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PROBLEMS RELATED TO THE PRESENCE OF NEW SPECIFIC BACTERIA (GENERE RALSTONI AND STENOTROPHOMONAS) INTO D_2O INTERFERIN WITH OVERLOADING OF DEUTERIUM INTO Pd BY ELECTROLITYC PROCEDURE.

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[FREEDOM COLLABORATION]

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A: PHYSICIST 4 + 1/2
B: CHEMISTRY 5

COLD FUSION

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OVERLOADING of DEUTERIUM
in the PALLADIUM

and SIMULTANEOUSLY

MOVEMENT of DEUTERIUM
in the PALLADIUM

without LOSING of OVERLOADING

The principle problem of experiments has caused from 1989 up to the present :

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Unreproducibility of results.

The tipical excess power (when it works) was about 5~10% and it also arrived up to a maximum value of 200% for many hours (That value is macroscopic, 10~300 W)

These results have been controlled by several different experimental methodes.

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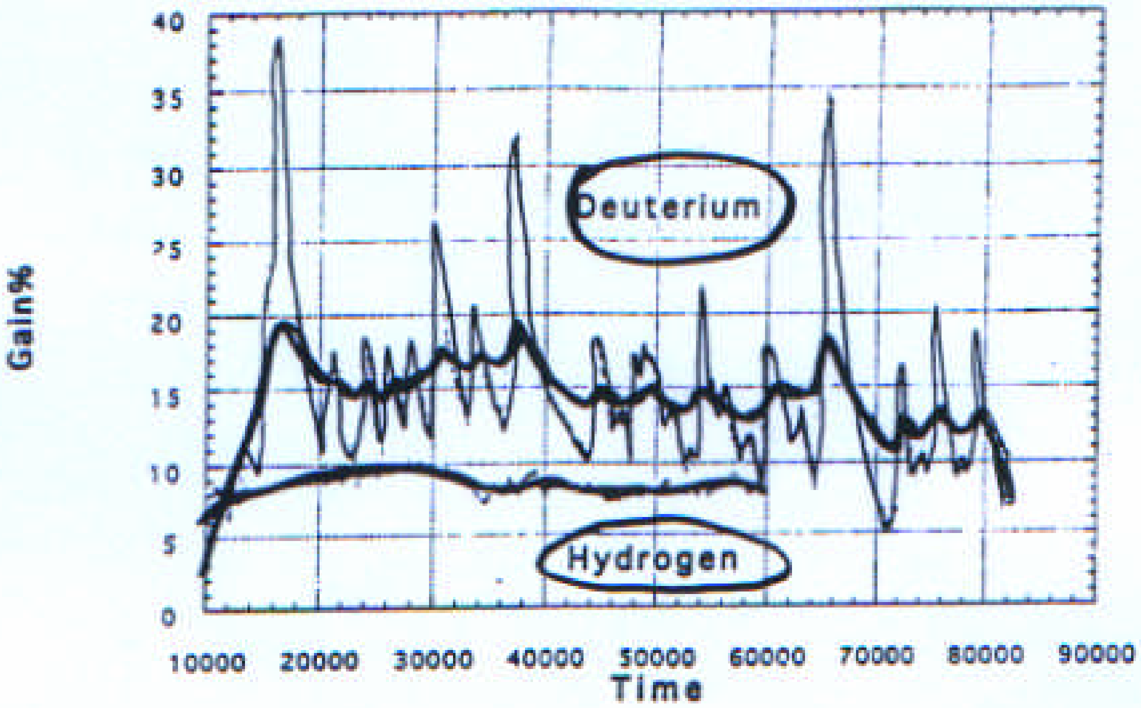
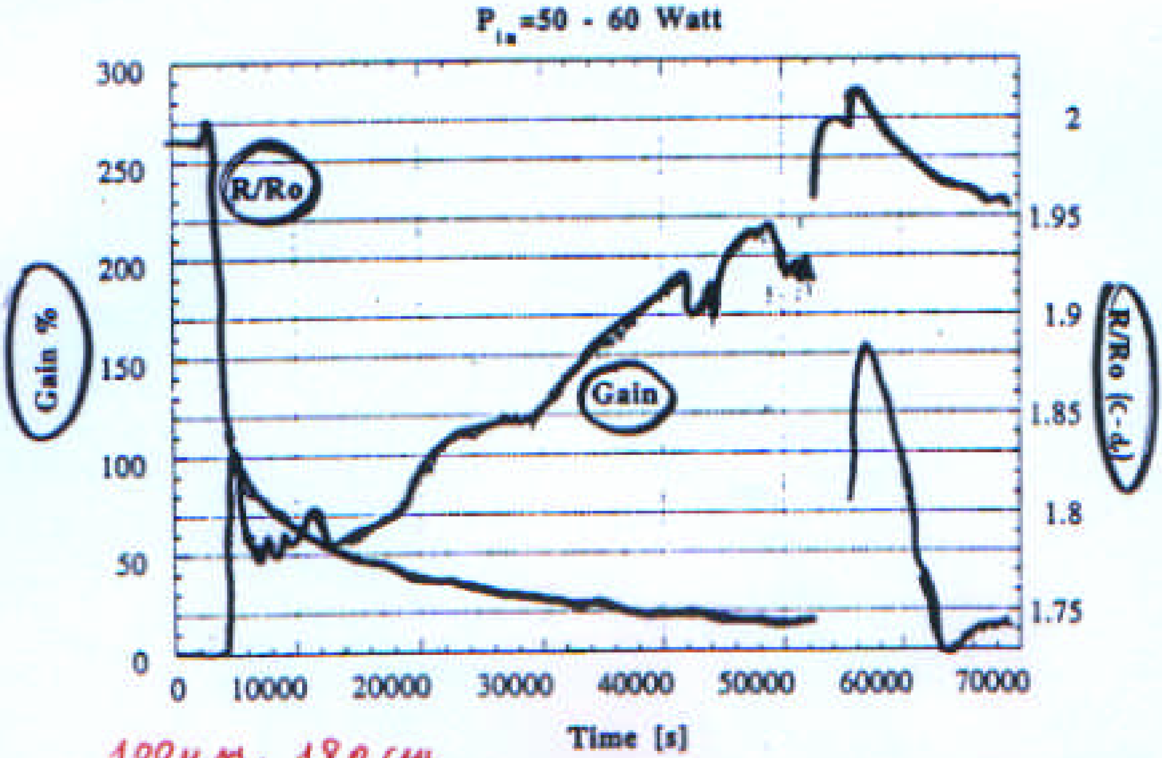
Therefore, it's NO mistake of measurements.

Excess Heat



Pd-Y alloy, 100µm, 180 cm

Fig.6: Isotopic behaviour of normal wire geometry set-up using flow-calorimetry (1% accuracy). We used the same wire, before loaded by D and later by H. We notify that after six loading/deloading procedure (washing) the excess heat of Pd/Pt/H system decreased to about 3%.



100µm, 180 cm

Fig.7: Pure Pd "central wire geometry" results. Isoperibolic calorimetry (15% accuracy).

Selected criteria for "suitable new technique" were below:

a) $H/Pd > 0.95 \Rightarrow R/R_0 < 1.5$
b) Time,
to get $H/Pd > 0.95$: < 50 hours
c) Stability of overloading $>$ 4 hours
d) Success rate $>$ 60%

The measurement of loading, H/Pd, D/Pd were performed, on the line, in situ, by variations of resistance of Palladium, which change depending on the ratio of H/Pd and D/Pd.

According to a literature, it's given that the maximum ratio of H/Pd and D/Pd is 0.97 with the pressure of 50,000 atm (Baranowsky graph).

The necessary condition, but not sufficiently, in order to achieve anomalous excess heat is to surpass one certain level of value of the D/Pd ratio.

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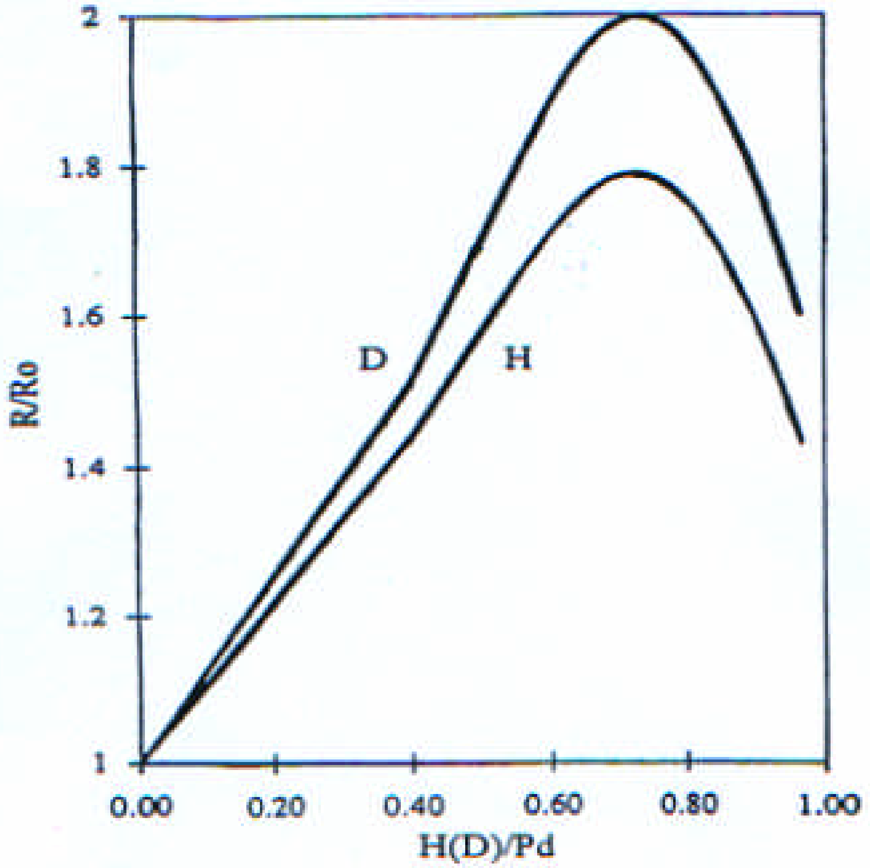

Figura 1. – Variazione della resistività dei sistemi Pd-H, Pd-D in funzione della concentrazione atomica (x = H/Pd, D/Pd), a temperatura ambiente. Il picco di resistività (R/Ro = 1,78 e 2,0) corrisponde a x = 0,75. I massimi valori di caricamento noti sono: R/Ro = 1,43 (H) e 1,6 (D) corrispondenti a x = 0,97, 50'000 Atm. -

do il ben noto grafico di Baranowsky (fig. 1). In questo grafico i rapporti atomici x=D/Pd e x=H/Pd, sono posti in relazione con la variazione di resistività (R/Ro) dei sistemi Pd-D e Pd-H. Si osserva che la

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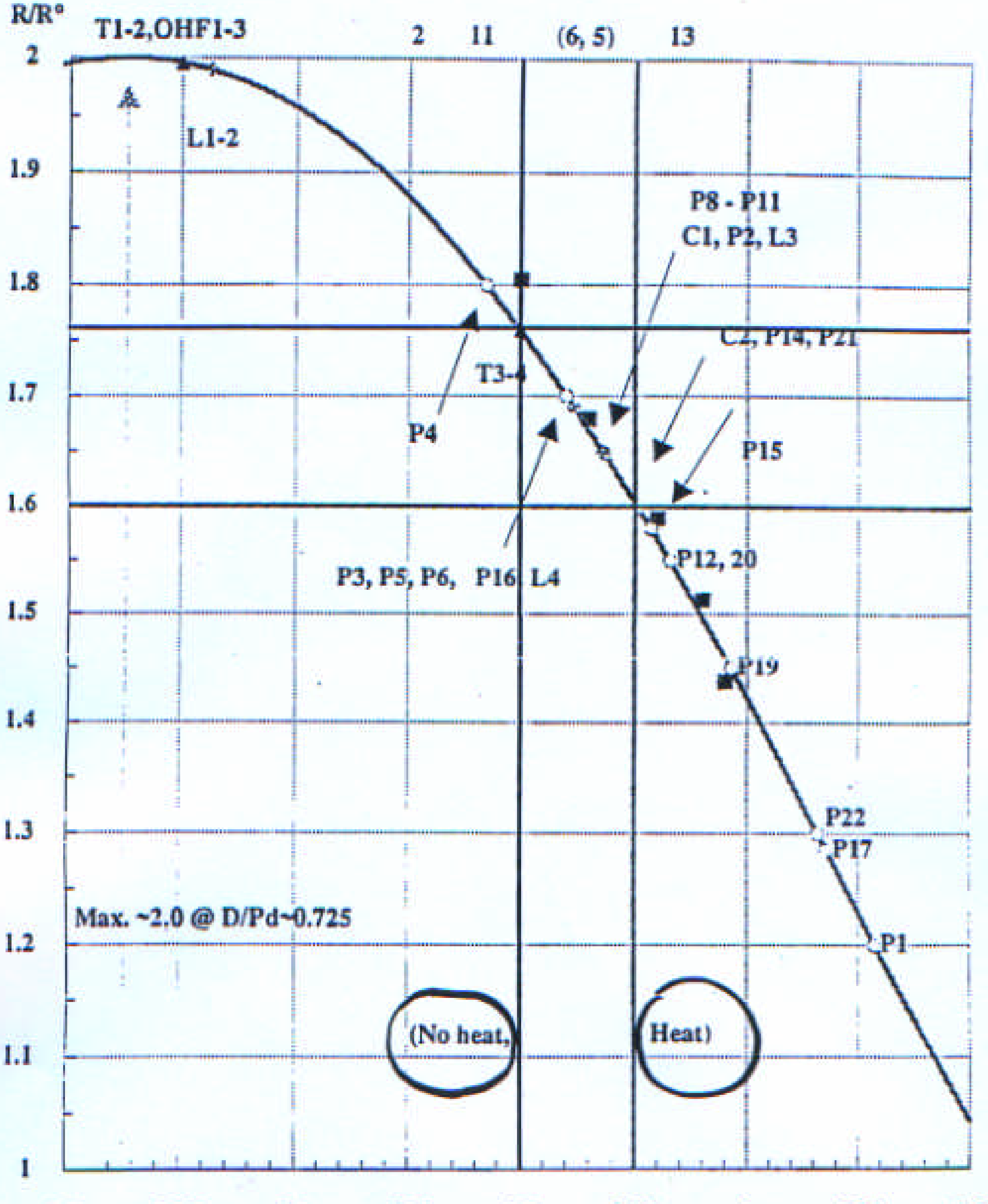

Figure 1 Maximum loading, D/Pd, attained in experiment; determined by R/R°.

Procedure at INFN-LNF

In situ, during loading Pd wire, a semipermeabile (like a "DIODE"), thin and homogeneous barrier is made, which allows the entrance of Hydrogen or Deuterium into Pd like H or D (under the action of electric field due to electrolysis), is unfavorable for ricombination :

$$H+H \rightarrow H_2$$

$$D+D \rightarrow D_2$$

[The pressure of bubbles's gas is at low atmosphere.]

Moreover, the experimental evidence exists that H or D behaves like H^+ or D^+ once it goes inside Pd . Therefore, it can be moved by application of electric field along the wire (electro-migration[1] , it was discovered in1929 in Germany by Alfred Cöehn and later, it was proved and improved by Franzini in 1932 in Italy).

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[1] The diffusion coefficient dipends on the concentration of H or D in Pd.

***Another, strange, peculiarity of H(D)/Pd system is that the diffusion speed of H(D) changes in a remarkable way upon loading, according to the following Tab. 1

Phase	Concentration	Diffusion speed (cm^2/s)
α	<0.1	10^{-5}---10^{-6}
β	0.1--0.75	10^{-6}---10^{-7}
γ	0.75--1.0	10^{-4}---10^{-3}
super-γ	>1	$>10^{-3}$ (only indication)

Tab.1 Diffusion speed of α, β, γ, super-γ, H(D)-Pd phases.

***According to the electromigration formula, it is necessary to maximise the ratio V/T in order to increase the concentration gradient of H(D) in Pd.

The voltage drop (V), according to the Ohm law, is:

$$V=I*R=I*\rho*l/s=I*\rho*l/(\pi*r^2) \quad \text{(Eq.2)}$$

The *Effective Wire Temperature* (T_*ew*) depends on the ratio between the total surface area (S) and the volume (Vol.); if l>>r, we get Eq.3:

$$T\_we=1/(\tau*S/Vol.)\sim 1/[\tau*(2*\pi*r*l)/(\pi*r^2*l)]=r/(2*\tau) \quad \text{(Eq.3)}$$

As final result, the ratio V/T as the following expression, Eq.4:

$$V/T=[I*\rho*l/(\pi*r^2)]/(r/2)=(2*I*\rho*\tau*l/\pi)*(1/r^3) \quad \text{(Eq.4)}$$

where:

ρ=resistivity; l=length; s=surface of cross section; r=radius; τ=heat exchange constant.

It results that, given some material resistivity ρ and heat exchanging constant (τ) of the bath where the wire is immersed, the ratio depends only on: $1/r^3$.

**In Fig.1 it is shown the behaviour of ratio V/T versus current flowing inside pure Pd wire (r=25μm), in pure water at 20^C.

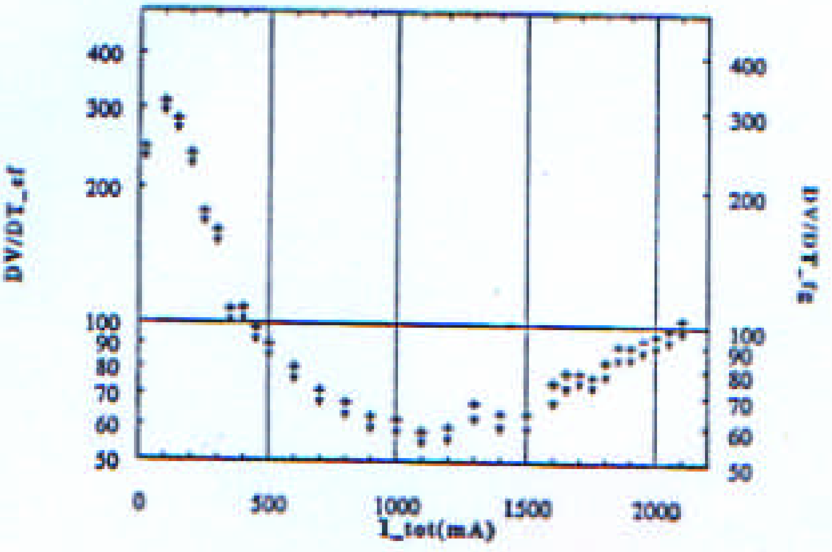

Fig.1 ΔV/ΔT ratio vs. electromigration current.

ensues in a rapid increase of the pH value. This generates a rapid increase of CO_3^{2-} concentration (proportional to the square of the OH^- concentration). At the same time there is also an enrichment of Sr^{2+} ions, attracted by the cathodic field. In these conditions the ionic concentration product $[Sr^{2+}]$ $[CO_3^{2-}]$ becomes higher than the solubility product and there is precipitation. As the time goes on, more CO_3^{2-} ions are rejected by the cathode than Sr^{2+} ions are attracted, because the mobility of the former is higher than the mobility of the latter. So it happens that the ionic concentration product drops back to the solubility product, thereby arresting the precipitation. This latter process is faster the higher is the current density so, at higher current densities, the precipitation stops earlier and the correspondent thickness of the precipitated layer is smaller.

3 – EXPERIMENTAL APPARATUS

The schematic diagram of the experimental set-up is shown in Fig. 4. The electrolytic cell is a glass cylinder containing 2.4 litres of electrolyte. The cathode is a thin Pd wire (diameter 50 μm, length 30 cm) while a Pt wire with a diameter of 500 μm and a length of 30 cm is the anode. The anode was set at a distance of 5 cm from the cathode. The cell was kept at the fixed temperature of 20°C.

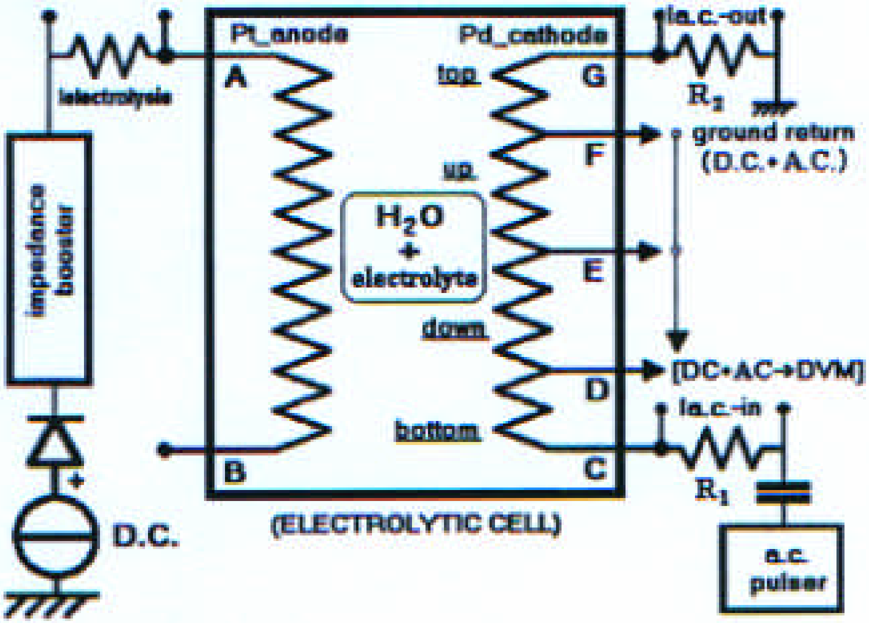

Figure 4 - Apparatus set-up.
Electrolysis cell is composed by anode-cathode electrodes fully immersed into an electrolytic solution (H_2O+HCl+Alkaline-Earth soluble salt): resistance is measured for "up" and "down" Pd segment wires. External electronic devices are divided in power supply block (DC generator and impedance booster) and measurement block (ac-pulser, ground-return connection and several picks-up connected to Digital VoltMeter). High precision and stability resistances have been used to acquire circuit currents (of D.C. and a.c. generators).

For a sake of experimental simplicity in these experiments, we adopted the parallel geometry of the wire instead of the coaxial one, as reported in the numerical simulation. It can be shown that the electrical paths, about electric field homogeneity at the cathode, are similar to coaxial geometry when the anode-cathode distance is >>20 radius of the cathode.

