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HCM Large Facilities Access Programme Soft X-ray Radiation Effects on Sacaromices Cerevisiae Yeast Cells

D Batani et al

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SOFT X-RAY RADIATION EFFECTS ON *SACAROMICES CEREVISIAE* YEAST CELLS

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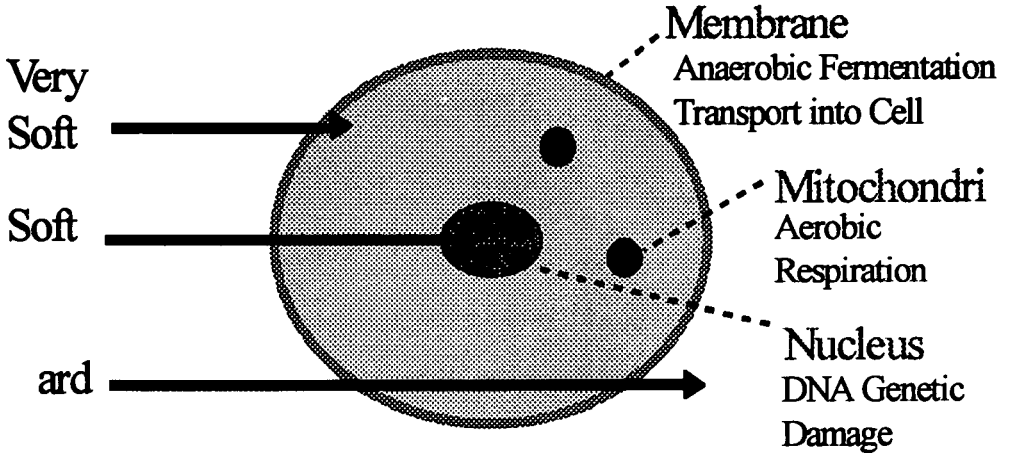

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SUMMARY

This report describes the experiment entitled "Soft X-ray Radiation Effects on Sacaromices Cerevisiae Yeast Cells", carried out in the Central laser Facility (CLF) at the Rutherford Appleton Laboratory over a five week period during September/October 1995. The experiment, funded by the Framework III Large Facilities Access Scheme, was conducted by a team consisting of both young and established Italian scientists derived from the Universita' di Milano and Universita' di Modena, together with UK scientists from the CLF.

Key Points and Observations

- Yeast cells provide the ideal biological sample as they can be regarded as a "living laboratory".
- Irradiation of yeast cells by soft x-rays (0.5-0.9 keV) allows selective interference of different cellular structures due to the short mean free path of the soft x-rays.
- A novel technique for assessing response to x-ray irradiation is employed in which the cell metabolism is measured by monitoring the production of carbon dioxide using high accuracy semiconductor pressure sensors.
- Monotonic responses of cell activity as a function of radiation dose should not be taken for granted.
- Non-monotonic responses evidence the capacity of living systems to exhibit a response that is not detectable by macroscopic methods (i.e. averaged on time and sample number).
- The results indicate that a novel research trend could be pursued in the study of soft x-ray interactions with biosystems.

Arising Publications

Three fully refereed journal publications together with three conference presentations have already arisen from the work described in this report.

Refereed Publications

D. Batani, M. Milani, A. Conti, A. Masini, M. Costato, A. Pozzi, F. Musumeci, A. Triglia, E. Turcu, R. Allott, N. lisi, "Biosystem response to soft x-ray irradiation: non-monotonic effects in the relevant biological parameters of yeast cells", **IL Nuovo Cimento D**, Vol D18, No 5, 657-662,1996.

D. Batani, M. Milani, G. Leoni, A. Conti, A. Masini, F. Previdi, R. Casati, R. Bonadio, N. Correale, M. Costato, A. Pozzi, E. Cotelli, C. Lora Lamia Donin, M. Moret, E. Turcu, R. Allott, N. lisi, "Soft x-ray radiation effects on saccharomyces cerevisiae yeast cells", **Laser and Technology**, in press September 1996.

D. Batani, M. Milani, G. Leoni, A. Conti, A. Masini, F. Pisani, M. Costato, A. Pozzi, E. Cotelli, C. Lora Lamia Donin, M. Moret, E. Turcu, R. Allott, N. lisi, "Yeast cells response to soft x-rays from laser plasmas" **Vuoto, Science and Technology**, in press September 1996.

Conferences

13th National Conference on Vacuum science and Technology, Milan, Italy ,February 1996.

A. Masini, A.Conti, D. Batani, M. Milani, M. Costato, A.Pozzi, I.C.E. Turcu, R. Allott and N.Lisi, "Study of yeast cells by CO_2 production and their response to soft x-ray irradiation from laser plasmas".

A. Masini, A.Conti, D. Batani, M. Milani, F. Pisani, G. Leoni, M. Costato, A.Pozzi, I.C.E. Turcu, R. Allott, N.Lisi and M. Koenig, "Characterisation of a soft x-ray laser plasma source for studies on yeast biophysics".

15th General Conference of EPS Condensed Matter Division, Stresa, Italy, April 1996.

I.C.E. Turcu, R. Allott, N.Lisi, A.Conti, D. Batani, A. Masini, , F. Pisani, M. Milani, G. Leoni, M. Costato, A.Pozzi, and M. Koenig,"Characterisation of a soft x-ray laser plasma source for yeast biophysics studies".

Reports

E. Turcu, R. Allott, N. Lisi, M. Milani, G. Leoni, A. Conti, A. Masini, R. Bonadio, N. Correale, M. Costato, A. Pozzi. "Metabolic Interference of Soft X-rays on Yeast Cells", Central Laser Facility Annual Report 1995-96, RAL Report TR-96-006, p 152.

Aims of the Research:

- **Biological**

Selective damaging of enzyme metabolic activity at the membrane level (responsible for fermentation) without interfering with respiration (taking place in mitochondria), see figure 1.

- **Technological**

Easy non invasive on line monitoring metabolism in yeast cells

- **Biophysical**

Search for oscillations of enzymatic cycle and its regulatory mechanisms with a physical probe on line, that is active on many cells, and can give quantitative information in a continuous way. The system has many interesting features from the physical point of view being the glycolytic cycle a model system for nonlinear science.[1]

- **Bioengineering**

Techniques are made available for increasing respiration rate (by damping fermentation ability); thus biomass production is increased without damaging the biomolecules that are produced inside the cell. A feature certainly interesting for biotech and genetech fabbrication.

