Pipelines Used for Injection of Seawater into Oil Fields. *Appl Environ Microbiol* 74:2841-2851.
- Shyu C, Soule T, Bent SJ, Foster JA, Forney LJ. 2007. MiCA: A Web-Based Tool for the Analysis of Microbial Communities Based on Terminal-Restriction Fragment Length Polymorphisms of 16S and 18S rRNA Genes. *J Microbiol Ecol* 53:562-570. URL:<http://mica.ibest.uidaho.edu/digest.php>
- Siefert WM, Moldowan JM. 1978. Applications of steranes, terpanes, and non-aromatics to the maturation, migration, and source of crude oils. *Geochimica et Cosmochimica Acta* 42:79-96.
- Sigiura K, Ishihara M, Shimauchi T, Harayama S. 1997. Physicochemical Properties and Biodegradability of Crude Oil. *Environ Sci Technol* 31:45-51.
- Singer ME, Finnerty WR. 1984. Microbial metabolism of straight-chain and branched alkanes. A Atlas RM ed. *Petroleum Microbiology*. New York: Macmillan Publishing Company pp. 355-398.

- Singleton DR, Powell SN, Sangaiah R, Gold A, Ball LM, Aitken MD. 2005. Stable-Isotope Probing of Bacteria Capable of Degrading Salicylate, Naphthalene, or Phenanthrene in a Bioreactor Treating Contaminated Soil. *Appl Environ Microbiol* 71:1202-1209.
- Sipilä TP, Keskinen AK, Akerman ML, Fortelius C, Haahtela K, Yrjälä K. 2008. High aromatic ring-cleavage diversity in birch rhizosphere: PAH treatment-specific changes of I.E.3 group extradiol dioxygenases and 16S rRNA bacterial communities in soil. *ISME J* 2:968-981.
- Snape I, Ferguson SH, McA Harvey P, Riddle MJ. 2006. Investigation of evaporation and biodegradation of fuel spills in Antarctica: II-Extent of natural attenuation at Casey Station. *Chemosphere* 63:89-98.
- Solanas AM, Parés R, Bayona JM, Albaigés J. 1984. Degradation of aromatic petroleum hydrocarbons by pure microbial cultures. *Chemosphere* 13:593-601.
- Sorokin DY, Tourova TP, Muyzer G. 2005. *Citricella thiooxidans* gen. nov., sp. nov., a novel lithoheterotrophic sulfur-oxidizing bacterium from the Black Sea. *Syst Appl Microbiol* 28:679-687.
- Speight JG. 1991. Classification. The chemistry and technology of petroleum. 2a ed. New York: Marcel Dekker pp. 192-227.
- Staley JT, Konopka A. 1985. Measurements of in situ activities of nonphotosynthetic microorganisms in aquatic and Terrestrial Habitats. *Ann Rev Microbiol* 39: 321-346.
- Stephen JR, Chang YJ, Gan YD, Peacock A, Pfiffner SM, Barcelona MJ, White DC, MacNaughton SJ. 1999. Microbial characterisation of a JP-4 fuel contaminated site using a combined lipid biomarker/polymerase chain reaction-denaturing gradient gel electrophoresis (PCR-DGGE)-based approach. *Environ Microbiol* 1:231-241.
- Sutiknowati LI. 2007. Hydrocarbon degrading bacteria: isolation and identification. *Makara sains* 11: 98-103.
- Suzuki MT, Giovanni SJ. 1996. Bias caused by template annealing in the amplification of mixtures of 16S rRNA genes by PCR. *Appl Environ Microbiol* 62: 625-630.
- Swannell PJ, Croft BC Grant AL, Lee K. 1995. Evaluation of Bioremediation Agents in Beach Microcosms. *Spill Sci Technol Bull* 2:151-159.
- Swannell RPJ, Lee K, McDonagh M. Field evaluations of marine oil spill bioremediation. 1996. *Microbiol Rev* 60:342-365.
- Tam NFY, Guo CL, Yau WY, Wong YS. 2002. Preliminary study on biodegradation of phenanthrene by bacteria isolated from mangrove sediments in Hong Kong. *Mar Pollut Bull* 45:316-324.
- Tamura K, Dudley J, Nei M, Kumar S. 2007. Molecular evolutionary genetics analysis (MEGA) software version 4.0. *Mol Biol Evol* 24:1596-1599.
- Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673-4680. [Citat el 06 d'octubre de 2008] Disponible a URL:<http://www.ebi.ac.uk/clustalw/index.html>.
- Throne-Holst M, Markussen S, Winnberg A, Ellingsen TE, Kotlar HK, Zotchev SB. 2006. Utilization of *n*-alkanes by a newly isolated strain of *Acinetobacter venetianus*: the role of two AlkB-type alkane hydroxylases. *Appl Microbiol Biotechnol* 72: 353-360.
- Tissot BP, Welte DH. 1978. Petroleum formation and occurrence. Berlin: Springer-Verlag.
- Torsvik V, Øvreås L, Thingstad TF. 2002. Prokaryotic diversity-magnitude, dynamics, and controlling factors. *Science* 296:1064-1066.
- Tsutsumi H, Kono M, Takai K, Manabe T. 2000. Bioremediation on the shore after an oil spill from the *Nakhodka* in the Sea of Japan. III. Field test of a bioremediation agent with microbiological cultures for the treatment of an oil spill. *Mar Pollut Bull* 40:320-324.
- USEPA. 2004. USEPA Oil spills emergency management. [citat el 06 d'octubre de 2008] Disponible a URL:<http://www.epa.gov/oilspill/refined.htm>