The low value for the cathode diameter was chosen both to enhance the H absorption by

New experimental approach at INFN-LNF

Date	Main Experiment	Side Experiment
1994~ 1998 Mar	Wire : 100 μm, ϕ 100~300cm ℓ D_2O Li OD (10^{-1}~10^{-4} mol)	Wire 100 μm, ϕ 30~100cm ℓ H_2O, Li OH+ different electrolytes
1998 Apr~ 1999 Nov	Wire : 50 μm, 30cm H_2O $CaCl_2$, $SrCl_2$ + HCl (10^{-5}~10^{-4} mol) + $HgCl_2$ (10^{-6}~10^{-5} mol)	Wire : 50 μm, 30cm D_2O $SrCl_2$ + HCl (10^{-5}~10^{-4} mol) + $HgCl_2$ (10^{-6}~10^{-5} mol)

Based on the study of overloding of H, or D in Pd and of movement of it inside Pd, we selected some reliable technique in order to apply them to the Pd-D system.

Experimental problems

During the loading, the Pd also distorted 8% in volume with production of :

✱Superficial cracks
(paths of dispelled H or D)

✱✱Dislocations, internal vacances
($H+H \rightarrow H_2$ gas)

a) Grade of purity of Pd
(if purity is too much, 99.99%, it doesn't work well.)

b) Metallurgical preparation and procedure of loading
(Current density, time pattern, addition of proper impurities like INFN-LNF method, impurities are anti-cracking agent whose amount is 10^{-6} mol).

Procedure of Experiment
at INFN-LNF

a thin wire (50 μm)

a) Small diameter, superficial bubbles (H_2, D_2) of small dimentions

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large pressure

b) Large gradient of electric field ($\propto 1/r$) like in a proportional, coaxial, wire chamber

c) Possibility of changing, in large amount, the range of pH of solution, from 9 to 13, surrounding the cathode, (It was given by Computer simulation, just changing the electrolysis's current density).

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Simultaneously, it's possible to obtain a situation in which there is a dissoluble salt in the electrolyte and another indissoluble salt,which is formed by the same element, deposits on the wire.

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Control the thickness of deposit in feedback by means of acid in order to dissolve the salt in "excess".

[ex.]

$SrCl_2$	soluble in the solution
HCl	acid
$SrCO_3$	insoluble over the cathode

Table 1 – Equivalent ionic conductance at infinite dilution, in water, at 25°C.

CATHIONS	Λ_{0+} (Ω^{-1} cm^2 eq.$^{-1}$)	ANIONS	Λ_{0-} (Ω^{-1} cm^2 eq.$^{-1}$)
H^+	349.8	OH^-	198.3
Li^+	38.6	F^-	55.4
Na^+	50.1	Cl^-	76.35
K^+	73.5	Br^-	78.1
Mg^{2+}	53.0	I^-	76.8
Ca^{2+}	59.5	HCO_3^-	44.5
Sr^{2+}	59.4	SO_4^{2-}	80.0
Ba^{2+}	63.6	CO_3^{2-}	69.3

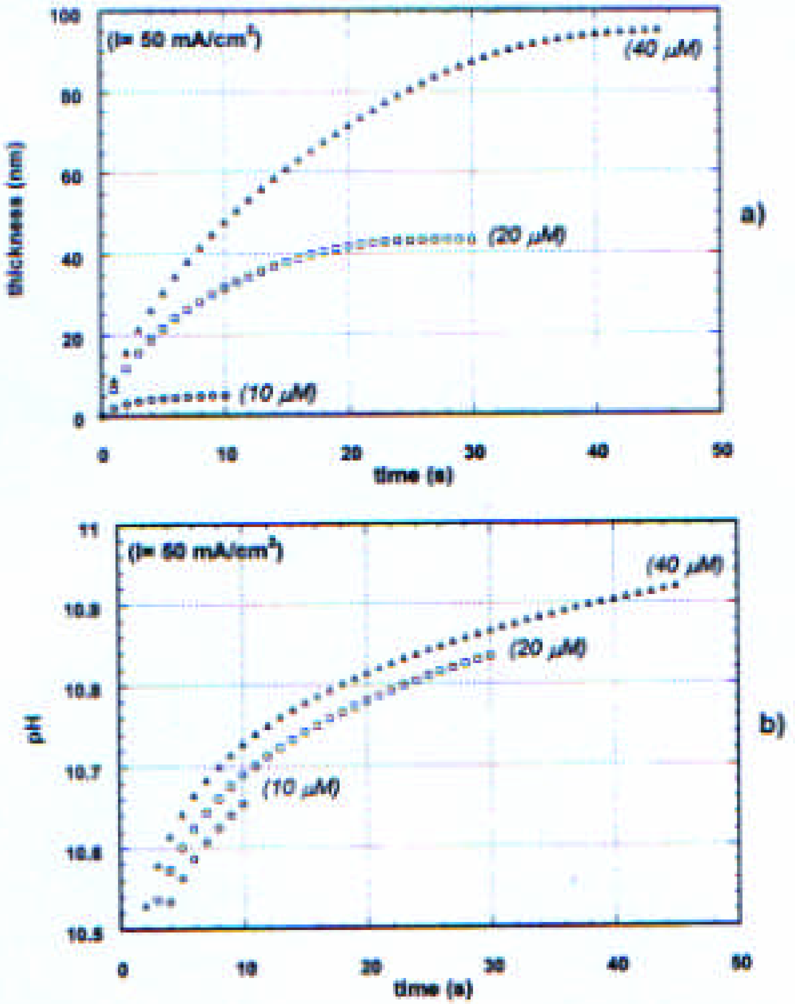


***Figure 2** – Computer simulation at constant current density of 50 mA/cm², wire diameter 50 µm. **a)** $SrCO_3$ deposition thickness, versus time, at 10, 20, 40 µM of Sr^{2+} ions concentrations;*
***b)** pH variation versus time at 10, 20, 40 µM of Sr^{2+} ions concentrations.*

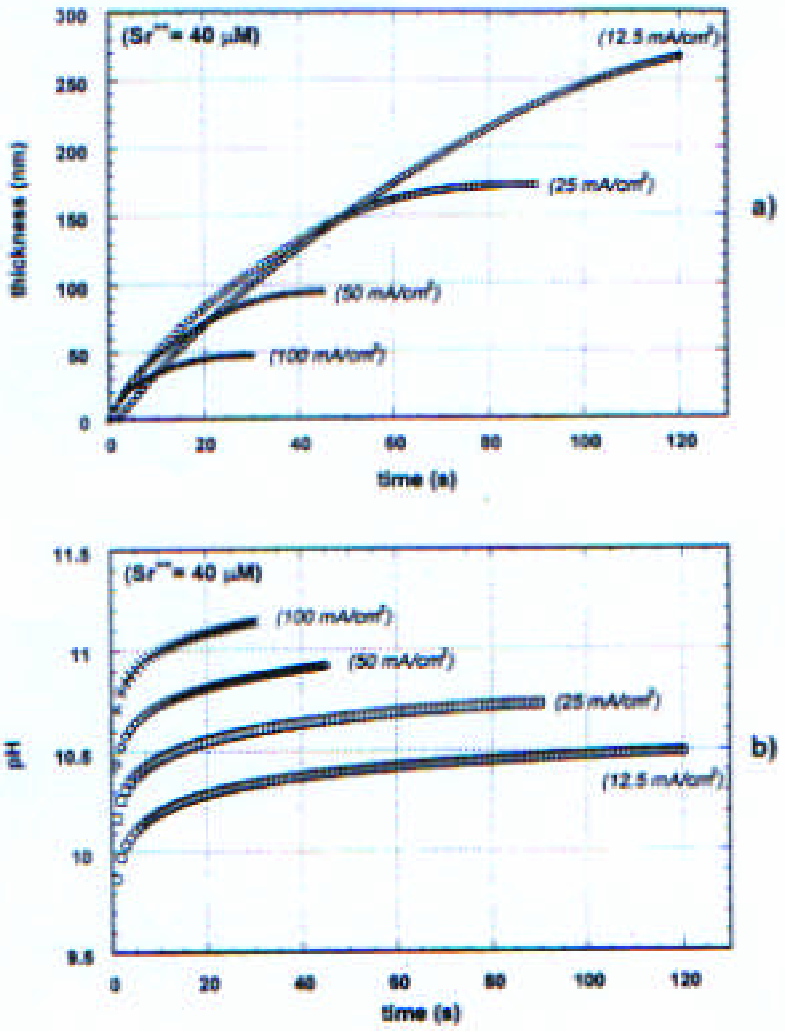


Figure 3 *– Computer simulation at constant concentration of 40 µM Sr^{++} ion concentration, Pd wire diameter 50 µm.* ***a)*** *$SrCO_3$ deposition thickness versus time at 12.5, 25, 50, 100 mA/cm² current density;* ***b)*** *pH variation versus time at 12.5, 25, 50, 100 mA/cm² current density.*

The results can be summarised as following:

a) the pH value in the immediate proximity of the cathode depends on the electrolytic current density and in a few seconds raises to a stable asymptotic value;

b) the thickness of the layer reaches, versus time, an asymptotic value which decreases exponentially by increasing the electrolytic current. The density of the coating was assumed equal to the bulk density of $SrCO_3$;

c) the thickness of the layer increases by increasing the Sr concentration in the electrolyte before the beginning of the electrolytic process; no (practically) useful precipitation (about 4.8 nm) occurs when this concentration is lower than 10^{-5} M, in our experimental conditions (Pd wire: length 30 cm, diameter 50 µm);

d) it is possible to achieve a very fine control of the thickness.

The not-intuitive behaviours reported at points **a)** and **b)** can be explained through the following cross-linked considerations.

At the cathode there is a strong subtraction of H^+ ions because of their discharge, which best results were obtained with Ca and Sr and are reported hereafter.

In Fig.s 5 and 6, measurements of electric resistance ratios of the "up" (EF) and "down" (DE) wire sections (see fig. 4) are presented as function of time, adopting the Ca^{++} ($7.0\ 10^{-5}$ M) and Sr^{++} ($3.5\ 10^{-5}$ M) electrolytes, respectively. The graphic representations of the loading process (ρ versus log time) show a first stage, lasting about 300 seconds, during which ρ goes from 1 up to a maximum of 1.78-1,80, corresponding to a loading level x=H/Pd=0.75, according to the Baranowski diagram. Afterwards, as the loading proceeds the value of ρ decreases. The course of this decrease shows a "shoulder", after which ρ drops asymptotically toward minimum values, usually different in the various segments of the wire and depending on the alkaline-earth ion added to the "ground solution".

In Ca-based electrolyte after 12 hours, loading corresponding to ρ_up=1.28 and ρ_down=1.38 were obtained (see Fig. 5).

In Sr-based electrolyte the course is very similar, the final values obtained were even better: ρ _up= 1.13 and ρ _down=1.15 (Fig. 6).

At these values, the effective cathode loading cannot be determined directly from the Baranowski curve. A reasonable and conservative extrapolation indicates exceptionally high loading levels, around x=0.99 or even higher.

In order to check the correctness of the results, the electrolytic process was stopped to allow the complete de-loading of the cathode and to control that the ρ values go back to the maximum and then decrease to the initial ρ=1 value. Fig. 7 shows a complete de-loading of a cathode previously loaded down to ρ=1.13. The complete de-loading required about 16 hours. Several loading/de-loading cycles were performed and substantially confirmed the obtained results.

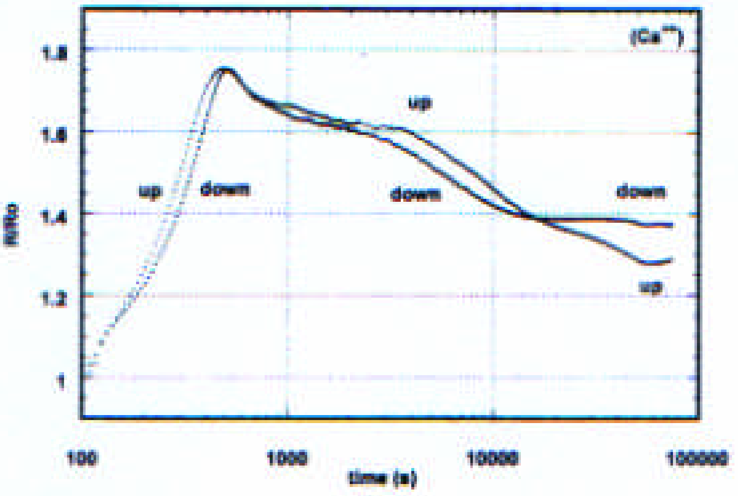


***Figure 5** – Calcium loading trend.*
Loading trend curve (R/Ro vs log time) occurred when Ca ions ($70*10^{-6}$ M) have been added to the electrolyte; in about 300s resistivity peak was achieved. It is visible a shoulder lasting for about a hour after the peak; the highest loading (R/Ro=1.28 for "up" and 1.38 for "down" at 23 °C) reached stable values in about one day. ("up" and "down" labels are corresponding to up (EF) and down (DE) Pd wire segments, see Figure 4).

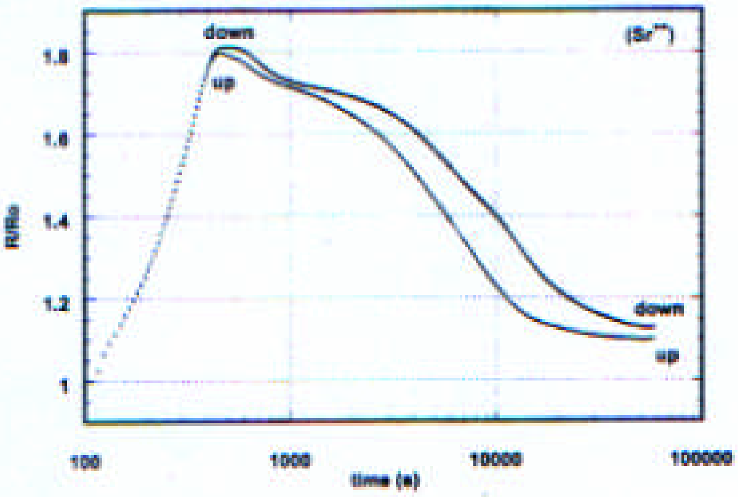


Figure 6 *– Strontium loading trend.*
Loading trend curve (R/Ro vs log time) occurred when Sr ions ($35*10^{-6}$ M) have been added to the electrolyte; in about 300s resistivity peak was achieved. The shoulder occurring lasts about half hour; loading higher than Ca test (R/Ro=1.13 for "up" and 1.15 for "down" at 27 °C) were reached in less than one day.

4.1 – Evidence of a new phase in the system Pd-H

Experiments with the Sr containing electrolyte have shown a remarkable phenomenon: once the ultimate loading level was reached, corresponding to ρ=1.13, the electrolysis was interrupted and the wire was allowed to de-load toward the maximum value of ρ : the electrolysis was restarted at R/Ro=1.7 (H/Pd= 0.8). It was observed that the re-loading was so rapid that in about 20 minutes the value of ρ dropped to 1.2. The subsequent lowering to the starting value of 1.13 was reached within the next 40 minutes (Fig. 8).

The occurrence of very rapid re-loading after the first loading is highly reproducible, in the sense that it happens every time ρ becomes lower than 1.4.

If the wire is made to lose H beyond the ρ =1.80, that is under x=0.75, a clear "shoulder" appears in the course of the de-loading (Fig 7), meaning that some process which slows down the de-loading is taking place. If the de-loading is carried out beyond this "shoulder" the re-loading process is found to be as slow as any first loading. Furthermore, such a "shoulder" is observable only when the loading has been carried out down to ρ <1.4 (i.e. H/Pd>0.97).

The described behaviour suggests that, during the first loading, a slow process of nucleation of a solid phase in the Pd-H system may take place. This new phase should be able to absorb hydrogen at a relatively high rate. Metastable nuclei of this phase would then "survive" a partial de-loading (before the above said "shoulder") and then allow for the rapid re-loading, while if the wire is de-loaded further-on, they disappear and the whole process, including their slow formation, has to start again.

This is also supported by the presence of another "shoulder" referred to in Fig. 6, and characteristic of the first loading. It appears reasonable to think that in the "first-loading shoulder time" there is a slow nucleation of this new phase at x>0.9. The fact that an analogous "shoulder" presents itself during de-loading at x = 0.7, strongly suggests the occurrence of a hysteresis which can be just bound first to the nucleation and growth of a phase and then to its disappearance.

R_t is the resistivity at the temperature t expressed in °C;
R_0 is the resistivity at 0°C;
α is the resistivity thermal coefficient (RTC).

Previous assessments in the range of x (=H/Pd) between 0 and 0.70, have shown that the RTC starts with a value of $4.1 \cdot 10^{-3}$ °C $^{-1}$ at x=0 and increases between x=0 and x=0.08, up to $4.35 \cdot 10^{-3}$ °C $^{-1}$, then decreases monotonically as x increases (Fig. 9). Our measurements are substantially consistent with those in the literature [ref. 7]. At x=0.75 the value found for α is $1.7 \cdot 10^{-3}$ °C $^{-1}$, which is very close to the value of (1.5--1.6) 10^{-3} obtainable by extrapolating the values of Fig. 9.

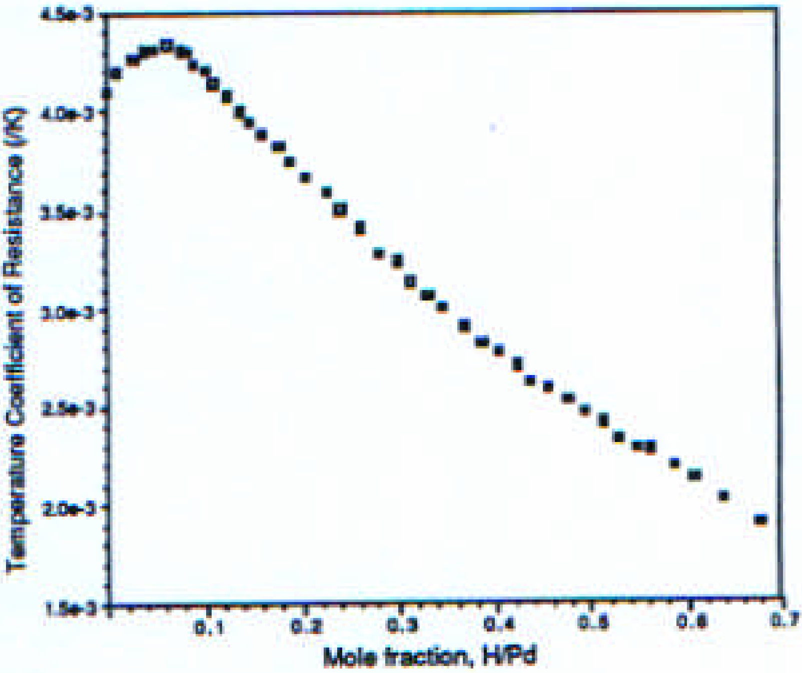


***Figure 9** - H/Pd resistance temperature coefficient.*
According to the literature data (Baranowsky et al.) this coefficient almost linearly decreases from 4.1 10^{-3} K^{-1} (end of α phase) to 1.8 10^{-3} K^{-1} (end of $\alpha+\beta$ phase) because H loading. At H/Pd >0.70 no data are allowable.

If in the Pd-H system a new phase nucleated at higher loading levels, it is probable that also the RTC would undergo a significant variation. In other words, if beyond certain H/Pd values there is a significant change in the RTC course, it would be reasonable to think that a new phase has nucleated and grown.

A correct assessment of the value of α requires that the value of x= H/Pd remains stable while the temperature is made to change and the correspondent measurements of the resistance are effected.

The stability of the loading values has been obtained by profiting of the barrier effect produced by electrolytic coatings on the cathode, with metals which were found to strongly inhibit the intake of H (see reaction *Ib*, above), therefore able to hinder its outgoing as well. Among the possible metals the choice fell over Hg because it forms *amalgams* with Pd (0.06 wt% at 20 °C, Ref. 9). Such room-temperature formation of solid solutions allows for complete and rather uniform coatings of the "physical" surface and, as extra bonus, the covering of

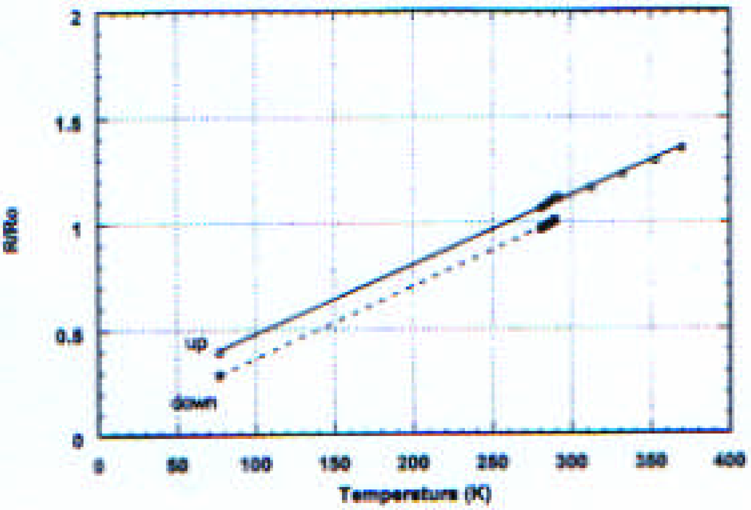


Figure 11 – Hg coated wire, test at very low temperatures.
After achieving R/Ro=1.1, the Pd surface was coated with Hg and a cycle of low temperature test (up to liquid nitrogen) was performed. For sector "up" both high and low temperatures data are available: they are roughly on the same line. In the range 77÷300 K the temperature coefficient, α_T, was estimated to be $3.3 \div 3.4\ 10^{-3}$ °C^{-1}.

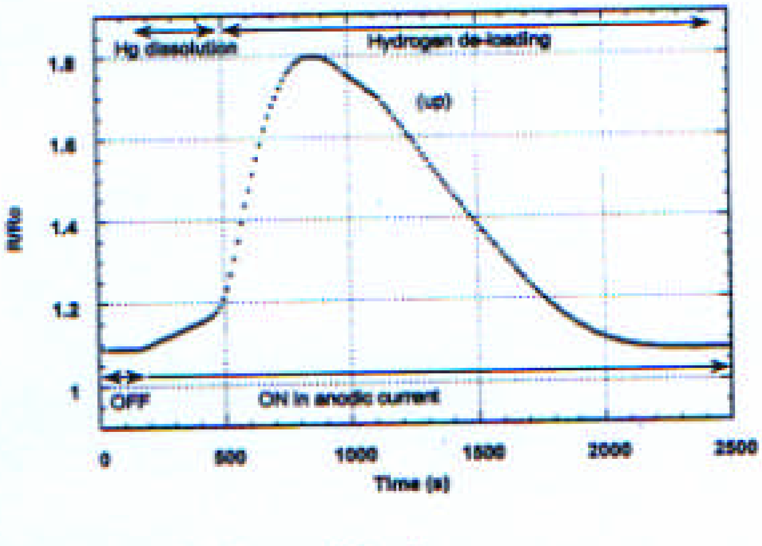


Figure 12 – Hg removing and Hydrogen de-loading.
Starting from very high loading (R/Ro=1.1), anodic electrolytic current was applied: in few minutes Hg deposit (about 4÷6 µm) at the Pd surface was time-linearly removed. Later on, hydrogen come out from the Pd following the expected trend (peak at about R/Ro= 1.8, shoulder at R/Ro=1.7) up to the initial resistance value (R/Ro≅ 1).

