DOCKET	
08-AFC-8	
DATE	APR 19 2010
RECD.	APR 21 2010

19 April 2010

California Energy Commission docket@energy.state.ca.us
Rod Jones, Project Manager
Systems Assessment & Facility Siting Division
James D. Boyd, Commissioner, Presiding Member
Jeffrey Byron, Commissioner, Associate Member
Raoul Renaud, Hearing Officer
1516 Ninth Street, MS-15
Sacramento, CA 95814
Phone: 916-654-5191
RJones@energy.state.ca.us PAO@energy.state.ca.us
HYDROGENENERGY-request@listserver.energy.ca.gov

RE: Hydrogen Energy California (HECA) Permanency and Impacts on Bacteria #2
Docket Number: 08-AFC-8 Hydrogen Energy Power Plant Licensing

Dear Sirs;

On 14 April, 2010, I sent an email to the Commission through Mr. Rod Jones, Project Manager, about the concern with the claim by HECA of the permanency of the HECA process and the concern about the impact of the HECA process on bacteria, if the HECA process worked as specified and if it failed. (Exhibit A) I have not received an acknowledgement of the receipt of the email.

I am most concerned about the claim that the HECA process will result in the permanent storage of CO2 in the ground of the Elk Hills oil field. Nothing is permanent. This claim is impossible to believe. (See Exhibit A)

How would the HECA process impact the micro-organisms/bacteria (Zoogloea sp.) that are living in the ground in crude oil and are known to digest crude oil and are known to purify soil contaminated by polycyclic aromatic hydrocarbons? How will the increase in ground-born CO2 impact these bacteria?

I'm referring to the bacteria that live in crude oil deposits thousands of feet down and break down lighter hydrocarbons into methane and the crude oil eating bacteria that live near the surface that are used to treat hazardous waste spills. How would these bacteria be affected by the placement of liquid CO2 into the interstitial spaces thousands of feet down?

Knowledge about the range of ecosystem services performed by bacteria is still unfolding. Some say that the oil companies would rather people not know that they are exterminating rare forms of life in the process of bringing them their gasoline.

Others see the value of these bacteria and are investigating the effects. Some discussion of the value of bacteria in crude oil is presented in the attached five files. (Exhibit B, Exhibit C, Exhibit D, Exhibit E, and Exhibit F)

"One group of microorganisms that is able to live from the reduction of the chemically quite inert sulfate by producing aggressive hydrogen sulfide is the guild of sulfate reducing prokaryotes.
In this context it is appropriate to use the term prokaryotes, since sulfate-reducers are found in the domain Bacteria, as well as within the domain Archaea.
The history of microbial sulfate reduction is quite long. There is good isotopic (Shen 2001) and molecular (Wagner 1998) evidence that microbial dissimilatory sulfate reduction is a very ancient process, at least in our perception. It is most likely older than 3.47 billion years. This makes sulfate-respiration an evolutionary very successful metabolic pathway, a pathway that enables the sulfate reducer to gain energy in environment where the redox potential is low, energy conservation is hard and growth is slow. This successful reduction of the highest oxidized state of sulfur in nature and the participation in the last steps of microbial decomposition near the endpoint of possible energy conservation is a reason for the widespread distribution of sulfate-reducers in various environments on planet earth." (Michael Dieter Klein, 2004, Lehrstuhl für Mikrobiologie der Technischen Universität München - Dissimilatory (bi-) sulfite reductase as a marker for phylogenetic and ecological studies of sulfate-reducing prokaryotes) (http://tumb1.biblio.tu-muenchen.de/publ/diss/ww/2005/klein.pdf)(7.5 MB)

The questions must include Archaea and their existence in crude oil and the impacts of the HECA process on the Archaea and their ecosystem services.
http://www.ucmp.berkeley.edu/archaea/archaea.html

Respectfully submitted,

Mr. Ara Marderosian
P.O. Box 988
Weldon, CA 93283
(760) 378-4574
ara@sequoiaforestkeeper.org

Exhibit A – 100414-3. CEC HECA
Exhibit B – 100412-7.Crude Oil
Exhibit C – 100412-7.Bacteria
Exhibit D – 100416-6.4.Crude Oil
Exhibit E – 100416-5.Crude Oil
Exhibit F – 100415-6.UC Berkeley

14 April 2010

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1516 Ninth Street, MS-15
Sacramento, CA 95814
Phone: 916-654-5191
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Hydrogen Energy California (HECA) Permanency and Impact on Bacteria
Docket Number: 08-AFC-8 Hydrogen Energy Power Plant Licensing

Dear Sirs;

I am most concerned about the claim that the HECA process will result in the permanent storage of CO2 in the ground of the Elk Hills oil field. Nothing is permanent. I find this claim impossible to believe.

The following statements about the permanence of the CO2 storage in Elk Hills were made with absolute surety by HECA spokespersons:

George Minter, said, “CO2 permanent sequestration in Elk Hills is not a question – there is 40 years of history.”

