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Differential Two Colour X-Ray Radiobiology of Membrane/Cytoplasm Yeast Cells

TMR Large-Scale Facilities Access Programme

M Milani et al

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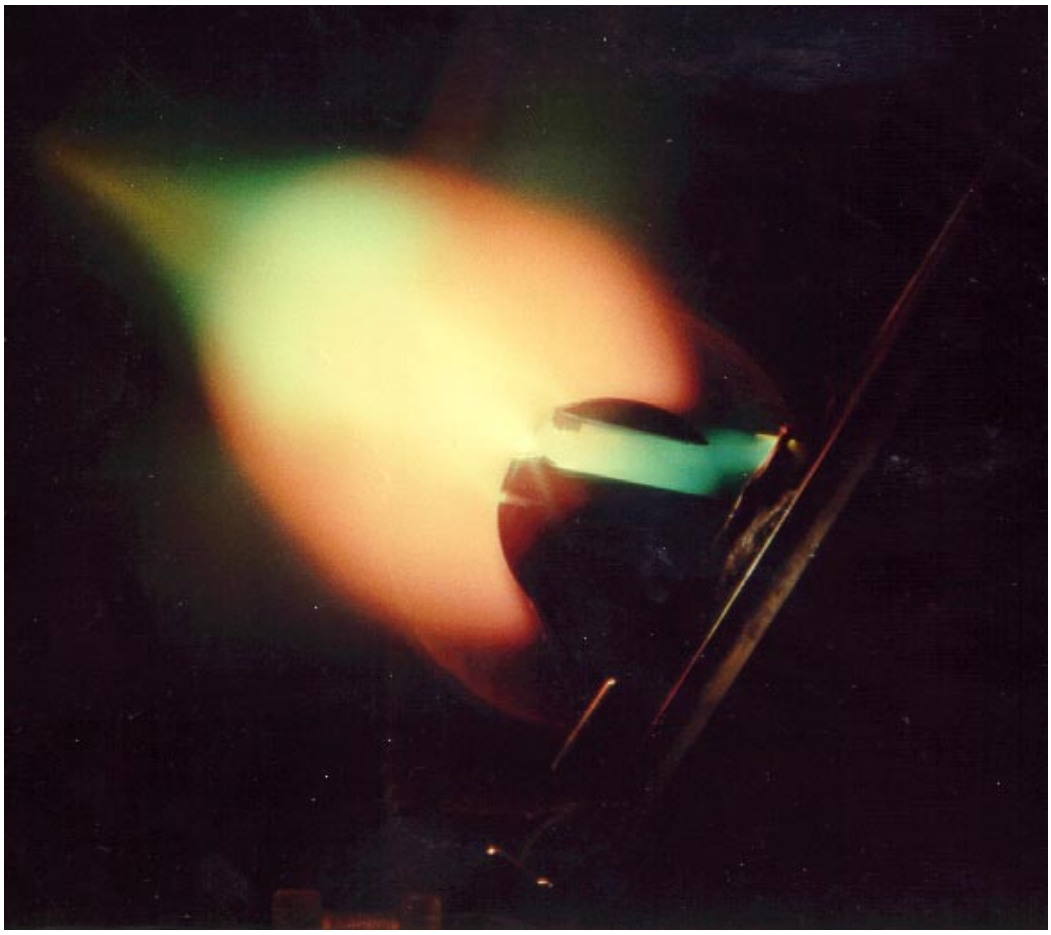

Differential Two Colour X-Ray Radiobiology of Membrane/Cytoplasm Yeast Cells

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Access to Lasers at the Central Laser Facility
Rutherford Appleton Laboratory
Contract No. ERBFMGECT950053

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SUMMARY

This report describes the experiment entitled 'Differential Two Colour X-Ray Radiobiology of Membrane/Cytoplasm Yeast Cells'; carried out at the Central Laser Facility (CLF) from the 6th January to the 3rd February 1997. The experiment, funded by the Framework IV Large-Scale Facilities Access Scheme, was proposed by Dr M Milani, Dipartimento di Scienza dei Materiali, University of Milan, Italy, and carried out by visiting researchers from his institute, the University of Modena and with technical support from the Central Laser Facility, Rutherford Appleton Laboratory.

Experimental Highlights

- The development of an investigation technique based on the use of very soft X-ray in order to damage specific structures inside the cell structure (specifically cell wall and membrane);
- The use of pressure sensors as a diagnostics of cell response which allows to monitor cell response over a large range of times from fast response up to several hours;
- The use of dry yeast cells as an "easy to handle" type of sample;
- The development of a simple model for X-ray dosimetry of the different cell compartments.
- The study of metabolic oscillation in yeast cell suspension and the observation of the oscillation frequency shift following an exposure to soft X-rays.

The CLF makes beam time at its facilities available to European Researchers with funding from DG-XII, CEC under the Large Scale Facilities Access Scheme. For further information contact Dr. Chris Edwards at the CLF. Tel: (0)1235 445582, e-mail: c.b.edwards@rl.ac.uk

From Left to Right:

Top row: F Bortolotto, E Turcu, M Costato, M Milani, N Lisi, R Allott

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ARISING PUBLICATIONS

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Proc. Conf. of the Italian Physical Society (SIF), Como, Italy, p. 170 (1997).

Differential Two Colour X-Ray Radiobiology of Membrane/Cytoplasm Yeast Cells

ABSTRACT

Saccharomyces Cerevisiae yeast cells were irradiated using the soft X-ray laser-plasma source at Rutherford Laboratory. The aim was to produce a selective damage of enzyme metabolic activity at the wall and membrane level (responsible for fermentation) without interfering with respiration (taking place in mitochondria) and with nuclear and DNA activity. The source was calibrated by PIN diodes and X-ray spectrometers. Teflon stripes were chosen as targets for the UV laser, emitting X-rays at about 0.9 keV, characterised by a very large decay exponent in biological matter. X-ray doses to the different cell compartments were calculated following a Lambert-Bouguet-Beer law. After irradiation, the selective damage to metabolic activity at the membrane level was measured by monitoring CO_2 production with pressure silicon detectors. Preliminary results gave evidence of pressure reduction for irradiated samples and non-linear response to doses. Also metabolic oscillations were evidenced in cell suspensions and it was shown that X-ray irradiation changed the oscillation frequency.

INTRODUCTION

We present the results of an experiment concerning the irradiation of Saccharomyces Cerevisiae Hansen yeast cells with soft X-rays produced with the laser-plasma source at Rutherford Laboratory. The main idea behind the experiment was to use very soft X-rays in order to allow the production of selective damages to the cell structures (specifically cell wall and membrane, responsible for part of the cell metabolic activity, in particular fermentation) without interfering with respiration (taking place in mitochondria) and with nuclear and DNA activity. For this purpose, Teflon (CF_2) stripes were chosen as targets which, when irradiated with high intensity UV laser radiation, transform to a plasma of C and F He- and H-like ions. Then X-rays are emitted with a K-shell spectrum centred around 0.9 keV. Such X-rays are characterised by a very large decay exponent in biological matter, and hence by very large absorption. X-ray doses to the different cell compartments were calculated following a Lambert-Bouguet-Beer law. The structure of yeast, characterised by a large and massive cell wall, contributed to stop most X-rays before the cell nucleus.

After irradiation, the selective damaging of metabolic activity at the membrane level was measured by monitoring the production of CO_2 through pressure measurements performed with silicon detectors. Indeed CO_2 is related to energy production (ATP-ADP reactions) through fermentation and respiration. Preliminary results gave an evidence of pressure reduction for all irradiated samples and of a non-linear cell response to irradiation doses [1]. Also metabolic oscillations were evidenced in yeast cell suspensions thorough the metabolic related CO_2 production, and it was shown that soft X-ray irradiation at given doses was changing the oscillation period [2]. This result is very important in biophysics and physics, the glycolytic cycle of yeast cells being a model system for non-linear science. Also at the technological level, it may allow the development of an easy non-invasive on-line technique for monitoring the metabolism in cells since oscillations of enzymatic cycle and its regulatory mechanisms have been studied with a physical probe, which is active on many cells and can give quantitative information in a continuous way.

