MANGALORE UNIVERSITY

STUDY OF DOSE RATE EFFECTS ON THE INDUCTION OF BIOLOGICAL DAMAGES USING ELECTRON BEAM ACCELERATOR

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FINAL REPORT

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1. Study of Gamma Radiation Induced Damages and Variation of Oxygen Enhancement Ratio with Radiation Dose using *Saccharomyces cerevisiae*, *Journal of Radioanalytical and Nuclear Chemistry*, Vol. 302, 1027-1033, 2014.

2. Evaluation of Micronuclei Induced by Energetic Electrons Delivered at Different Dose Rates Per Pulse, *Journal of Radioanalytical and Nuclear Chemistry*, Vol. 302, 993-997, 2014.

In Symposia / Seminars

3. Study of Variation of Oxygen Enhancement Ratio with Radiation dose using *Saccharomyces cerevisiae*, International Conference on Application of RadiotraCers and Energetic Beams in Sciences (ARCEBS-14), Saha Institute of Nuclear Physics, Kolkata, Jan. 12-18, 2014.

4. Protective effect of caffine against radiation induced cytotoxicity and genotoxicity in yeast strains. International conference on Radiation Biology-Frontiers in Radiobiology : Immunomodulation, Countermeasures & Therapeutics (ICRB-2014), INMAS, Delhi, November 11-13, 2014.

Preface

The biological effects of radiation observed in a complex multi-cellular organism are the result of the effect at molecular and cellular level. Radiation absorption may lead to cell killing, modification in sub cellular levels without losing viability or different cellular abnormalities like mutations. In case of cell killing, the cells lose their ability to divide whereas in case of cell modification, cells retain their viability but their characteristics will be modified which would cause hereditary alterations.

Many sensitive, accurate methods and techniques have been developed to quantify the radiation induced biological damages and explored them to analyse the damages, which are manifested at different levels in organisms. The levels of damages can be at organism level, organs or tissues level, cellular level and at molecular level. These levels of damages were analysed quantitatively and qualitatively by studying different endpoints such as induction of cell lethality, genetic damages and cytogenetic damages. Radiobiological investigations suggest that the radiation induced damages are greatly influenced by physical, chemical and biological factors. The cell type, repair process, dose rate, and the presence of modifying agents and the endpoint studied are few important factors.

At present, use of electron beam is constantly gaining importance in therapy applications. Since its physical absorption properties offers many advantages over conventional ^{60}Co teletherapy, such as better dose profile and drastic reduction in dose to the normal tissues beyond the tumor. The clinical experiences with different type of radiations and with new type of treatment procedures suggest that the use of accelerated charged particles might be optimal for the treatment of tumors.

The present investigation is aimed to compile and analyze the radiobiological data associated with 8 MeV electrons on yeast strains. The present work involving systematic dosimetry study and assessment of biological damages induced by 8 MeV electrons at DNA level using different doserates.

STUDY OF DOSE RATE EFFECTS ON THE INDUCTION OF BIOLOGICAL DAMAGES USING ELECTRON BEAM ACCELERATOR

1. Introduction

In physics, radiation describes the process in which energetic particles or waves travel through a medium or space. Presently, radiation is used as an important tool in research, medical diagnosis, radiotherapy, materials processing, non-destructive testing, food preservation, and so on. Radiation exposure to people handling radiation sources in these activities, will be high in case of any mishaps. Therefore, a detailed understanding of the effects of elevated levels of radiation on human population is essential.

Radiation can be classified according to the effects it produces on matter, into ionizing and non-ionizing radiation. Ionizing radiation includes cosmic rays, X-rays, and radiation from radioactive materials. Non-ionizing radiation includes ultraviolet light, radiant heat, and microwaves. The word radiation is commonly used in reference to ionizing radiation only, but it may also refer to non-ionizing radiation such as radio waves or visible light. Both ionizing and non-ionizing radiation can be harmful to organisms and the natural environment. However, the harmful effects of these radiations was not known for many years. This led to unusual applications of radium, and a number of people got exposed until the harmful effects began to appear.

The biological effects originates from two basic types of interaction namely, the direct and indirect interaction. In Direct action, radiation interacts directly with critical target within the cell and produces excitation or ionization and in indirect action radiation interacts with other molecules, mainly with water molecules within the cell and produces chemically active free radicals and other chemically reactive species that eventually produce the biological damage. At the molecular level, the most important effects are Single Strand Breaks (SSBs), Double Strand Breaks (DSBs), Base damages, DNA-protein, and DNA - DNA cross links. These effects lead to lethality, mitotic inhibition, division delay, Chromosomal Aberrations (CAs), and induction of mutations. In multi-cellular organisms, these changes reflect as radiation

sickness or delayed somatic effects, which could result in the death of the organism. Mutations that occur in germ cells could result in hereditary alterations; hence, a qualitative as well as quantitative knowledge of the action of several modifiers of radiation damage is an obligatory prerequisite for the evaluation of radiation risk, and also, for the improvement of radiotherapy.

At present, the use of electron beam is constantly gaining importance in therapeutic applications. It offers many advantages over conventional ^{60}Co teletherapy, such as better dose profile and drastic reduction in dose to the normal tissues beyond the tumor. Electron beam with 12 MeV energy will deliver 80% of the dose at a depth of 4 cm, hence studies in the energy range of about 5-20 MeV have relevance in radiation therapy application.

2. Aims and objectives:

- To study the survival fraction of yeast cells of different radiation sensitivity using electron radiation.
- To study the dose rate effect of electron radiation on yeast cells of different radiation sensitivity under euoxic and hypoxic condition.
- Gene mutation analysis in yeast cells under euoxic and hypoxic conditions for different doses and dose rates.

3. Materials and Methods

3.1 Dosimetry

In the present study, four chemical dosimeters such as Fricke, FBX, alanine and glutamine were used to calibrate ^{60}Co-gamma chamber-900 and 8 MeV plused electron beam Accelerator. These dosimeters were also used to measure dose distribution and dose details in radiobiological studies. For Proton beam two solid state detector were used to calibreate Folded tendom ion accelerator.