Michael Cox, Hydrogen Energy California, said, “[Elk Hills is] the most secure container in the world” “an impervious barrier” and “it is a stable container for liquids and gasses” and “the phase trapping, capillary action, and mineralization in the container can hold it there in perpetuity.”

HECA provides a close-up perspective of the geologic structure in the Elk Hills area as justification for the claims of stability and permanence of the proposed project plan.

HECA should provided the wide-angle, long distance, long-term view of the forces acting on the Earth and project area as well as any calculations that show HECA considered the long-term cumulative impact effects of all the forces on which their conclusions were based.

The long-term view should include presentations by independent scientists and professors who speak to the issues of the geologic structure as well as all the cyclical and random extraterrestrial forces acting on the area from planetary influences and gravitational effects by Jupiter and the Sun and Moon in their various arrangements and orientations around the Earth (fluctuations caused from apogee to perigee and from solar to lunar eclipse positions and their permutations), as well as meteorites and asteroids (in particular the catastrophic, large bolides (asteroids) impacts on the Earth) and their effects on plate tectonics, continental drift, magma flows, rift zone pressures, and uplifts, folding, and faulting plates, earthquakes, and tidal effects, and on the

Earth's magnetic field. All of these forces and effects must be cumulatively analyzed to reach any conclusion on the relative stability or instability of the Elk Hills area.

Many examples of the relative instability, over time, on the Earth, are clear. Based on their similar magnetic properties, some geologists have concluded that West Africa and eastern South America have matching geologic structures that drifted apart. Whether the separation of those continental structures occurred rapidly or slowly is not a question. But those structures over time separated and no force prevented that separation.

The Himalaya Mountains were caused by the lateral movement of the floating crust with buckling at the collision edge of the India and the Asia plates.

Likewise, if some geologic force is applied to the disassociation of the continent at the point where the purported "impervious" rock domes of Elk Hills are located, they will disassociate in time, eventually releasing their stored CO_2.

Not only are geologic forces in effect which could cause the disassociation of the Elk Hills area, but there are large astronomical forces in effect. The Earth and the moon are in a shooting gallery of sorts with asteroids as large as a mile in diameter taking aim.

There are thirty thousand asteroids of significant size for which the Earth is a target. Throughout history, meteorites have had more than close encounters with the Earth and the Moon as the scars on both will testify. There is evidence of impacts with the Moon and Earth by larger asteroids (bolides).

The Earth's thin crust is about 0.26 percent of the radius of the Earth. "Chunks of crust bob like so many corks on the surface of the viscose mantle, smashing into each other at various rates." [1]

Nels Winkless III and Iben Browning confirm the instability and impermanence of the Earth and its crust in their book *Climate and the affairs of Man*.

> Tidal forces cause changes in ocean levels of up to 50 feet in a day. Planetary forces are cyclical and can be additive or subtractive depending on where they are in the cycles. The cumulative effects of the planetary changes distort the diameter of the earth
>
> Meteorite hits come to mind as the other likely source of round structures, and Barringer Crater near Winslow, Arizona, is the famous example. We began to search for other documented meteorite craters and turned up a number of them, such as Lake Bosumtwi in West Africa and Chubb Crater in Canada.
>
> One begins to wonder after a while why the Moon is so chewed up with meteorite (or, preferably, *bolide)* strikes and the Earth is not. In fact, Earth *is* scarred by such strikes, but our atmosphere protects the surface by burning up the smaller incoming objects. Only the big ones make it to the surface and the craters that they have created tend to be

[1] (Nels Winkless III and Iben Browning, Climate and the affairs of Man, Harper and Row 1975)

masked by erosion and vegetation. The biggest ones are so big that they are difficult to detect from the ground.

The Rieskessel crater in Germany has a floor roughly fourteen miles in diameter surrounded by a thirteen-hundred-foot wall. The crater is very old, weathered, and overgrown, but it is visible under the overlying roads and human structures. During the 1940s the crater became visible to people who had occasion to fly over it in B-29s while on other business. Sixteen-inch chunks of material from this crater have been found forty kilometers away from the site. Something exciting obviously occurred there at one time and the conviction is growing that the event involved the fall of a big, heavy thing onto the Earth.

Rieskessel is but one of an increasing number of such structures identified on the Earth.

Let us recall the thirty thousand asteroids of significant size for which the Earth is a moving target. This notion of the celestial shooting gallery is dramatized by a passage from Baldwin's *The Face of the Moon:*

> "At first, the meteorite would plunge into the Earth moving faster than the shock waves and pushing ahead of it an ever increasing plug of compressed rock and probably a similar plug of compressed air. When the speed of the meteorite becomes less than that of the elastic waves, the vast amount of compression produced finds a shoulder against which to push, and the mass is soon stopped. With the stoppage of motion the meteorite is sitting on top of a tremendously compressed, tremendously hot plug of matter. Naturally, an explosion of the utmost violence follows." [2]

Violent, indeed!
A one-mile-diameter asteroid, Hermes, shot past the Earth on October 28, 1937, missing us by only seventy-five Earth diameters (600,000 miles) and passing through the Earth's orbit. This space-borne mountain would have blown out over 4,000 cubic miles from a crater 80 miles across and perhaps 25 miles deep. If it had hit an ocean, it would have made a tidal wave four miles high and it would have evaporated 3,800 cubic miles of water—enough for a 1.25-inch rain over the entire Earth.