- Van Beilen JB, Funhoff EG, van Loon A, Just A, Kaysser L, Bouza M, Holtackers R, Röthlisberger M, Li Z, Witholt B. 2006. Cytochrome P450 alkane hydroxylases of the CYP153 family are common in alkane-degrading Eubacteria lacking integral membrane alkane hydroxylases. *Appl Environ Microbiol* 72:59-65.
- Van Hamme, JD, Singh A, Ward OP. Recent advances in petroleum microbiology. 2003. *Microbiol Mol Biol Rev* 67:503-549.
- Vaneechoutte M, De Beenhouwer H, Claeys G, Verschraegen G, De Rouck A, Paepe N, Elaichouni A, Portaels F. 1993. Identification of *Mycobacterium* species by using amplified ribosomal DNA restriction analysis. *J Clin Microbiol* 8:2061-2065.
- Velázquez F, de Lorenzo V, Valls M. 2006. The *m*-xylene biodegradation capacity of *Pseudomonas putida* mt-2 is submitted to adaptation to abiotic stresses: evidence from expression profiling of *xyl* genes. *Environ Microbiol* 8:591-602.
- Venkateswaran K, Hoaki T, Kato M, Maruyama T. 1995. Microbial degradation of resins fractionated from Arabian light crude oil. *Can J Microbiol* 41:418-424.
- Venosa AD, Suidan MT, Wrenn BA, Strohmeier KL, Haines JR, Eberhart BL, King DW, Holder E. 1996. Bioremediation of experimental oil spill on the shoreline of Delaware Bay. *Environ Sci Technol* 30:1764-1775.
- Venosa AD, Zhu X. 2003. Biodegradation of crude oil contaminating marine shorelines and freshwater wetlands. *Spill Sci Technol Bull* 8:163-178.
- Venosa AD. 1998. Oil spill bioremediation on coastal shorelines: a critique. A: Sikdar SK, Irvine RI eds. Bioremediation: principles and practice. A Bioremediation Technologies, vol. III. Lancaster PA: Technomic. pp. 259-301.
- Viñas M, Grifoll M, Sabaté J, Solanas AM. 2002 Biodegradation of a crude oil by three microbial consortia of different origins and metabolic capabilities. *J Ind Microbiol Biotechnol* 28:252-260.
- Viñas M, Sabaté J, Solanas AM. 2005a. Culture-dependent and -independent approaches establish the complexity of a PAH-degrading microbial consortium. *Can J Microbiol* 51:897-909.
- Viñas M, Sabaté J, Solanas AM. 2005b. Bacterial community dynamics and PAHs degradation during bioremediation of a heavily creosote-contaminated soil. *Appl Env Microbiol* 71:7008-7018.
- Wang B, Lai Q; Cui Z, Tan T, Shao Z. 2008. A pyrene-degrading consortium from deep-sea sediment of the West Pacific and its key member *Cycloclasticus* sp. P1. *Environ Microbiol* 10:1948-1963
- Wang Z, Fingas MF, Page DS. 1999. Oil spill identification. *J Chromatogr A* 843:369-411.
- Wang Z, Fingas MF, Blenkisnopp S, Sergy G, Landriault M, Sigouin L, Foght J, Semple K, Westlake DWS. 1998. Comparison of oil composition changes due to biodegradation and physical weathering in different oils. *J Chromatogr A* 809:89-107.
- Wang Z, Fingas MF, Sigouin L, Owens EH. 2001. Fate and persistence of long-term spilled 'Metula' oil in the marine salt marsh: degradation of biomarkers. A *Proceedings of the 2001 International Oil Spill Conference*. Washington DC: American Petroleum Institute. pp. 115-125.
- Wang Z, Fingas MF. 1995. Use of methyl dibenzothiophenes as markers for differentiation and source identification of crude and weathered oils. *Environ Sci Technol* 29:2842-2849.
- Wang ZD, Fingas MF. 2003. Development of oil hydrocarbon fingerprinting and identification techniques. *Mar Pollut Bull* 47:423-452.
- Ward DM, Weller R, Bateson MM. 1990. 16S rRNA sequences reveal numerous uncultured microorganisms in a natural community. *Nature* 345, 63- 65.
- Weisburg WG, Barns SM, Pelletier DA, Lane DJ. 1991. 16S ribosomal DNA amplification for phylogenetic study. *J Bacteriol* 173:697-703.