After the measurement campaign the wire has been put back in the cell and anodically de-loaded. In Fig. 12 the ρ value is reported against time at constant anodic current. It can be seen how, at the beginning of the electrolysis, there is an interval of time during which there is an increase of ρ between 1.1 up to 1.18 with a slope proportional to the current. This is clearly due to the dissolution of the Hg coating. When this is completely gone, the slope becomes steeper, due to H de-loading.

Going back to fig. 9, in the range of x= H/Pd between 0.1 and 0.7, the value of α possible surface defects like, for example, micro-crevices.

In practice, once a sufficiently high loading level was achieved (ρ=1.16), 10^{-3} moles of $HgCl_2$ have been added to the electrolyte, while the current was maintained at about 20mA. When the value of ρ had dropped to 1.10, due to the contribution of the Hg coating (notice that the resistivity of Hg is ten fold higher than that of the pure Pd; the thickness of the Hg film was estimated to be around 5 μm), the current was cut-down. It was observed that the ρ value remained stable for several hours at room-temperature, showing that the Hg coating reduces strongly the H de-loading. The temperature of the cell was then raised up to 100 °C and the electrolyte made to gently boil for one hour. After cooling down to 20 °C it was found that the value of ρ differed from the value reached before heating of a few percent. The wire was then taken out of the cell and put into liquid nitrogen for storage, before the measurements for the assessment of the value of α at ρ=1.16.

In Fig. 10 there are reported the values of ρ between 20 and 100 °C, while in Fig. 11, the temperature range is extended down to 77 °K. It can be seen that the results are well aligned one another. The average value for α was found to be $3.2\cdot10^{-3}$ °C^{-1},which is definitely higher than the minimum value reported in Fig. 9 correspondent to a value of $\rho=1.8\cdot10^{-3}$ °C^{-1}, beyond which no value for α is available. The contribution of the Hg film can be easily estimated to be of a few percent and therefore cannot significantly affect the validity of the results.

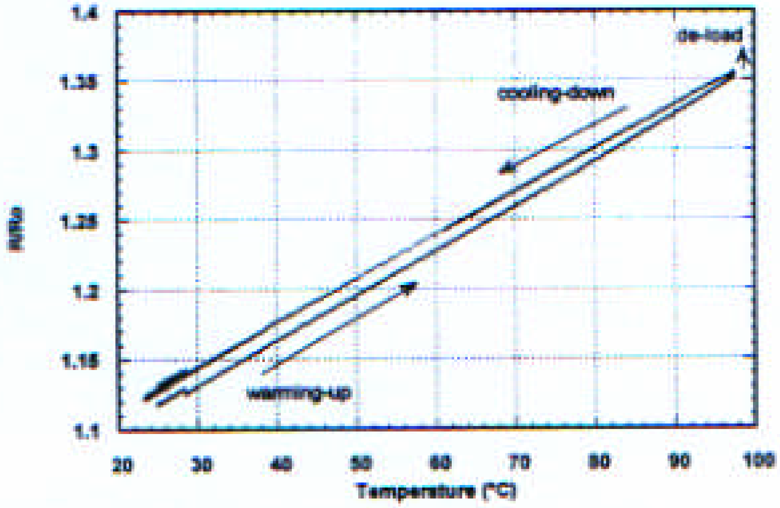


Figure 10 *– Hg coated wire, high temperature measurements.*
After achieving R/Ro=1.1, the Pd surface has been coated with Hg (by $HgCl_2$ *electrolysis) and a cycle of "high temperature test" was performed.*
Only a weak de-loading occurred at 100 °C (2 hours at ebullition state). In this experiment the temperature coefficient ($\alpha_{T=}$*) was estimated to be* $3.2\,10^{-3}$ °C^{-1}.

The limit of technique :

the purity of H_2O and D_2O

Impurity

- "inorganic substance"
- "organic substance"
- "something living"

[ex.]

Conditions of LNF experiment are (Pd=50 μm, 30cm)

H_2O=1200 cc→66.6 mol

HCl=2×10^{-5} mol

$SrCl_2$=2×10^{-5} mol

(1 mol Sr = 88g⇨1.8mg[Sr])

Anti Cracking Agent=10^{-6} mol

Impurity

H_2O

① **Inorganic substance**

Deionized ≦ 10 mg/ℓ **Not good**

Bi-**Distillated ≦ 0.3 mg/ℓ** **good**

② **Organic substance < 0.2 mg/ℓ** [TOC]

③ **Living things = 0**

if they are "sterilized "

D_2O

① **10～20 mg inorganic substance**

⇩

There is the necessity of further distillation.

② **～10 mg organic substance** [TOC]

③ **"Living things"**

(60 bacteria/ml was confirmed by INFN-LNF)

According to Biological investigation of INFN-LNF,(*) we have found one deleterious bacterium in that :

- **a) It makes the pH of solution change.**

- **b) It is deposited on the surface of cathode competing with useful deposits** (to be experimentally confirmed)

ONL
INDIC
TION

- **c) It inhibits the deposit of Anti-cracking agent on the cathode in the cause of reducing valences.**

(*) CLOSE COLLABORATION WITH
ENEA - CR CASACCIA - ITALY
GROUP OF G.D'AGOSTARO

III. SPECIFICATION

HEAVY WATER GRADE: REACTOR GRADE

Technical Specifications

D_2O Isotopic Purity	**99.92 wt %**
$KMnO_4$ Demand	**< 10 mg/kg**
Conductivity	**< 1.5 mS/m**
Tritium	**< 2 µCi/kg**
Turbidity	**< 2 FTU**
pH	**6 - 10**
Odour	**None**
Visible Oil	**None**

MASAO SUMI & NHE PROJECT - SAPPORO - JAPAN
NAOTO ASAMI
KAZUAKI MATSUI
1998

FAX (39)06 9403 2548

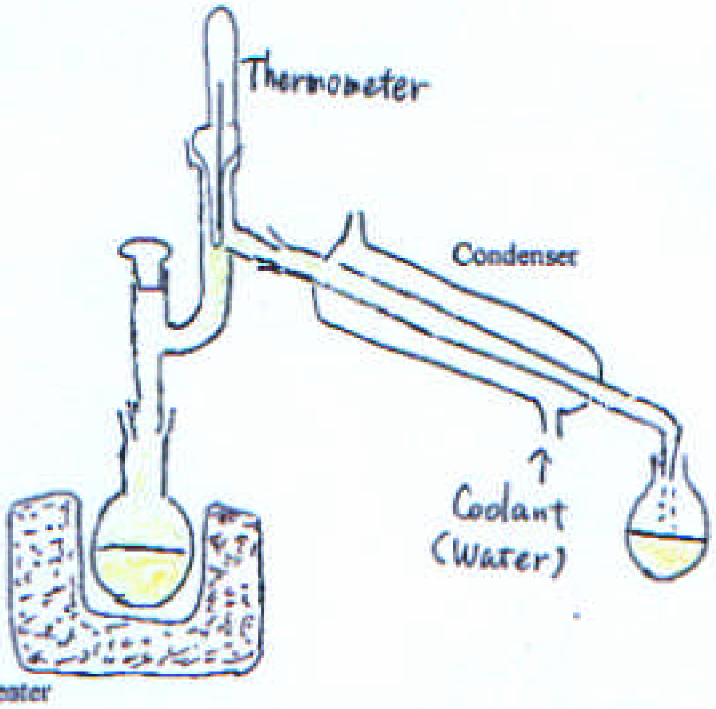
Dear Dr. Celani

29 July, 1999

The follow is our results on NHE Project.

	as received		Distillate					Analysis
Item	ISOTEC	Aldrich	forerun	A	B	Remainder	unit	Method
H/D	0.17	0.09	0.23	0.12	0.10	0.15	%	NMR
Si	0.82	0.07	D.L.>	D.L.>	D.L.>	2.5	ppm	ICP-AES
Ca	0.28	D.L.>	D.L.>	D.L.>	D.L.>	D.L.>	ppm	ICP-AES
Na	2.1	0.21	0.16	0.08	0.08	2.2	ppm	ICP-AES
TOC	12	9.3	19	D.L.>	D.L.>	1.4	ppm	TOC analyzer
Al	2.5	0.22	0.9	D.L.>	D.L.>	59	ppb	ICP-MS
Ni	0.85	0.41	D.L.>	D.L.>	D.L.>	0.93	ppb	ICP-MS
Cu	1.0	0.20	D.L.>	D.L.>	D.L.>	1.1	ppb	ICP-MS
Sr	5.9	0.58	0.20	0.11	0.10	0.16	ppb	ICP-MS
Zr	0.48	0.23	0.24	0.16	0.17	0.25	ppb	ICP-MS
Sn	0.60	D.L.>	0.17	D.L.>	D.L.>	0.18	ppb	ICP-MS
Ba	0.58	14	D.L.>	D.L.>	D.L.>	1.0	ppb	ICP-MS

TOC means total organic carbon. Volatile organic material was included in the heavy water. Because the forerun had the largest TOC.



Sincerely yours

Masao Sumi

```
{21;7} (Idc_in   )=    0.0000 [_A  ]      {23;7} (DC_tub   )=    1.8971 [_V  ]

{22;0} (AC_top   )=    1.6818 [_V  ]      R_top_  =  0.092    R/Ro_t  = 92.249
>{22;1} (AC_up    )=    1.6769 [_V  ]      R_up__  =  0.074    R/Ro_u  = 74.259
{22;2} (AC_mid   )=    1.6739 [_V  ]      R_down  =  0.000    R/Ro_d  =  0.219
{22;3} (AC_dow   )=    1.6739 [_V  ]      R_bot_  = 30.218    R/Ro_b  =  1.786
{22;4} (AC_bot   )=    0.4376 [_V  ]      R_tube  =  0.190    RRoTub  =190.094
{22;5} (Iac_in   )=    0.0409 [_A  ]
{22;6} (Iac_ou   )=    0.0410 [_A  ]
{22;7} (AC_tub   )=    0.4298 [_V  ]      (Rsol=      0.00)
```

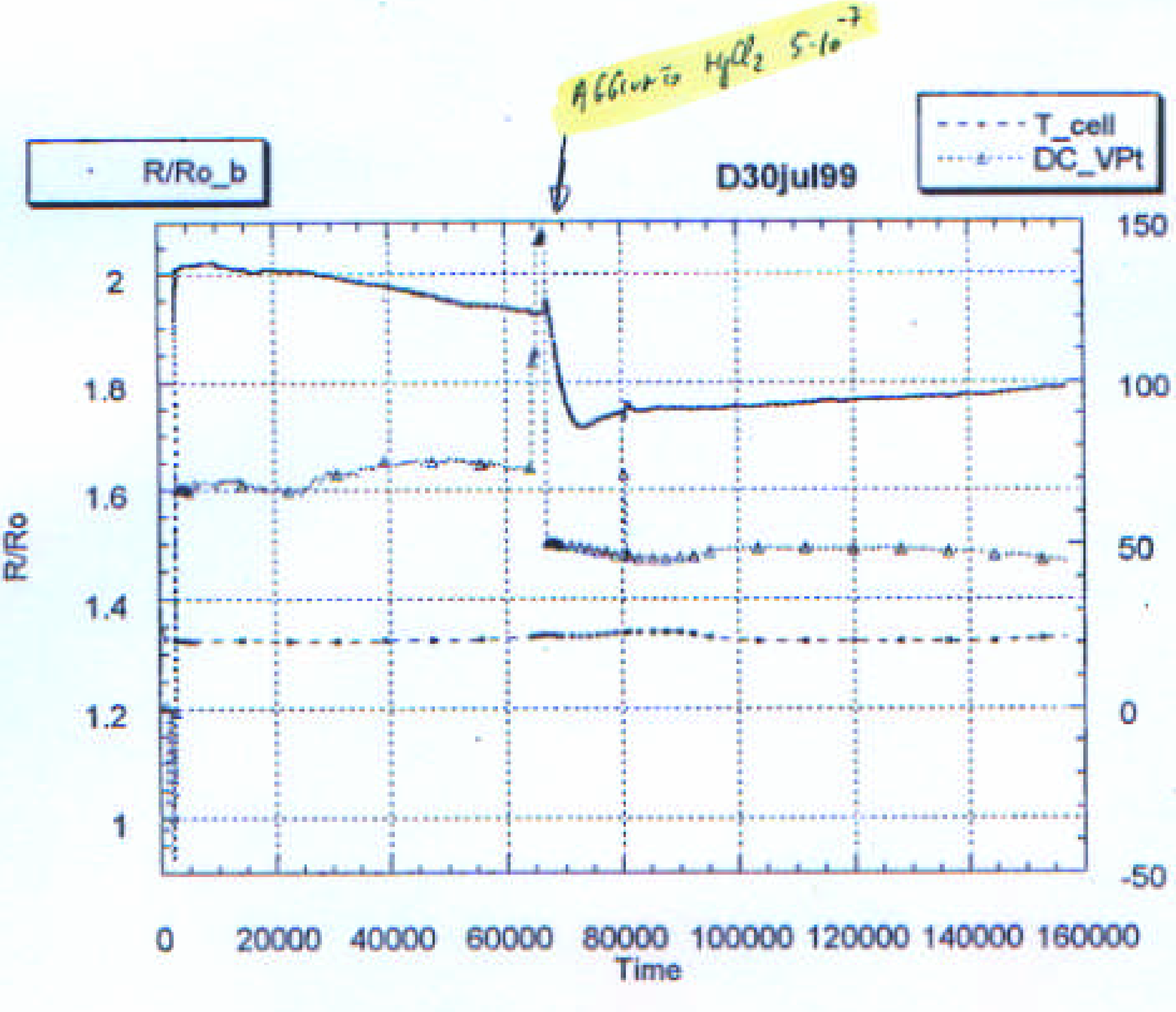
RESTART

```
Type: W =WAIT/Comment; T =Time_step; F =Flux; R =New_Ro; Z =END

TOTev:   6941;     File: C:\FILOFUS\DATA\R01aug99.14h             Step:[   1.0
Nev:   2942;  at 12:44:30  ;  dt:      5.4      h:12:44:30  ; Timer [s]: 23754
TOTev:   7002;     File: C:\FILOFUS\DATA\R01aug99.14h             Step:[   1.0
```

VIRGIN D_2O

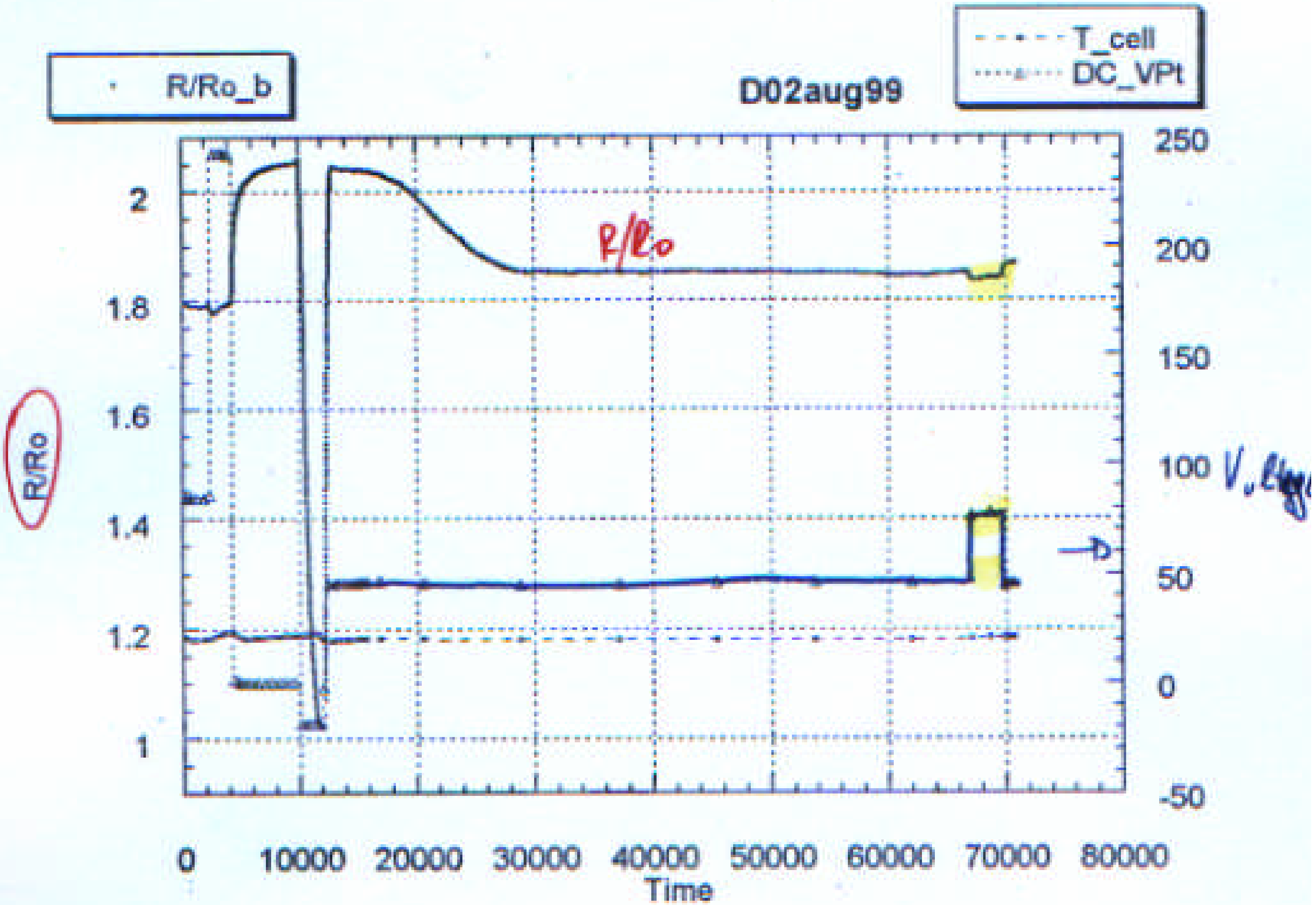
NO FILTERING



AGGIUNTO H_2O_2 $5 \cdot 10^{-7}$

```
TOTev:    6562;    File: C:\FILOFUS\DATA\R03aug99.11m              Step:[   1.0
Nev:    2563;  at 16:16:25  ;  dt:       4.6      h:16:16:25  ; Timer [s]:   8806
```

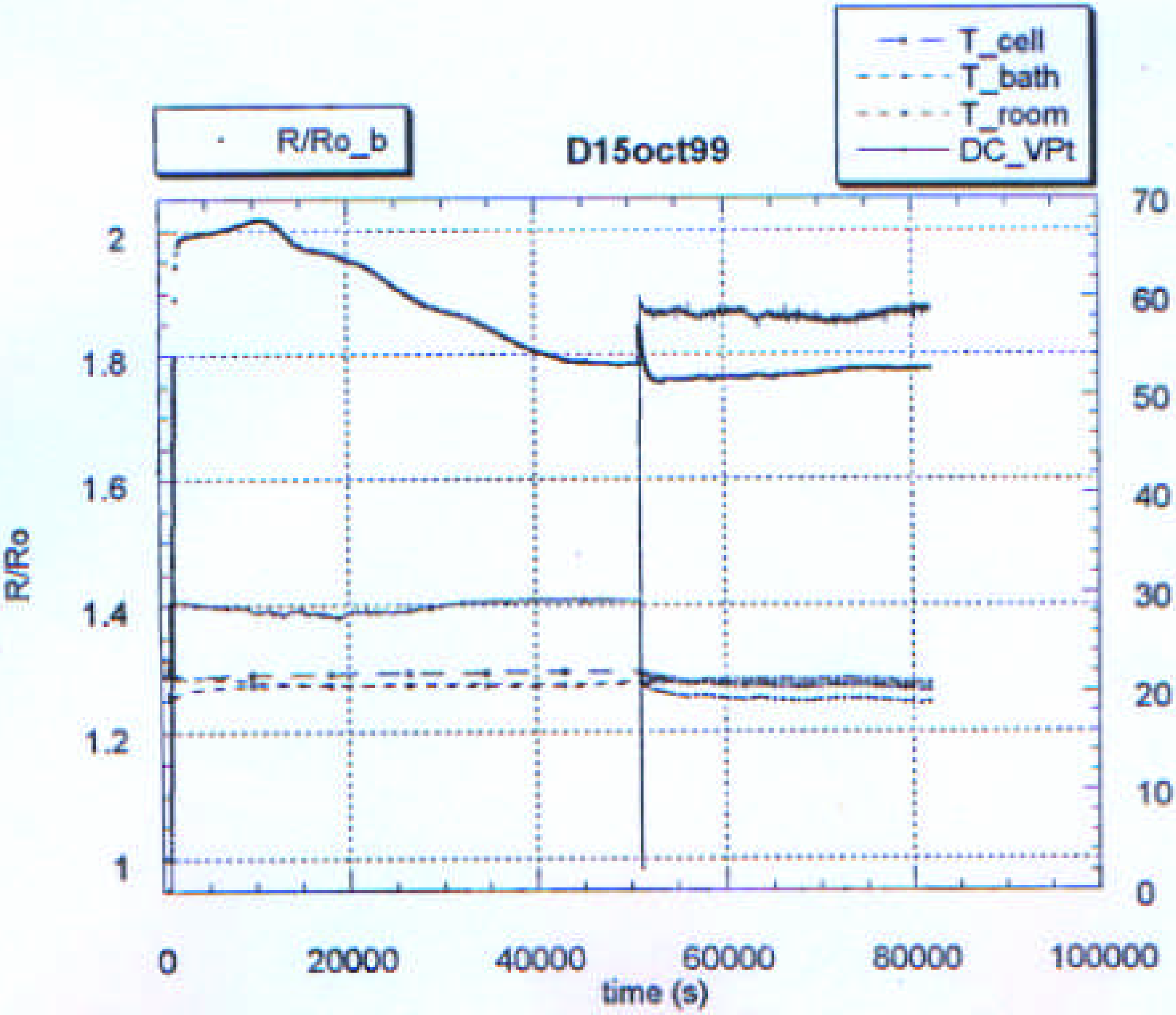
VIRGIN D_2O
NO FILTERING



FILTERED D2O

WITH:

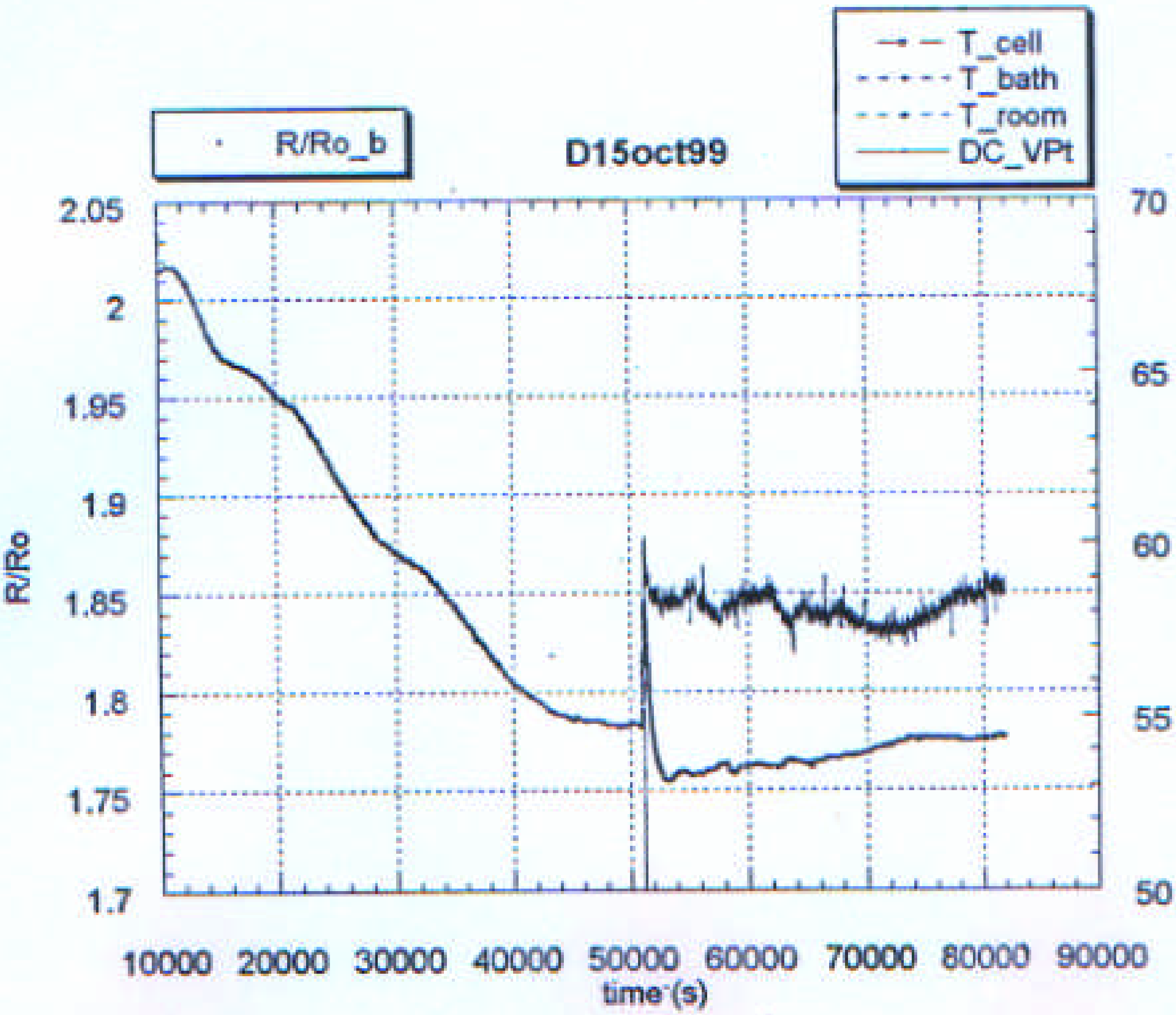
3 X 100 mm IN SERIES, MILLIPORE

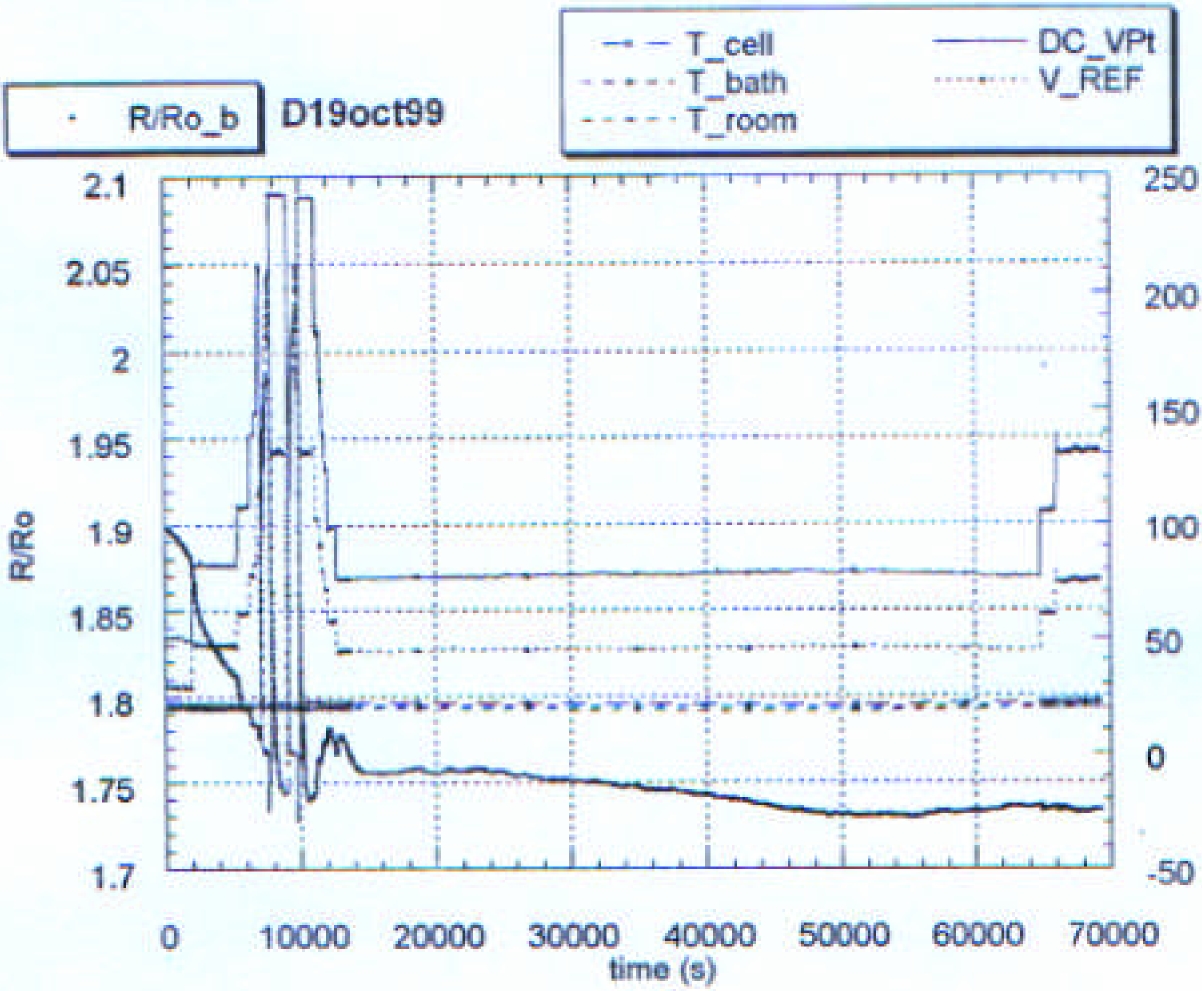


FILTERED D_2O

WITH

3X100 nm IN SERIES FILTERS, MILLIPORE





FILTERED D_2O
WITH
3x100 mm FILTERS IN SERIES, MILLIPORE -

N.B. R/R_0 EFFECTIVE*, STABLE (> 12^h) IS
1.69 at 12 mA (ϕ=50 μm, l=30 cm)

* CORRECTED FOR R_{max}: 2.05

NEW TREATMENT AND ELECTROLISYS PROCEDURE FOR D_2O.

① BACTERIA "DEPLETION" BY

- ADDITION 1 g/ℓ-D_2O of $KMnO_4$ + H_2SO_4(96%)
  ($pH \simeq 1.3$). T= 80°C, 1^h.
- VACUUM DISTILLATION, ∂ T= 30°C
- ADDITION OF LiOD up to pH = 13
  T= 80°C, 30^m
- VACUUM DISTILLATION, ∂ T= 30°C
  pH ~ 8 R_{AC} > 400 KΩ/cm

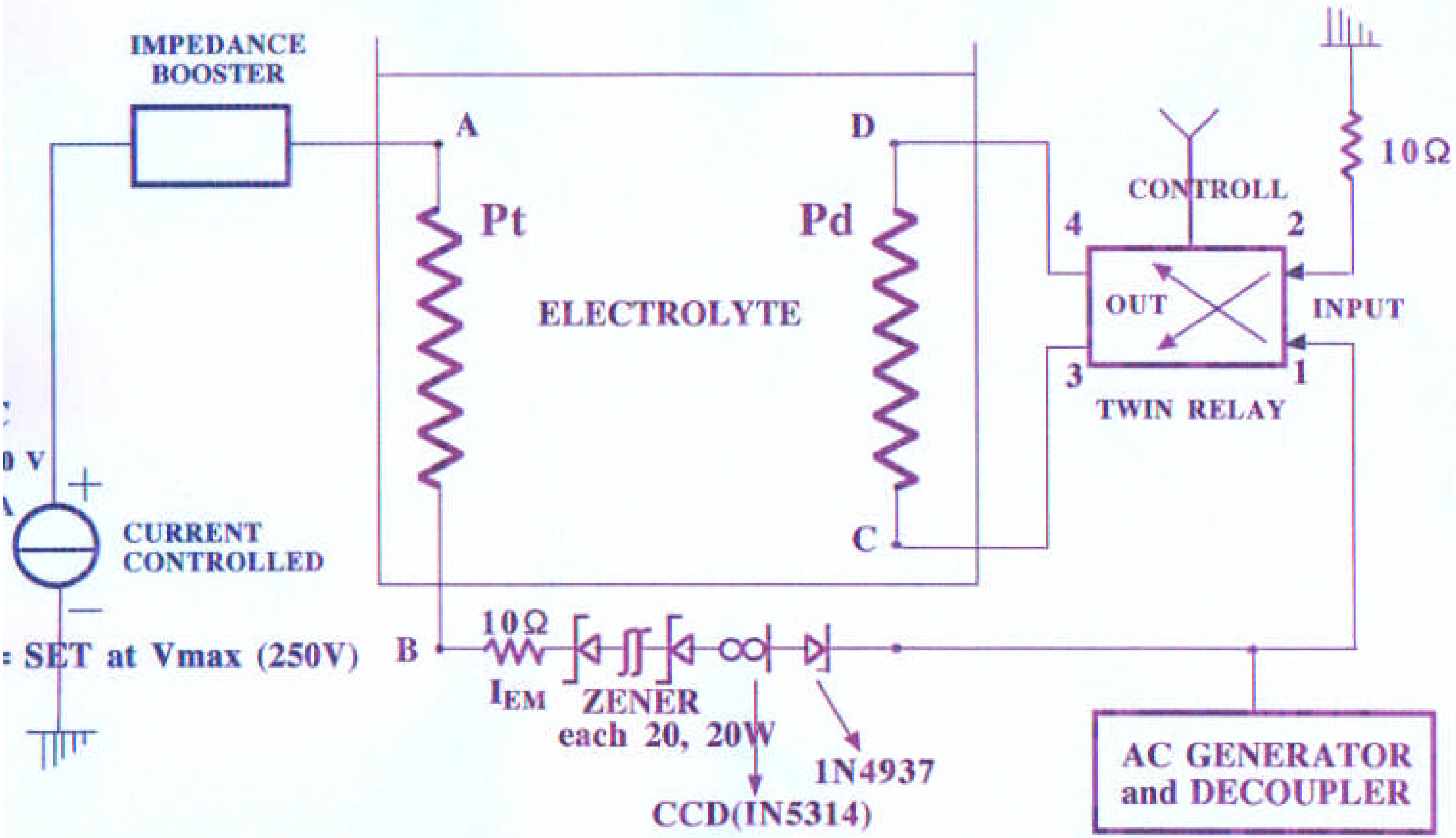
② NEW CIRCUITRY TO STRONGLY REDUCE DELOADING, AFTER REACHING HIGH VALUES OF D/Pd.

③ ADDITION OF SILICA (12 mM) + "BACTERICIDE" TO REDUCE THE FURTHER GROWING OF BACTERIA AFTER PURIFICATION

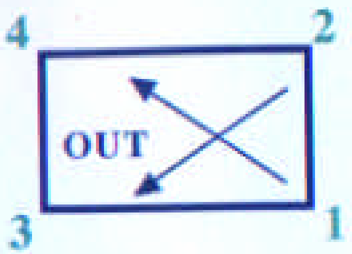
④ ADDITION OF $HgCl_2$ (10^{-5} M) when D/Pd > 0.90

CELL ELECTRIC CONNECTIONS

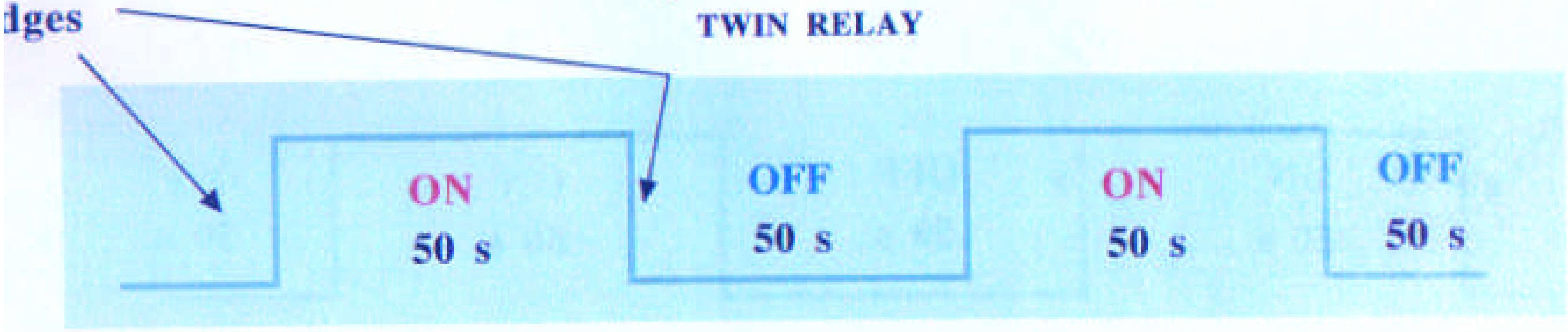
wire : $\phi = 50\,\mu m$, $\ell = 33cm$, Surface $\fallingdotseq 0.5cm^2$



RELAY CONTROL



TWIN RELAY



ON $1 \longleftrightarrow 3$
$2 \longleftrightarrow 4$

OFF : $1 \longleftrightarrow 4$
$2 \longleftrightarrow 3$

teady Conditions (Typical operative Condition) :

$I_{Electrolysis} = 20 \sim 60$ mA, $I_{EM} = 5$ mA

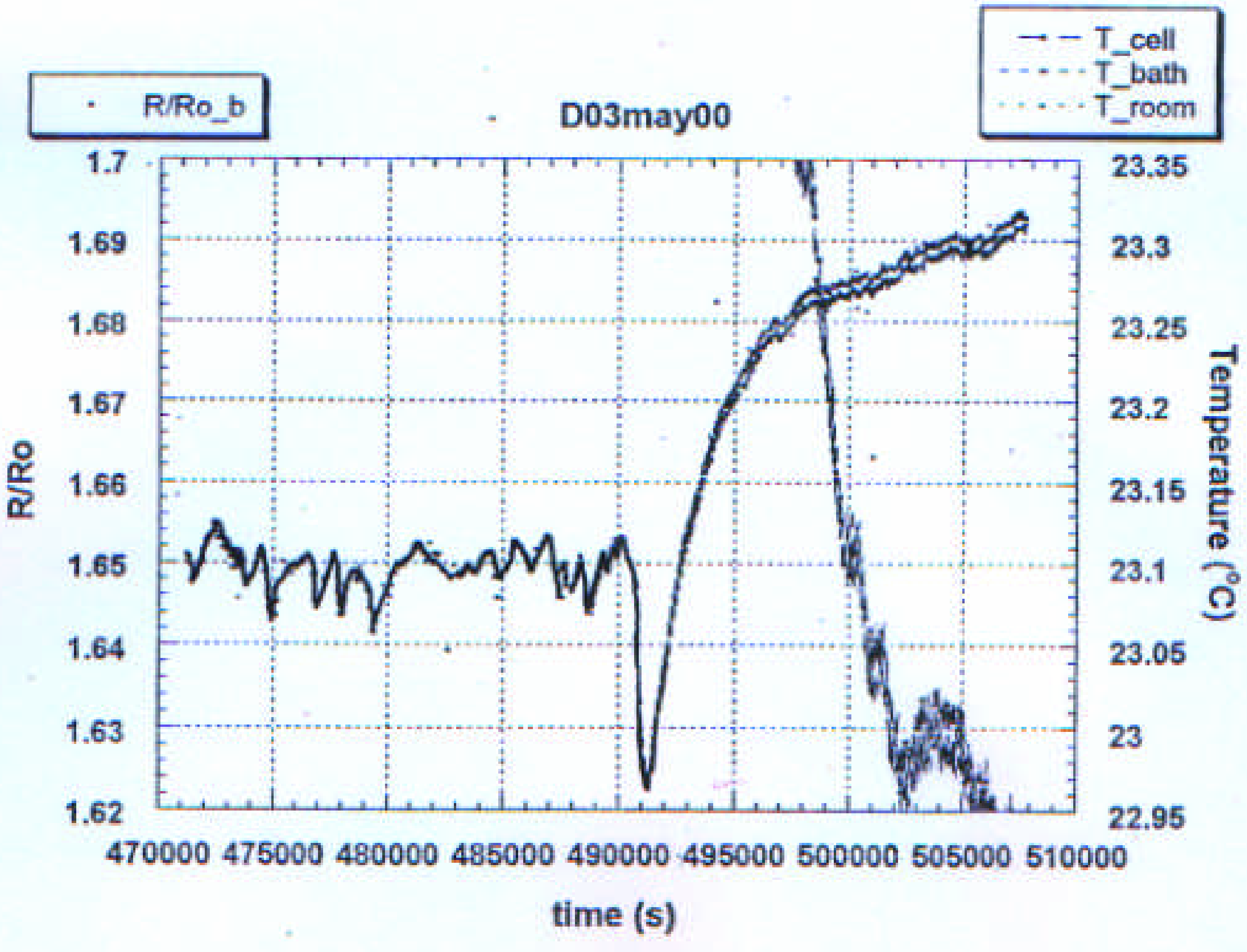
dges : Effective time is about 2 ms. The Pd is not connected. ⇨ NO ELECTROLYSIS

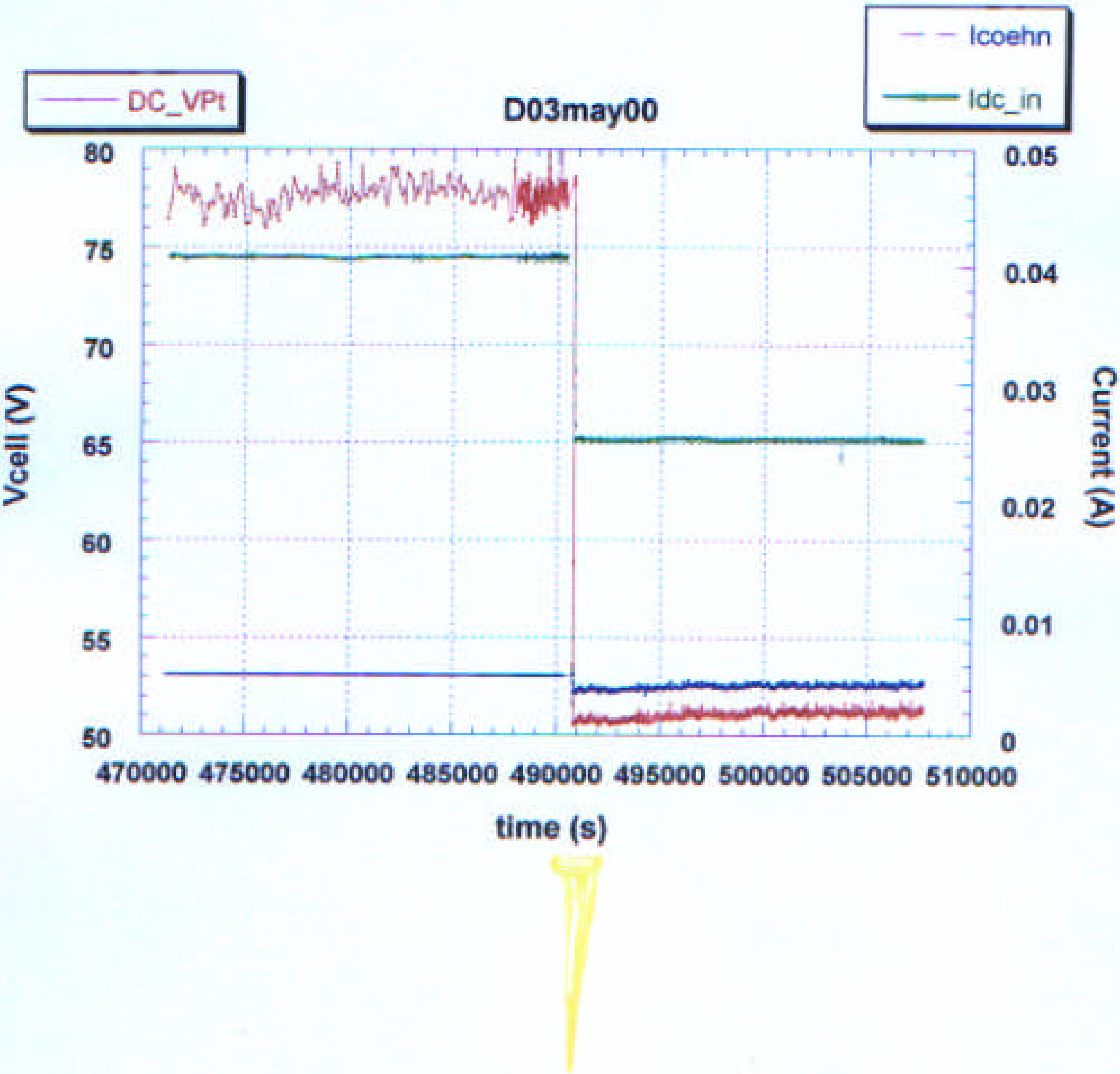
- V_{DC} increases toward the MAX value allowed from the P.S.
- At connection, we have :
  - $V_{Pd\text{-}Pt} = V_{Max}$ reached after 2 ms ⇨ $I_{Electrolysis} \fallingdotseq 100$ mA
  - $I_{EM(Max)} = \dfrac{(V_{Max} - V_{ZENER} - 70)}{10 + 10 + R_{Pd}} \longrightarrow$ Ex. $\dfrac{(250 - 40 - 70)}{20 + 25} \longrightarrow 3\text{ A}$