Hitting at a skewed angle, its colossal earthquake-like waves would have vibrated the Earth like a gong. The P-waves (pressure waves) would have spread out like earthquake waves, while the S-waves (shear waves) would have been absorbed by the liquid core. The turbulence would have set up a fluid flow in the core of more than a hundred miles an hour and this enormous rotating iron armature would have altered the Earth's magnetic field.

A fireball would have blossomed from thirty to fifty miles high; and there, meeting no more atmospheric resistance, it would have thrust laterally, creating a fire funnel perhaps a hundred miles in diameter at its top. A part of the 4,000 cubic miles of crustal materials blown out would have been thrust laterally to spew out and reenter the atmosphere up to a thousand miles away as tektites. A part would have been reduced to dust and put into

[2] (Ralph B. Baldwin, ***The*** *Face of* ***the Moon,*** Chicago: University of Chicago Press, 1949)

orbit, cutting off half or more of the Earth's sunlight for years. A part would have been shot into space and would have constituted showers of stony meteorites for millennia to come. [3]

Watson calculates that the ". . . Earth probably goes at least 100,000 years between collisions with them . . . [4] (asteroids).[5]

"There is a 1-in-10,000 chance that a large (~2-km diameter) asteroid or comet will collide with the Earth during the next century, disrupting the ecosphere and killing a large fraction of the world's population. Although impacts of this magnitude are so infrequent as to be beyond our personal experience, the long-term statistical hazard is comparable to that of many other, more familiar natural disasters, raising the question of whether mitigation measures should be considered." [6]

"One of the most significant discoveries of the Space Age, the ubiquitous cratering of planetary surfaces by remnant debris from the origin of the planets, encourages a re- examination of the geological evolution of the Earth's surface. The bombardment by comets and asteroids is not complete and large, K/T-Boundary-scale impacts may be expected in the future. Impacts on Earth by 1 - 2 km diameter objects happen on a time scale of several hundred thousand years and have a small, but non-zero probability of creating a global ecological catastrophe within our lifetimes. The recent crash of fragments of tidally disrupted Comet Shoemaker-Levy 9 into Jupiter has dramatized the serious atmospheric consequences of impacts of comparatively small cosmic objects." [7]

The Earth's geological history exhibits many examples of geologic changes, some of them catastrophic and tumultuous, where nothing stayed the same. I find the permanence claim infeasible and illogical in the long term.

The benefits do not outweigh the possible, catastrophic costs. We already have too many problems, which are not being resolved, to create another.

Also, how would the HECA process impact the micro-organisms/bacteria that are living in the ground and the bacteria living in crude oil that are known to digest crude oil? How will the increase in ground-born CO2 impact these bacteria?

---

[3] (Nels Winkless III and Iben Browning, *Climate and the affairs of Man*, Harper and Row 1975)
[4] (F. G. Watson, *Between the Planets.* New York Doubleday, 1962)
[5] (Nels Winkless III and Iben Browning, *Climate and the affairs of Man*, Harper and Row 1975)
[6] (*Nature* 367, 33 - 40 (06 January 1994); doi:10.1038/367033a0
http://www.nature.com/nature/journal/v367/n6458/abs/367033a0.html
CLARK R. CHAPMAN & DAVID MORRISON, *Impacts on the Earth by asteroids and comets: assessing the hazard* (65 references cited)
Planetary Science Institute, Science Applications International Corporation, 620 N. 6th Avenue, Tucson, Arizona 85705, USA. Space Science Division, NASA Ames Research Center, Moffett Field, California 94035, USA.)
[7] THE RISK TO CIVILIZATION FROM EXTRATERRESTRIAL OBJECTS and Implications of the Shoemaker-Levy 9 Comet Crash, Clark R. Chapman
http://www.boulder.swri.edu/clark/papers.html
Southwest Research Institute, Boulder, Colorado USA
In press, Abhandlungen der Geologischen Bundeanstalt, Wien, Vol. 53, 51-54, 1996

Respectfully submitted,

Mr. Ara Marderosian
P.O. Box 988
Weldon, CA 93283
(760) 378-4574
ara@sequoiaforestkeeper.org

ENVIRONMENTAL ENGINEERING SCIENCE
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Degradation of Phenanthrene and Pyrene in Soil Slurry Reactors with Immobilized Bacteria *Zoogloea* sp.