All the investigated processes, being driven by ATP-ADP universal energetic cycle, are linked to replication of cells.

To decouple glycolysis, and respiratory cycles from replication it is important to irradiate the biological target with X-rays that, because of wavelengh and dose, damage selectively cellular compartments, such as the membrane or cytoplasmatic structures, without giving rise to significant DNA damage (figure.1).

- **Educational**

Training of graduate and postgraduate students in Physics and Biology. Some students have actively taken part in the experiment on which their thesis work will be based.

Soft X-rays selective action on yeast cells

- **The biological target:**

Yeast cells have many fundamental characteristics of higher eukaryotic cells, whilst being relatively simple objects in the kingdom of living beings. Yeast is also viewed as a microorganism of major economic and social significance. In fact, not only does it provide fermentation for most breads and alcoholic beverages, but it is increasingly used in the new field of biotechnology to produce heterologous proteins and other molecules mainly but not exclusively for pharmaceutical use [2].

- **Points of interest:**

1. Glycolysis vs cancer cell growth

Glycolysis and oxidative phosphorilation are among the most widely exploited biochemical pathways, but the spatio-temporal regulation of their interactions in vivo is not yet completely understood, and may enlight mechanisms peculiar of cancer cells. In fact rapidly growing tumors are found to exhibit a capacity to utilize more glucose than normal tissues, and seem to obtain significant amounts of ATP from sources involving both mitochondrial (glycolytic) reactions and mitochondrial oxidative phosphorilation [3].

2. Recovery mechanism

Saccharomyces cerevisiae yeast cells have recently permitted to evidence the role played by AMP in the homeostatic mechanism which is responsible for the capacity exhibited by cells to activate a recovey mechanism after damage [4]. Furthermore yeast has permitted rapid progress in the problem of damaged DNA[5].

3. Ageing

Genetic analysis of ageing in yeast revealed that a protein complex known to play an essential role in transcriptional silencing at mating-type loci and telomers also control ageing and stress resistance [6].

4. Protein transport

The vacuole of yeast *Saccharomyces cerevisiae* is functionally equivalent to the mammalian lysosome. Protein transport to the yeast vacuole provides new insight into the mechanisms of vacuolar protein localisation [7]

5. Morphogenesis

Pheromones produces by mating types in yeast have been used to elucidate the fundamental components of the molecular machinery controlling morphogenesis in eukaryotic cells [8]. The proven capacity of yeast to permit genetic manipulation has greatly improved the understanding of the fundamental mechanism of intra-cellular signal transduction associated with cellular division and differentiation [9].

6. Antifungal agents

Interest in the assembly of the yeast cell wall has been recently growing from a pharmaceutical point of view. In fact, since the extracellular matrix of mammalian cells strongly differs from

the cell wall of yeast, the assembly of the wall represents in principle an ideal target for new antifungal agents [10].

7. Neurodegenerative disorders

Yeast has recently permitted to identify prions, a type of infectious protein responsible for causing several neurodegenerative disorders in mammals. The propagation of prion phenotype in yeast involves pathways related to control of nitrogen catabolism [11].

8. Incorporation of drugs

Yeast has been found to lead to a better understanding of electropermeabilisation, which is generally utilized for incorporation of drugs, DNA, tec. into cells for gene engineering, the mechanisms of which have not yet benn completely established [12].

9. Cell signal transduction

Yeast has proven to be a useful tool to elucidate the mechanism of transduction of stimulatory signals from the cell surface to cytoplasmic nuclear targets. Such a mechanism is seen as a cascade involving a series of protein kinase (MAP, mitogen activated protein) which are present in eukaryotic cells from yeast to mammals [13].

Structure of Yeast Cells

Yeast cells have been analysed with with different techniques: optical microscope, SEM microscope (figure.2), X-ray microscopy (figure.3), Coulter Counter:

Average Radius (3 - 4 microns), Membrane Thickness (100 - 200 nm), Nucleus Size (about 2 microns). In figure 2 and 3 we show respectively SEM and X-ray microscope images.

Typical Metabolism: Fermentation and respiration

CO_2 and energy production (ATP-ADP pool) [14] Schemes of the major reactions and enzyme; pivotal role of PFK [14]

In our experiment CO_2 production is obtained by means of Pressure measurements.

Preparation of the biological samples

The biological target is made of yeast cells (*Saccharomyces Cerevisiae*). The cells are commercial dry ones that are hydrated just before the irradiation procedure. A suspension ($2x10^7$ cells/ml is the typical concentration) is obtained by adding deionized water. Then the suspension is filtered in a Venturi tube and cells are deposited on a paper filter giving rise to a monolayer.

After a short period for drying the filters, they were deposited onto a Hostaphan film so that yeast cells are hosted by the sandwich made of Hostaphan from one side and paper filter on the other. The sandwiches were then deposited into a robot [19] (the Hostaphan film facing the X-ray source in order to filter unwanted X-rays) and the dose could be delivered onto the target.

The radiation source

The experimental arrangement [19] can generate X-rays by laser-plasma interaction.

Why soft X-rays

Keeping in mind that we want to reduce DNA damage down to a minimum and that the genetic material is contained within the nucleus, and that cellular biological material attenuates X-rays following a Bouguet-Beer laws with a decay exponent that is very large, the suitable procedure consists of making use of X-rays with $h\nu < 1$ KeV. On the other hand X-Rays in the "water window " region (~ 300-500 eV) should also be avoided as these have a low attenuation coefficient. Recall that X-rays with $h\nu \sim 1.3$ KeV are allowed to travel only 5 μm through biological material.