3.1.1 Fricke Dosimeter

Fricke dosimeter provides a reliable means for measurement of absorbed radiation dose based on the process of oxidation of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}) in acidic aqueous solution. The amount of ferric ion produced in the solution is measured by absorption spectrophotometry at a wavelength of 304 nm; the wavelength strongly absorbed by the ferric ions. Fricke solution shows two absorption peaks at 224 nm and 304 nm wavelengths. Since absorption peak at 224 nm is due to a mixture of both ferric as well as ferrous ions, peak at 304 nm which is purely due to the concentration of ferric ions, was considered for measurements. Fricke dosimetry depends on an accurate knowledge of the radiation chemical yield (G value) of ferric ions, measured in moles produced per 1 joule of energy absorbed in the solution. The G value is defined as the number of ferric molecules produced in the ferrous sulphate solution by 100 eV of absorbed radiation energy. An accurate value of the chemical yield is difficult to ascertain because the chemical yield is affected to a certain degree by the energy of the radiation, dose rate and temperature of the solution during irradiation and readout. The best G value for ^{60}Co gamma rays is 15.6 molecules per 100 eV, corresponding to a chemical yield of 1.607×10^{-6} mol J^{-1}. The typical dynamic dose range for Fricke dosimeter is between 40 Gy and 400 Gy.

Preparation of Dosimetric Solution

To prepare 1000 cm^3 of Fricke dosimetric solution, 22 cm^3 of concentrated sulphuric acid (0.1 N H_2SO_4) was transferred to a clean 1000 cm^3 standard flask containing about 750 cm^3 of double distilled water (ddw). The solution was allowed to cool to room temperature. Then 392.14 mg of ferrous ammonium sulphate [1 mM $FeSO_4$ $(NH_4)_2$ $(6H_2O)$] and 58.44 mg of sodium chloride salt (1 mM NaCl) was transferred to the solution and dissolved in it. Sodium chloride was used to reduce any adverse effects on the response of the dosimeter due to traces of organic impurities. The volume was made up to 1000 cm^3 by adding ddw. The solution was aerated and stabilized by allowing in room temperature for about an hr.

Preparation of Irradiation Vials

Set of pre-cleaned polypropylene vials were filled with dosimetric solution after rinsing at least three times with the same. Vials with no air bubbles were taken and labeled for irradiation. Minimum two vials were kept as reference samples. During irradiation with gamma rays, vials were inserted in a 5 mm thick perspex build up cap to achieve electronic equilibrium conditions. For 8 MeV electrons, cylindrical polypropylene vials of 1 cm diameter were used. Similar irradiation geometry was maintained for both dosimetry and radiobiological irradiation experiments.

Irradiation and Measurement Procedures

The dosimeters were exposed to graded doses between 50 Gy to a maximum of 400 Gy at room temperature. Irradiated vials were kept at room temperature for minimum 30 min to complete the radiation induced chemical reaction. Optical absorbance measurements of the dosimeter were done at 304 nm wavelength using a UV-Visible spectrophotometer (SECOMOM). A quartz cuvette of 1 cm path length pre-cleaned first with sulphuric acid and then with ddw was used for spectrophotometric measurement of the Fricke solution. Before the absorbance measurement of the irradiated Fricke solution, the cuvette was rinsed and filled with un-irradiated (control) Fricke solution to appropriate level and placed in sample position of spectrophotometer and measured zero absorbance (100% transmission) reading. After setting the spectrophotometer to zero absorbance, optical density (absorbance) of the irradiated dosimetric solution was measured starting from low dose to high doses.

Calculation of Absorbed Dose

Absorbed dose corresponding to changes in the optical density of the Fricke solution, when measured at 25°C, was calculated using the relation;

$$\text{Absorbed Dose (Gy)} = \frac{1.04 \text{ x } \Delta OD}{G\ \rho\ \varepsilon_t L}$$

where, ΔOD is the difference in absorbance between irradiated and un-irradiated Fricke solution. G is equal to 1.607×10^{-6} mol J^{-1} for ^{60}Co γ- rays. ε_t is the molar extinction coefficient, which is equal to 219 m^2 mol^{-1} at 25 °C (ICRU 1984). ρ is the density of dosimetric solution which is equal to 1024 kg m^{-3}. L is the optical path length of quartz cuvette used for spectrophotometric measurement (0.01 m). Molar extinction coefficient increases with rise in temperature at a rate of 0.7 % per °C. When absorbance measurement is carried out at any other temperature 'T', a correction of 0.7 % per °C has to be applied to obtain the value of the molar extinction coefficient, ε_T at temperature T. It implies that ε_t should by replaced by $\varepsilon\ [1 + 0.007(T-25)]$. Therefore, the absorbed dose corresponding to optical density of the Fricke solution, when measured at other than 25 °C, was calculated using relation;

$$\text{Absorbed Dose (Gy)} = \frac{1.04 \text{ x } \Delta OD}{G\ \rho\ \varepsilon\ [1 + 0.007\ (T - 25)]\ L}$$

where, T is the temperature of the solution during the measurement of optical absorbance. A graph of optical absorbance *vs* time of exposure was plotted to find the slope of the graph, which gives the average optical density (ΔOD) per time. Then, the dose rate of the gamma irradiator was calculated using the relation;

$$\text{Dose Rate}\,(\text{Gy min}^{-1}) = \frac{1.04 \text{ x slope}}{G\ \rho\ \varepsilon\ [1 + 0.007\ (T - 25)]\ L}$$

3.1.2 FBX Dosimeter

It is a low dose level, sensitive and accurate dosimeter containing Ferrous ammonium sulphate, Benzoic acid and Xylenol Orange (XO) in acidic aerated aqueous solution. The limitation of the Fricke dosimeter for the measurement of low doses has been overcome by the development of FBX dosimeter (Gupta 1973) and the dosimeter has been studied extensively (Bhat *et al.,* 1996). The FBX dosimeter is capable of measuring doses in the range of 0.1 to 30 Gy. In FBX dosimeter, the ferrous ions get oxidized to ferric ions by absorbing energy. The benzoic acid acts as a catalyst to increase the radiolytic oxidization of ferrous ions through a chain reaction and the chain reaction is controlled by XO, making the system accurate and reproducible. In addition, XO forms a complex with ferric ions and this ferric-XO complex has a high molar linear absorption coefficient at 540 nm wavelength. This complex is measured by using spectrophotometer. FBX dosimeter is a secondary standard dosimeter which requires calibration against a reference standard dosimeter or a standard irradiator. In the present investigation, this dosimeter was mainly used for calibrating the Microtron and Gamma Chamber-900 in the low dose ranges.

Preparation of Dosimetric Solution

To prepare 1000 cm^3 of FBX solution, 610 mg (5 mol m^{-3}) of benzoic acid was weighed and transferred to a clean 1000 cm^3 standard flask, containing about 250 cm^3 of ddw. The flask was heated to about 70 °C to dissolve the benzoic acid. The solution was allowed to cool to the room temperature and then 16 cm^3 (5 N H_2SO_4) of sulphuric acid was transferred to a flask containing benzoic acid. Then 78.4 mg (0.2 mol m^{-3}) of ferrous ammonium sulphate was added and dissolved by mixing. Finally, 153.4 mg (0.2 mol m^{-3}) of tetra sodium salt of XO was added to the flask. Since XO is sparingly soluble in acidic solution, it was weighed in a small beaker and then transferred into the flask by washing the beaker repeatedly. The volume was made up to 1000 cm^3 by adding ddw and aerated and stabilized by allowing in room temperature for about an hr.