Peijun Li,[1] Xin Wang,[2] Frank Stagnitti,[3,*] Ling Li,[4] Zongqiang Gong,[1] Hairong Zhang,[1] Xianzhe Xiong,[1] and Chris Austin[3]

[1]Institute of Applied Ecology
Chinese Academy of Sciences
Shenyang 110015, People's Republic of China
[2]School of Science
Shenyang University of Technology
Shenyang 110023, People's Republic of China
[3]School of Ecology and Environment
Deakin University
Warrnambool, Vic. 3280, Australia
[4]Centre for Eco-Environmental Modelling
Hohai University
Nanjing 210098, People's Republic of China

ABSTRACT

The environmental fate of polycyclic aromatic hydrocarbons (PAHs) in soils is motivated by their wide distribution, high persistence, and potentially deleterious effect on human health. Polycyclic aromatic hydrocarbons constitute the largest group of environmental contaminants released in the environment. Therefore, the potential biodegradation of these compounds is of vital importance. A biocarrier suitable for the colonization by micro-organisms for the purpose of purifying soil contaminated by polycyclic aromatic hydrocarbons was developed. The optimized composition of the biocarrier was polyvinyl alcohol (PVA) 10%, sodium alginate (SA) 0.5%, and powdered activated carbon (PAC) 5%. There was no observable cytotoxicity of biocarriers on immobilized cells and a viable cell population of 1.86×10^{10} g^{-1} was maintained for immobilized bacterium. Biocarriers made from chemical methods had a higher biodegradation but lower mechanical strengths. Immobilized bacterium *Zoogloea* sp. had an ideal capability of biodegradation for phenanthrene and pyrene over a relative wide concentration range. The study results showed that the biodegradation of phenanthrene and pyrene reached 87.0 and 75.4%, respectively, by using the optimal immobilized method of *Zoogloea* sp. cultivated in a sterilized soil. Immobilized *Zoogloea* sp. was found to be effective for biodegrading the soil contaminated with phenanthrene and pyrene. Even in "natural" (unsterilized) soil, the biodegradation of phenanthrene and pyrene using immobilized *Zoogloea* sp. reached 85.0 and 67.1%, respectively, after 168 h of cultivation, more than twice that achieved if the cells

*Corresponding author: School of Ecology and Environment, Deakin University, P.O. Box 423, Warrnambool, Victoria, Australia 3280. *Phone*: 61 3 5563 3535; *Fax*: 61 3 5563 3143; *E-mail*: frankst@deakin.edu.au

Environmental Engineering Science

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Peijun Li

Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110015, People's Republic of China.

Xin Wang

School of Science, Shenyang University of Technology, Shenyang 110023, People's Republic of China.

Frank Stagnitti

School of Ecology and Environment, Deakin University, Warrnambool, Vic. 3280, Australia.

Corresponding author: School of Ecology and Environment, Deakin University, P.O. Box 423, Warrnambool, Victoria, Australia 3280. Phone: 61 3 5563 3535; Fax: 61 3 5563 3143; E-mail: frankst@deakin.edu.au.

Ling Li

Centre for Eco-Environmental Modelling, Hohai University, Nanjing 210098, People's Republic of China.

Zongqiang Gong

Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110015, People's Republic of China.

Hairong Zhang

Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110015, People's Republic of China.

Xianzhe Xiong

Institute of Applied Ecology, Chinese Academy of Sciences, Shenyang 110015, People's

Keywords:

immobilization
biodegradation
PAH

Republic of China.

Chris Austin

School of Ecology and Environment, Deakin University, Warrnambool, Vic. 3280, Australia.

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Shenyang University of Technology
Shenyang 110023, People's Republic of China
[3]School of Ecology and Environment
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*Corresponding author: School of Ecology and Environment, Deakin University, P.O. Box 423, Warrnambool, Victoria, Australia 3280. *Phone*: 61 3 5563 3535; *Fax*: 61 3 5563 3143; *E-mail*: frankst@deakin.edu.au

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Shuyan Zang, Peijun Li, Bin Lian, Frank Stagnitti, Monique R. Stagnitti, Jing Chen, Lijing Xie
Environmental Engineering Science. March 2008: 247-254.
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Plasmid Incidence in Four Species of Hydrocarbonoclastic Bacteria Isolated from Oil Polluted Marine Environment

[1]R. Thavasi, [1]S. Jayalakshmi, [2]R. Radhakrishnan and [1]T. Balasubramanian
[1]CAS in Marine Biology, Annamalai University, Parangipettai-608 502, Tamil Nadu, India
[2]Sri S.R.N.M. College, Sattur-606 203, Tamil Nadu, India

Abstract: The present investigation is on evaluation of biodegradation potential of four species of hydrocarbonoclastic bacteria, *B. megaterium*, *C. kutscheri*, *L. delbrueckii* and *P. aeruginosa* and screening for plasmids. For biodegradation study, the strains were cultured in mineral salt medium with 0.1% of crude oil for seven days. Biodegradation results inferred that, *P. aeruginosa* showed 85.15% of crude oil degradation, followed by *B. megaterium* (78.5%), *C. kutscheri* (76.4%) and *L. delbrueckii* (71.6%). Strains were grown for 24 h in Luria-Bertani broth for plasmid study. Plasmid study revealed that, *P. aeruginosa* harbored two plasmids with molecular weight of 4.2 and 3.8 kb. The other strains namely *L. delbrueckii*, *C. kutscheri* and *B. megaterium* were found to have single plasmid with respective molecular weight of 3.8, 4.2 and 4.1 kb. Complete loss of biodegradation potential was observed when the plasmids of these strains cured with acridine orange.