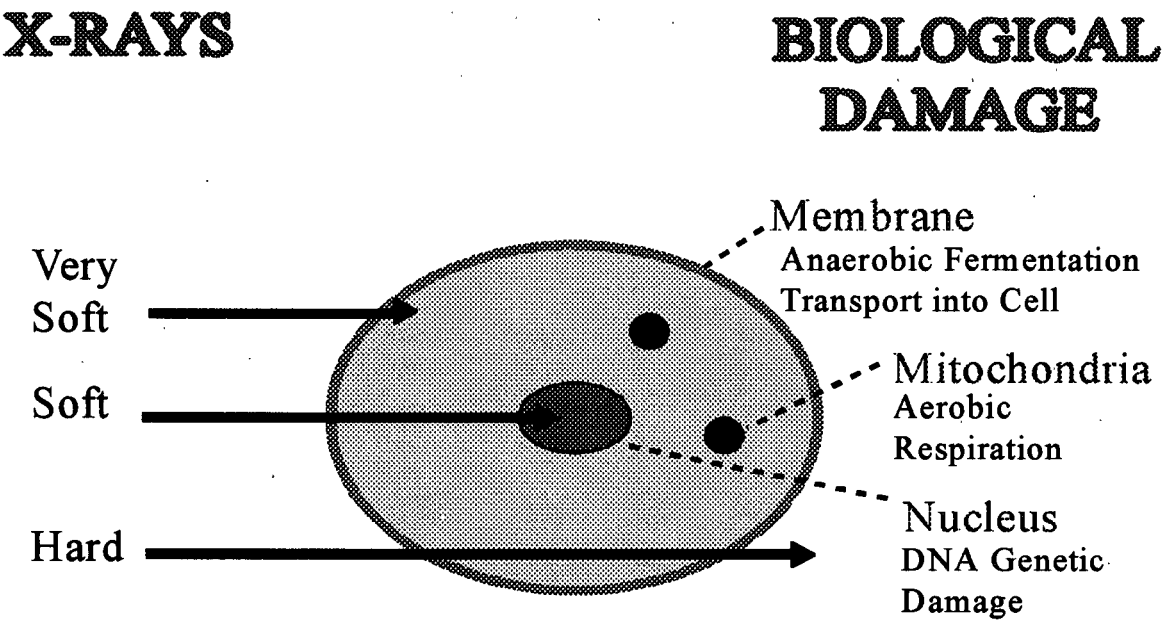


Figure 1: Yeast cell irradiation at different soft x-ray wavelengths

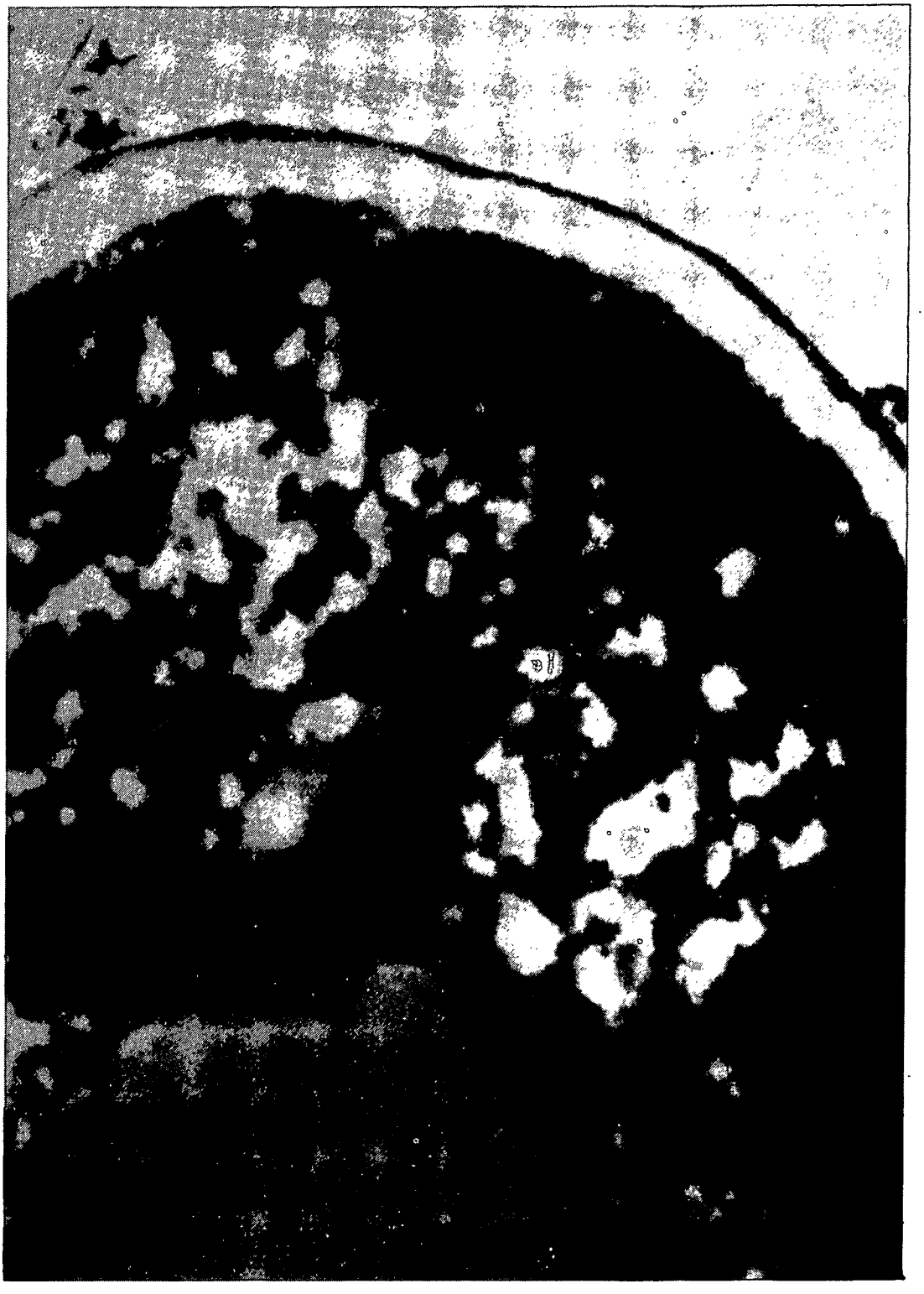


Figure 2: Electrom microscope image (TEM) of a yeast cell. Magnification x82500. It is possible to see the cell nucleus and some vacuola and measure the membrane thickness (~150-200nm)