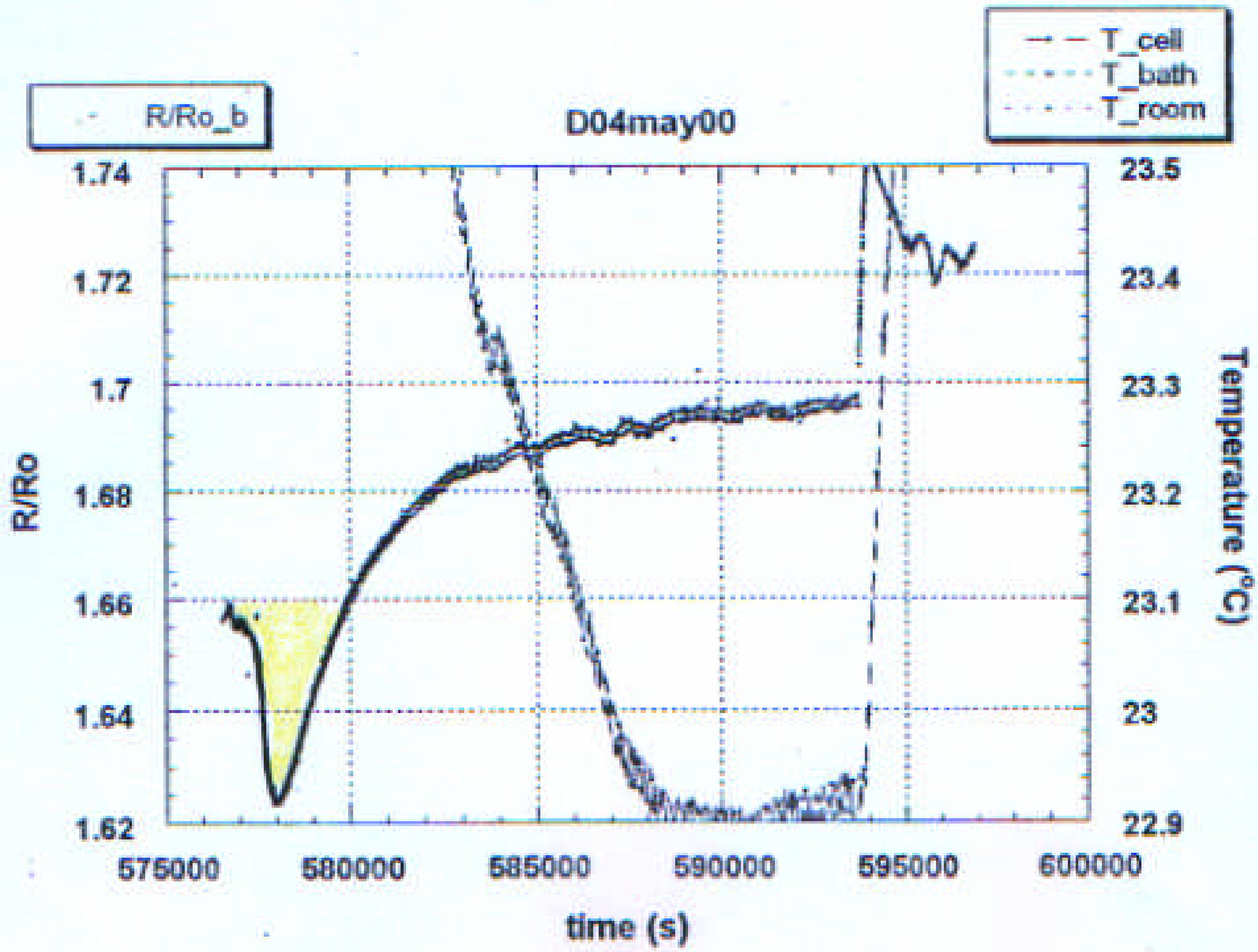
R/Ro MAX= 2,08 , 23°C

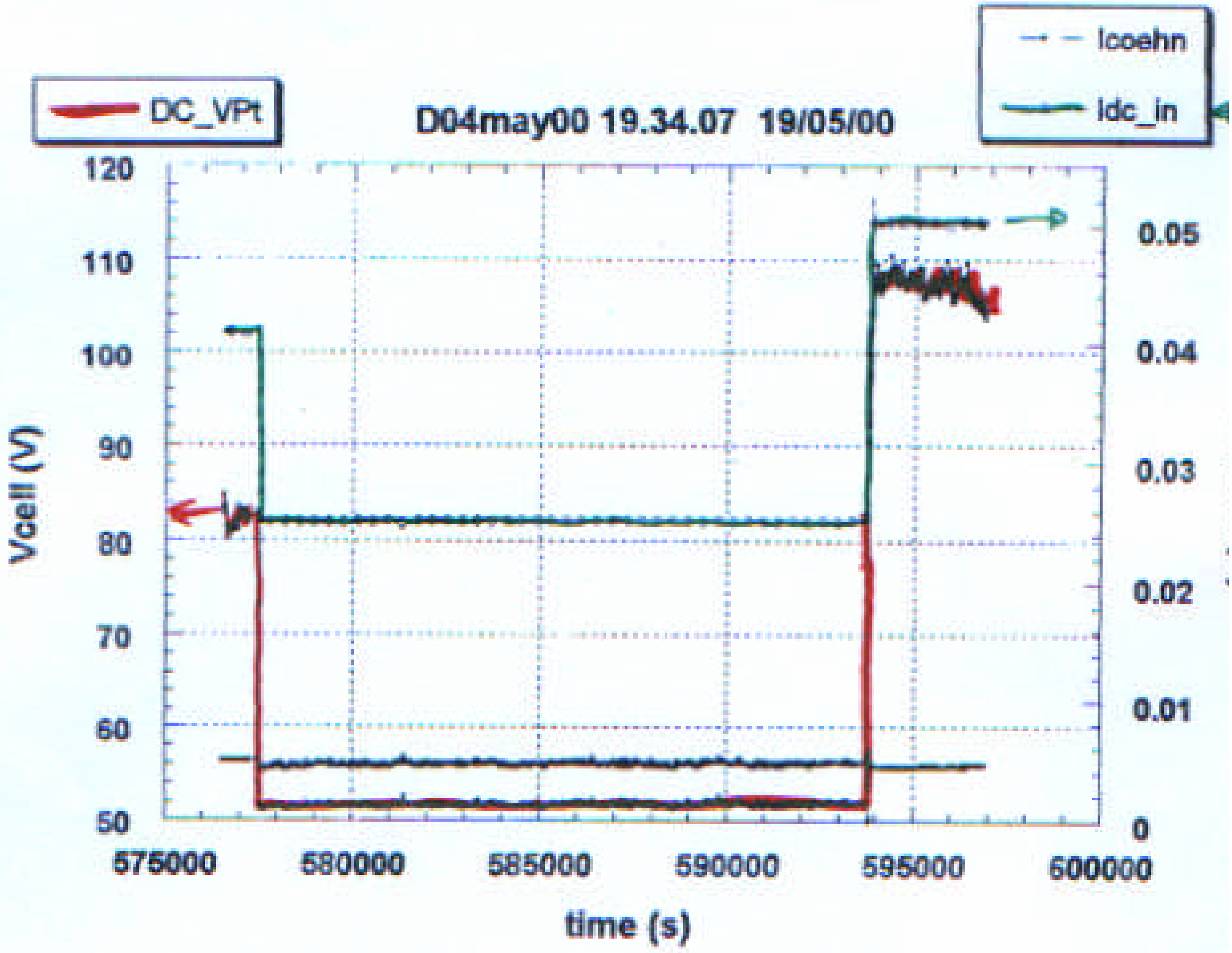
R/Ro 1.65 => 1.586

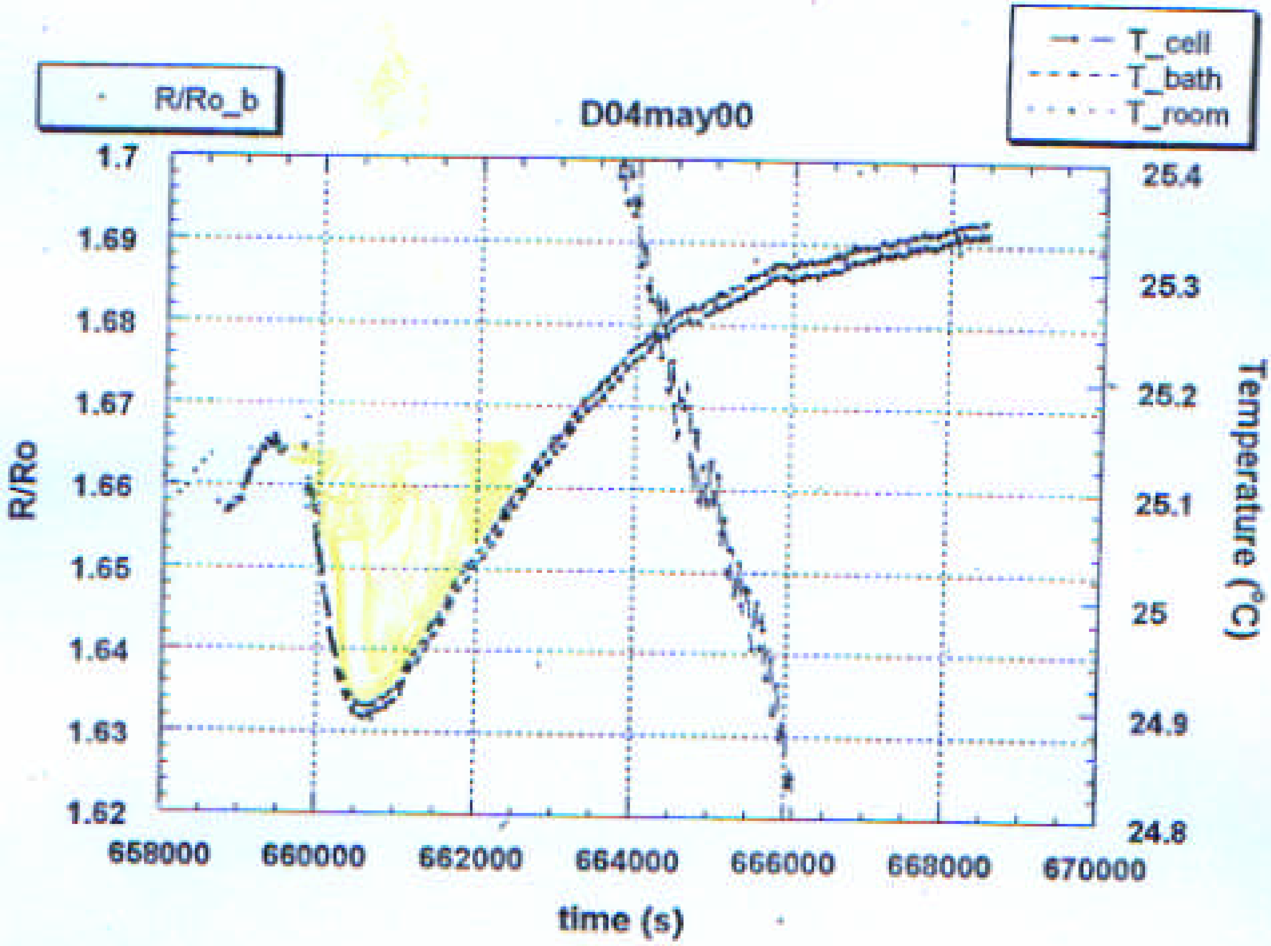
R/Ro 1.62 => 1.558

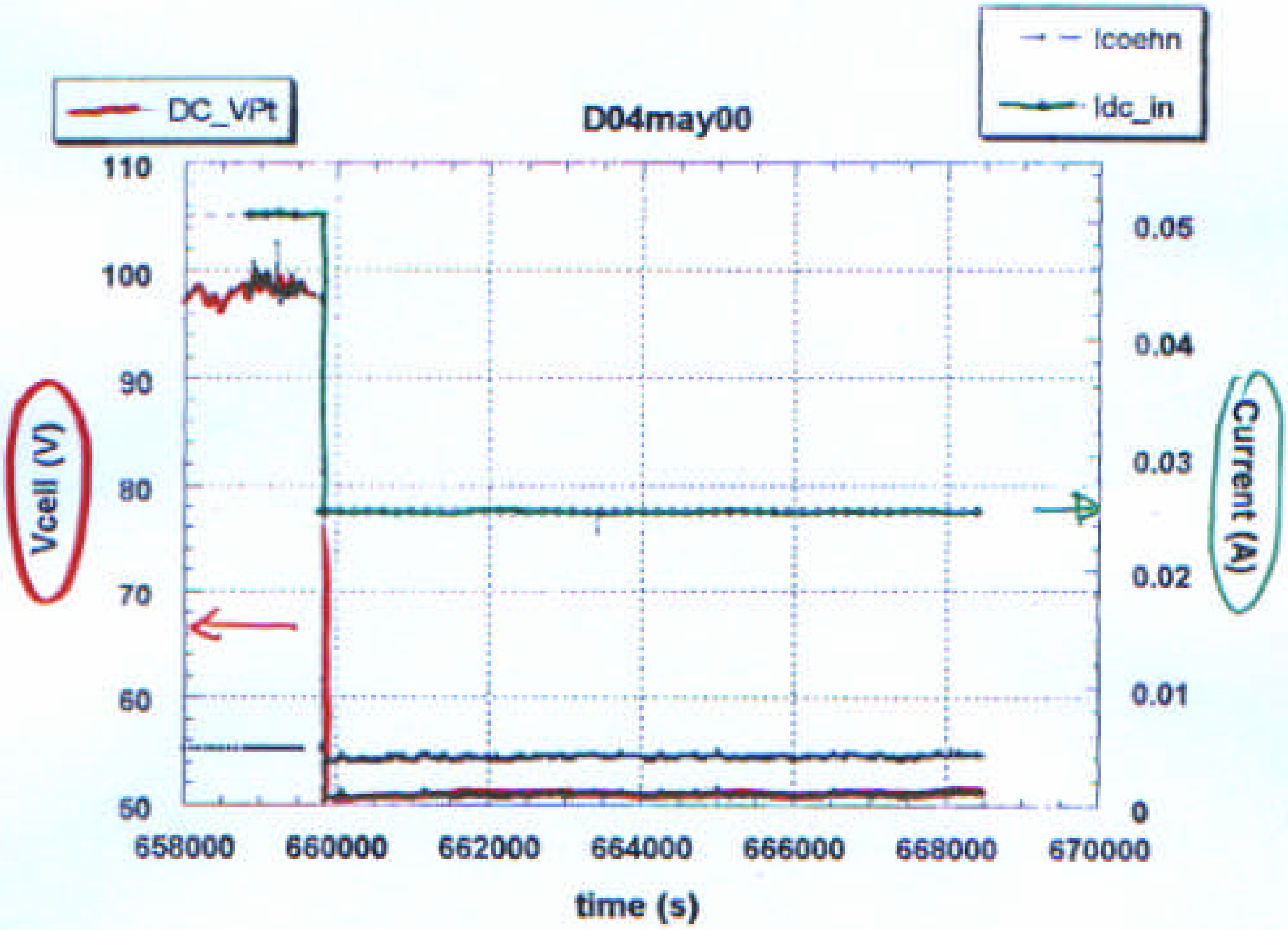


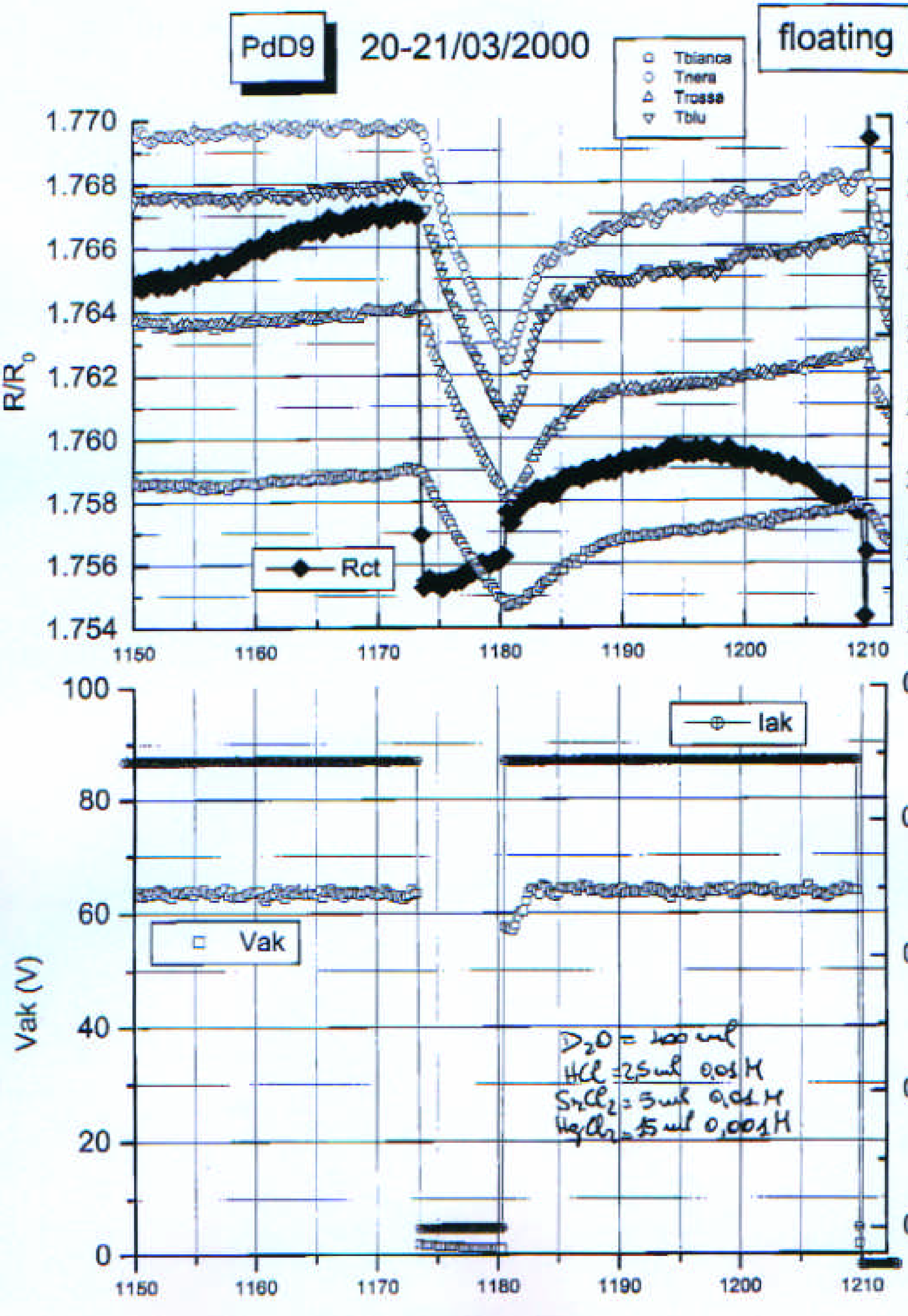

DC_VPt
D03may00
Icoehn
Idc_in
Vcell (V)
80
75
70
65
60
55
50
Current (A)
0.05
0.04
0.03
0.02
0.01
0
470000
475000
480000
485000
490000
495000
500000
505000
510000
time (s)


R/Ro_b
D04may00
T_cell
T_bath
T_room
1.74
1.72
1.7
1.68
1.66
1.64
1.62
R/Ro
23.5
23.4
23.3
23.2
23.1
23
22.9
Temperature (°C)
575000
580000
585000
590000
595000
600000
time (s)


DC_VPt
D04may00 19.34.07 19/05/00
Icoehn
Idc_in
Vcell (V)
120
110
100
90
80
70
60
50
0.05
0.04
0.03
0.02
0.01
0
575000
580000
585000
590000
595000
600000
time (s)


R/Ro_b
D04may00
T_cell
T_bath
T_room
1.7
1.69
1.68
1.67
1.66
1.65
1.64
1.63
1.62
R/Ro
25.4
25.3
25.2
25.1
25
24.9
24.8
Temperature (°C)
658000
660000
662000
664000
666000
668000
670000
time (s)


DC_VPt
D04may00
Icoehn
Idc_in
110
100
90
80
70
60
50
Vcell (V)
0.05
0.04
0.03
0.02
0.01
0
Current (A)
658000
660000
662000
664000
666000
668000
670000
time (s)



D_2O = 100 ml
HCl = 2,5 ml 0,01 M
$SnCl_2$ = 5 ml 0,01 M
$HgCl_2$ = 5 ml 0,001 M

D/Pd Loading

--- Preliminary Tests:

TEST	MAX LOADING (R/Ro)		SOLUTION D_2O +HCl + Sr
	LOW current	HIGH current	
(A)	2.00 (peak)	1.95 (Tafel)	no-Sr *(blank)*
(B)	1.90 - 1.85	1.80 - 1.75	Organic impurities
(C)	1.90 - 1.80	1.75	Commercial water
(D)	1.70	1.60	Pirelli water *(pure)*
(E)	1.80 - 1.67	1.63	LNF-purification

Tab. – Loading tests using different heavy waters for the electrolysis solution; in (A) no Sr addition to the solution (blank test). OFF/ON cycles performed for all tests.

HTTP://www.* biap.vt.EDU/BRARG/BRASYM96/KUSHNER96.H

①

BIOTECHNOLOGICAL POTENTIAL OF HEAVY WATER AND DEUTERATED COMPOUNDS

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SUMMARY

Some of the environmental, medical, biochemical and other commercial uses of deuterium (D) and heavy water (D_2O) are briefly reviewed. Many microorganisms can grow in "pure" (99.6-99.8%) D_2O, usually after a period of adaptation. Such organisms can produce a very large number of deuterated compounds.