The importance of the work is also related to the fact that yeast are a classical organism for investigating cell dynamics. They have indeed many fundamental characteristics of higher eukaryotic cells, whilst being relatively simple objects in the kingdom of living beings. Finally yeast is viewed as a micro-organism of major economic and social significance, of interest to biotechnological industry too. In fact, not only it provides fermentation for most breads and alcoholic beverages, but it is increasingly used to produce proteins and other molecules mainly, but not exclusively, for pharmaceutical use.

YEAST CELLS AND THEIR STRUCTURE

As already said, yeast cells were chosen as the biological target because they are eukaryotic cells, being anyway simple objects. Moreover the structure of yeast, characterised by a large and massive cell wall, contributed to stop most X-rays before the cell nucleus, contributing to the success of the idea of inducing differential damages [3, 4]. Finally dry yeast cells were used (commercial type produced by "Aboca") as an "easy to handle" type of sample.

In order to precisely characterise their morphological structure before irradiation (hence allowing a precise dosimetry), yeast cells have been analysed with different experimental techniques including: optical microscopy, TEM, X-ray microscopy and Coulter Counter. We wanted to measure the cell average radius r_O, the total membrane and wall thickness DS, and the nucleus radius r_N. Also we wanted to characterise the distribution of cell radii in order to assess the variation in absorbed X-ray dose as a function of individual cell size.

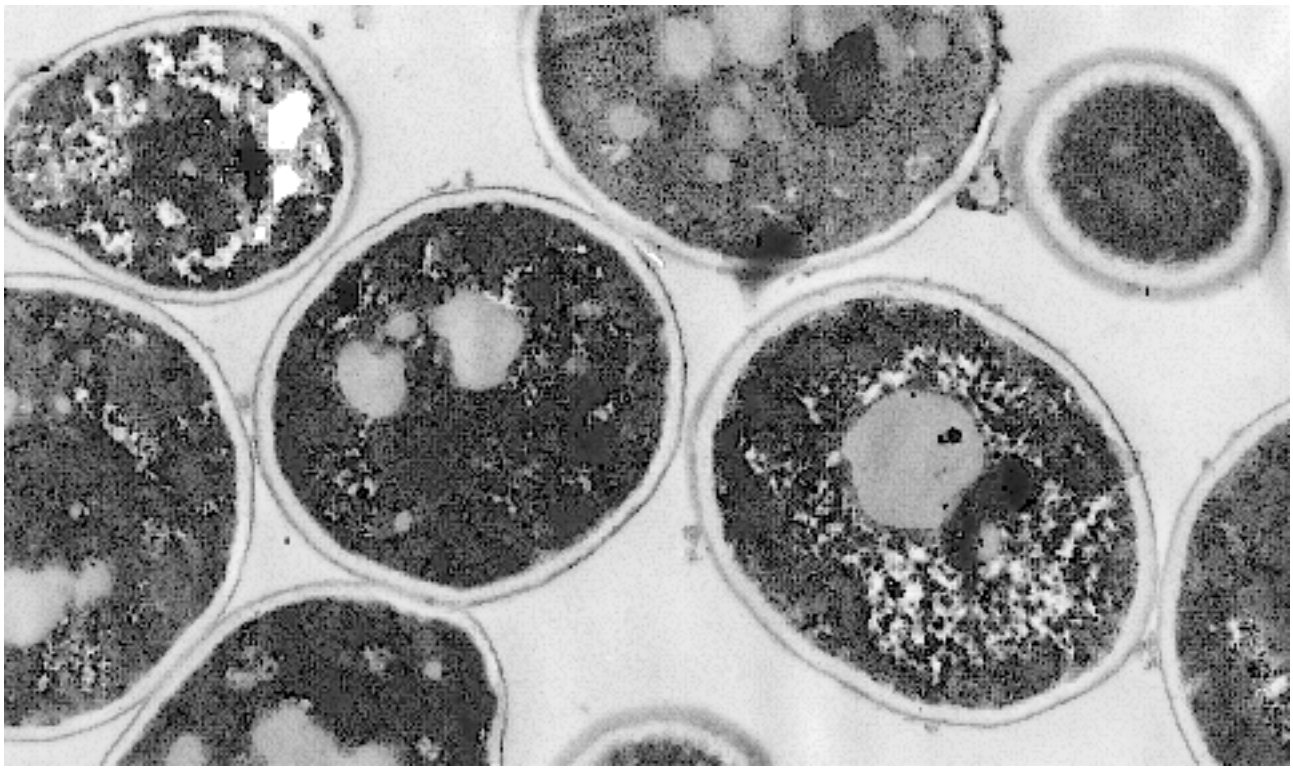

Fig. 1: Picture of yeast cells obtained with TEM at x 6000 magnification.

Fig. 1 shows a picture of yeast cell obtained with the Transmission Electron Microscope and a ×6000 magnification. It is clearly possible to see the cell nucleus and some vacuole and measure the membrane thickness (about 150 - 200 nm). The TEM advantages are its very high resolution (of the order of 10 Å in biological material) and the possibility of studying the internal structure. On the other side there are disadvantages connected to the long preparation required (including fixation and dehydration of samples). In particular very thin sections of the cells must be cut which may bring to DS to be overestimated, while r_O and r_N are underestimated.

By taking the arithmetic media of the results obtained with the different experimental techniques (including coulter counter and optical microscopy not presented here for sake of brevity) we got the following average values:

$$r_O = 2.58\ \mu m \qquad \Delta S = 0.18\ \mu m \qquad r_N = 0.95\ \mu m$$

Finally fig. 2 shows a distribution of cellular radii obtained with optical microscopy. Data are easily interpolated with a gaussian distribution with $r_O = 2.58\ \mu m$ and $\sigma = 0.54\ \mu m$ (such value has been obtained by averaging with the results of the other techniques too). Even if there is no physical background for such a choice, the use of a gaussian distribution proves to be an easy way to perform analytical calculations.

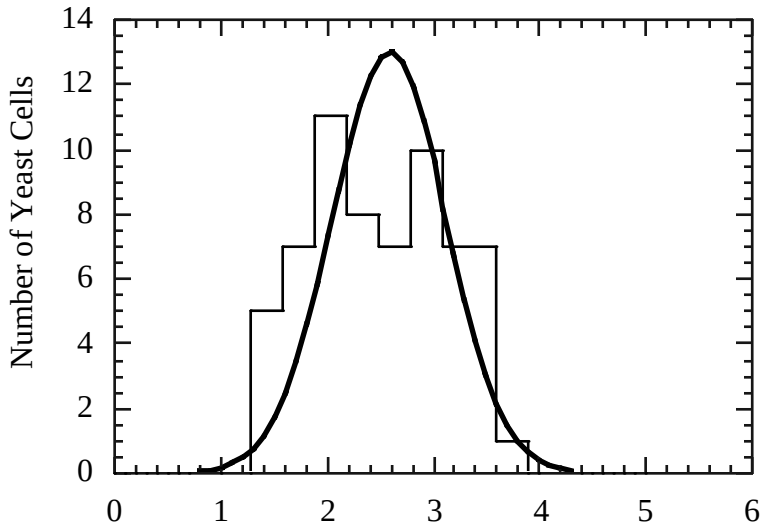

Fig. 2: Distribution of cellular radii as a function of cell radius (in µm) obtained with optical microscopy and interpolation with a gaussian function with $r_O = 2.58\ \mu m$ and $\sigma = 0.54\ \mu m$.

Typical metabolism of yeast cells includes respiration and fermentation, the first one being the production of energy by sugar degradation in presence of oxygen, the second one in anaerobic conditions. In both cases, CO_2 production is related to and energy production (ATP-ADP pool, glycolytic cycle) being one of the final products (the other one is ethanol) of a complicated series of enzymatic reactions in which the pivotal role is played by the enzyme PFK [5]. Hence measuring CO_2 production is a direct (and non-invasive!) way of following the cell metabolic activity.

EXPERIMENTAL SET-UP

The experimental technique is based on: i) the use of laser-plasma soft X-rays, and ii) the use of pressure sensors as a diagnostics of cell response which allows to monitor cell response over a large range of times from fast response (a few minutes after irradiation) up to several hours.