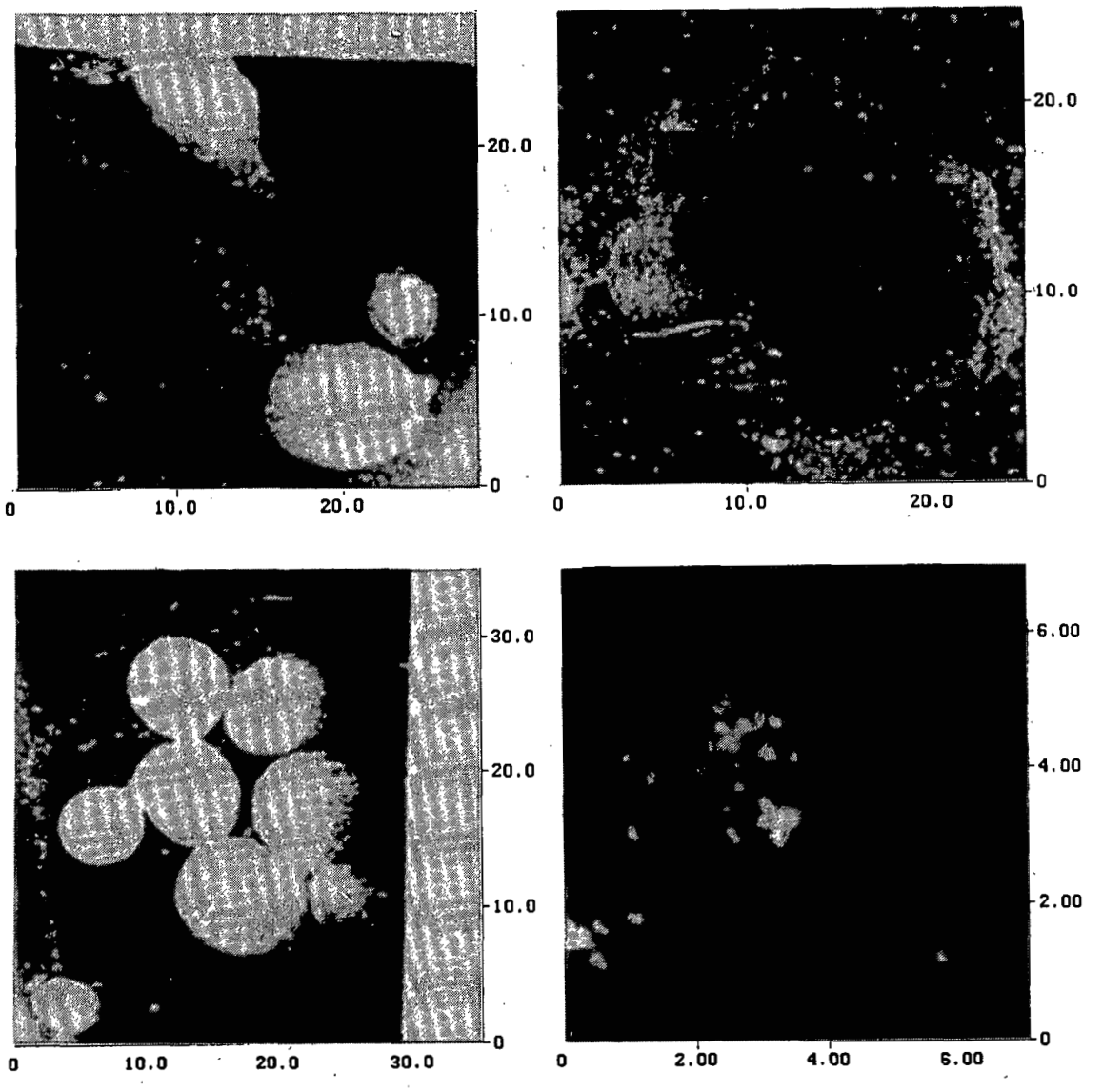


Figure 3: X-ray microscope images of living yeast cells. The technique (soft X-ray contact microscopy) uses a laser plasma source and shows with high contrast carbon rich structures.

Complimentary but fundamental elements of the generation of X-rays are the Hostaphan filter, together with the Al filter to cut off the unwanted portion of the spectrum (and UV light) and the use of a teflon target. The X-ray spectrum is shown in figure.4.