Preparation of Irradiation Vials

Dosimetric solutions were filled in a pre-cleaned polypropylene vials without air bubbles. Two vials were kept as reference samples and the remaining vials were used for irradiation. During gamma irradiation, vials were exposed using sample holder to get uniform dose distribution. For 8 MeV electrons, cylindrical polypropylene vials of 1 cm diameter were used.

Irradiation and Measurement Procedures

The dosimetric solutions were exposed to graded doses, from 0.1 to a maximum of 40 Gy, at room temperature. Irradiated vials were kept at room temperature for minimum 30 min to complete the chemical reactions. An optical absorbance measurement of the irradiated dosimetric solution was done at 548 nm wavelength using a UV-Visible spectrophotometer. The FBX dosimeter was calibrated against a standard ^{60}Co gamma irradiator.

3.1.3 Alanine Dosimeter

Alanine has been used as a free radical dosimeter using spectrophotometric readout methods. In the present study, a simpler method developed by Gupta *et al.*, (2000) was used for the dose measurement. When DL-alanine powder was exposed to radiation, free radicals are produced, which gets trapped in a solid matrix and are stable for a long period. The total numbers of free radicals formed are proportional to the absorbed dose. In spectrophotometric readout method, a known amount of irradiated alanine powder was dissolved in an aqueous acidic FX solution containing ferrous ammonium sulphate and XO. The free radicals formed in irradiated alanine oxidize ferrous ions into ferric ions, in FX solution. Ferric ions thus formed, form a complex with XO. This complex was measured spectrophotometrically at 548 nm, the wavelength of maximum absorption of the complex. The change in the absorption is a function of dose for the dose range of 0.01 kGy to 4 kGy.

Preparation of FX Dosimetric Solution

To prepare 1000 cm^3 of FX solution, 28 cm^3 (5 N H_2SO_4) of sulphuric acid was transferred to an empty clean standard flask. Then, 78.4 mg (0.2 mol m^{-3}) of ferrous ammonium sulphate was added to the flask followed by 152 mg (0.2 mol m^{-3}) of XO salt. The solution was adjusted for the final volume of 1 litre by adding ddw and kept for about an hour to stabilize. The solution was freshly prepared, aerated and used within one day.

Preparation of Irradiation Vials

DL-alanine powder (about 60 mg) was filled into small plastic pouches for electron irradiation. The distribution of alanine powder within the plastic pouches was maintained almost uniform thickness to get a uniform dose of electrons. The thickness of the pouches was maintained within 3 to 4 mm.

Irradiation and Measurement Procedures

The alanine powder taken in small pouches were exposed to graded doses, between 0.01 kGy and 4 kGy, at room temperature. After irradiation, 50 mg of the sample was weighed and transferred to a clean, dry 50 cm^3 standard flask. 10 cm^3 of FX solution was added to the flask. The solution was mixed well and allowed to complete radiation induced reaction for about 30 min. The optical absorbance at 549 nm was measured against the control FX solution with un-irradiated alanine. For each dose point, at least three trial measurements were carried out and averaged to get better reproducibility and accuracy. The dosimeter was calibrated against a standard ^{60}Co gamma irradiator. Fricke dosimeter was also used to calibrate the alanine dosimeter in the case of 8 MeV electrons from Microtron.

3.1.4 Glutamine Dosimeter

In the present dosimeter study using glutamine, a simpler spectrophotometric method developed by Gupta *et al*., (2000) was used. The glutamine dosimeter is capable of measuring

doses in the range of 0.1 kGy to 100 kGy. Analysis of the glutamine dosimeter is similar to that of alanine dosimeter.

Preparation of FX Dosimetric Solution

1000 cm^3 of dosimetric (FX) solution for glutamine consists of;

Sulphuric acid	…	14 cm^3 (5 N H_2SO_4)
Ferrous ammonium sulphate	…	78.4 mg (0.2 mol m^{-3})
XO salt	…	76 mg (0.1 mol m^{-3})

After exposure of glutamine powder to graded doses, 20 mg of irradiated glutamine sample was dissolved in 10 cm^3 of FX solution, taken in the standard flask. The solution was kept for an hr to complete the chemical reactions. The optical absorbance at 549 nm was measured against the control FX solution with un-irradiated glutamine.

All chemicals used in the present study were of ANALAR grade. In the first step, all glasswares were rinsed and kept immersed in 1:1 mixture of concentrated nitric acid and sulphuric acid for one day. In the second step, glasswares were immersed in chromic acid followed by liquid detergent Exalin. Glasswares were rinsed several times with distilled water before use.

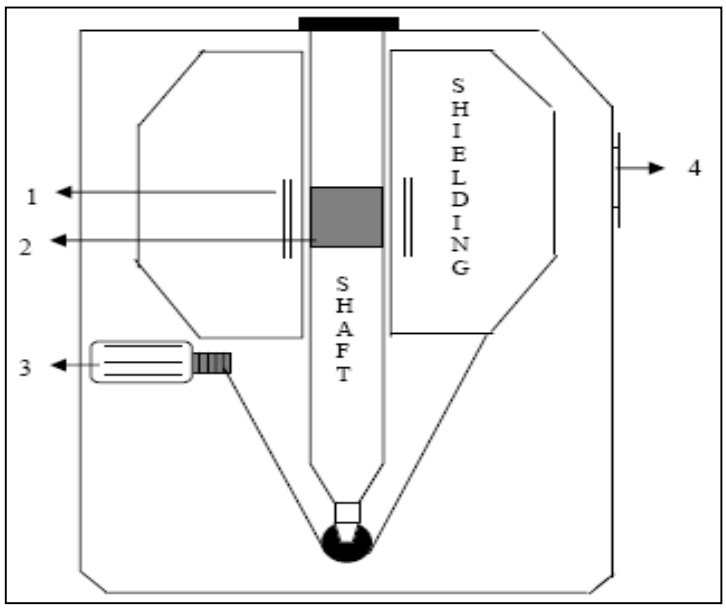
3.2 Radiation source used for Study

In the present study, Gamma chamber 1200 and Microtron accelerator accelerator was used for radiobiological studies.