- Weissenfels WD, Beyer M, Klein J, Rehm HJ. 1991. Microbial metabolism of fluoranthene: isolation and identification of ring fission products. *Appl Microbiol Biotechnol* 34:528-535.
- Wenderoth DF, Rosenbrock P, Abraham WR, Pieper DH, Höfle MG. 2003. Bacterial community dynamics during biostimulation and bioaugmentation experiments aiming at chlorobenzene degradation in groundwater. *Microb Ecol* 46:161-176.
- Wang L-M, Liu P-W G, Ma C-C, Cheng S-S. 2008. Application of biosurfactants, rhamnolipid, and surfactin, for enhanced biodegradation of diesel-contaminated water and soil. *J Hazard Mater* 151:155-163.
- Whiteley AS, Thomson B, Lueders T, Manefield M. RNA stable-isotope probing. *Nature protocols* 2007; 2:838-844.
- Whitman WB, Coleman DC, Wiebe WJ. 1998. Prokaryotes: the unseen majority. *Proc Natl Acad Sci USA* 95:6578-6583.
- Whyte LG, Hawari J, Zhou E, Bourbonnière L, Inniss WE, Greer CW. 1998. Biodegradation of variable-chain-length alkanes at low temperatures by a psychrotrophic *Rhodococcus* sp. *Appl Environ Microbiol* 64:2578-2584
- Whyte LG, Smits THM, Labbé D, Witholt B, Greer CW, van Beilen JB. 2002. Gene cloning and characterization of multiple alkane hydrolase systems in *Rhodococcus* strains Q15 and NRRL B-16531. *Appl Environ Microbiol* 68:5933-5942
- Wilson SC, Jones KC. 1991. Bioremediation of soils contaminated with polynuclear aromatic hydrocarbons PAHs: A Review. *Environ Pollut* 81:229-249.
- Winniford RS, Bersohn, M. 1962. Structure of petroleum *asphaltenes* as indicated by proton magnetic resonance. 1962 [citat el 06 d'octubre de 2008]. Disponible a URL: http://www.anl.gov/PCS/acsfuel/preprint%20archive/Files/Merge/Vol-06_2-0003.pdf
- Wrenn BA, Venosa AD. 1996. Selective enumeration of aromatic and aliphatic hydrocarbon-degrading bacteria by a most-probable-number procedure. *Can J Microbiol* 42:252-258.
- Yakimov MM, Golyshin PN, Lang S, Moore ERB, Abraham WR, Lunsdorf H, Timmis KH. 1998. *Alcanivorax borkumensis* gen. nov., sp. nov., a new, hydrocarbon-degrading and surfactant-producing marine bacterium. *Int J Syst Bacteriol* 48:339-348.
- Yakimov MM, Gentile G, Bruni V, Capello S, D'Auria G, Golyshin PN, Giuliano L. 2004. Crude oil-induced structural shift of coastal bacterial communities of rod bay (Terra Nova Bay, Ross Sea, Antarctica) and characterization of cultured cold-adapted hydrocarbonoclastic bacteria. *FEMS Microb Ecol* 49:412-432.
- Yakimov MM, Denaro R, Genovese M, Cappello S, D'Auria G, Chernikova, TN Kenneth N. Timmis KN, Golyshin PN, Giuliano L. 2005. Natural microbial diversity in superficial sediments of Milazzo Harbor (Sicily) and community successions during microcosm enrichment with various hydrocarbons. *Environ Microbiol* 7:1426-1441
- Yakimov MM, Timmis KN, Golyshin PN. 2007. Obligate oil-degrading marine bacteria. *Environ Biotechnol* 18:257-266.
- Yang S-J, Cho J-C. 2008. *Gaetbulibacter marinus* sp. nov., isolated from coastal seawater, and emended description of the genus *Gaetbulibacter*. *Int J Syst Evol Microbiol* 58:315-318.
- Yu Z, Morrisson M. 2004. Comparisons of different hypervariable regions of *rrs* genes for use in fingerprinting of microbial communities by PCR-Denaturing Gradient Gel Electrophoresis. *Appl Environ Microbiol* 70:4800-4806.
- Yuste L, Corbella ME, Turiegano MJ, Karlson U, Puyet A, Rojo F. 2000. Characterization of bacterial strains able to grow on high molecular mass residues from crude oil processing. *FEMS Microbiol Ecol* 32:69-75
- Zhou HW, Guo CL, Wong YS, Tam NF. 2006. Genetic diversity of dioxygenase genes in polycyclic aromatic hydrocarbon-degrading bacteria isolated from mangrove sediments. *FEMS Microbiol Lett* 262:148-157.