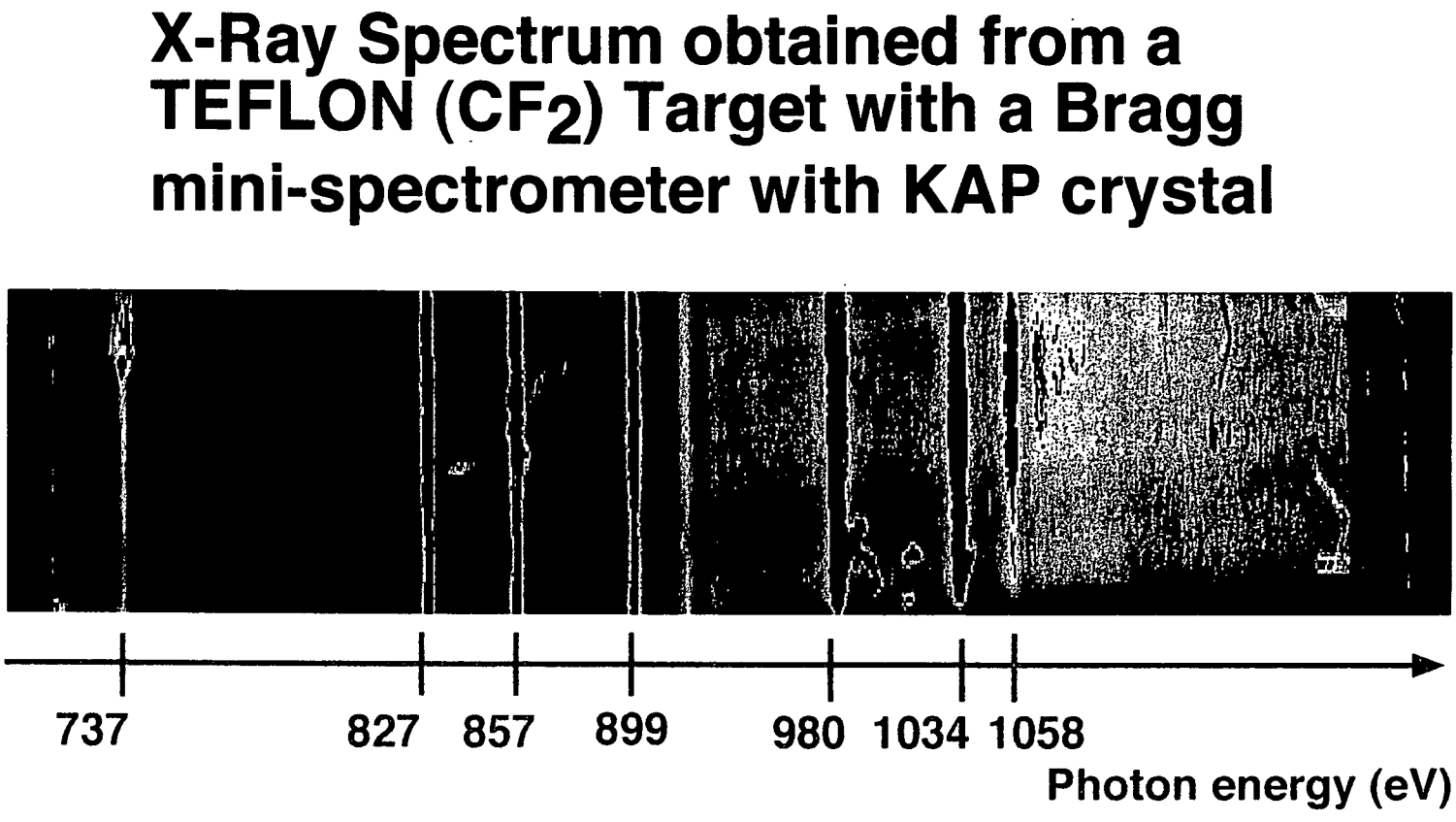
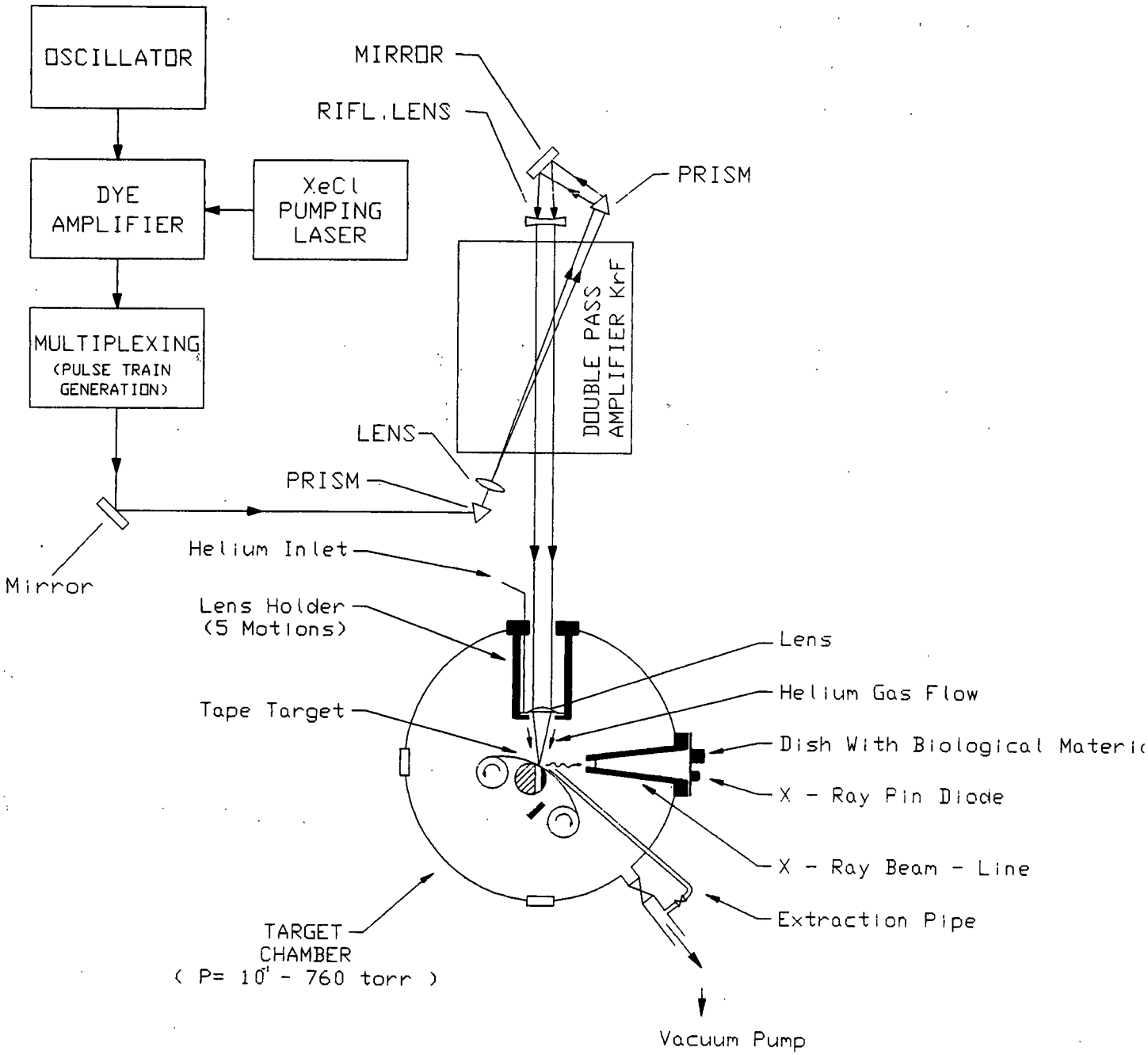


Figure 4: Soft x-ray spectrum of the teflon target



X-RAY SOURCE EXPERIMENTAL SET-UP

Figure 5: CLF /RAL x-ray plasma source schematic.

Why at RAL

A very important aspect of the experiment is the computer assisted robot and dose control system. [18]

A PIN diode, placed at a short distance from the target measures the x-ray dose. Since a single laser shot is not enough to give the required dose, a sequence of laser shots is required (laser repetition rate is set to 20 Hz).

For many technical and biological reasons the x-ray flux changes, therefore a system has been used [19] that monitors all the necessary processes, blocks the laser beam when the required dose is delivered and provides a histogram of the x-ray dose distribution. A diagram of the experimental set-up is in figure.5.

Another fundamental point is the fact that the x-ray source is absolutely calibrated, by PIN diodes and x-ray spectrometer.