Deuterated glycerol has interesting potential uses as a precursor of transparent plastics and other compounds. We report experiments on the formation of deuterated glycerol by *Dunaliella* spp, salt-tolerant algae growing in D_2O. These algae produce glycerol as a "compatible solute" when growing in high NaCl concentrations. Although the different species studied did not grow as well in D_2O as in H_2O, the total glycerol production of at least one species was just as high. Our results indicate a new and interesting biotechnological potential for such algae.

Key words: deuterium, heavy water, D_2O, glycerol, algae, *Dunaliella*.

INTRODUCTION

Deuterium (^2H or D), the isotope of hydrogen containing two neutrons and one proton, was separated by Urey *et al.* (1932). D_2O makes up a small but significant fraction (*ca.* 0.015 mol %) of natural water. Its ability to act as a moderator of nuclear reactions led to major industrial efforts to produce it in large quantities. It may be concentrated from natural water by several different methods. These include exchange between deuterated hydrogen (HD), hydrogen sulfide (in the Girdler-Sulfide process, which has been most used), ammonia or methylamine and liquid water or water vapor, coupled with the use of continual enrichment processes to make the end product, 99.8% D_2O (Benedict *et al.*, 1981; Miller and van Alstyne, 1994; Rae, 1991). By 1991, sixty years after its discovery, about 29,000 tonnes of D in the form of D_2O had been produced, 60% of this in Canada (Rae, 1991).

All methods are carried out on a very large industrial scale, involving large energy utilization. The increase of the cost of D_2O from $60 US/kg in 1960 to about $240 US/kg in 1980 was mainly due to the increased costs of energy (Rae, 1991).

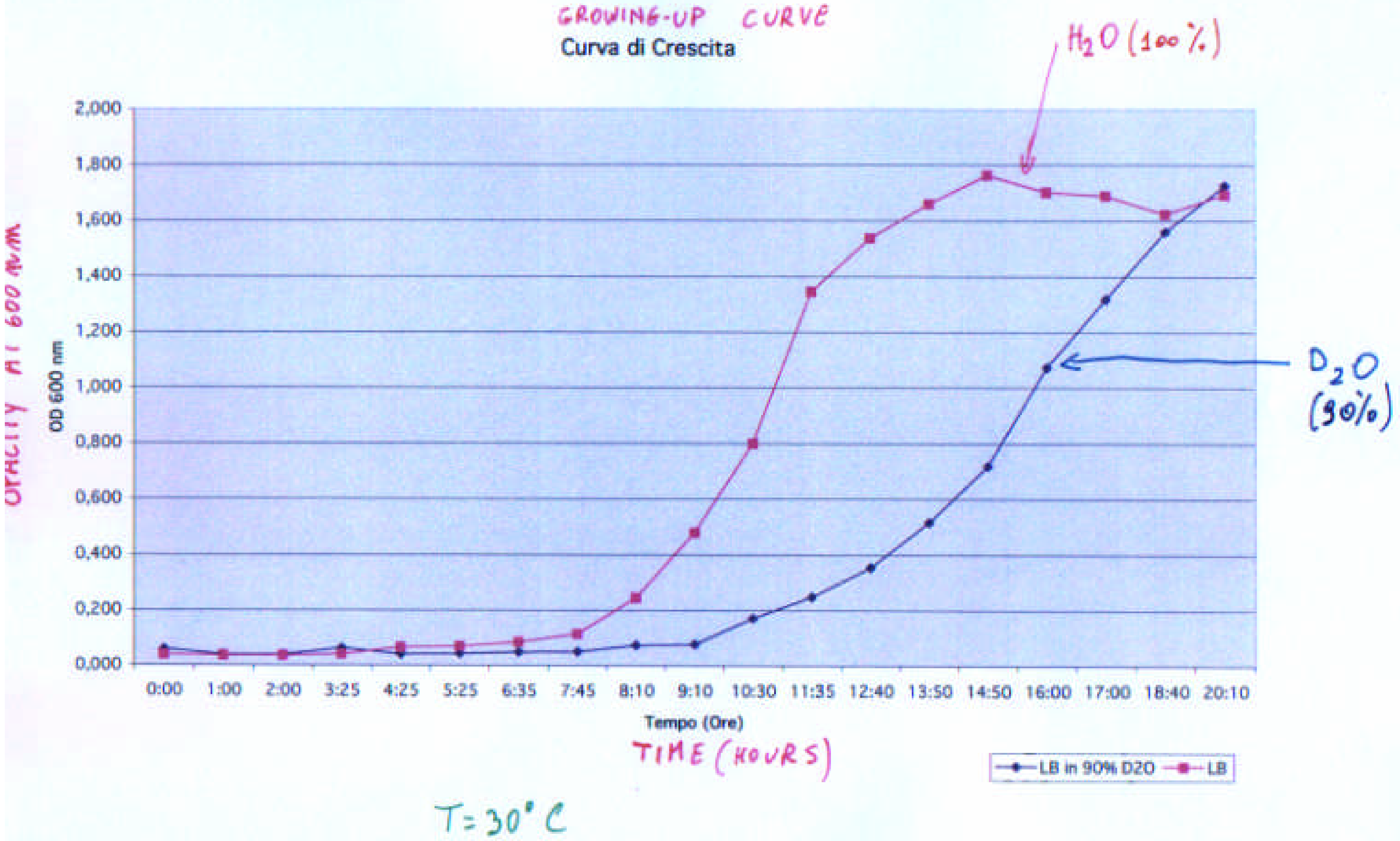
Since this conference is largely concerned with the risks of releasing different substances into the environment, it should be stated at the beginning that D_2O itself poses little such risk—so long as it has not been used in nuclear reactors. As will be can be greatly increased if the patient's brain is "loaded" with heavy water (Hatanaka, 1989, 1991).

Heavy water has been widely used in measurements of body water space, in children and in adults at various stages of nutrition and exercise. Many human subjects have been injected with or have swallowed a few ml (often 0.1 ml per kg body weight) of heavy water. This increases the D_2O content in the blood from 150 to about 300 ppm, which subsequently decays to the normal level with a half-life of a few days (Coward, 1979). The water space of animal bodies may be determined by the degree of dilution of the heavy water, which can be measured with great sensitivity.

The widespread use of heavy water in clinical studies and treatments without reported adverse effects implies that it is not very toxic to humans. This is also suggested by animal experiments, in which toxic effects did not begin to appear until the D content of blood and body fluids and tissues was over 20% (Katz, 1960; 1965; Thomson, 1963). To attain such a concentration and adult human weighing about 70 kg would have to drink rapidly more than 10 L D_2O.

Microbial growth in D_2O. D_20 is even less toxic to microorganisms than to multicellular creatures. Some bacteria and algae can grow in "pure" (normally 99.6-99.8%) D_2O, though usually more slowly than in H_2O, and after a period of adaptation. Even more complex cells, such as protozoa can grow in quite high concentrations (70-100% D_2O) (Katz, 1965; Thomson, 1963). Microorganisms that can grow in pure D_2O include, but are not limited to, the algae, *Chlorella* and *Scenedesmus* (Katz, 1965; Unno *et al.*, 1987, 1989) and, as this report shows, the salt-tolerant *Dunaliella* species. Bacteria include *Escherichia coli, Pseudomonas putrefaciens, Serratia marcescens* (Katz, 1965; Thomson, 1963; Vanatulu *et al.*, 1993), and extremely halophilic archaebacteria (Crespi, 1982). Among yeasts and fungi, *Torula* and *Aspergillis* spp. have been grown in pure D_2O (Thomson, 1963), as have methylotrophic *Candida* spp. (Haon *et al.*, 1993). In most cases, a period of "training" or adaptation is required at lower D_2O concentrations before cells can grow in the pure substance; mechanisms involved in such adaptation have hardly been studied. Microorganisms growing in D_2O can provide a large number of deuterated compounds. Algae that have grown in D_2O can provide biomass to serve as feedstock for the growth of heterotrophic microorganisms (Crespi, 1988).

Environmental and pollutant studies. Determination of the natural occurrence of deuterium and other stable isotopes is a powerful means for investigating past and current environmental changes in the Earth's atmosphere and hydrosphere. The isotopic composition of trace atmospheric constituents provides information on sources, sinks and transformations of these compounds and, since the distribution of environmental isotopes is governed by environmental conditions, they can be used to examine both natural and anthropogenic influences on climatic variation (Kaye, 1992; Rozanski and Gonfiantini, 1990; Bowen *et al.*, 1990). Stable isotopes of H, O and C have been used to assess the atmospheric methane inventory (Levin and Doerr (1991), to evaluate the source of water vapour in the upper troposphere


GROWING-UP CURVE
Curva di Crescita

Genetic Identification

- Sequence Analysis of the 16 S rRNA gene

  APF isolates : fall within the genus Ralstonia of the β subclass of proteobacteria

- Amplified Fragment Length Polymorphism (AFLP) of the entire bacterial genome

  APF isolates : have a unique genome distinct from the genome of Ralstonia pickettii

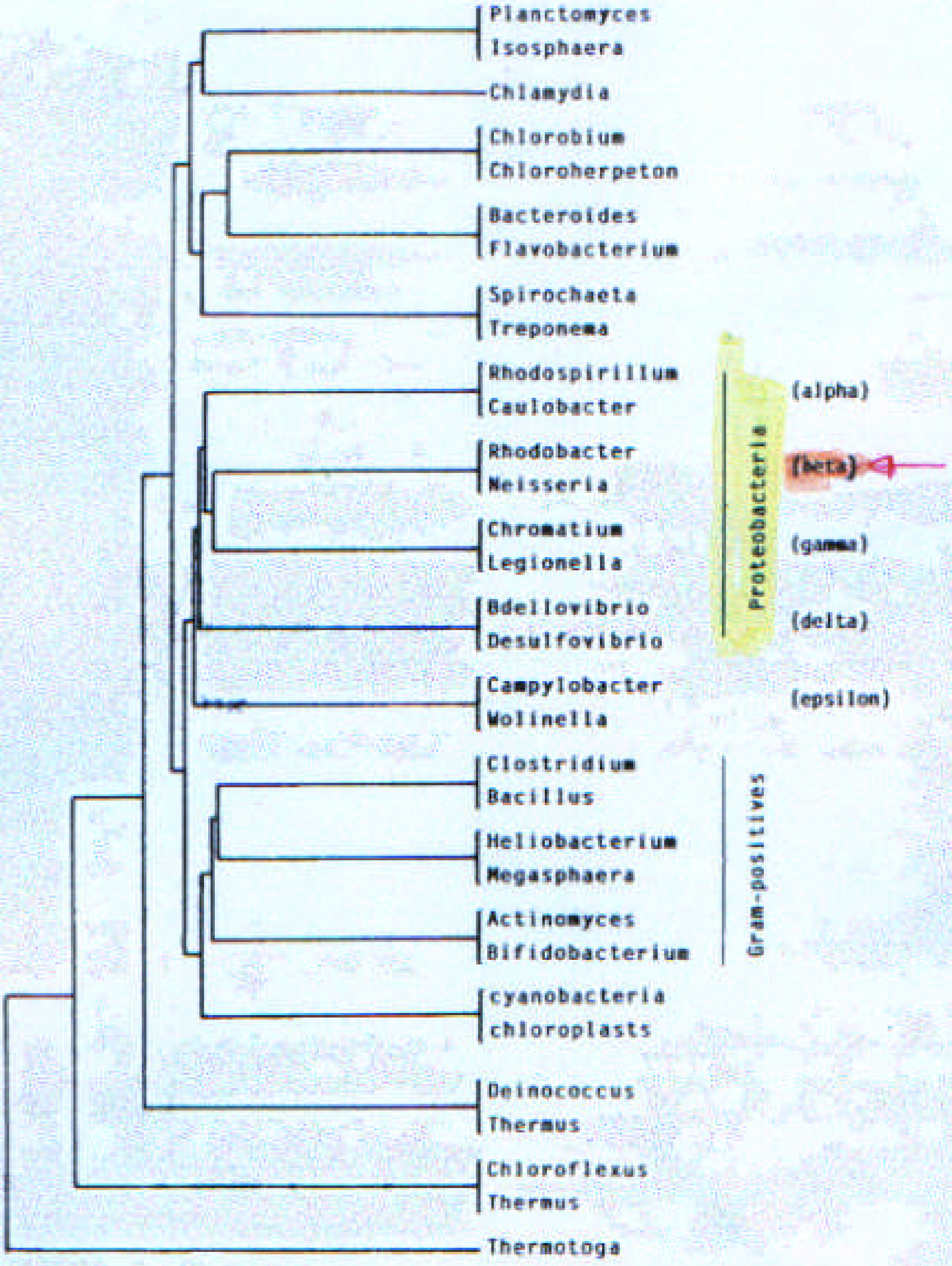
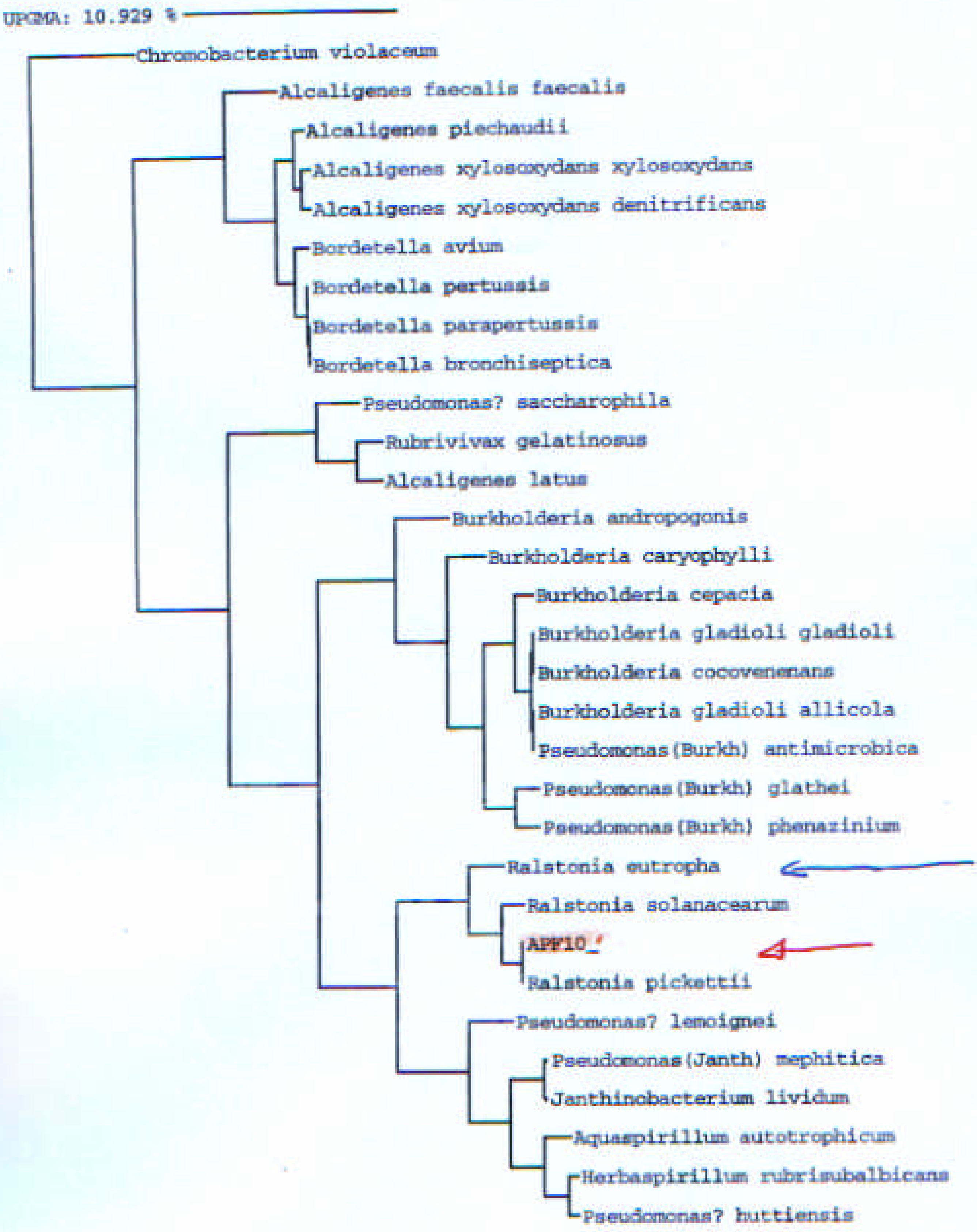


Fig. 1. Topology tree of the eubacteria based upon a number of methods used to determine phylogenetic relationsh The order of branches that constitute the main lines of descent and the relative distances separating them are based 16S rRNA sequence comparison and were adapted from Fig. 11 of Woese (1987). The branch lengths do not ref evolutionary distances. Except for the *Chlamydia* and *Thermotoga* lines of descent, each major line is represented two taxa; for a detailed composition see Table 1. The allocation of the epsilon group as a fifth subclass of proteobact has not been confirmed as yet. The branching point of the lines embracing *Fibrobacter* (Montgomery et al. [1988]) *errucomicrobium* (Albrecht et al. [1987]) (not shown) is not settled.


UPGMA: 10.929 %
Chromobacterium violaceum
Alcaligenes faecalis faecalis
Alcaligenes piechaudii
Alcaligenes xylosoxydans xylosoxydans
Alcaligenes xylosoxydans denitrificans
Bordetella avium
Bordetella pertussis
Bordetella parapertussis
Bordetella bronchiseptica
Pseudomonas? saccharophila
Rubrivivax gelatinosus
Alcaligenes latus
Burkholderia andropogonis
Burkholderia caryophylli
Burkholderia cepacia
Burkholderia gladioli gladioli
Burkholderia cocovenenans
Burkholderia gladioli allicola
Pseudomonas(Burkh) antimicrobica
Pseudomonas(Burkh) glathei
Pseudomonas(Burkh) phenazinium
Ralstonia eutropha
Ralstonia solanacearum
APF10_'
Ralstonia pickettii
Pseudomonas? lemoignei
Pseudomonas(Janth) mephitica
Janthinobacterium lividum
Aquaspirillum autotrophicum
Herbaspirillum rubrisubalbicans
Pseudomonas? huttiensis

```
Alignment:  1534 APF10_
 0.13 % 1533 Ralstonia pickettii
 2.25 % 1531 Ralstonia solanacearum
 3.60 % 1527 Ralstonia eutropha
 6.81 % 1528 Pseudomonas? lemoignei
 7.26 % 1528 Aquaspirillum autotrophicum
 7.53 % 1528 Herbaspirillum rubrisubalbicans
 7.66 % 1528 Burkholderia gladioli allicola
 7.72 % 1528 Pseudomonas(Burkh) antimicrobica
 7.73 % 1527 Burkholderia cepacia
 7.79 % 1528 Burkholderia gladioli gladioli
 7.82 % 1528 Burkholderia cocovenenans
 7.85 % 1528 Pseudomonas? huttiensis
 8.06 % 1526 Pseudomonas(Burkh) glathei
 8.52 % 1526 Burkholderia andropogonis
 8.58 % 1526 Pseudomonas(Janth) mephitica
 8.62 % 1526 Janthinobacterium lividum
 8.75 % 1526 Pseudomonas(Burkh) phenazinium
 9.17 % 1526 Alcaligenes xylosoxydans xylosoxydans
 9.27 % 1526 Bordetella avium
 9.34 % 1526 Alcaligenes xylosoxydans denitrificans
 9.40 % 1526 Burkholderia caryophylli
 9.53 % 1526 Alcaligenes piechaudii
 9.61 % 1519 Pseudomonas? saccharophila
 9.61 % 1524 Rubrivivax gelatinosus
 9.70 % 1526 Bordetella parapertussis
 9.70 % 1526 Bordetella bronchiseptica
 9.76 % 1526 Bordetella pertussis
 9.99 % 1532 Alcaligenes faecalis faecalis
10.04 % 1524 Alcaligenes latus
10.06 % 1531 Chromobacterium violaceum
```

↑ NUCLEOTIDE ~~NUMBER~~ LENGTH (IN BASE PAIR)

ANALYSIS FROM : GENEBANK (APRIL 2000)

Identification and characterisation

RALSTONIA PICKETTII is a non-fermentative gram-negative bacillus formerly known as Pseudomonas pickettii and Burkholderia pickettii.

In 1995, a new genus, Ralstonia was reproposed on the basis of phenotypic characterization, cellular lipid and fatty acid analyses, phylogenetic analysis of 16S rDNA nucleotide sequences and rRNA-DNA hybridization.

Since 1972, Ralstonia pickettii has been detected as a contamination of several solutions (e.g., saline, deionized water, "sterile" water, and intra venous ranitide). Previous laboratory studies have shown low number (1-10 colony-forming units) of Ralstonia pickettii inoculated into 0.9% sodium chloride solution can proliferate over a wide range of temperature. Although the filter size used to terminally sterilize this product is not known, previous studies have shown that Ralstonia picketti can pass 0.2 micrometer filter.

Growth of APF isolates on nutrient-agar plates containing $Hg(CH_3COO)_2$

	1 μM	10 μM	100 μM	1 mM	10 mM
APF 3	100	100	100	0	0
APF 10	100	100	100	0	0

At each concentration, the percent of the number of bacterial colonies on control plates is reported

Growth of APF isolates on nutrient-agar plates containing $SrCl_2$

	1 μM	10 μM	100 μM	1 mM	10 mM
APF3	100	100	100	100	42
APF10	100	100	100	100	45

At each concentration, the percent of the number of bacterial colonies on control plates is reported

BROOKHAVEN NATIONAL LABORATORY **Genomics** BROOKHAVEN NATIONAL LABORATORY

Alcaligenes eutrophus Sequencing Project:

Image: Bacterial induced formation of Cd crystals by A. eutrophus on Zirfon M5 membranes in the continuous tubular membrane reactor. The figure shows the bacterial formed CdCO3 crystals. The bar represents 1 mm. (S.Taghavi) We thank S. Taghavi for providing this image.

GENERAL INFORMATION

Alcaligenes eutrophus* CH34**, recently renamed ***Ralstonia eutrophus is a gram-negative, non-spore forming bacterium which thrives in the presence of millimolar concentrations of several heavy-metals (**Zn, Cd, Co,Pb,Hg, Ni & Cr**). The heavy-metal resistance is conferred by two large megaplasmids (*pMOL28*=180 kbp and *pMOL30*=240 kbp) carrying gene clusters that encode cation-efflux machinery spanning both bacterial membranes. These low-copy number plasmids are stably maintained in the presence or absence of selective pressure and are self-transferable at relatively low frequencies.