Key words: Biodegradation, plasmids, hydrocarbonoclastic bacteria

INTRODUCTION

Environmental pollution is a cause of major concern affecting ecosystems globally. Oil spill, an offshoot of this environmental pollution caused by spillage from tankers, release of effluents and offshore drilling activities is adversely affecting the aquatic ecosystems (Atlas, 1981). Chemical or physiological means of controlling oil spill are rapidly being developed, but they have the disadvantage that, these chemicals leave toxic wastes which may not be biodegradable. Physical means are only useful in clearing small areas. Use of eco-friendly methods such as bioremediation using microbes is the recent concern for environmental pollution. The adaptation of microbes to survive in the polluted environment is mainly based on their genetic make up. Plasmids that have been found to harbor genes encoding the transformation of environmental pollutants are known as catabolic plasmids. From single step reaction to multi step pathways, plasmids appear to be versatile mean for microorganisms to gain metabolic capacities for the exploitation of otherwise unavailable resources (Anthony *et al*., 2000). Heterotrophic bacteria have been observed to exhibit a higher incidence of plasmid DNA in hydrocarbon contaminated environments, such as offshore fields (Hada and Sizemore, 1981), riverine sediment polluted by coking plant discharges (Burton *et al*., 1982; Day *et al*., 1988) ground water contaminated by aromatic hydrocarbons (Ogunseitan *et al*., 1987) and a dystrophic lake containing naturally high concentrations of aromatic humic compounds (Schutt, 1989). Some of the plasmid mediated bacterial utilization of various carbon compounds which could be found in the complex mixture of crude oil (Chakrabarty, 1976). Presence of catabolic genes responsible for the degradation of naphthalene in plasmid of *P. putida* was reported by Park *et al*. (2003). With the information in above literature, the present study was carried out to investigate the presence of plasmids in four species hydrocarbonoclastic bacteria and their biodegradation ability of crude oil.

MATERIALS AND METHODS

Isolation and identification of hydrocarbonoclastic bacteria (HCB): *Bacillus megaterium*, *Corynebacterium kutscheri*, *Lactobacillus delbrueckii* and *Pseudomonas aeruginosa* were isolated from water samples collected in Tuticorin harbor, South East Coast of India (Lat. 08°44'N; Long. 78°13'E). Further analysis of the sample was carried out at Centre of Advanced Study in Marine Biology, Annamalai University, Parangipettai, India. Bushnell Haas agar (g L^{-1} of $MgSO_4$-0.2, $CaCl_2$-0.2, KH_2PO_4-1.0, K_2HPO_4-1.0, NH_4NO_3-1.0, $FeCl_3$-0.05 and agar-15 g) supplemented with 0.1% crude oil was used for the isolation of bacteria.

Corresponding Author: S. Jayalakshmi, CAS in Marine Biology, Annamalai University, Parangipettai-608 502, Tamil Nadu, India

The strains were identified to species level by following Bergy's manual of determinative bacteriology (Buchanan *et al*., 1974).

Estimation of crudr oil degradation: *B. megaterium*, *C. kutscheri*, *L. delbrueckii* and *P. aeruginosa* were cultured in 250 mL culture flasks containing 50 mL of mineral salt medium (g L^{-1} NH_4Cl 0.5, K_2HPO_4 0.5, NaH_2PO_4 0.5, pH 7.5, natural seawater 750 mL and 250 mL of distilled water) supplemented with 0.1% of crude oil (Juwarkar and Khirsagar, 1991) and the cultures were maintained in shaker at 150 rpm for 7 days at 30°C. After 7 days, cell free culture broth was extracted with three volume of toluene and extract was made up to 10 mL and the OD was measured at 420 nm. The percentage of degradation was calculated from the standard curve. The standard graph was obtained with different concentration of crude oil in toluene (Rahaman *et al*., 2002). The experiment was carried out in duplicate and the mean values were expressed.

Isolation of plasmids: Strains were grown in Luria-Bertani (LB) broth and cells were pelleted by centrifugation at 5000 rpm for 15 min at 4°C. Plasmids from the cells were isolated using alkaline lysis method (Sambrook and Russel, 2001). Isolated plasmids were stored in TE buffer until analysis. 0.8% of agarose gel was casted by dissolving 0.8 g of agarose in 100 mL of 1X TAE buffer and the plasmid DNA was loaded along with the loading buffer into the wells. Electrophoresis was carried out with 50 mA of current and the DNA bands were viewed under UV trans-illuminator. Molecular weight of the isolated plasmids was determined by using Total Lab Software. ECOR1 Hind-III double digest was used as molecular weight marker.

Plasmid curing: The role of plasmids in biodegradation process was confirmed by curing the plasmids with acridine orange at a concentration of 500 µg mL^{-1} which was added to the culture broth and incubated for 12 h (Fujji *et al*., 1997). The plasmid cured strains were screened for biodegradation.