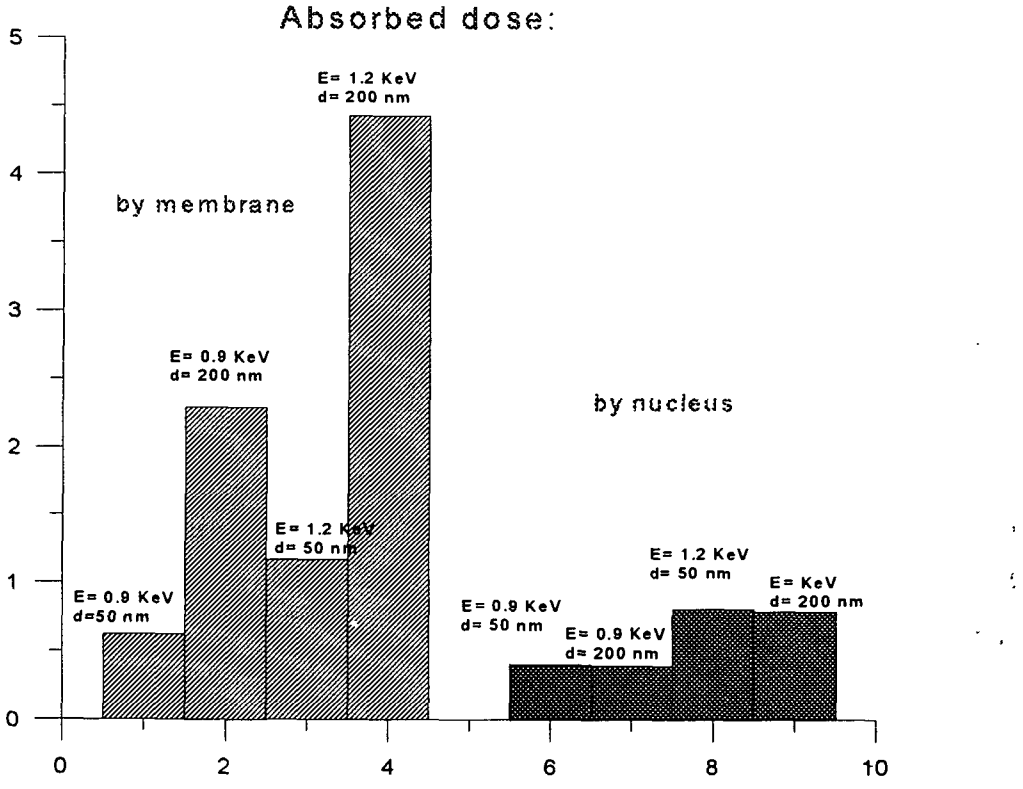


Figure 6: Integral dose absorbed by the nucleus and by the membrane for two typical X-ray energies (Teflon and Copper X-rays) and two different d (= membrane thickness)

Experimental techniques

- *Overall process*
- CO_2 production by glycolysis in brewer's yeast which makes ethanol
- *Detailed process*

Observation of a time dependent process: only in 1957 Duysens and Amesz obtained the first hint that glycolysis does not always produce energy in a steady DC mode but that it sometimes goes AC, the cell oscillating with a period of approximately one minute [14].

Intermediate investigations

1. Optimization of the biological target and its support
2. CO_2 production on line non invasive follow up [CO_2 PLNF] in aerobic and non aerobic regimes in cell cultures fed with glucose in the absence of irradiation.
3. Preparation of samples on filters for irradiation and [CO_2 PLNF] in aerobic and non aerobic regimes in cell cultures fed with glucose in the absence of irradiation. [17]
4. [CO_2 PLNF] in aerobic and non aerobic regimes in cell cultures fed with glucose irradiated at different doses and dose rates. (with "normal" Teflon target)
5. [CO_2 PLNF] in aerobic and non aerobic regimes in cell cultures fed with αD-glucose, L-glucose, and L- and D-mannose in the absence of irradiation
6. [CO_2 PLNF] in aerobic and non aerobic regimes in cell cultures fed with αD-glucose, L-glucose, and L- and D-mannose, irradiated at different doses and dose rates.
7. Irradiation of samples with Cu X rays [16]
8. Irradiation of samples with UV light
9. Morphological analysis of the samples before and after irradiation (optical microscope, SEM, TEM and Xray Microscope)

Results

Control samples: Role of ambient pressure

Differential pressure sensors (Low Pressure Sensor type # AM5305 DV and # AM5310 DV from Miteco, CH) of high sensitivity (from 0 to 50 or to 100 mbar, giving 25 mV output end of scale, with sensitivity of 0.1 mV) were used (figure.7), linked to a computer interface card.

From the graph it is evident that there is linearity in the pressure region of interest (0-100 mbar) and beyond.

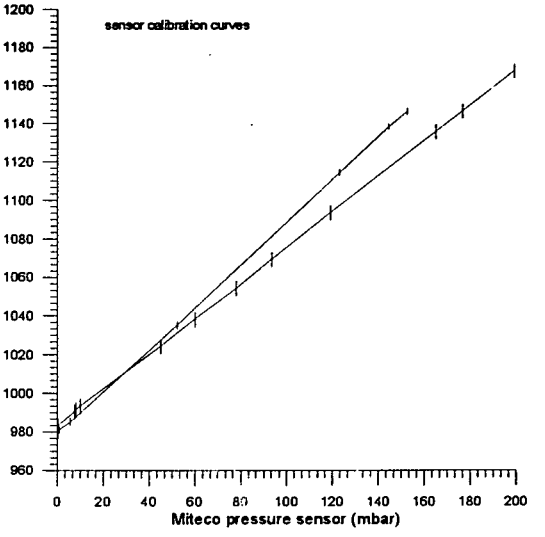


Figure 7: Pressure sensors calibration curves.