3.2.1 Gamma chambers-1200

Gamma chambers-1200 is a compact self-shielded ^{60}Co gamma irradiator supplied by the Isotope Division, Bhabha Atomic Research Centre. The source pencils were placed concentrically in a cylindrical geometry. The samples for irradiation were placed in an irradiation chamber, which is cylindrical in geometry and located in the vertical shaft as shown

in Plate 1. This shaft can be moved up and down with the help of a motorized drive, which enables the precise positioning of the irradiation chamber at the centre of the radiation field. Since the samples were exposed from all sides, it gave better uniformity in dose distribution within the samples, as it reduced the depth dose and build up effects. A sample hoe by Perspex with 2 sample positions was used to get the precise dose measurements (Plate 3).



1. Source
2. Irradiation chamber
3.Shaft drive mechanism
4. Irradiation

Plate 1: Schematic diagram of Gamma chambers-1200

Using the upward and downward switches, the shaft can be moved vertically downward for irradiation and upward to remove the irradiated samples. During this to and fro movement, the samples receive a small quantity of dose called transit dose, T_d. The time at which the transit dose is received is called the transit time, T_t.

Plate 2: The photograph of Gamma Chamber-2000 irradiator

Plate 3: A photograph of the sample holder

Although the transit dose is very small compared to the total doses given in the studies, correction is applied to get precise doses. T_d was evaluated by the intercept of the graph drawn by dose *vs.* exposure time. T_t was calculated using the relation:

$$T_t = \left(\frac{T_d}{\text{Dose Rate}} \right) \quad \ldots \tag{1}$$

For a given dose, say D Gy, total exposure time T was calculated using the relation:

$$T = \left(\frac{D}{\text{Dose Rate}} - T_t \right) \ldots \tag{2}$$

The dose rate after n days (DR_n) from the calibration date was calculated using the relation:

$$DR_n = DR_0 \exp\left(-\frac{0.693 \times n}{T_{1/2} \times 365} \right) \quad \ldots \tag{3}$$

Where, DR_o is the initial dose rate, n is the number of days after calibration, and $T_{1/2}$ is the half-life of the ^{60}Co - gamma source (5.27 years). Decay correction was applied from time to time to arrive at the exact dose rate during the experiments.

3.2.2 The Microtron

The Microtron is a compact cyclic accelerator intended solely for accelerating the electrons to relativistic energies. In the Microtron, the electrons are accelerated tangentially by an alternating Radio Frequency (RF) electric field of constant frequency, in a constant uniform magnetic field. The vacuum chamber used to accelerate the electrons is called the Microtron chamber as shown in Plate 4. The electron trajectory in the Microtron is a system of increasing diameter circles with a common point, where the accelerating RF cavity is placed, which supplies the high frequency electric field needed to accelerate the electrons. The high frequency pulsed microwave, generated by the magnetron, is coupled with the RF cavity through a wave guide to make the electron source emit electrons thermionically and to produce

high potential difference within the cavity to accelerate the emitted electrons. The electron source used in the Microtron is a lanthanum hexa-boride (LaB_6) crystal.

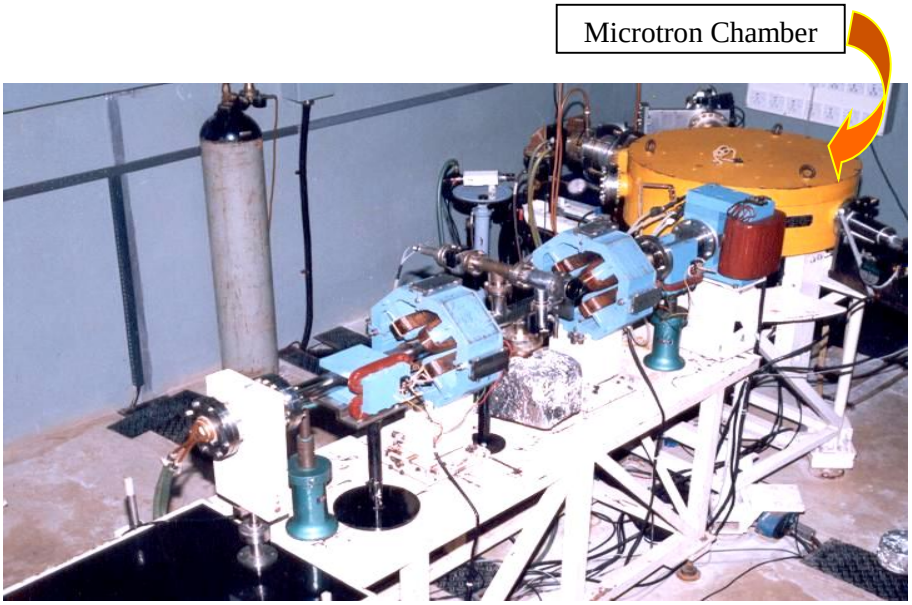


Plate 4: Photograph of the Variable Energy 8/12 MeV Microtron Accelerator

After each passage through the cavity, the electrons gain a certain amount of energy and move circularly, under the influence of an electromagnet with higher radius, and returns to the cavity with an integral number of times the original period so that they are again accelerated. Thus, the electron path is a sequence of circles of every increasing radius, all tangent at the resonator cavity. The electrons attain relativistic velocities in the initial orbits, and hence, further increase in velocity with successive acceleration is negligible. However, the orbit size increases with successive accelerations due to increase in the relativistic mass and the momentum of the electrons. The schematic diagram of the electron orbits in the Microtron chamber is shown in Plate 5.

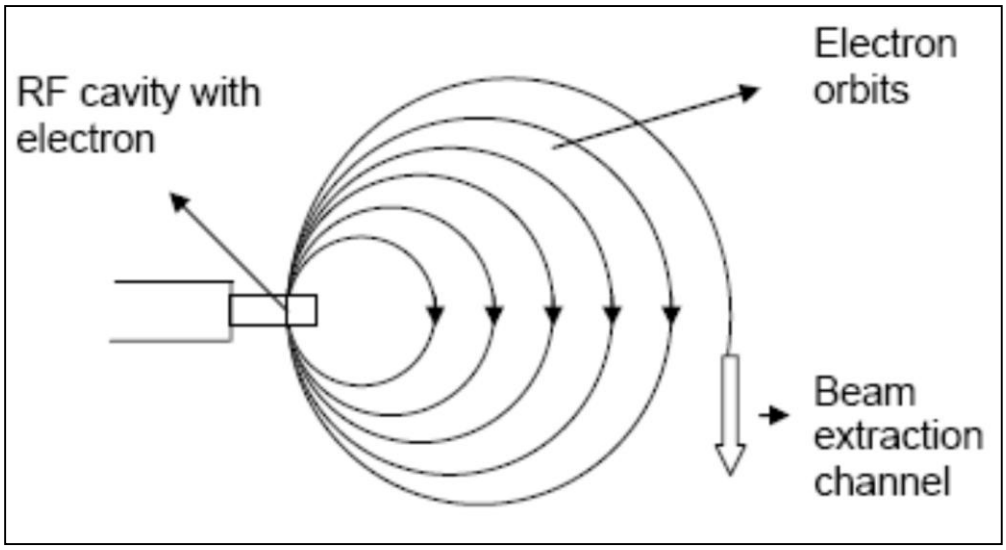


Plate 5: Schematic diagram of electron orbits passing through the RF cavity inside the Microtron chamber