The pressure sensors and the biological target

Differential pressure sensors (Low Pressure Sensor type # AM5310 DV from Miteco, CH) of high sensitivity (from 0 to 100 mbar, giving 25 mV output end of scale, with accuracy of 0.1 mV) were used, linked to a computer input card. We calibrated the sensors showing that there is linearity in the region of interest (0-100 mbar) [6]. Before moving to the irradiation experiment, we also tested the sensors by measuring CO_2 production from cell suspensions fed with glucose.

The biological samples were commercial dry Saccharomyces Cerevisiae yeast cells are ones that are hydrated about one hour before irradiation. The use of dry yeast cells was important since they are an "easy to handle" type of samples. Suspensions with 2×10^7 cells / ml typical concentration were obtained by adding deionized water. Then they were filtered in a Venturi tube so that the cells deposited on a paper filter giving rise to a monolayer. After a short period for drying, the filters 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 (the Hostaphan film facing the X-ray source in order to filter unwanted X-rays, i.e. those in the water window emitted by Carbon ions) and the dose could be delivered.

Before starting the irradiation experiment we performed some intermediate investigations including the optimisation of the support of the biological target (trying different paper filters and different preparation procedures) and measuring CO_2 production not only in cell suspensions fed with glucose, but also verifying that there were no substantial change in CO_2 production after the paper filters with the cells were put again in the water-glucose solution.

The radiation source

The radiation source was obtained with a complex laser system. A Nd laser converted to 2ω was used to pump a dye oscillator followed by dye amplifiers, pumped by an excimer laser, and by frequency converter crystals. The pulse so obtained was transformed in a train of laser pulses (each 7 ps long) produced by spatial and temporal multiplexing, which underwent a final large amplification in KrF amplifiers (producing UV radiation at λ = 248 nm). Such laser radiation was focused onto the target with a f=9 cm focusing lens in order to produce an intensity at target of the order of $5\ 10^{15}$ W/cm^2. The overall system is very similar to that already described in [7].

An advantage of the radiation source was that it was also rather easy to irradiate the samples with harder X-rays or with UV light (by putting a mirror to reflect the laser beam directly onto the biological sample). Indeed we irradiated some samples with Cu X rays or with UV light in order to directly verify that radiation of different type were indeed producing a different response in biological cells. Such preliminary results are still being analysed.

Why at RAL?

A relevant element for the success of the experiment was the availability of a computer driven robot for sample exposure and of a dose control system. The exposure robot has been previously prepared at RAL in order to irradiate mammal cells with Cu L-shell X-rays [8].

The dose control system was based on a silicon PIN diode which measured the X-ray energy in each laser pulse. In order to exactly measure the same radiation which was exposing the biological samples, it was placed just beside them and filtered with exactly the same filters which were before the samples. A single laser shot was in general not enough to give the required dose, and since for many technical and physical reasons the X-ray flux changes substantially from shot to shot, successive PIN signals were integrated and summed until the required dose was delivered. The computer then automatically stopped the laser, also giving the number of laser shots and the histogram of the dose distribution.

PIN diodes also allowed a calibration of the source, provided the spectra (or at least the average frequency of photon emitted) was known, by some theoretical considerations or better by X-ray spectrometry. The result of such calibration was a conversion factor which was used to convert the integrated signal of the PIN diode (corresponding to a collected electrical charge measured in nC) to an absorbed dose value. Such conversion factor was dependent on photon energy because photons of different energies have a different penetration both in the PIN diode dead layer (the detector entrance window, giving a variation of PIN sensitivity with energy) and in biological material (hence giving different doses, measured in rad). When Teflon targets were used, the average energy of emitted photons was $h\nu = 0.9$ keV and such calibration factor turned out to be 3.58 rad/nC (we recall that 1 Gray = 100 rad).

Why soft X-rays?

As we have already said, very soft X-rays were used in order to damage specific points inside the cell structure (specifically cell wall and membrane). From experiments reported in literature we knew that X-rays with $h\nu = 1.3$ keV go through 5 µm of biological material and hence can deposit a large percentage of their energy into the cell nucleus. Indeed they were used for radiobiology and DNA recovery experiments [8]. On the other side, also X-Rays in the "water window " region ($h\nu = 300$-500 eV) should be avoided giving a low attenuation coefficient too, as shown in fig. 3. Hence we tried to choose a target material which could give emission at energies as close as possible to the end of water window (O-absorption edge). Also a K-shell spectrum, characterised by only a few lines, would have been preferable, making the dose calculations much easier. Unfortunately there are not many elements with this characteristics. Our choice has been Fluorine or rather Teflon (CF_2) which has been produced in thin (100 µm thick) stripes in our workshop with the height of normal tape for cassette recorders. Such tapes were then driven by a continuous electric motor.

The choice of stripe targets, as already described in many works, has been made since such targets drastically reduce debris emission which could damage the optics and/or the biological samples [9]. Unfortunately CF_2 tapes also contain carbon which exactly emits in the water window. In our experiment, such undesired K-shell C-emission has been removed by putting appropriate filters (in total 2 µm mylar plus 0.2 µm Al) before the biological samples. This also removed scattered UV light which is able of producing important biological effects. Also a buffer gas (He at 1 atmosphere) was present in the interaction chamber with the main role of further reducing debris effects, but also contributing to stop the much softer Carbon emission.

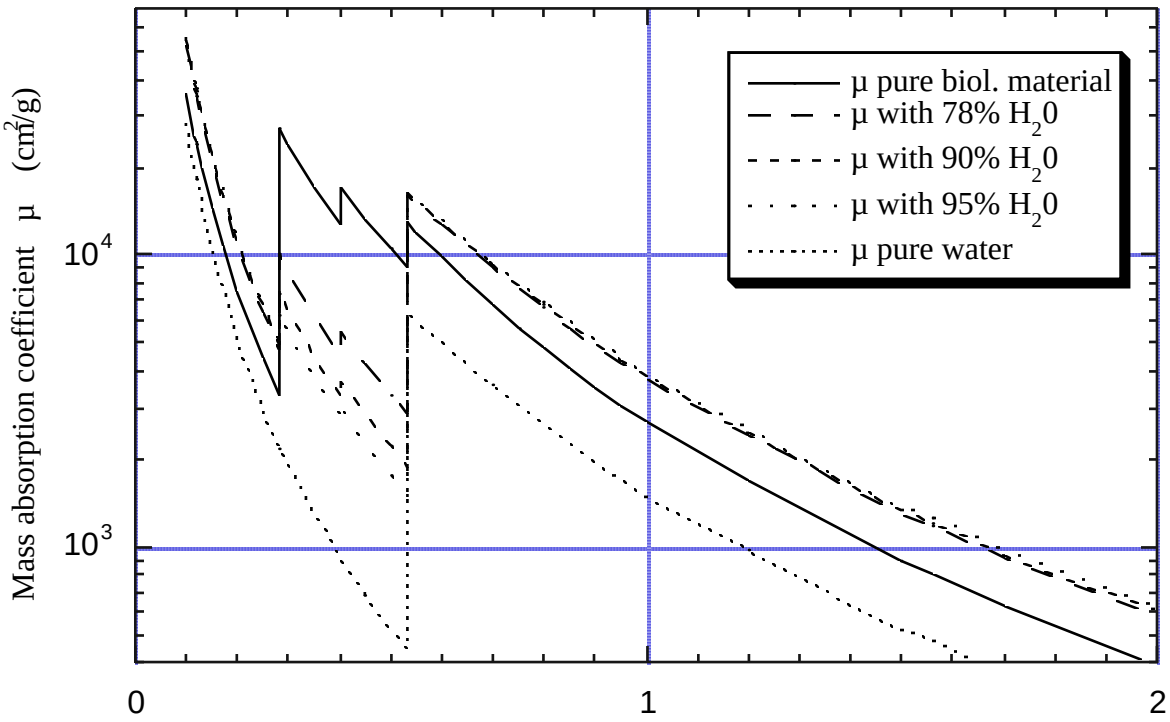

Fig. 3: Mass absorption coefficient of pure water, pure biological material and biological material with various percentages of water, vs. photon energy in keV.