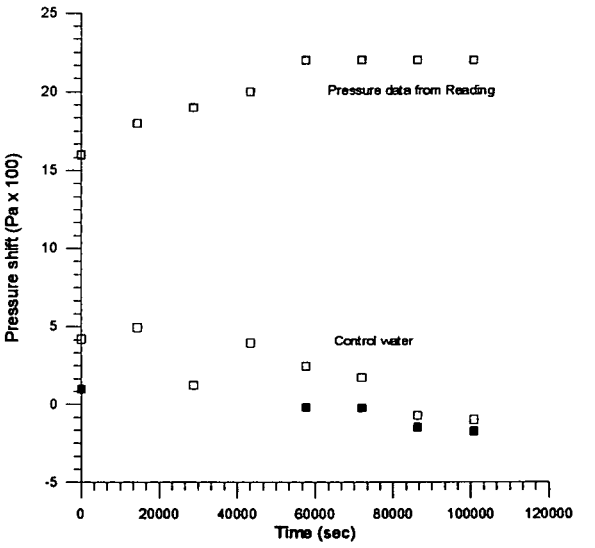


Figure 8: Atmospheric pressure (data from Benson and Reading) and control samples.

The control water perfectly reflects atmospheric pressure comportament (1000 mbar off-set). Three experimental points are absent because of problems with unipolar acquisition mode.

CO_2 production and O_2 consumption: Mass spectrometry

In figure 9 it is possible to see that the produced gas, during fermentation, is CO_2 (mass number 44) that wasn't in the initial blank run and in the bottle where air was substituted with Argon (mass number 40- anaerobic condition).

Figure 10 represents the analysis of the same sample after 24 hours and in which we allowed air to flow inside the bottle.

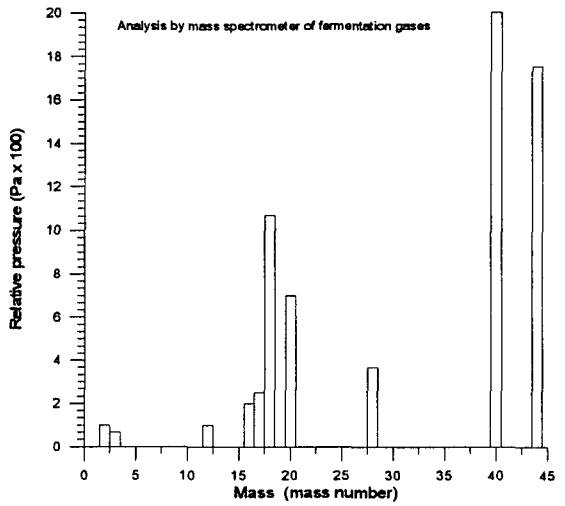


Figure 9: Gas produced by yeast cell activity in anaerobic conditions.

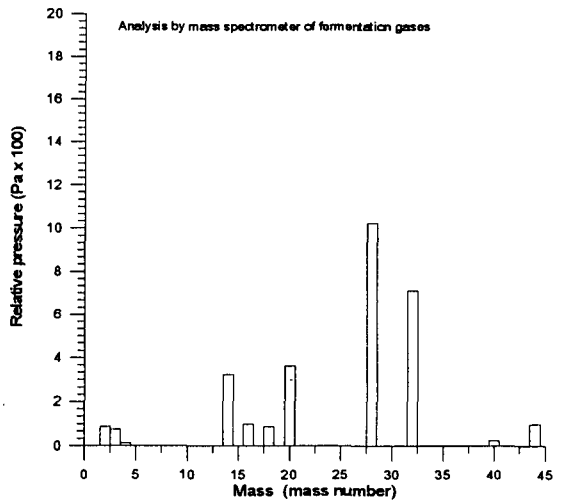


Figure 10: Same sample as figure 9, 24 hours after air was allowed into the bottle

Role of sugar

In Figures 11and 12 it is possible to observe the different behaviour of some sugars: αD-glucose, glucose, L-glucose. Fig. 12 represents the situation 36 hours after the start of the measurement (compare with the curves in fig. 1-2-3 [17]).

The metabolic rate depends on the chirality of the sugar used for fermentation.

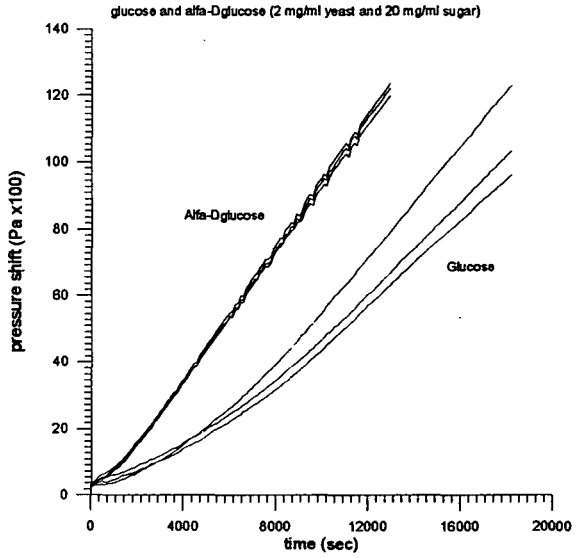


Figure 11

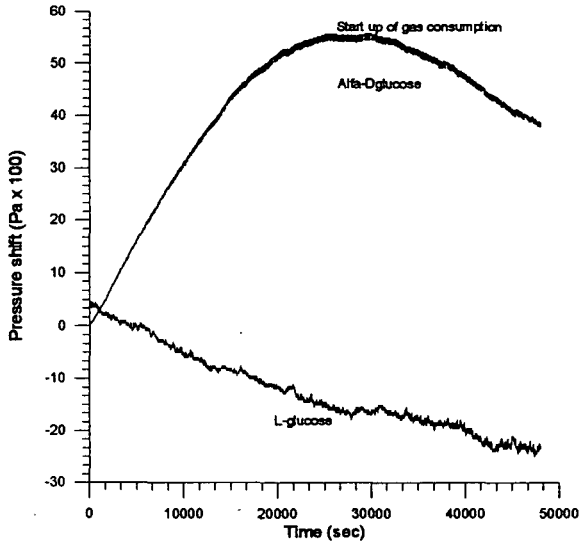


Figure 12

Preliminary Results with Irradiated Cells

Pressure shift vs Xray doses (action spectrum):

These graphs indicate a reduction in pressure for all irradiated samples. A reduction is seen also in the efficiency of the pressure production. Even more Figures 13 and 14 show a nonlinear response to irradiation doses. Figure 15 indicates different pressure production rates at different times.