RESULTS AND DISCUSSION

Biochemical and physiological characteristics of the strains are given in Table 1. The existence of hydrocarbonoclastic bacteria in the marine environment was already documented by the researchers (Bhosle and Mavinkurve, 1980; Baruah and Deka, 1995) supported the findings of the present study on isolation of HCB from marine environment. Hydrocarbon pollution in a particular area may increase the fraction of hydrocarbon-utilizing microorganisms and it may also increase the capacity of the microbial community to degrade hydrocarbons (Leahy *et al*., 1990). It was evidenced by the results obtained in the present study on biodegradation of crude oil. *P. aeruginosa* showed maximum degradation of crude oil (85.15%), followed by *B. megaterium* (78.5%), *C. kutscheri* (76.4%) and *L. delbrueckii* (71.6%). Similar results were obtained with bacterial strains namely *Rhodococcus rhodochrous* KUCC 8801 (93.1%), *Rhodococcus* sp. ISO1 (81.1%), *Acinotebacter calcoaceticus* IRO7 (91.2%) and *Pseudomonas putida* IR32 (47.6%) with 5 days of incubation (Sorkhoh *et al*., 1990) also complemented the results of the present study on biodegradation.

Table 1: Biochemical and physiological characteristics of hydrocarbonoclastic bacteria

Test	*B. megaterium*	*C. kutscheri*	*L. delbrueckii*	*P. aeruginosa*
Gram reaction	+	+	+	-
Cell shape	Rod	Rod	Rod	Rod
Spore formation	+	-	-	-
Catalase	-	+	-	+
Motility				
Hydrolysis of				
Starch	+	-		+
Gelatin		-		+
Fat	+			+
Carbohydrate utilization				
Adonitol				-
Arabinose	+		-	-
Cellobiose			-	-
Fructose			+	+
Galactose				-
Glucose			+	-
Mannitol	+		-	+
Mannose			+	-
Rhamnose			-	-
Ribose			-	+
Sorbitol			-	-
Sucorse		+	-	-
Trehalose			-	-
Xylose	+		-	-
Dnitrification	+	-		+

+ Positive result, -: Negative result

Results obtained in the plasmid analysis revealed that, *P. aeruginosa* was found to harbor two plasmids with molecular weight of 4.2 and 3.8 kb. The other bacterial strains namely *L. delbrueckii*, *C. kutscheri* and *B. megaterium* were found to have single plasmid with respective molecular weight of 3.8, 4.2 and 4.1kb (Fig. 1). Presence of catabolic genes responsible for the degradation of naphthalene in plasmid of *P. putida* was reported by Park *et al*. (2003). This finding supported the present study on plasmid mediated degradation of crude oil. Plasmids that have been found to harbor genes encoding the transformation of environmental pollutants are known as catabolic plasmids. From single step reaction to multi step pathways, plasmids appear to be versatile mean for microorganisms to gain metabolic

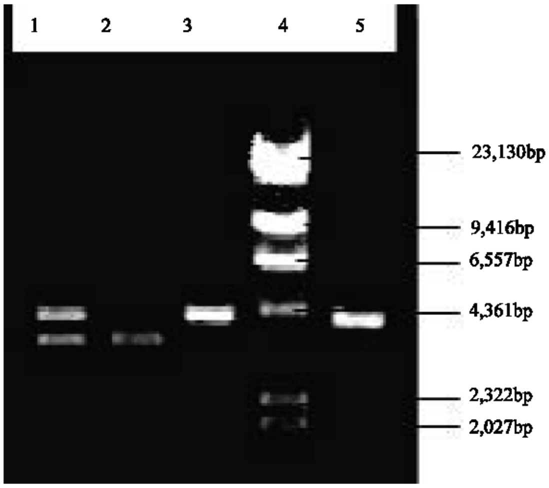

Fig. 1: Plasmids isolated from four species of HCB, Lane 1. *Pseudomonas aeruginosa*, Lane 2. *Lactobacillus delbrueckii*, Lane 3. *Corynebacterium kutscheri*, Lane 4. λ DNA/Hind III marker, Lane 5. *Bacillus megaterium*

capacities for the exploitation of otherwise unavailable resources (Anthony *et al*., 2000). The incidence of plasmids in oil degrading bacteria had been already reported by many researchers. According to Devereux and Sizemore (1982), plasmids were detected in 21% of the strains isolated on crude oil and 17% on polynuclear aromatic hydrocarbons. They also observed multiple plasmids in 50% of the plasmid containing strains are similar to the two plasmid observed in *P. aeruginosa* in the present study. Bacteria isolated from oil polluted environments have previously been shown to be more effective in degrading crude oil than bacteria from unpolluted environments (Colwell *et al*., 1973) and plasmid frequency increased in various hydrocarbon-contaminated environments (Burton *et al*., 1982; Day *et al*., 1988; Hada and Sizemore, 1981; Ogunseitan *et al*., 1987; Schutt, 1989). Small plasmid was obtained from *Pseudomonas* strain with a molecular weight 3.2 MDa in sediments from Campeche Bank (Leahy *et al*., 1990) which complemented the present result on plasmids with molecular weight of 3.8 to 4.2 kb in oil degrading bacteria.