SOFT X-RAY SPECTRUM

The actual X-ray spectrum is reported in fig. 4 The spectra were recorded with flat crystal minispectrometers (described in [10]) using RbAP crystals (2d = 26.121 Å) and kodak DEF film. A 2 μm mylar filter with a 0.2 μm Al layer was used as filters. While recording some spectra we also added a Cu layer on half the slit. Since Cu is characterised by the L-absorption edge at hν = 0.993 keV, this gave a wavelength fiducial on the film. Such wavelength fiducial was useful for spectra interpretation, even if we knew already that at our laser intensity and in the observed wavelength range, the Teflon spectrum was a rather easy to interpret K-shell spectrum (comprised between the fluorine He-α at 731 eV and the ionisation edge for the H-like ion at 1103 eV). Densitometries of the spectra were obtained with a Joyce Laoebl 3CS Microdensitometer at RAL which used calibrated wedges in order to obtain optical densities (OD) which were then deconvoluted to get absolute X-ray intensities. Such deconvolution took into account film sensitivity [11], filter and buffer gas transparency [12], and crystal reflectivity [13].

Fig. 5 shows a comparison between our experimental Fluorine spectrum and a theoretical spectrum which has been obtained using the code RATION developed at LLNL [14]. The theoretical spectrum has been obtained by using a temperature T_e = 140 eV and an electron density n_e =2 10^{22} cm^{-3}. Also we used a level of 30% carbon "impurities" (corresponding to the chemical composition CF_2) and a plasma size ≈ 10^{-3} cm (roughly the dimension of our focal spot).

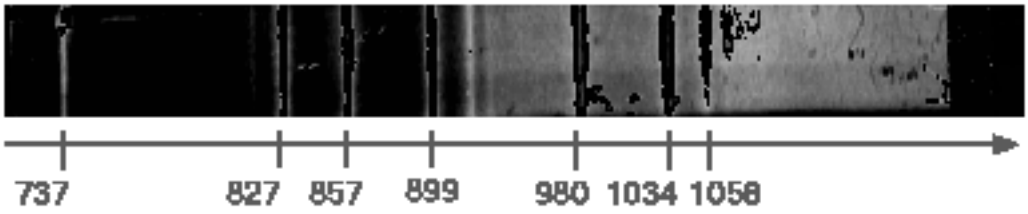

Fig. 4: Teflon spectrum recorded on kodak DEF film showing the He- and H-like line series (energies of main lines are indicated in eV).

It's worth noting that, as expected, the electron density is of the order of the critical density for KrF laser radiation (n_e =1.6 10^{22} cm^{-3}), and the temperature is practically the one which is experimentally measured through the bremsstrahlung slope.

Fig. 6 shows indeed the spectrum on a Log scale with the tail above the H-like ionisation edge well interpolated by an exponential function with temperature $T_e \approx 145$ eV. Despite such agreement, care should anyway be taken in considering the results of the simulations. Indeed while the experimental emission spectrum is the result of a plasma changing its temperature and density profiles continuously in time, the result obtained with RATION corresponds to a static and uniform plasma with the chosen plasma parameters. A full simulation of the experimental spectrum should imply the coupling to a hydrodynamic code which is anyway clearly beyond the scope of the present work. A more detailed theoretical analysis of our Teflon experimental spectrum has been performed by Magunov, Faenov et al. [15].

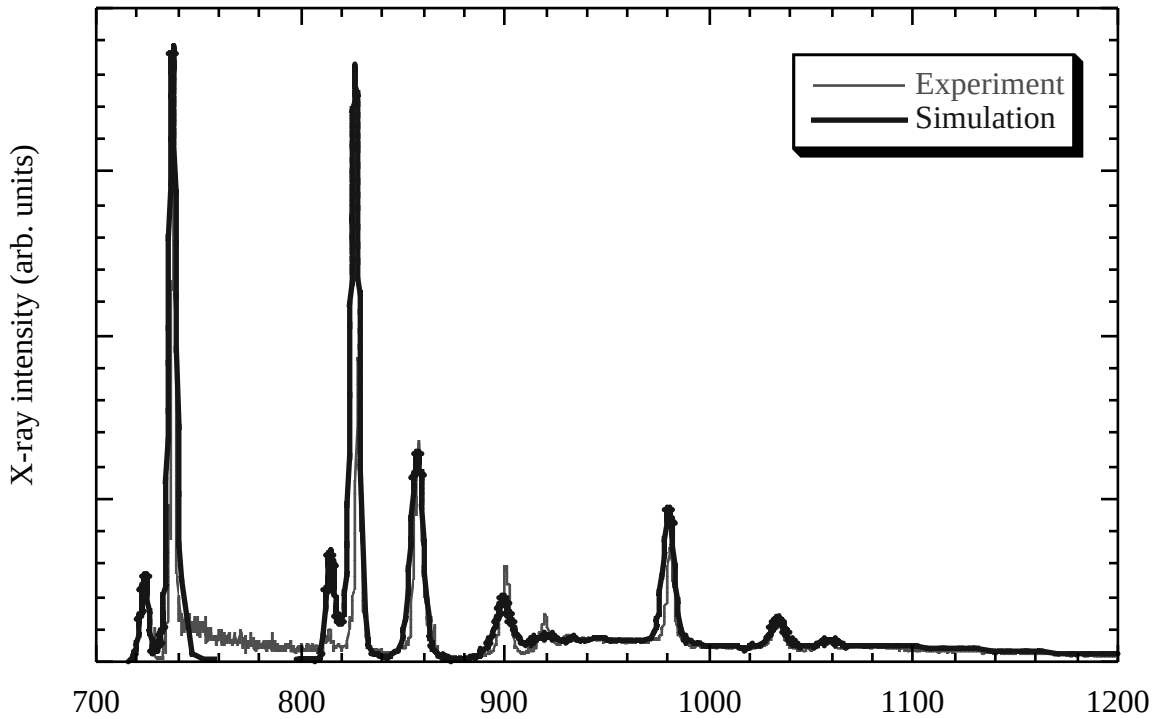

Fig. 5: Comparison between the experimental Fluorine spectrum and a simulation obtained with the code RATION (on x-asix: photon energy in eV).

Here the main objective of the simulations was to give an approximate characterisation of the source plasma and to deduce "average" plasma parameters which have then been used to "predict" the CF_2 spectrum due to carbon emission, which we could not record experimentally, in order to

verify that the filters used between the source and the sample were really enough to cut any radiation emitted in the water window region.

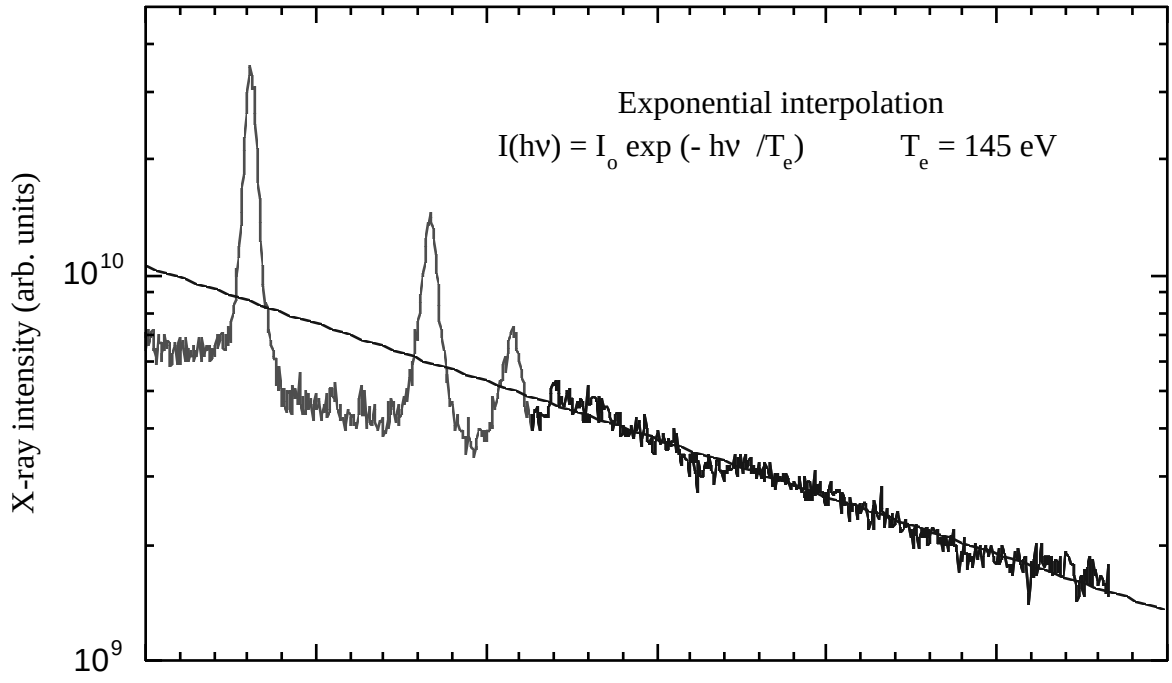

Fig. 6: High energy part of the experimental Teflon spectrum showing a bremstrahlung (exponential) tail corresponding to a temperature T_e = 145 eV (on x-axis: photon energy in eV).