A. eutrophus uses a variety of substrates as its carbon source or it can grow chemo-lithotropically using molecular hydrogen as the energy source and carbon dioxide as a carbon source. When nitrate is present ***A. eutrophus*** can grow anaerobically.

Regions of the megaplasmid DNA relevant to the cation-efflux pump were sequenced; however, to the best of our knowledge, none of the groups is systematically sequencing any of the plasmids. The complete sequence of the megaplasmids will be instrumental in understanding gene organization, especially mechanism of plasmid replication, partition and transfer, and allowing the construction of environmentally-friendly bacteria e.g. ***E.coli*** with stable and efficient mechanism for heavy-metal resistance.

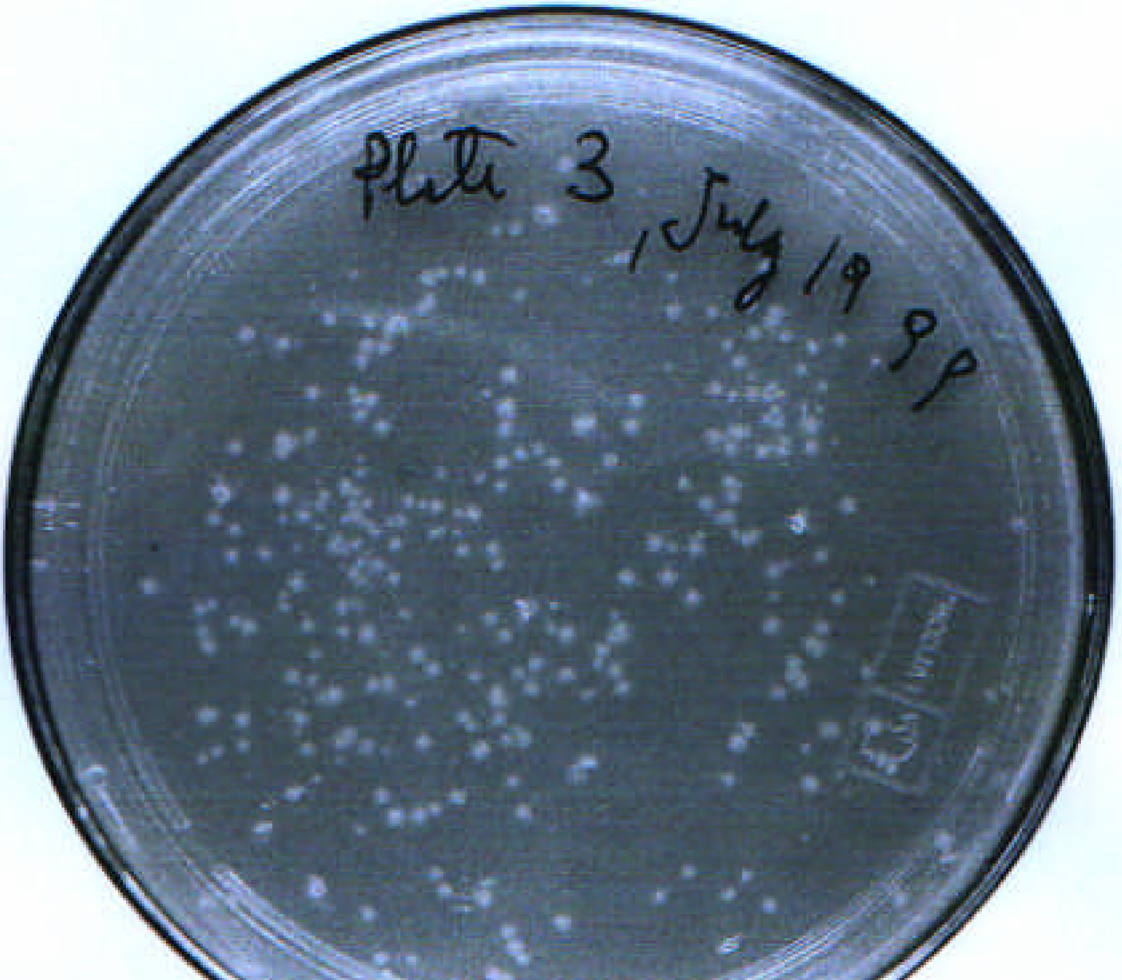
For more in depth information on biology of this bacterium, please refer to some of the following publications and references therein

1. **M. Mergeay et. al.** "*Alcaligenes eutrophus CH34* Is a Facultative Chemolithotroph with Plasmid-Bound Resistance to Heavy Metals" **J. Bact. Vol. 162 (1) 328-334, 1985.**
2. **A. Nies et. al.** "Nucleotide Sequence and Expression of a Plasmid-encoded Chromate Resistance Determinant from *Alcaligenes eutrophus*" **J. Biol. Chem. Vol. 265 (10):5648-5653, 1990.**
3. **S. Taghavi et. al.** "Genetic and Physical Maps of the *Alcaligenes eutrophus CH34* Megaplasmid *pMOL28* and its Derivative *pMOL50* Obtained after Temperature-Induced Mutagenesis and Mortality" **Plasmid Vol. 37:22-34, 1997.**

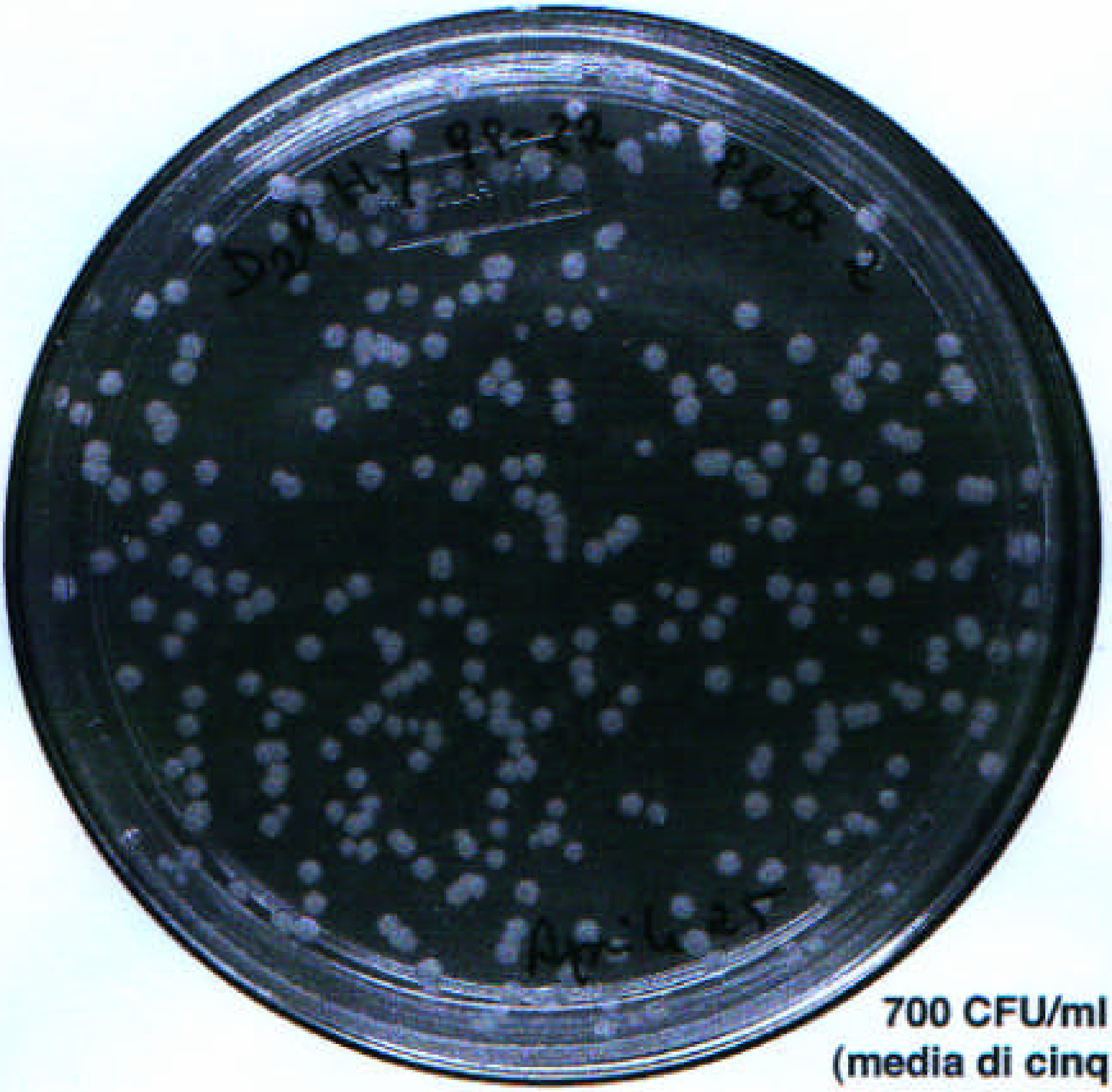
GOALS AND SEQUENCING INFORMATION

D_2O

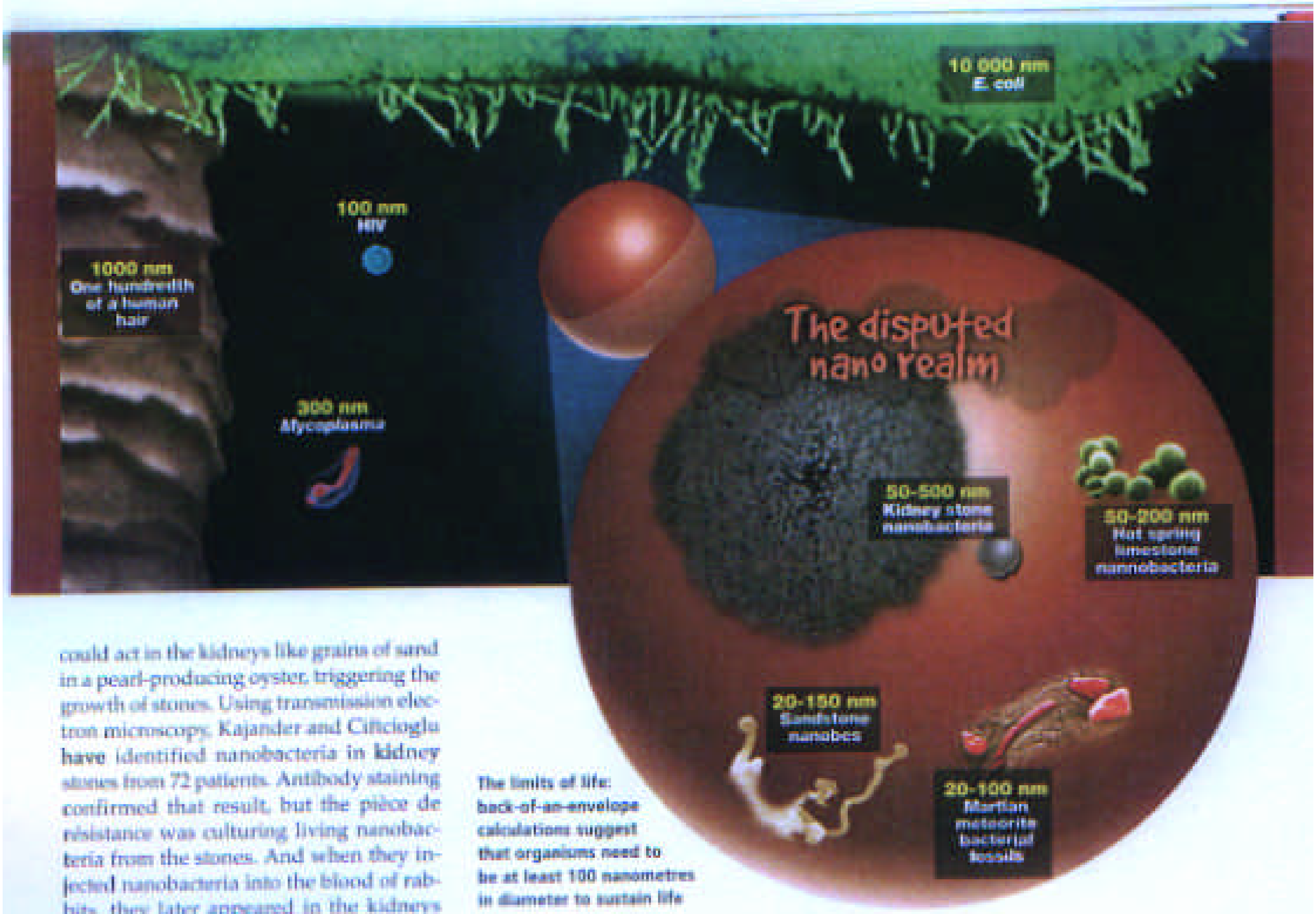
OBTAINED IN 1997, 25 l plastic bottle (HDPE)



120 CFU/ml (media di cinque piastre)



700 CFU/ml
(media di cinque
piastre)



The limits of life: back-of-an-envelope calculations suggest that organisms need to be at least 100 nanometres in diameter to sustain life

could act in the kidneys like grains of sand in a pearl-producing oyster, triggering the growth of stones. Using transmission electron microscopy, Kajander and Ciftcioglu have identified nanobacteria in kidney stones from 72 patients. Antibody staining confirmed that result, but the pièce de résistance was culturing living nanobacteria from the stones. And when they injected nanobacteria into the blood of rabbits, they later appeared in the kidneys and damaged the part of the organ where stones typically form.

To kidney specialists the idea that a bacterium—albeit a minuscule one—causes kidney stones doesn't seem so bizarre. Although the cause of most kidney stones has been a mystery, bacterial infections are known to cause a rarer kind of stone—the bacterium in question makes urine more acidic, encouraging mineral precipitation.

Small but deadly

Another kind of kidney disorder, polycystic kidney disease, may also be linked to nanobacteria, according to findings presented in June by Marcia Miller-Hjelle and J. Thomas Hjelle from the University of Illinois in Peoria at a Chicago meeting of the American Society for Microbiology. Worldwide, over 12 million people suffer from this inherited, incurable disorder in which the kidneys gradually swell up with cysts until they no longer function. Studies in animals suggest that the disease is part genetic, part infectious agent—mice that have been genetically engineered to get the disease remain healthy as long as they are kept in a germ-free environment. What's more, people with polycystic kidney diseases are far more likely to develop kidney stones than other people, so it would seem possible that the same agent causes both diseases.

Miller-Hjelle and Hjelle, working with Kajander, have cultured nanobacteria from 10 out of 12 kidneys from patients with polycystic kidney disease, and detected the nanobacteria in all the cystic kidneys they have examined with electron microscopy. What's more, Miller-Hjelle and Hjelle claim that the nanobacteria make a toxin that they have identified in the cyst fluids, and that they have found nanobacteria in the blood and urine of a 23-year-old patient with rapidly enlarging cysts in his kidneys. Even so, they admit that they have yet to complete comparable studies in people without kidney disease, and at this stage it's impossible to say whether the nanobacteria are causing the disease or are innocent bystanders.

"This opens a new door if it's true," says David Chan, a urologist at Johns Hopkins Medical Institutions in Baltimore. But, he cautions, "no one has repudiated it or confirmed it. We have to be excited, but also careful."

And what about those claims that nanobacteria may have come from outer space? Most people are familiar with the hypothesis that life on Earth is descended from extraterrestrial microbes that reached our planet on a comet or asteroid (*New Scientist*, 12 September 1998, p 24). Kajander says his nanobacteria would be ideal for such a trip. Ensconced in their "castles"—a mineral coat similar to the heat shields on the space shuttle, he says—nanobacteria could withstand the extremes of heat and pressure, as well as the radiation exposure, that such a journey would entail. Uwins says her nanobes are equally robust. What's to say, muses Kajander, that these microbes that defy the rules of life on Earth didn't arrive here from Mars?

It's reflections like this that perhaps best illustrate why the nano-enthusiasts stay fired up in the face of widespread scepticism and not a little ridicule. They know that if what they suspect turns out to be true, it would not only guarantee them scientific fame and fortune, but also challenge everything we think we know about life on Earth. ❑

Further reading: "Novel nano-organisms from Australian sandstones" by P. J. R. Uwins, R. I. Webb and A. P. Taylor, *American Mineralogist*, vol 83, p 1541
Nano in Australia **www.uq.edu.au/ nanoworld/ uwins.html**
Nano in Texas **www.geo.utexas.edu/litite/**
Nano in Finland **www.uku.fi/laitokset/biokem/ olli.html**
Nano from Mars **www.jpl.nasa.gov/snc/**

Antibiotic Resistance

	APF isolates 9, 10, 11	Ralstonia Pickettii
Ampicillin	S	S
Tetracycline	S	Ⓡ
Kanamicin	Ⓡ	Ⓡ
Streptomycin	Ⓡ	S
Chloramphenicol	S	S
Rifampicin	S	nd

S: sensitive ; R: resistant ; nd : not determined

CONCLUSIONS (AT ICCF 8 MAY 2000)

① The problem to get H/Pd >> 0.97 REPRODUCIBLY seems solved by the use of S_2CO_3 deposition.
EXPERIMENTS REPRODUCED **SUCCESFULLY**
AT PIRELLI (ITALY) and SRII (USA).

② THE SAME PREVIOUS PROCEDURE, ADOPTED FOR D_2O, WAS NOT EFFICENT BECAUSE BACTERIA CONTAMINATION OF D_2O.

③ PROPERLY PURIFIED D_2O AND NEW CIRCUITRY ALLOWED US TO GET LARGE LOADING ($D/Pd >> 0.9$ AND STRONG INDICATIONS OF ANOMALOUS "EXCESS HEAT" (≈ 0.5 W OVER 2 W of INPUT POWER) IN A REPRODUCIBLE WAY (3 TIMES).
SOME "MOVING", EXTERNAL, OF D INTO OVERLOADED Pd, ACCORDING TO US, IS THE ORIGIN OF EXCESS HEAT.

④ WE HAVE TO INCREASE THE ANOMALIES: NOW ONLY 2 SPOTS, 4 mm long over 15 cm of WIRE INSPECTED, WERE ACTIVE.

⑤ FURTHER WORK ON "PURIFICATION" OF D_2O NEEDED; "STERILE" ELECTROLYSIS SEEMS NECESSARY.

⑥ THE BACTERIA IDENTIFIED (PERHAPS NEW), BY ITSELF CAN BE USED TO "ABSORB" DANGEROUS HEAVY METALS (Cd, Cr, Pb, Hg) FROM THE ENVIROMENT POLLUTED:

UNEXPECTED PRESENT DUE TO
COLD FUSION STUDIES

FURTHER RESULTS (JUNE–OCTOBER 2000)

① REGISTERED, AT "GENBANK" (BETHESDA, USA) AND "DDBJ" (TOKYO, JAPAN)* ON 21 JUNE 200

TWO NEW BACTERIA

(1A) RALSTONIA DETUSCULANENSE COD. AF 280433

(1B) STENOTROPHOMONAS DETUSCULANENSE COD. AF 280434

DE TUSCULANENSE
⇓
DE => DEUTERIUM
TUSCULANENSE => ROMAN NAME OF FRASCATI (TUSCULUM)

REGISTERED AS NEW BECAUSE:

(1AB, C) DNA SEQUENCING DIFFERENT FROM ANY KNOWN BACTERIA (AS AT $23^{h}\,59^{m}$ OF JUNE 20, 20

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* DNA DATA BANK OF JAPAN "CENTER FOR INFORMATION BIOLOGY, NATIONAL INSTITUTE OF GENETICS" (MISHIMA, SHIZUOKA, JAPAN)

1AB,D FATTY ACID ANALYSIS;

1AB,E ANTIBYOTIC PATTERN SENSITIVITY;

1AB,F EXPERIMENTAL BEHAVIOUR IN RESPECT TO METAL TOLERANT AND HYDROGEN (DEUTERI
PRODUCTION UNDER SOLAR IRRADIATION.

2 MADE EXPERIMENTS ABOUT H_2 (OR D_2) GAS PRODUCTION, IN WATER SOLUTION, BY ADDITION OF CO_2 AND SOLAR LIGTH IRRADI
TION (ONLY ~ $80 W/m^2$, $400 \div 900$ nm; SUN ~ $1000 W/m^2$)

2A TYP 1 EXP.:

$H_2O + D_2O$ (450 + 50 cc), JUST BACTERIA USUAL FOUND IN D_2O (700 RALSTONIA AND 1 STENOTROPHOMONAS IN 1 cc)

2B TYP 2 EXP.:

RALSTONIA "CONCENTRATED" (~ 10^7 BACTERIA/cc) IN H_2O SOLUTION (NUTRIENT "L.B.")

RESULTS

H_2 PRODUCTION

(AS COMPARISON, RESULTS AT AAAS OF MARCH 2000, WASHINGTON, MEAN VALUE, 0.8 ul/l/h)

(2A.1) AFTER 48^h INCUBATION (AND ADDITION OF PROPER, INNOVATIVE, "FOOD") WE OBTAIN

> 10 ml/l/h

FOR OVER 5 DAYS.

LATER, IMPOSSIBLE TO MEASURE, WITH PROPER ACCURACY, H_2 PRODUCTION BECAUSE DAMAGE OF Pd USED TO MEASURE H_2 (BACTERIA "COVERED" Pd WIRE, SEE FIGG.).

THE H_2 PRODUCTION LASTED OVER 1 MONTH (WE STOPPED, FOR FURTHER EXPERIMENTS, THE REACTION => NO LIGTH; T=4°C)

- IN PROGRESS UPGRADING MEASUREMENT SYSTEM

(2A.2) AFTER, ONLY, 1 MINUTE OF TURNING-ON LIGTH:

$$\boxed{3 \div 5 \ ml/l/h}$$

(5 EXPERIMEN

FOR OVER 2^h. LATER IMPOSSIBLE TO MEASURE BECAUSE DAMAGED INSTRUMENTATION (BACTERIA

③ HEAVY METAL TOLERANCE CONFIRMED.

FURTHER TEST WITH Pb, Cd.

USED RADIOACTIVE ISOTOPES IN ORDER TO STUDY THE TOLERANCE OR "METABOLISM" PATHWAY.