- Zhu X, Venosa AD, Suidan MT. 2004. Literature review on the use of commercial bioremediation agents for cleanup of oil-contaminated estuarine environments. EPA/600/R-04/075.
- Zobell CE. 1946. Action of microorganisms on hydrocarbons. *Bacteriology Review* 10:1-49.
- Zucchi L, Angiolini S, Borin L, Brusetti N, Dietrich C, Gigliotti P, Barbieri P, Sorlini C, Daffonchio D. 2003. Response of bacterial community during bioremediation of an oil-polluted soil. *J Appl Microbiol* 94:248-257.

D'entre els nombrosos contaminants que arriben al mar, el petroli és un dels que més atenció capta. Així mateix, si bé els accidents de petroliers no són la principal font de contaminació per hidrocarburs en el medi marí, són la que més interès suscita.

El darrer dels accidents de gran magnitud esdevingut a les costes peninsulars fou el del *Prestige*, un buc monocasc, construït el 1976, que transportava fuel pesant. La nau s'accidentà el 13 de novembre del 2002 davant les costes de Galícia i acabà enfonsant-se uns dies més tard a unes 135 milles de la costa, alliberant més de 60.000 tones de producte.

La marea negra vingué acompanyada d'un fort impacte ambiental, social, econòmic i mediàtic. A més, arran de l'accident, la Unió Europea adoptà diverses mesures, entre les quals destaca la prohibició del transport de petrolis pesants en bucs monocasc. A nivell estatal, Ministerio de Ciencia i Tecnologia endegà dues línies d'ajuts destinats a mesurar-ne els efectes a curt i llarg termini.

El conjunt d'estudis, dins el qual s'inclou el present treball, va pretendre conèixer el destí tant del buc enfonsat com del fuel que se n'alliberà, avaluar els efectes del vessament als recursos pesquers i ecològics i proporcionar eines per millorar la gestió d'hipotètics accidents futurs.