DOSIMETRY

We calculated the integral doses absorbed by the nucleus, by the cytoplasm and by the membrane, as well as by an "average" undifferentiated cell, using Bouguet-Lambert-Beer law and the calculated absorption coefficients for water and for biological matter (already shown in fig. 3). Here we note that the case of biological matter with 78 % water corresponds to the cell nucleus, that with 95 % water to the cytoplasm, 90 % water to the average cell biological material. For the cell wall we have used the absorption coefficient for pure biological material since this is formed by tightly-packed protein [4]. Moreover the absorption coefficient of pure biological material has been calculated by assuming that it is made of 7 % hydrogen, 22.5 % oxygen, 52 % carbon, 16.5 % nitrogen, 1.5 % sulphur (as deduced from [22]) where the percentages are given in weight. Also the density of biological material has been calculated to be ρ = 1.6118 g/cm^3 (rather in agreement with what reported in [22]) and that of the average cell ρ = 1.06 g/cm^3.

Initially for sake of simplicity, in order to calculate the absorbed doses, we have used a "cubic cell" model as shown in fig. 7. Even if this seems quite far from the actual cell shape, it allows a simple parametric study of the influence of the various parameters (compartment sizes, cell composition, radiation wavelength) on the absorbed dose. Some results are shown in fig. 8 for two typical X-ray energy (approximately those of Teflon and Copper X-rays), two different radii and two values of the membrane thickness.

Even if this model is rather crude it allows to calculate separately the doses in each cell compartments (wall, cytoplasm, nucleus) and to draw a picture of where X-rays are absorbed and hence of where and what biological damages are likely to occur. This is a clear progress with

respect to the normal approach used in radiobiology (see for instance [22]) in which at most only the radiation penetration depth in the undifferentiated biological material and the average dose to the whole cell (or equivalently the surface dose) are calculated. We note anyway that in our case the penetration depth for undifferentiated biological material for $h\nu = 0.9$ keV (90 % water content or an absorption coefficient $\mu \approx 5000$ cm^2/g) is $(\mu\ r)^{-1} = 1.9$ µm.

In order to remove the hypothesis of the cubic cell, we have written a computer program which calculates the absorbed doses in the case of a spherical cell (also divided in three compartments). This case cannot be treated analytically because the integrated functions are exponential functions with different exponents in the different cell compartments and because the symmetry of the problem is cylindrical and hence involves two different variables (for instance it is useful to choose z, or the direction of propagation of X-rays, and r, the distance from the central ray, while the variable j does not compare and the distance from the centre R, from which µ and r are dependent, can be written as a function of r and z). The results obtained with the computer program (see fig. 9) show that the doses calculated with the simple cubic cell model are not too far from reality.

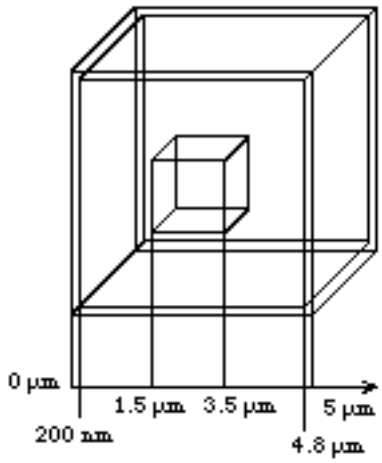

Fig. 7: "Cubic" model for dosimetry of yeast cells.

In order to take into account the variation of absorbed doses due to the variation in biological samples (different sizes) we have also calculated the absorbed doses in cell with a 2 µm radius (since the average radius is 2.58 µm and the distribution width is s Å 0.5 µm, such cells are easily found in the actual cell suspension). Fig. 9 shows how in this case the dose absorbed by the nucleus becomes much bigger. Also for normal size cells, fig. 8 shows already that even if most radiation is absorbed in the cell wall and in the cytoplasm, there is still a non negligible absorption in the cell nucleus. In reality the situation is even worse because the emitted spectrum has several lines characterised by different absorption coefficients. Hence the lower energy ones are mainly absorbed in the cell wall, while the harder ones are characterised by a lower absorption coefficient and hence by a larger penetration in biological material. This means that the higher energy lines can travel through the cells with much lower attenuation and hence, on one side, the X-ray spectrum becomes harder and harder while radiation travels through the cells and, on the other side, the higher energy lines are more likely to deposit their energy in the cell nucleus. The hardening effect is shown in fig. 10 (this may somewhat overestimate the effect since is drawn in a Log scale, but anyway show how the problem is real).

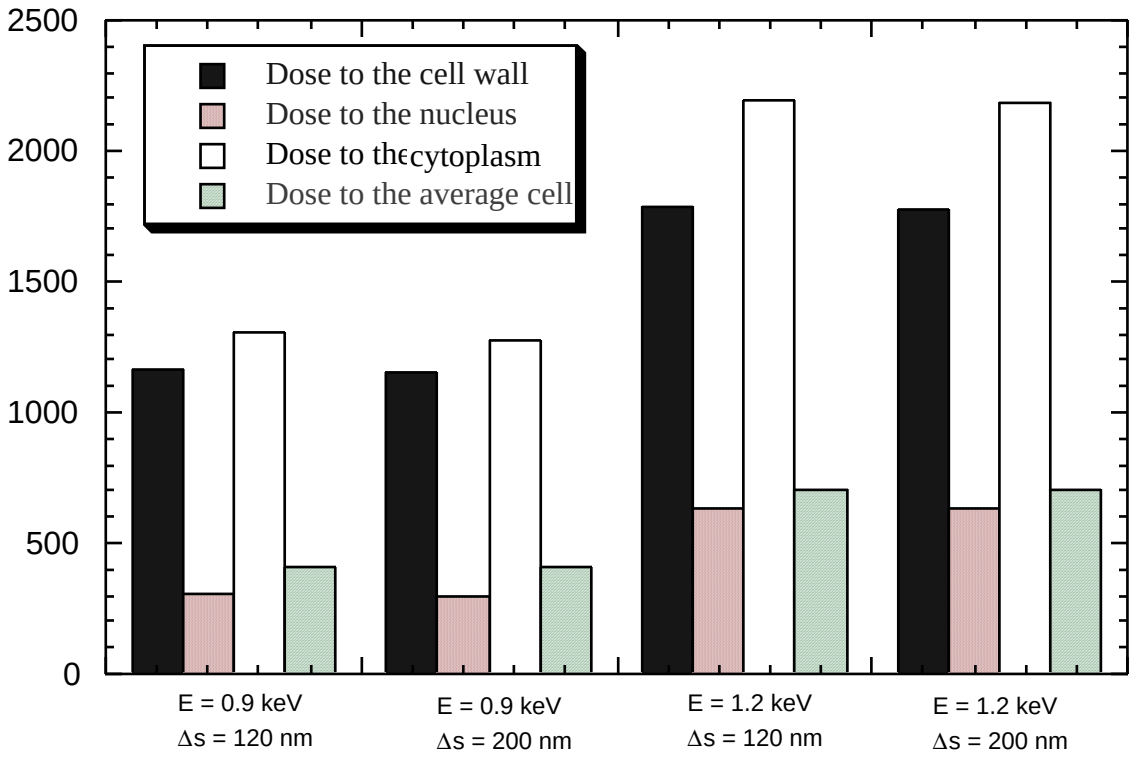

Fig. 8: Result of the "cubic" model for dosimetry of yeast cells. Doses are in Gray (J/Kg), and calculated assuming a X-ray FLUX equal to 1 mJ/cm^2.