The Variable Energy Microtron used in the present study allows up to 14 orbits with corresponding electron energy of 8 MeV. It was indigenously developed at the Raja Ramanna Centre for Advanced Technology, India, and offers electron beam with excellent beam parameters suitable for radiobiological research applications. It facilitates the extraction of electrons from different orbits to the beam line to vary the energy of the electrons. A set of quodrapole-steering magnets were used to focus and steer the extracted electron beam. Finally, the beam was extracted out of the beam line through a titanium window (called as beam exit window of the accelerator), as shown in Plate 4.

The machine operates in pulsed mode with maximum pulse duration of 2.5 μs and pulse current of 50 mA. A radiation field size of 4 x 4 cm is available for irradiation with uniform dose distribution at 30 cm from the titanium window. The details of the Microtron are given elsewhere [Siddappa *et al.*, 1998]. The electron beam current was measured using a Fast Current Transformer (FCT) and the signal from the FCT coil was digitized to get corresponding electron numbers using an analogue to digital converter and the numbers were displayed in the Current Integrator (CI). These electron numbers in the CI were calibrated against the absorbed dose using chemical dosimeters.

3.3 Model Systems used for the Investigation

3.3.1 Yeast Strains (*Saccharomyces Cerevisiae*)

Yeast is a simple, unicellular eukaryotic organism, which offers many advantages as a model test system for estimating the mutagenic potential of different chemicals and ionizing radiation. It has a short generation time, whereby a large number of cells can be handled at a time, and the cells grow into colonies on nutrient media plates. Higher eukaryotes and yeast cells exhibit striking similarities in the molecular mechanisms of fundamental cellular processes, such as transcription, translation, replication, and DNA repair. The genetic events occurring in eukaryotic diploid yeast are important as similar events may occur in man. In the present study, three yeast strains were selected from the view of radiobiological endpoints and their radio-sensitivity [Reddy *et al*., 1981]. The strains are:

1. *Saccharomyces cerevisiae* D7: It is a mutant strain donated by Prof. F. K. Zimmermann (Darmstadt, Germany). The genotype of the strain is as follows:

$$\frac{a}{\propto}\frac{ade2-40}{ade2-119},\frac{trp5-12}{trp5-27},\frac{ilv1-92}{ilv1-92}$$

2. *Saccharomyces cerevisiae rad52*: It is a repair deficient radio-sensitive mutant.

3. *Saccharomyces cerevisiae* X2180: It is a wild type diploid cell.

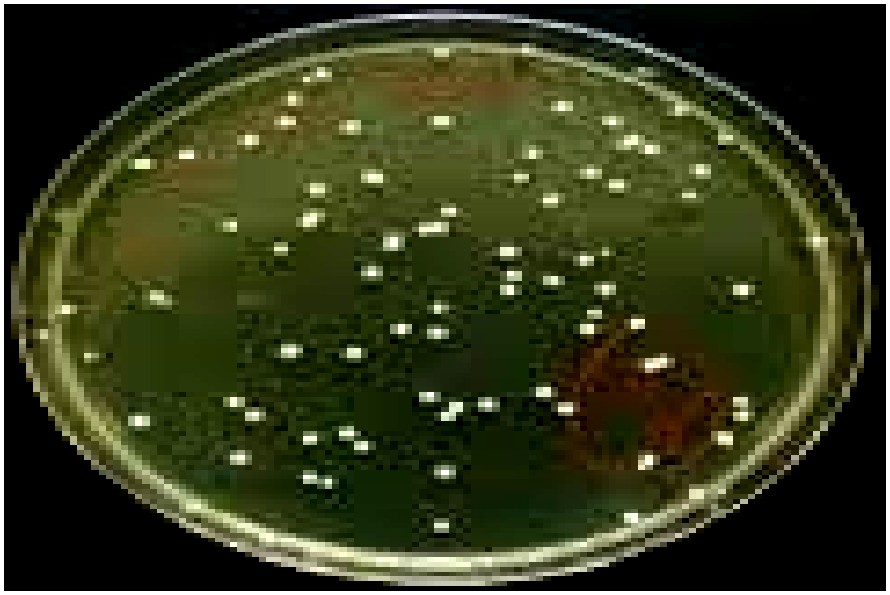


Plate 6: Photograph of the colonies of *Saccharomyces cerevisiae* cells formed in the growth medium

3.3.2 Sample Preparation for Irradiation

The yeast strains were sub-cultured and grown in a broth medium. The single cell stationary-phase cultures were obtained by growing the cells in Yeast extract: Peptone: Dextrose (YEPD) (1%:2%:2%) medium for several generations in a stationary phase to a

density of approximately 3×10^8 cells/ml [Nairy *et al.,* 2014]. The cells were washed thrice by centrifugation (2000g for 5 min) using d.d.w. and re-suspended to a cell concentration of 1×10^8 cells/ml (by counting in heamocytometer) in a sterile polypropylene vial for irradiation.

Hypoxic conditions were achieved by incubating the samples in air tight vials at 30 °C for 30 min prior to irradiation [Nairy *et al.,* 2014]. For euoxic samples, a cell suspension of 1×10^6 cells/ml was prepared and was thoroughly aerated by mixing before irradiation. For proton irradiation, the cell suspension was mixed well and exactly 1×10^6 cells were filtered using the millipore filter assembly in aseptic condition. The cells containing the filter paper were placed in a sterile 3 cm diameter petri dish and irradiated for different radiation doses. The cell suspensions were maintained at 0-4 ºC before and after irradiation, until plating.