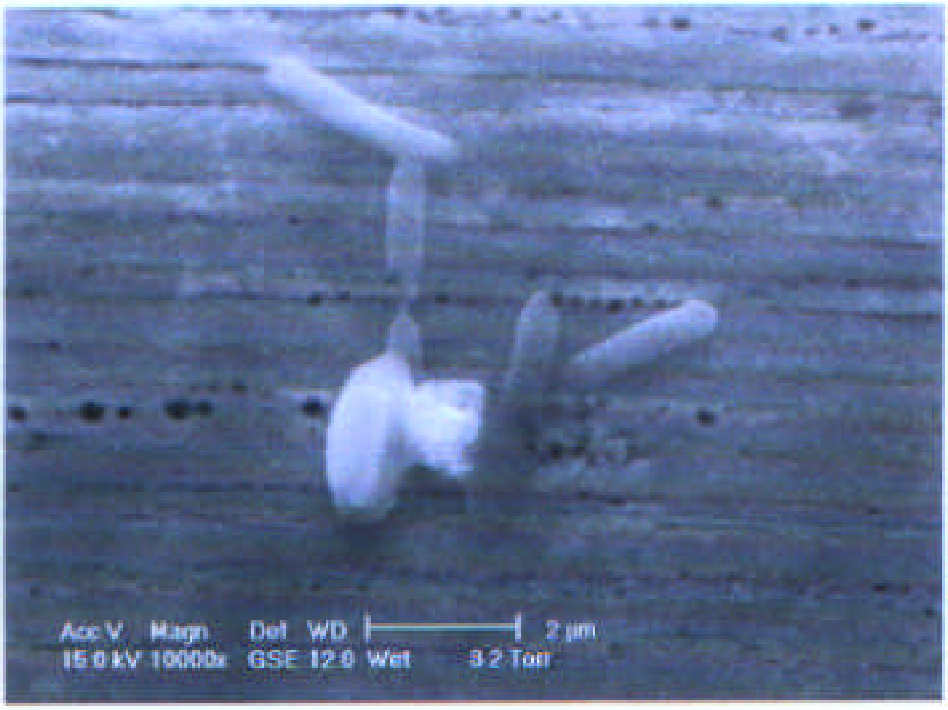
- IN PROGRESS

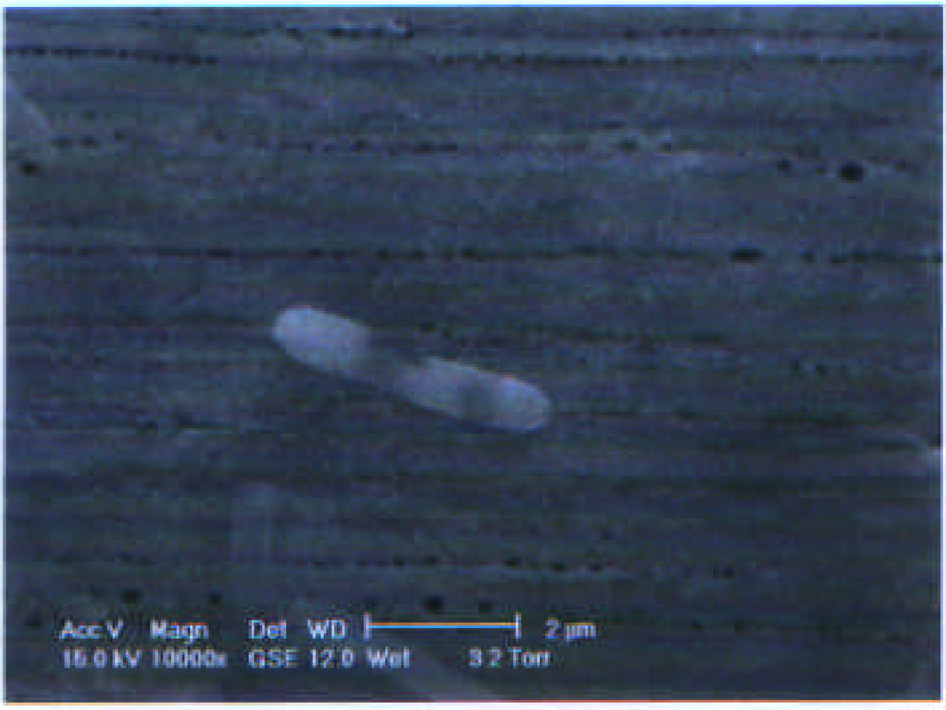
④ FURTHER STUDIES TO FIND "SIMPLE" PROCEDURES TO ERADICATE BACTERIA FROM D_2O - ELECTROLYSIS SYSTEM {COLLABORATION WITH PIRELLI, ITALY).

⑤ RADIATION TOLERANCE: 30 M Rad ARE ENOUGH TO KILL ALL BACTERIA (4 MONTHS TEST)

SOURCE: ^{60}Co.

[AS REFERENCE, ~500 REM IS THE DOSE TO KILL HUMAN]


Acc.V Magn Det WD 2 µm
15.0 kV 10000x GSE 12.0 Wet 3.2 Torr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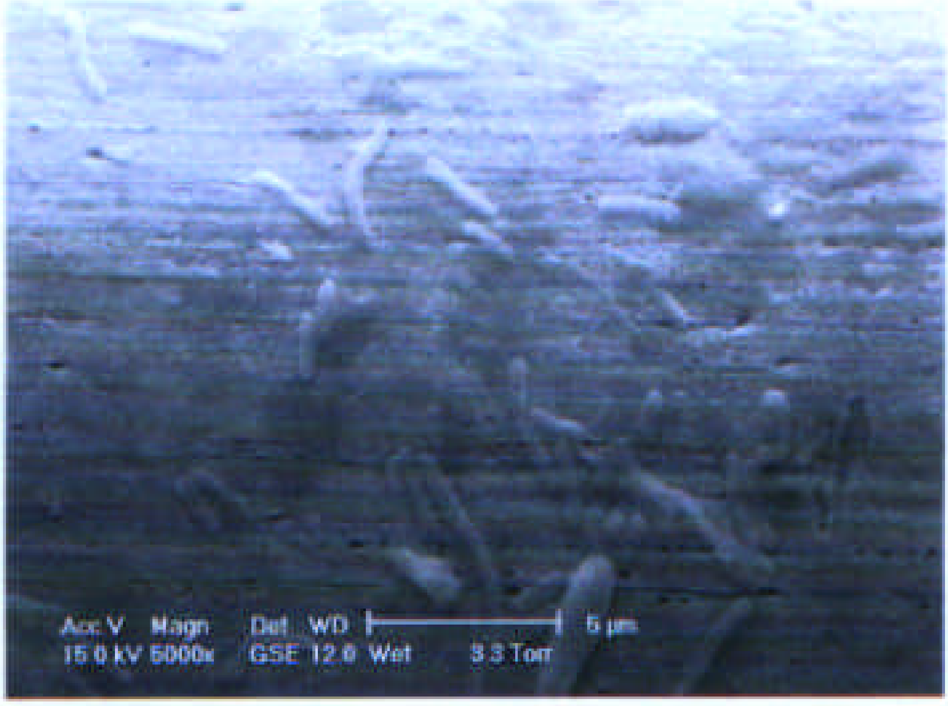
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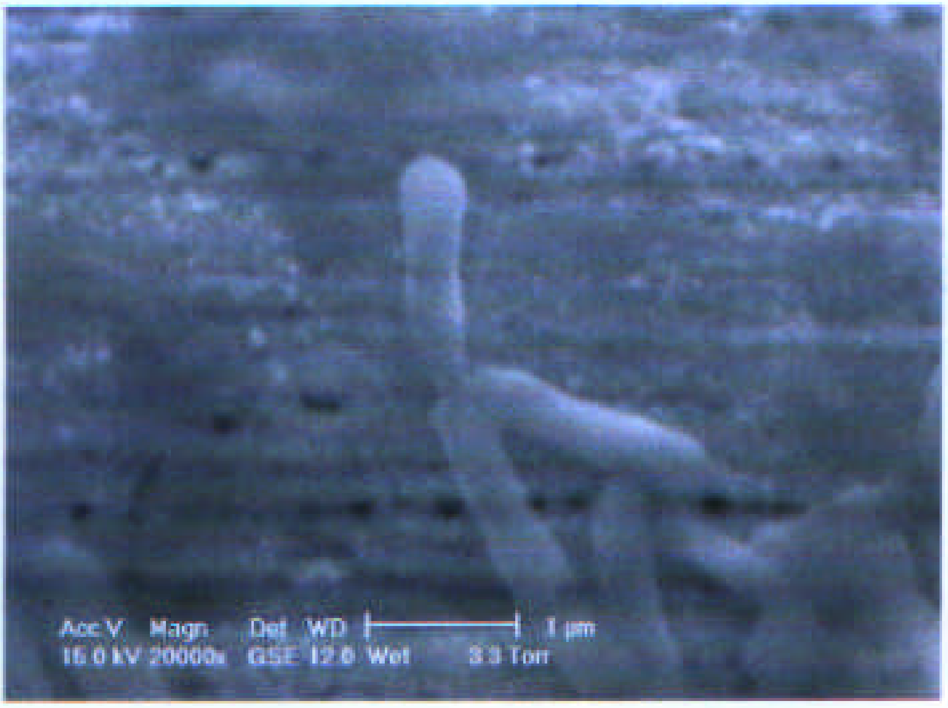
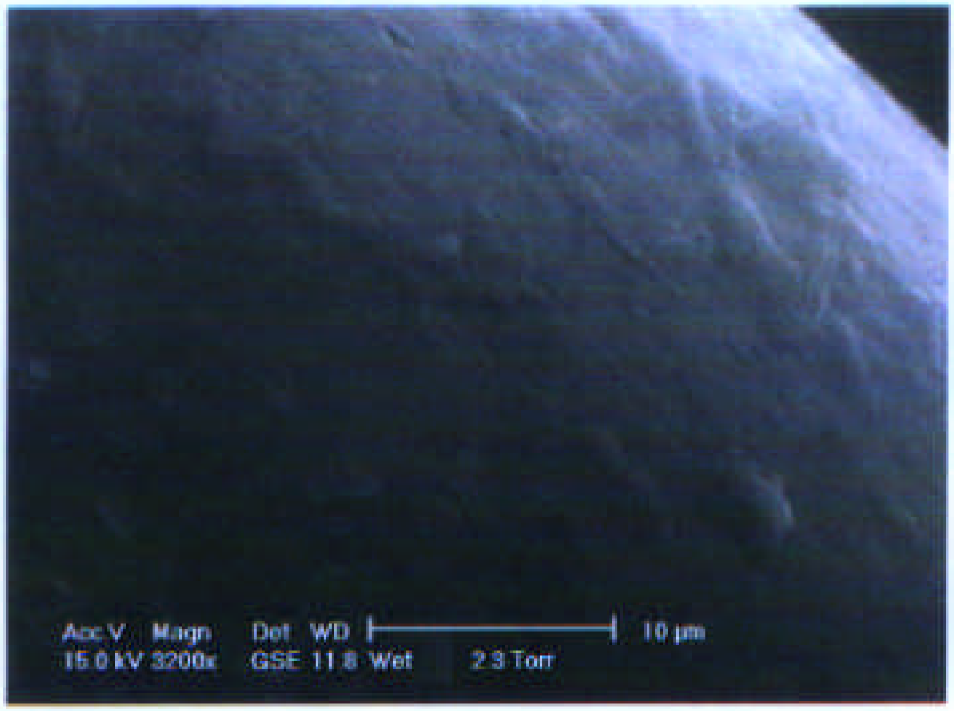
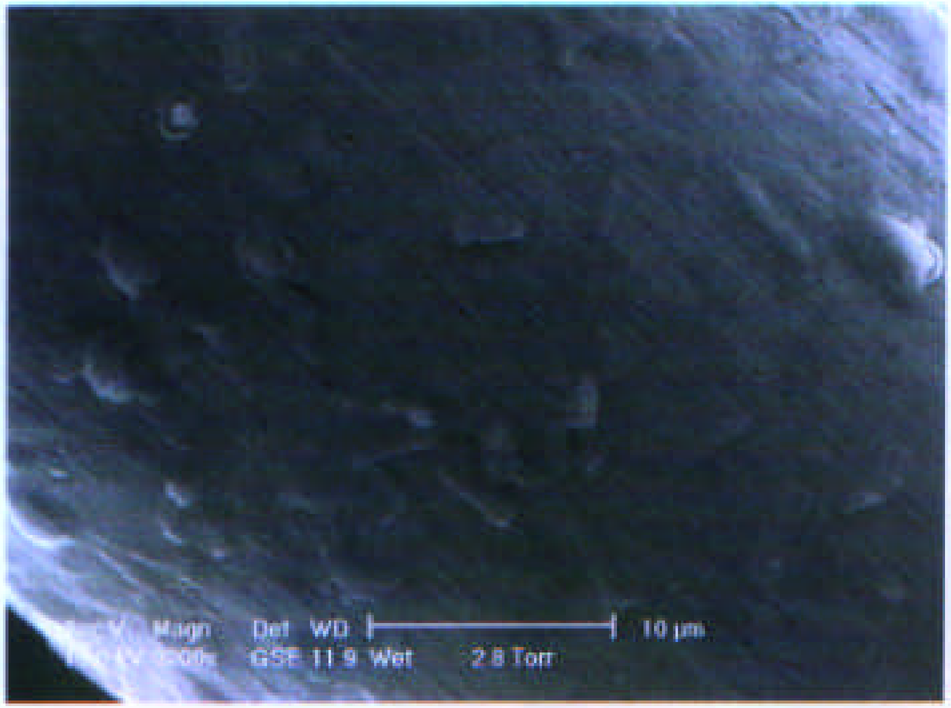
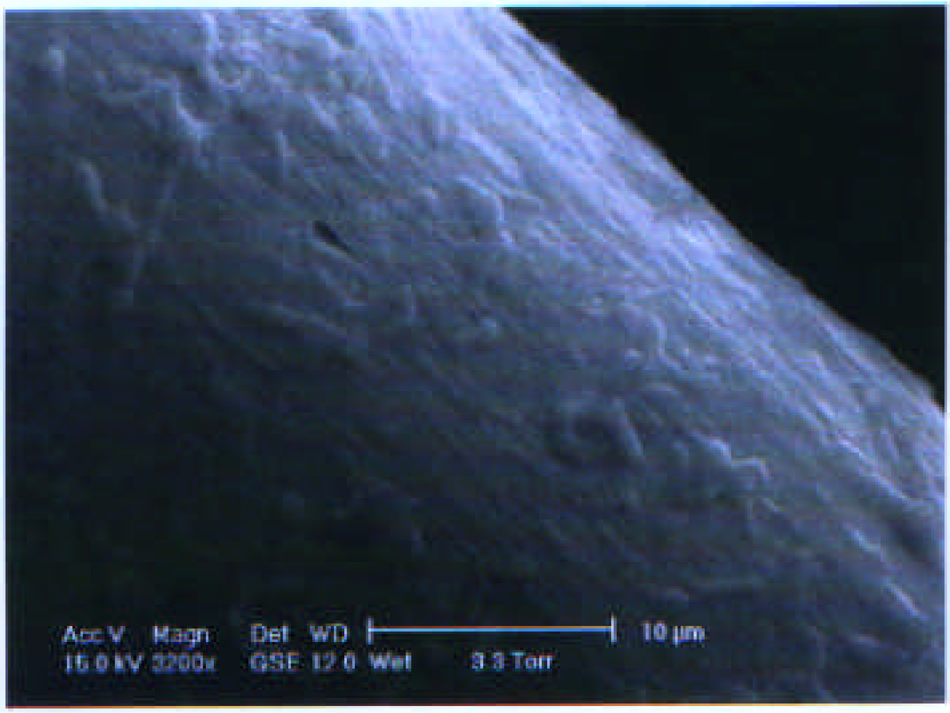
15.0 kV 5000x GSE 12.0 Wet 3.3 Torr


Acc.V Magn Det WD 1 µm
15.0 kV 20000x GSE 12.0 Wet 3.3 Torr


Acc.V Magn Det WD 10 µm
15.0 kV 3200x GSE 11.8 Wet 2.3 Torr


Magn Det WD 10 µm
GSE 11.9 Wet 2.8 Torr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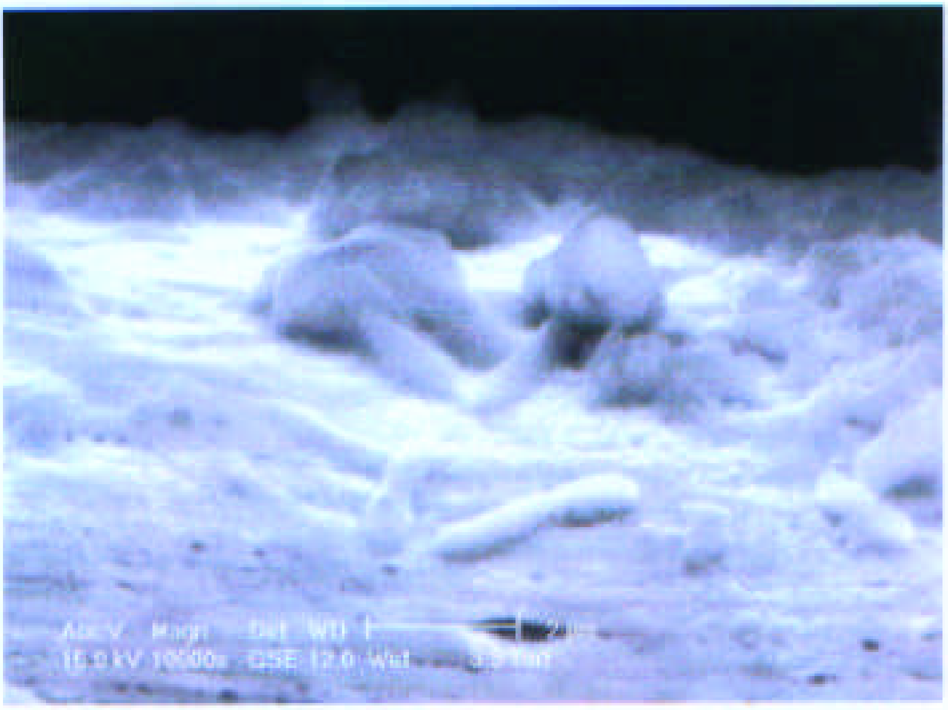
15.0 kV 3200x GSE 12.0 Wet 3.3 Torr


Acc.V Magn Det WD 2 µm
15.0 kV 10000x GSE 12.0 Wet 3.3 Torr


Acc.V Spot Magn Det WD
5 µm
00 kV 4.0 4356x GSE 7.5 Wet 2.4 Torr

SOME COMMENTS ABOUT BACTERIA IN D_2O

(AS AT OCT. 21, 2000)

① THE BACTERIA ARE DELETERIOUS TO H, D UPTAKE INTO Pd: IT IS MANDATORY TO AVOID CONTAMINATION OR, AT LEAST, KEEP THE "POPULATION" AT LOW LEVEL (E.G.: $< 10^2$/ml FOR 1 l SOLUTION, WIRE: 50 μm ϕ, 30 cm

② WE HAVE FOUND ALL THE USED D_2O SOLUTIONS CONTAMINATED BY BACTERIA AND THEIR METABOLITES (pH≤

THE USED D_2O-LiOD SOLUTION IS LONG TIME (> 1 y) STABL

IF LiOD CONCENTRATION IS $> 10^{-2}$ M/l.

③ EVEN SOME OF EXCESS HEAT, AT LOW POWER (< 1 W), CAN BE ASCRIBED TO SOME KIND OF BACTERIA "FERMENTATION" IF: THE VOLUME OF SOLUTION WAS LARGE (~ 1 l), LIGTH CAN PENETRATE INSIDE THE CELL, THE EXCESS HEAT VANISH AFTER ~ 1 WEEK.

④ THE RESULTS AT HIGH POWER ARE FULLY ACCOUNTABLE FOR " NUCLEAR ANOMALOUS EXCESS HEAT".

⑤ SOME OF THE RESULTS ABOUT ELEMENT FOUND ON Pd SURFACE CAN BE DUE TO THE SPECIFIC ABILITY OF RALSTONIA GENE TO CONCENTRATE POLLUTANT AND "ADHERE" ITSELF AT THE CATHODIC SURFACE OF Pd (RALSTONIA IS CHEMOLITOTROPHIC AND CAN USE H^+ AS ENERGY SOURCE).

⊙ MORE ACCURATE ANALYSIS OF D_2O USED ARE NEEDED TO RULE OUT THIS POSSIBILITY.

⑥ CAN BE INTERESTING, SPECULATIVELY, TO MAKE SOME SPECIFIC EXPERIMENTS TO EXPLORE THE POSSIBILITY THAT THIS KIND OF BACTERIA CAN "AGEVOLATE" THE TRASMUTATION (IF ANY) OF ELEMENTS.

⊙ NAA AND ICPMS NEEDED TO PROPERLY EVALUATE THE RESU SIMS IS REQUIRED TO CHECK ABOUT ISOTOPIC SELECTIVITY CAPABILITY (⇒ INTERESTING APPLICATIONS).

⑦ IT SEEMS REALISTIC TO USE, IN THE NEAR FUTURE, THIS NEW KIND OF BACTERIA TO CLEAN-UP WATER POLLUTED WITH SOLUBLE HEAVY ELEMENTS (Hg, Pb, Cd, U, S_2?):

BIOREMEDIATION

⑧ THE APPLICATION OF BACTERIA TO GENERATE H_2 FROM WATER, USING SUN LIGTH AND CO_2, NEEDS MORE SYSTEMATIC STUDIES.

FINAL STATEMENT

I AM CONVINCED THAT IF THE BACTERIA (AND EVEN ALGAE) CONTAMINATION OF HEAVY WATER WERE KNOWN (TROUGH D_2O PRODUCERS) FROM THE BEGINNING OF COLD FUSION STUDIES, SOME OF THE PROBLEMS RELATED TO POOR REPRODUCIBILITY OF D OVERLOADING CAN BE AVOIDED.

⊙ MOREOVER, WE TESTED THAT $KMnO_4$ (usually 10 mg/l) ADDED FROM THE PRODUCER TO D_2O, LIMITS ITSELF THE MAXIMU LOADING (LNF PROCEDURE, H_2O EXPERIMENT: $\phi\ KMnO_4 \Rightarrow R/R_0 \simeq 1.3$ T

10 mg/l $KMnO_4 \Rightarrow R/R_0 \simeq 1.50 \div 1.55$; 50 mg/l $KMnO_4 \Rightarrow R/R_0 \simeq 1.70$).

⊙ I AM SURE THAT THE EFFORTS ABOUT D OVERLOADING OBTAINE COULD BE MORE EFFECTIVE:

NHE LABORATORY, E.G., COULD PRODUCE BETTER RESULTS WITHOUT WASTING TIME BECAUSE THIS KIND OF "SECRET" PROBLEMATICS. I NOTE THAT THEY "SUSPECT", IN 1998, THIS PROBLEM (TOC ANALYSIS) BUT DIDN'T HAVE ENOUGH TIME