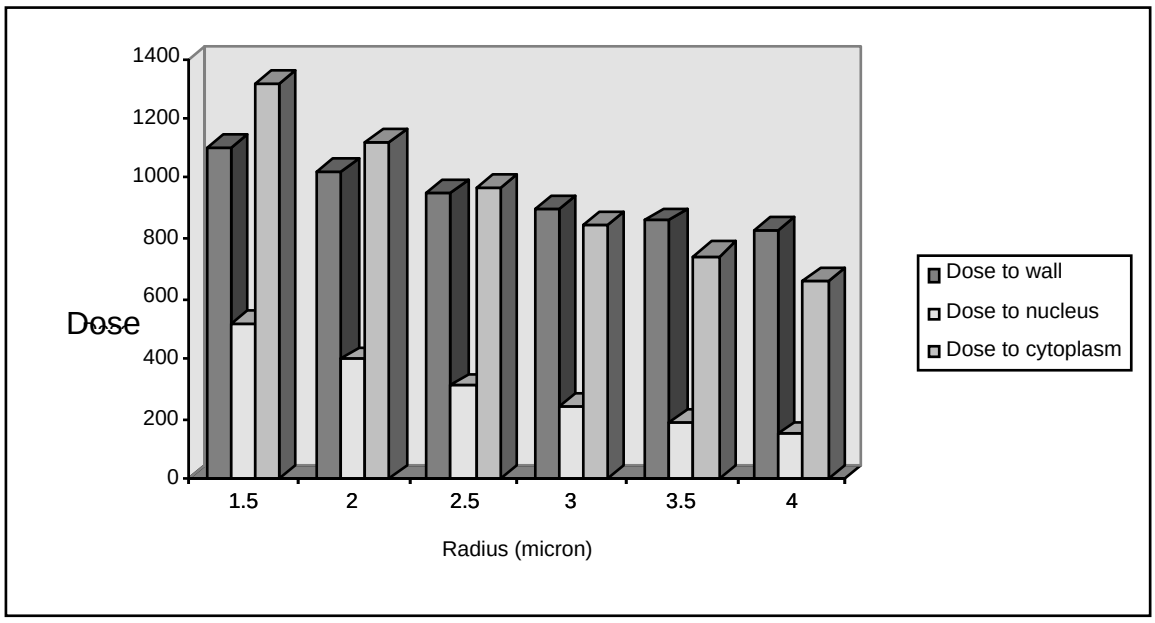

Fig. 9: Result of the "spherical" model for dosimetry of yeast cells as a function of cell radius (between 1.5 and 4 µm) calculated for 0.9 keV X-ray radiation and assuming a X-ray equal to 1 mJ/cm^2 before the filters and 0.23 mJ/cm^2 after the filters.

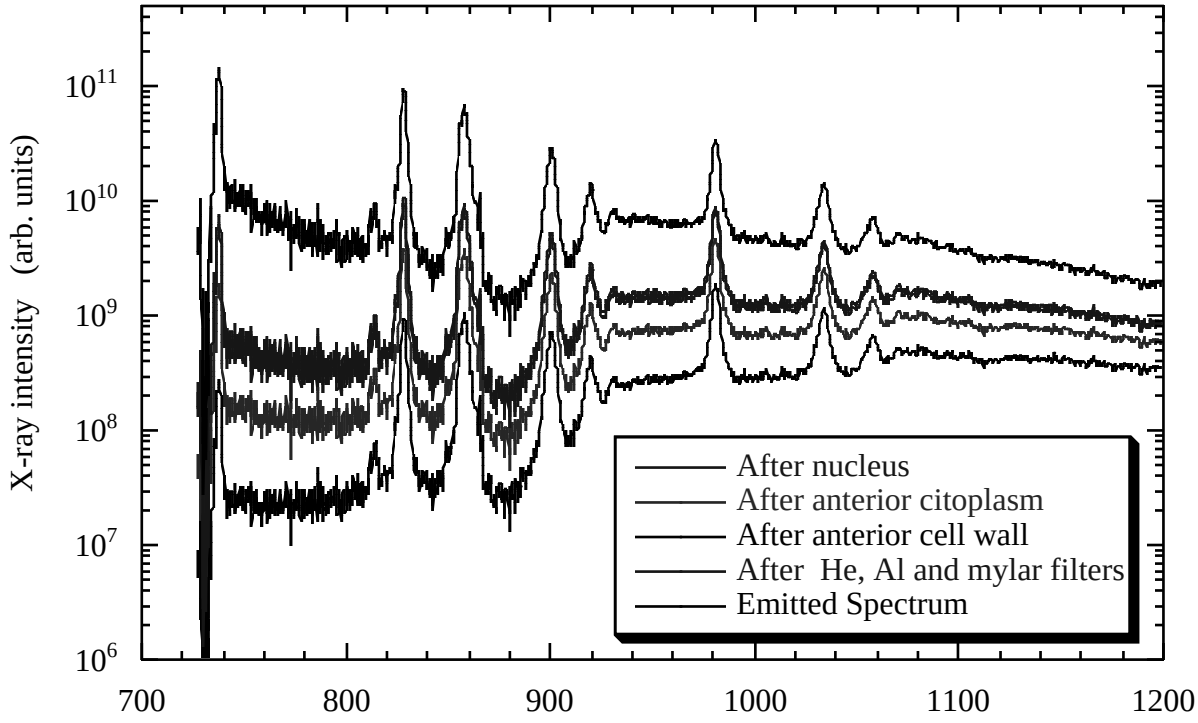

Fig. 10: Hardening effect on X-ray spectrum.

In order to solve the problems due to radiation hardening and to cell size variations, in the last experimental runs we have introduced a Cu filter before the cell samples (and before the nearby PIN diode which measures the dose). As we already said, Cu is characterised by an absorption L-edge at hν = 0.993 keV and hence may effectively remove most of the harder X-ray which are present in the CF_2 spectrum hence bringing to irradiation conditions which are much cleaner and much closer to what we really want for this kind of experiment.. In the following experiments we also plan to add some Neon to the buffer gas contained in the interaction chamber. This will improve the irradiation condition even more, being characterised by an absorption K-edge at 870 eV.

EXPERIMENTAL RESULTS

The experimental results we show here regard CO_2 production from biological samples as a function of time and irradiation dose. Before analysing such data, we took care of verifying that for control samples (made of a pure solution of water and glucose) the differential pressure sensor measurements perfectly reflected the atmospheric pressure behaviour. Also, in order to be sure that the pressure increase was due to CO_2 production, we used mass spectrometry. Fig. 11 shows that the gas produced during fermentation is CO_2 (mass number 44) that was not in present in the initial blank run.

We now present some very preliminary results obtained with irradiated (and non-irradiated) cells. The graphs in fig. 12 show how pressure in the bottle is naturally increasing with time as a results of cell metabolism. It is also evident a pressure reduction for all irradiated samples