3.4 Radiobiological Studies to Quantify the Radiation Effects

3.4.1 Cell Survival Analysis

The cell survival analysis of the yeast strains was carried out using YEPD agar plates. After autoclaving for about 30 min, the YEPD medium was allowed to cool to about 55 - 60 °C, and then, poured on to sterile petri plates under aseptic conditions. The plates were allowed for gel formation for about one day. The plates were checked for possible contamination and uncontaminated plates were used. For irradiation, the cell suspension of required concentration was prepared and about 2 to 3 ml of cell suspension was filled in the irradiation vials. Graded doses were delivered as per the sensitivity of the cell lines and plan of the experiments with an identical geometry as dosimetry. After irradiation, the cell suspension was further diluted to get the required cell concentration depending on the doses given so as to get 200 - 300 colonies per plate. About 0.25 ml of the cell suspension was plated on each YEPD agar plate. The plates were incubated at 30 °C for 48 hrs. Cells exposed to higher doses had taken more than 48 hrs to form visible colonies. The colonies were counted using the colony counter. Generally, for yeast studies, multi-target theory is used to explain the survival response. The multi-target single hit model can be represented as:

$$S = [1 - (1 - \exp(-kD))^n] \quad (4)$$

Where, S represents the survival fraction, k is a slope of the curve, which reflects the sensitivity of the organism, and n represents the number of targets at the exponential region. The mean

lethal dose D_0 is the dose required to reduce the survival to 37% in the exponential region of the survival curve, which can be calculated as the inverse of the slope, i.e., 1/k.

3.4.2 Gene Conversion Analysis using D7 Yeast Strain

The gene conversion analysis was carried out using the Tryptophan deficient (*Trp*$^-$) medium plates and the survival analysis was done using the Synthetic Complete (S.C) medium plates, while studying gene conversion. One litre of *Trp*$^-$ medium was prepared using 20 g of agar, 20 g of dextrose, 20 ml of *Trp*$^-$ amino acid stock solution, 6.67 g of yeast nitrogen base (without amino acid), and 980 ml of d.d.w. The prepared medium was autoclaved at 1.1 kg/cm^2 pressure for 30 min. The composition of the *Trp*$^-$ amino acid stock solution is given in Table 1. The contents given in Table 1 were dissolved by heating in a water bath with constant stirring to avoid clumping.

Estimation of Gene Conversion Frequency

The irradiated cell suspension was diluted to get a suitable concentration (1 x 10^6 cells/ml), and 0.25 ml of this suspension was plated per each *Trp*$^-$ plates to get 0.25 x10^6 cells per plate. The plates were incubated at 30 °C for 70 - 80 hrs to form colonies. The gene conversion (G.C.) per million cells plated was calculated using the relation:

$$\text{G.C.} = \left[\frac{\text{Number of colonies formed on Trp}^- \text{ plates} \times 10^{-6}}{\text{Number of cells plated}}\right] \ldots \quad (5)$$

The convertants per million survivors were calculated by normalizing the G.C. with the corresponding survival-fractions for every dose. The relation used is,

$$\text{Convertants per million survivors} = \left[\frac{\text{G.C.}}{\text{Survival Fraction}}\right] \ldots \quad (6)$$

To find the survival responses, YEPD agar plates were used, but while analyzing the gene conversion in D7, S.C. medium plates were used for the survival response.

Back Mutation Analysis using D7 Yeast Strain

Isoleucine deficient (*Ilv*$^-$) plates were used to carry out Back mutation (B.M.) analysis, and the survival analysis was done using S.C. media plates. One litre of *Ilv*$^-$ medium was prepared using 20 g of agar, 20 g of dextrose, 20 ml of *Ilv*$^-$ amino acid stock solution, 6.67 g of yeast nitrogen base (without amino acid), and 980 ml of d.d.w. The *Ilv*$^-$ amino acid stock solution composition is given in Table 1. The contents were dissolved and autoclaved.

Estimation of Back Mutation Frequency

The irradiated cell suspension was diluted to get a suitable concentration (1 x 10^7 cells/ml), and 0.25 ml of this suspension was plated per each *Ilv*$^-$ plates to get 0.25 x10^7 cells/ml. The plates were incubated at 30 °C for 5-6 days to form colonies. The B.M. per 10^7 cells plated was calculated using the relation:

$$\text{B.M.} = \left[\frac{\text{Number of colonies formed on Ilv}^- \text{ plates} \times 10^{-7}}{\text{Number of cells plated}}\right] \ldots \quad (7)$$

The convertants per 10^7 survivors were calculated by normalizing the B.M. with the corresponding survival-fractions for every dose. The relation used is,

$$\text{Convertants per million survivors} = \left[\frac{\text{B.M.}}{\text{Survival Fraction}}\right] \ldots \quad (8)$$

The data from several independent experiments were pooled, and the mean value and standard deviation of the mean were computed for each individual dose.

S.C. amino acid solution		*Trp*$^-$ amino acid solution		*Ilv*$^-$ amino acid solution	
Amino acid	Quantity (mg)	Amino acid	Quantity (mg)	Amino acid	Quantity (mg)
Adenine	25	Adenine	25	Adenine	25
Argenine	250	Argenine	250	Argenine	250
Histidine	250	Histidine	250	Histidine	250

Leucine	1500	Leucine	1500	Leucine	1500
Lysine	250	Lysine	250	Lysine	250
Methionine	250	Methionine	250	Methionine	250
Uracil	250	Uracil	250	Uracil	250
Isoleucine	1500	Isoleucine	1500	Isoleucine	-
Tryptophan	60	Tryptophan	-	Tryptophan	60

Table 1: Composition of 500 ml amino acid solution

4. Result and Discussion

4.1 Cell Survival, Gene Conversion, and Back Mutation Analysis under Euoxic and Hypoxic Irradiation using Gamma radiation

Diploid yeast strain *Saccharomyces cerevisiae* D7, X2180, and *rad52* were used to study the survival, gene conversion, and back mutation response after irradiation with ^{60}Co-gamma radiation and the results are presented in Figures 1 to 3. The survival response is fit to a multi target single hit model as explained in Equation 4. The observed parameter values from the theoretical fit is shown in Table 2. The theoretical values for the different parameters were obtained using Equation 4. In Figures 1 and 2, the survival response was found to be sigmoid, which is characteristic of the repair proficient diploid yeast strain due to the multi-track hit processes combined with the dose rate dependent molecular repair processes [Joseph *et al.,* 2011, Nairy *et al*., 2014].

The survival response of *Saccharomyces cerevisiae* D7 under euoxic condition is represented as $S = 1 – (1- \exp (-4.5 \times 10^{-3} D)^{3.01}$ ($chi^2 = 0.0002$, $R^2 = 0.99$) and for hypoxic condition as $S = 1 – (1- \exp (-1.75 \times 10^{-3} D)^{2.50}$ ($chi^2 = 0.001$, $R^2 = 0.99$). The calculated D_0 value, which is a reciprocal of the inactivation constant, is 222 and 571 Gy for euoxic and hypoxic conditions, respectively. The OER value at 37% survival in the exponential region can be

calculated by taking the ratio between the hypoxic and euoxic D_0 doses and was found to be 2.57.