The total loss of biodegradation activity after plasmid curing over emphasizes the role of plasmids in catabolic activity of hydrocarbon biodegradation. Rheinwald *et al.* (1973) reported the loss of isobutyrate degradation after plasmid curing in *Pseudomonas putida*. Stuart-Keil *et al.* (1998) observed the loss of naphthalene degradation after plasmid curing in marine heterotrophic bacterial population. These findings supported the results of present study on loss of biodegradation ability after plasmid curing.

In Conclusion, results obtained in the present study on biodegradation of crude oil inferred the possibility of applying these four hydrocarbonoclastic bacteria or their products (biosurfactants) for environmental cleaning of oil spill. Loss of biodegradation activity after plasmid curing indicated that, the genes responsible for biodegradation of crude oil may be plasmid mediated.

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Occurrence of bacteria in Pembina crude oil pipeline system and their potential role in corrosion process

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C. O. Obuekwe[1] and D. W. S. Westlake[2]

(1) Department of Microbiology, University of Benin, Benin City, Nigeria

(2) Department of Microbiology, University of Alberta, T6G 2E9 Edmonton, Canada

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Summary Crude oil stream from the Pembina fields of North Central Alberta, Canada, contained a relatively high detectable load of bacteria. The oil and produced water contained aerobic and anerobic microorganisms capable of producing sulphides from sulphates and sulphite, and ferrous ions from ferric compounds. The ability to produce S^{2-} and Fe(II) in solution is considered very important in corrosion phenomenon in the pipeline system. Apart from SO_4^{2-} — reducers (*Desulfovibrio* spp) the organisms found in the crude oil system and capable of generating corrosive environment were mainly members of the Genus *Pseudomonas*.

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Short contribution

Occurrence of bacteria in Pembina crude oil pipeline system and their potential role in corrosion process

C. O. Obuekwe[1] **and D. W. S. Westlake**[2]

[1] Department of Microbiology, University of Benin, Benin City, Nigeria
[2] Department of Microbiology, University of Alberta, Edmonton. T6G 2E9, Canada

Summary. Crude oil stream from the Pembina fields of North Central Alberta, Canada, contained a relatively high detectable load of bacteria. The oil and produced water contained aerobic and anaerobic microorganisms capable of producing sulphides from sulphates and sulphite, and ferrous ions from ferric compounds. The ability to produce S^{2-} and Fe(II) in solution is considered very important in corrosion phenomenon in the pipeline system.

Apart from SO_4^{2-} — reducers (*Desulfovibrio* spp) the organisms found in the crude oil system and capable of generating corrosive environment were mainly members of the Genus *Pseudomonas*.

Introduction

Relatively high levels of bacteria are present in water and crude oil produced from the Pembina oil field of North Central Alberta, Canada. Produced waters contain between 10^2 and 10^4 aerobes, and 10^1 to 10^3 sulphide-generating anaerobes per mililitre of crude oil. Representatives of both of these microbial groups are usually readily detectable in crude oil delivered to the storage tanks in Edmonton, Alberta, 152 km north-east of the Pembina oilfield. In fact, some of these organisms have been detected in the oil transported by pipeline as far as Montreal, a distance of over 4000 km, from the oilfield. Associated with the occurrence of these relatively high levels of bacteria is a high incidence of pipeline failure, in this field, due to corrosion.

Offprint requests to: C. O. Obuekwe

In this paper is summarized the general microbiology of the crude oil that may be pertinent to the corrosion problem, and how the corrosion process can be affected by the activities of the organisms present.

Materials and methods

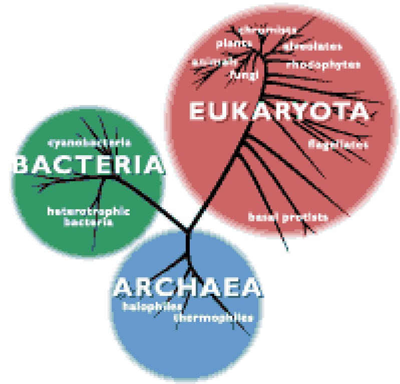
The crude oil samples were obtained from Edmonton Terminal of Interprovincial Pipeline Co. Ltd. The total heterotrophic aerobic bacteria in 0.1 ml aliquots were counted on plate count Agar after incubating for 14 days at 22°C. The number of sulphide-producing bacteria was determined by Most Probable Number (MPN) technique (Cochran 1950) in modified Butlin's medium containing iron finishing nails (Obuekwe and Okoronkwo 1982). The nails acted as poising agent by removing residual O_2 in the medium to allow the growth of sulphate-reducers and also served as indicator of S^{2-} production by forming black ferrous sulphide. Sodium sulphite was omitted from medium when sulphate-reducing bacteria were counted.