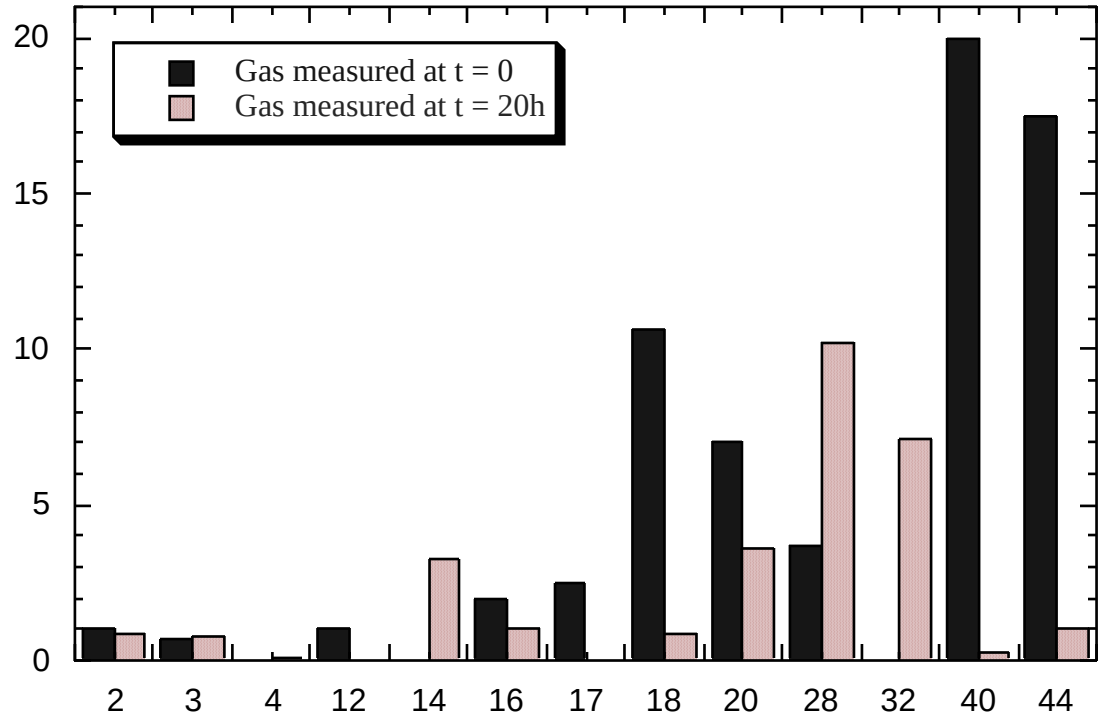

Fig. 11: Mass spectroscopy of produced gas (as a function of molecular number).

Oscillations were evidenced in both irradiated and normal samples. For instance, the graphs in fig. 12 clearly show, especially if analysed in close detail, a typical oscillatory pattern in carbon dioxide measures (the recorded output voltage from the pressure sensor in a time interval of 3000 sec). The considered sample was 4 ml of a solution of 2 g/ml of aD-glucose and 200 mg/ml of dry yeast cells (non irradiated).The precision of the measurements is limited by the sensitivity of the pressure sensor to 0.1 mV. The data acquisition set-up has a resolution of 0.0015 mV. In the experimental set-up, the connections between the sensor and the data acquisition board have caused a saturation in the input stage of the amplifier of the board which is visible in the "cuts" of the top sinuses of the oscillations. Even more fig. 12 and 13 show a non-linear response to irradiation doses.

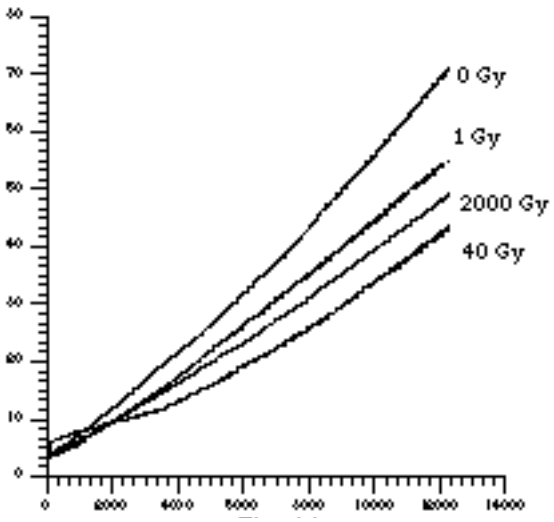

Fig. 12: $C0_2$ production as a function of time (form 0 to 14000 seconds) for various irradiation doses and for 4 ml of a solution of 2 g/ml of aD-glucose and 200 mg/ml of dry yeast cells.

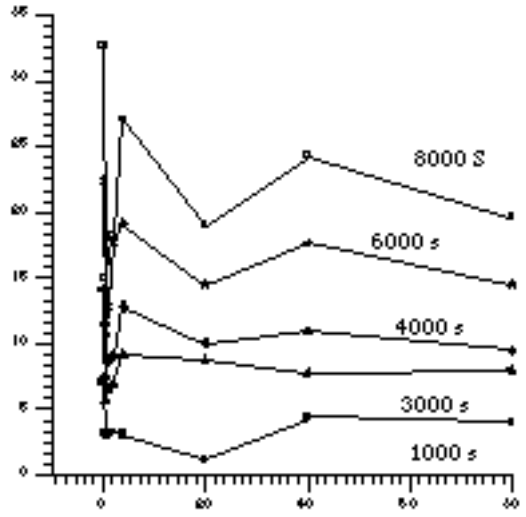

Fig. 13: $C0_2$ production as a function of dose (from 0 to 80 Grey) at various times and for 4 ml of a solution of 2 g/ml of αD-glucose and 200 mg/ml of dry yeast cells.

The data have been treated with different analysis techniques in order to study the oscillations in detail. The spectral analysis was performed estimating the Fourier transform of a given pressure measure as outcoming from the data acquisition board; the data were submitted to a piecewise linear detrending before undergoing the transform. The signal after the detrending procedure showed oscillations in the range ± 0.5 mV, i.e. greater than the precision of the pressure sensor ±0.05 mV.

Also, in order to obtain smoother spectra, during Fourier transforming each signal has been convoluted with a suitable regular function which contributes to smooth the resulting transform. The dependence from the function with whom the signal is convoluted before transforming it is visible as been evaluated as a function of the overlapping between the function and the signal and a suitable overlapping has been chosen in order to avoid the presence of a great deal of noise while at the same time avoiding information losses. The analysis technique is described in [16-18].

Fig. 14 shows the result of the spectral analysis: it's clearly visible the presence of a principal frequency component corresponding to a period of 35.7 ± 2.5 sec.

The most important point is that, since such oscillations are not found in the control water and their amplitude is larger than the sensor sensitivity, hence we can conclude that they are a real oscillatory phenomenon connected to cell metabolism. Also the evidence of the oscillatory behaviour is confirmed by the analysis of yeast cells suspensions fed by Λ-glucose. Having the wrong chirality, Λ-glucose is not absorbed by yeast cells and not used for metabolism. Fig. 14 also shows the spectra obtained from a sample of 4 ml of water only, and that obtained from 4 ml of solution with 2 g/ml of Λ-glucose and 200 mg/ml of yeast.

Finally we have repeated this kind of measurement with irradiated samples. Fig. 15 shows the frequency shifting and mixing as a consequence of irradiation. The samples were 4 ml of a solution of 2 g/ml of α-D glucose and 200 mg/ml of yeast cells. The irradiated sample was exposed with 1000 nC. The main frequency peak is shifted towards lower frequencies (giving now a period of 52.6 ± 5.5 sec) and the rising of other frequency components is clearly visible.

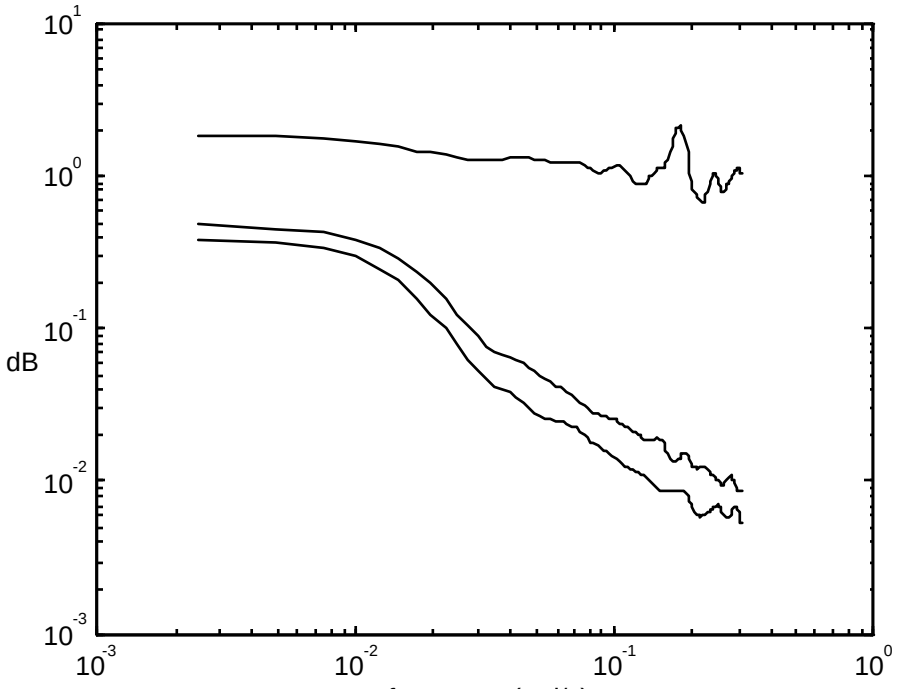

Fig. 14: Frequency spectra as a function of frequency (from 10^{-3} to 1 rad/s) obtained from a suspension of yeast cells fed with glucose (upper curve, sample was identical to those in fig. 12 and 13), from 4 ml of solution with 2 g/ml of Λ-glucose and 200 mg/ml of yeast (medium curve) and from a sample of 4 ml of water only (bottom curve). The upper curve clearly shows the presence of a principal frequency component corresponding to a period of 35.7 ± 2.5 sec.