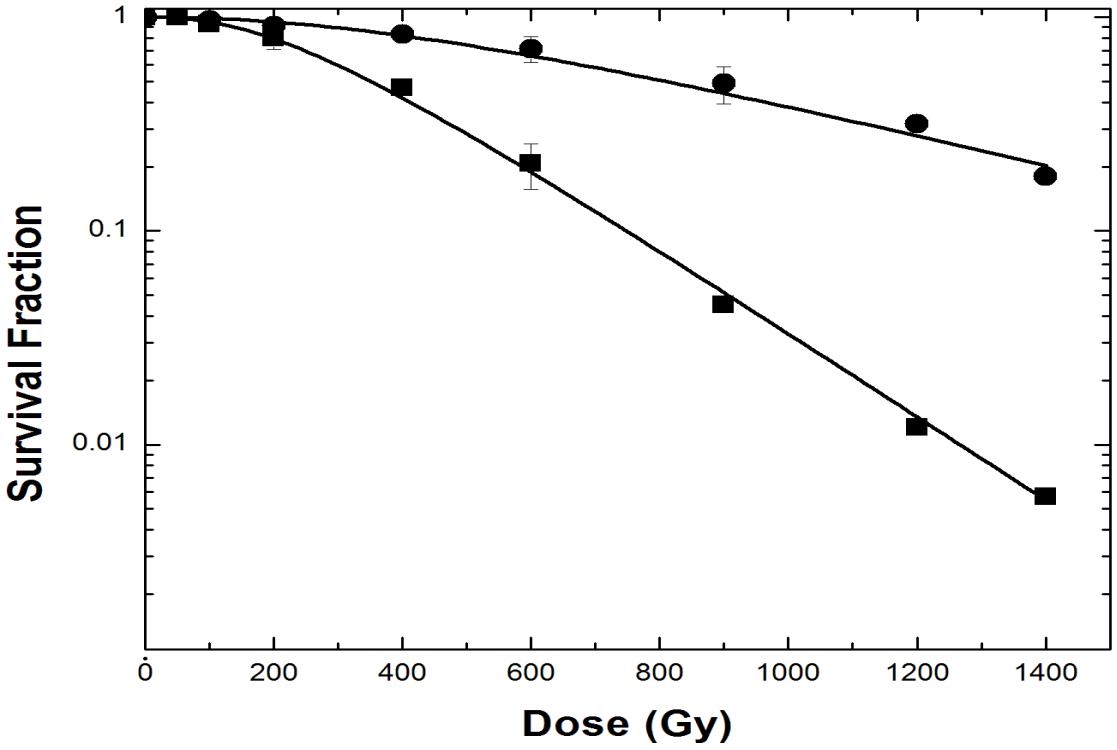


Figure 1: Survival - Dose response of *Saccharomyces cerevisiae* D7 strain under euoxic (■) and hypoxic condition (●) after irradiating with gamma radiation

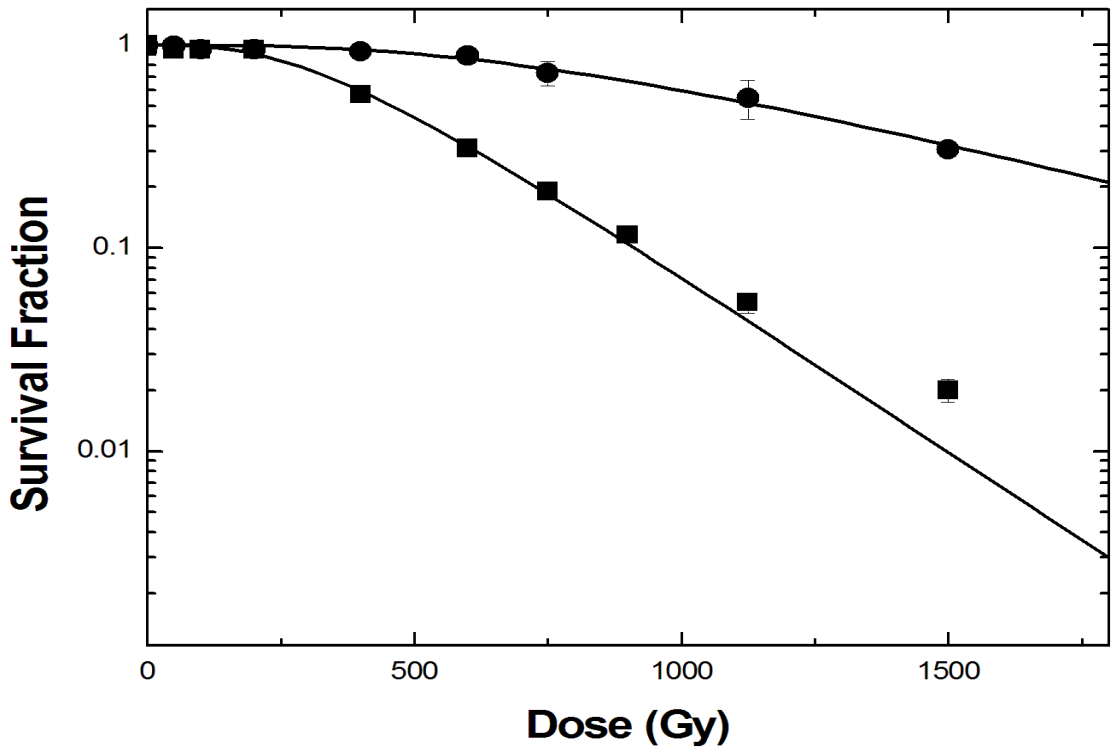


Figure 2: Survival - Dose response of *Saccharomyces cerevisiae* X2180 strain under euoxic (■) and hypoxic condition (●) after irradiating with gamma radiation

The survival response of *Saccharomyces cerevisiae* X2180 under euoxic condition can be represented as $S = 1 – (1- \exp (- 4.33 \times 10^{-3} D)^{4.24}$ ($chi^2 = 0.0007$, $R^2 = 0.99$) and for hypoxic condition as $S = 1 – (1- \exp (-1.48 \times 10^{-3} D)^{2.71}$ ($chi^2 = 0.001$, $R^2 = 0.99$). The D_0 values calculated from the equation are 230 and 676 Gy for euoxic and hypoxic conditions, respectively. From these values, the calculated OER value at 37% survival (in the exponential region) is 2.93. The present data agreeing well with the literature data, shows an OER value for low LET radiation is between 2.5 to 3 [Kiefer and Ebert, 1970, Kiefer and Bettina, 1979].