The bacteria isolated from the crude oil samples were tested for the ability to degrade crude oil, produce sulphide from SO_4^{2-} and SO_3^{2-}, reduce Fe(III) to Fe(II) and utilize a wide range of organic compounds as carbon/energy sources.

Results and discussion

The incidence of bacteria in Pembina crude oil samples at the Edmonton Terminal of Interprovincial Pipeline Co. Ltd. is shown in Figs. 1 and 2. Figure 1 shows the occurrence of total heterotrophic aerobic bacteria, while Fig. 2 shows the sulphide-producing bacteria which included all bacteria able to reduce both SO_4^{2-} and SO_3^{2-} to S^{2-}. Generally, the incidence of bacteria in the crude oil samples was greatest in the summer months and lowest in winter. It is noteworthy that the period of high incidence of sulphide-producing bacteria corresponded to the period when the occurrence of aerobes was equally high. This

Introduction to the Archaea



Life's extremists. . .

The Domain Archaea wasn't recognized as a major domain of life until quite recently. Until the 20th century, most biologists considered all living things to be classifiable as either a plant or an animal. But in the 1950s and 1960s, most biologists came to the realization that this system failed to accomodate the fungi, protists, and bacteria. By the 1970s, a system of Five Kingdoms had come to be accepted as the model by which all living things could be classified. At a more fundamental level, a distinction was made between the **prokaryotic** bacteria and the four **eukaryotic** kingdoms (plants, animals, fungi, & protists). The distinction recognizes the common traits that eukaryotic organisms share, such as nuclei, cytoskeletons, and internal membranes.

The scientific community was understandably shocked in the late 1970s by the discovery of an entirely new group of organisms -- the Archaea. Dr. Carl Woese and his colleagues at the University of Illinois were studying relationships among the prokaryotes using DNA sequences, and found that there were two distinctly different groups. Those "bacteria" that lived at high temperatures or produced methane clustered together as a group well away from the usual bacteria and the eukaryotes. Because of this vast difference in genetic makeup, Woese proposed that life be divided into three **domains**: Eukaryota, Eubacteria, and Archaebacteria. He later decided that the term Archaebacteria was a misnomer, and shortened it to Archaea. The three domains are shown in the illustration above at right, which illustrates also that each group is very different from the others.

Further work has revealed additional surprises, which you can read about on the other pages of this exhibit. It is true that most archaeans don't look that different from bacteria under the microscope, and that the extreme conditions under which many species live has made them difficult to culture, so their unique place among living organisms long went unrecognized. However, biochemically and genetically, they are as different from bacteria as you are. Although many books and articles still refer to them as "Archaebacteria", that term has been abandoned because they aren't bacteria -- they're Archaea.

Finding Archaea : The hot springs of Yellowstone National Park, USA, were among the first places Archaea were discovered. At left is Octopus Spring, and at right is Obsidian Pool. Each pool has slightly different mineral content, temperature, salinity, etc., so different pools may contain different communities of archaeans and other microbes. The biologists pictured above are immersing microscope slides in the boiling pool onto which some archaeans might be captured for study.

Archaeans include inhabitants of some of the most extreme environments on the planet. Some live near rift vents in the deep sea at temperatures well over 100 degrees Centigrade. Others live in hot springs (such as the ones pictured above), or in extremely alkaline or acid waters. They have been found thriving inside the digestive tracts of cows, termites, and marine life where they produce methane. They live in the anoxic muds of marshes and at the bottom of the ocean, and even thrive in petroleum deposits deep underground.

Some archaeans can survive the dessicating effects of extremely saline waters. One salt-loving group of archaea includes *Halobacterium*, a well-studied archaean. The light-sensitive pigment **bacteriorhodopsin** gives *Halobacterium* its color and provides it with chemical energy. Bacteriorhodopsin has a lovely purple color and it pumps protons to the outside of the membrane. When these protons flow back, they are used in the synthesis of ATP, which is the energy source of the cell. This protein is chemically very similar to the light-detecting pigment **rhodopsin**, found in the vertebrate retina.

Archaeans may be the only organisms that can live in extreme habitats such as thermal vents or hypersaline water. They may be extremely abundant in environments that are hostile to all other life forms. However, archaeans are not restricted to extreme environments; new research is showing that archaeans are also quite abundant in the plankton of the open sea. Much is still to be learned about these microbes, but it is clear that the Archaea is a remarkably diverse and successful clade of organisms.

Click on the four buttons below to learn more about the Archaea.

For even more archaeal information :

- An impressive set of links to all things Archaean may be found at Life in Extreme Environments: Archaea on the Astrobiology Web.
- Get a general introduction to the major groups of prokaryotes from Kenneth Todar at the University of Wisconsin--Madison.
- The Microbe Zoo features several methane-producing organisms, including some Archaea.
- For more information on Halobacteria, including lesson information for teachers, go to The HaloEd Project.

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Images of Yellowstone springs courtesy of Norman Pace at the University of Colorado, Boulder.

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