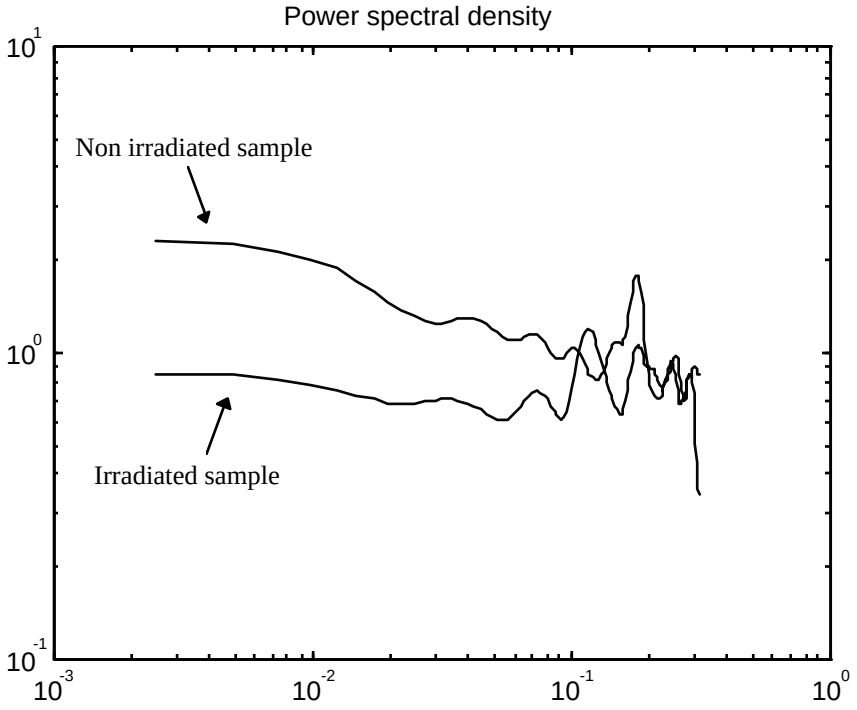

Fig. 15: Comparison between power spectra for identical samples of irradiated and non-irradiated cells (identical to fig. 14, upper curve) as a function of frequency (from 10^{-3} to 1 rad/s).

DISCUSSION

Yeast cells are an ideal laboratory for the analysis of the glycolytic cycle and consequently of dissipative structures, whose principal characteristic is the presence of different cyclic interconnected transformations where the PFK enzyme assumes a pivotal role [19-21]. The basic properties of the oscillations correlated to glycolysis are the stability of the period and phase resetting by an external stimulus. These enable the organism to adapt in a flexible way to external conditions and to keep, through suitable phase relations, an identical succession of various vital processes.

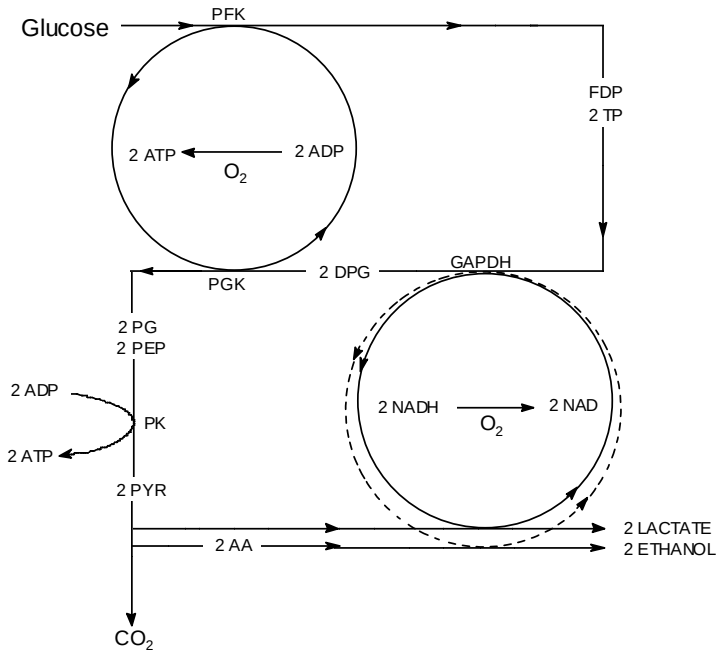

Fig. 16: The cycle for glucose degradation and $C0_2$, ethanol and energy production in yeast cells.

Oscillations in yeast cells appear to be a relevant tool for the investigation of control and feedback mechanisms at work in living organisms. Actually these cells have been studied for their enzymatic dynamics, for their genetic interest, and finally for lightning the mechanisms responsible for enzyme activity cooperation, allostericity (i.e. biological feedback), order parameters appearance and ruling, energetic charge conservation.

Glycolysis and, particularly in yeast cells, fermentation are the principal sources of energy for the organisms to develop, duplicate or only be alive. CO_2 production, being at the end of the metabolic cycle (see fig. 16) gives important indications on the cell good state, need of nourishment or afford to recovery after irradiation. Monitoring on line the variations of gas production it is possible to obtain a direct indication on the metabolism of different sugars, and on the cell recovery immediately after the induced radiation damage. Furthermore, an immediate response allows to study the modification to wall and cytoplasm, where glycolysis and fermentation take place, without taking into account duplication and possible DNA breaks or mutations.

Another great advantage of having an on-line response is the highlighting of the dynamics that play a role at various doses. While long term analysis usually provide a monotone response to increasing energy release, monitoring cell energy needs during the first phase of recovery may give indications on the mechanisms that are primed with a threshold and are more efficient at higher doses rather than at lower ones. It is fundamental the fact that in this kind of analysis we obtain an almost individual response: the irradiation consequences are directly evaluated on cells and not on daughter-cells, after many biological processes have already take place.

CONCLUSIONS

Several results of this experiment need to be pointed out, namely:

i) the development of an investigation technique based on the use of very soft X-ray in order to damage specific structures inside the cell structure (specifically cell wall and membrane);

ii) the use of pressure sensors as a diagnostics of cell response which allows to monitor cell response over a large range of times from fast response up to several hours;

iii) the use of dry yeast cells as an "easy to handle" type of sample;

iv) the development of a simple model for X-ray dosimetry of the different cell compartments.

v) the study of metabolic oscillation in yeast cell suspension and the observation of the oscillation frequency shift following an exposure to soft X-rays.

All these points prove that the availability of high brightness soft X-ray sources, like laser-plasma sources, makes it possible to think of new experiment and to realise new applications. Such experiment could not have been performed with conventional, low intensity, soft X-ray sources which require long exposure times. In this cases indeed cell reaction to the damaging factor would have started before the complete dose deposition, hence changing the biophysics of the process.

Further research is now progressing in order to get a more reliable data statistics and improve the comprehension of the biophysical phenomena involved.

With respect to point (ii), which again is possible because of the acquisition technique we used, but also because the high brightness of the source allow the required dose to be "instantaneously" deposited, we want to stress again that it may represent a powerful tool to investigate cell metabolism and the response to X-rays. Instead in cell radiobiology the usual approach ([22] for instance)is based on the study of survival curves, i.e. as the capability of individual cells of developing colonies after damage induced by X-rays, i.e. is based on a response at very long times after irradiation, thereby losing all the information on fast cell response to damages and also all information about cell damages which are not likely to be transmitted to the genetic descendants.

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