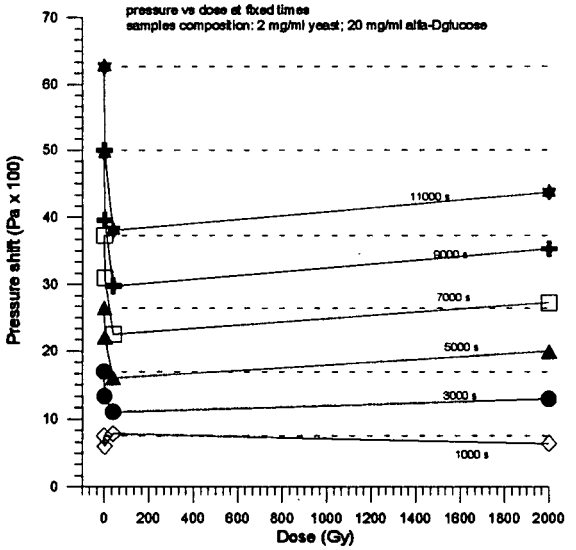


Figure 13: Irradiated cells. Pressure vs dose.

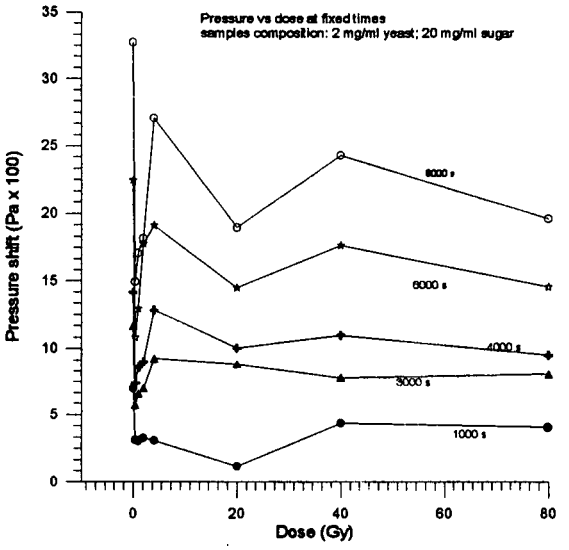


Figure 14: Irradiated cells. Pressure vs dose.

A preliminary idea of the powerful tool we are using to investigate yeast metabolism and the response to X-rays can be given by the comparison with data reported in works of D. Frankenberg, D.T. Goodhead [15] where survival curves are given as a probe for damage induced by X-rays.

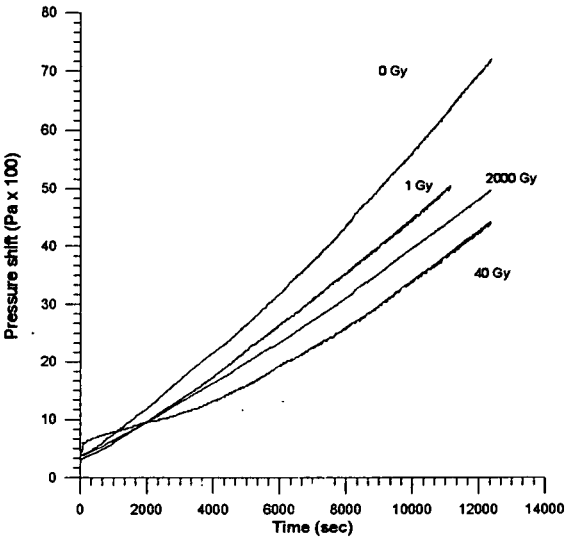


Figure 15: Pressure at different times (for different doses)

Recovery mechanisms

The technique gives information on the autorecovery of the cell system in particular between 0-4000s and seems very promising. A hypothesis is that mixing irradiated cells with cells from a fresh non-irradiated culture can rise to co-operative effects in the cell population.

Chiral properties of macromolecular units play an important role in the cell metabolism as can be observed in Fig.11 and 12. When α-D glucose is given to the samples prepared with L-glucose (curves with negative trend) the CO_2 production assumes a normal comportment again.

Oscillations

With both irradiated and non irradiated samples: The oscillations in the graphes (figures 16 and 17) are not found in the control water and their amplitude is larger than the sensor sensitivity (compare with curves in [14]).

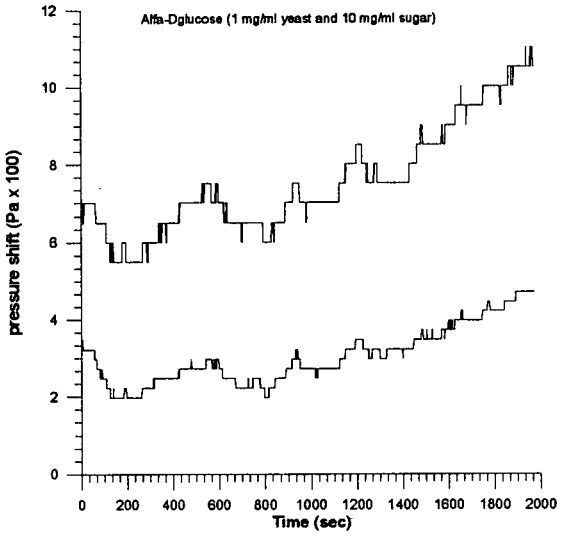


Figure 16: Oscillations for irradiated and non-irradiated samples

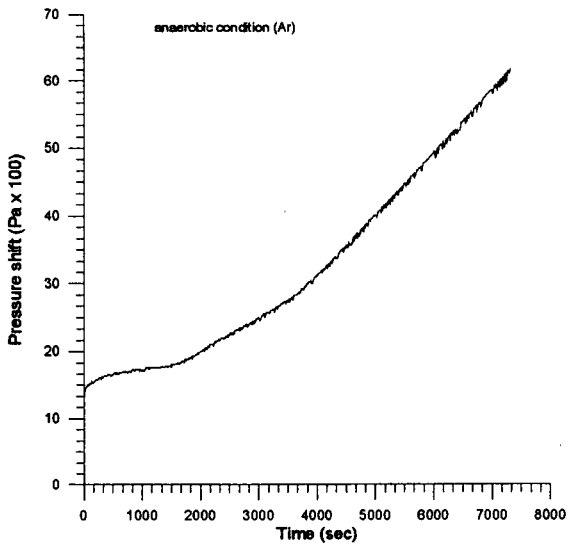


Figure 17: Oscillations in anaerobic condition (Ar)

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Armando Reale	Università de L'Aquila

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