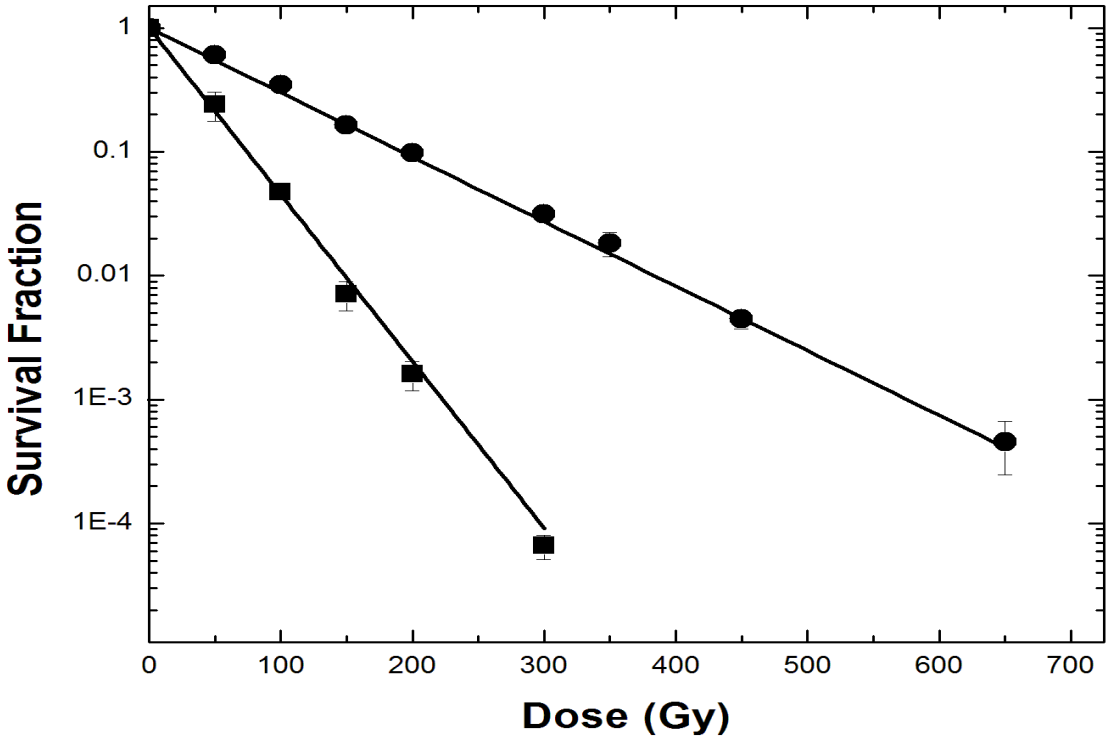


Figure 3: Survival - Dose response of *Saccharomyces cerevisiae rad52* strain under Euoxic (■) and hypoxic condition (●) after irradiating with gamma radiation

The survival response of *Saccharomyces cerevisiae rad52* was found to be exponential, confirming the absence of the sublethal repair mechanism. The survival response under euoxic condition can be represented as $S = 1 – (1- \exp (-3.1 \times 10^{-2} D)^{1}$ ($chi^2 = 1.15 \times 10^{-6}$, R^2

= 0. 99) and for hypoxic condition as $S = 1 – (1- \exp (-1.2 \times 10^{-2} D)^1$ (chi^2=0. 0001, R^2 = 0. 99). The D_0 values calculated from the equation are 32 and 83 Gy for euoxic and hypoxic conditions, respectively. From these values, the calculated OER value is 2.59.

Parameter	**Yeast Strains**					
	***Saccharomyces cerevisiae* X2180**		***Saccharomyces cerevisiae* D7**		***Saccharomyces cerevisiae rad52***	
	Euoxic	**Hypoxic**	**Euoxic**	**Hypoxic**	**Euoxic**	**Hypoxic**
n	4.24 ± 0.64	2.71 ± 0.65	3.01 ± 0.22	2.50 ± 0.40	1	1
k	$4.3 \times 10^{-3} \pm 3 \times 10^{-4}$	$1.48 \times 10^{-3} \pm 1 \times 10^{-4}$	$4.5 \times 10^{-3} \pm 1.9 \times 10^{-4}$	$1.75 \times 10^{-3} \pm 1.6 \times 10^{-4}$	$3.1 \times 10^{-2} \pm 5 \times 10^{-4}$	$1.2 \times 10^{-2} \pm 5 \times 10^{-4}$
Chi^2 (χ^2)	7.5×10^{-4}	1.1×10^{-4}	2.2×10^{-4}	1.1×10^{-3}	1.15×10^{-6}	9×10^{-5}
R^2	0.99	0.99	0.99	0.99	0.99	0.99

Table 2: Parameter values from the theoretical fit for *Saccharomyces cerevisiae* D7, X2180, and *rad52* after euoxic and hypoxic irradiation using gamma radiation [Nairy *et al.,* 2014].

The shoulder of the sigmoid curve as in the case of X2180 and D7 indicates the repair of sublethal damage, and no shoulder as in the case of *rad52* represents the absence of the sublethal repair mechanism. A wider shoulder observed in the case of hypoxic condition may be due to the decreased rate of production of the peroxy radicals. Under euoxic condition, the initial damaged DNA was higher than that under hypoxic condition [Joseph *et al.,* 2011, Nairy *et al.,* 2014]. In the presence of highly reactive oxygen molecules, the reactions produced by the ionizing radiation are different and are more harmful biologically. In the absence of oxygen, radicals react with one another or dimerise or polymerize. The presence of oxygen blocks the restoration process and enhances the radiation damage [Narayanan 2007, Joseph *et al.,* 2011, Nairy *et al.,* 2014]. In the presence of oxygen, the formation of peroxy radical is the predominant reaction. Therefore, a dose of radiation will be more destructive to the biological system in the presence of oxygen than in its absence.

The gene conversion and back mutation studies were carried out using the *Saccharomyces cerevisiae* D7 yeast cells at *trp* and *ilv* locus. The strain is heteroallelic at this locus having allele's *trp5-12/trp5-27* and *ilv 1-92/ilv 1-92,* which are non-complementing. As a result, the cells cannot synthesize tryptophan and isoleucine amino acids. Hence, they cannot grow on the Trp^- and Ilv^- medium. On a complete medium, they grow, divide, and form colonies. Exposure to radiation results in non-reciprocal recombination in *trp* and *ilv* locus resulting in conversion of the auxotrophic cell into prototropic cell.

The prototropic cell can synthesize its own tryptophan and isoleucine and grow to form a colony on a medium lacking tryptophan and isoleucine. Each colony represents a gene convertant and back mutant. The results are presented in Figures 4 - 5. The sublethal doses 25 Gy, 50 Gy, 75 Gy, and 100 Gy were selected for the experiment, and the results showed a linear increase in gene conversion and back mutation frequency with the dose.

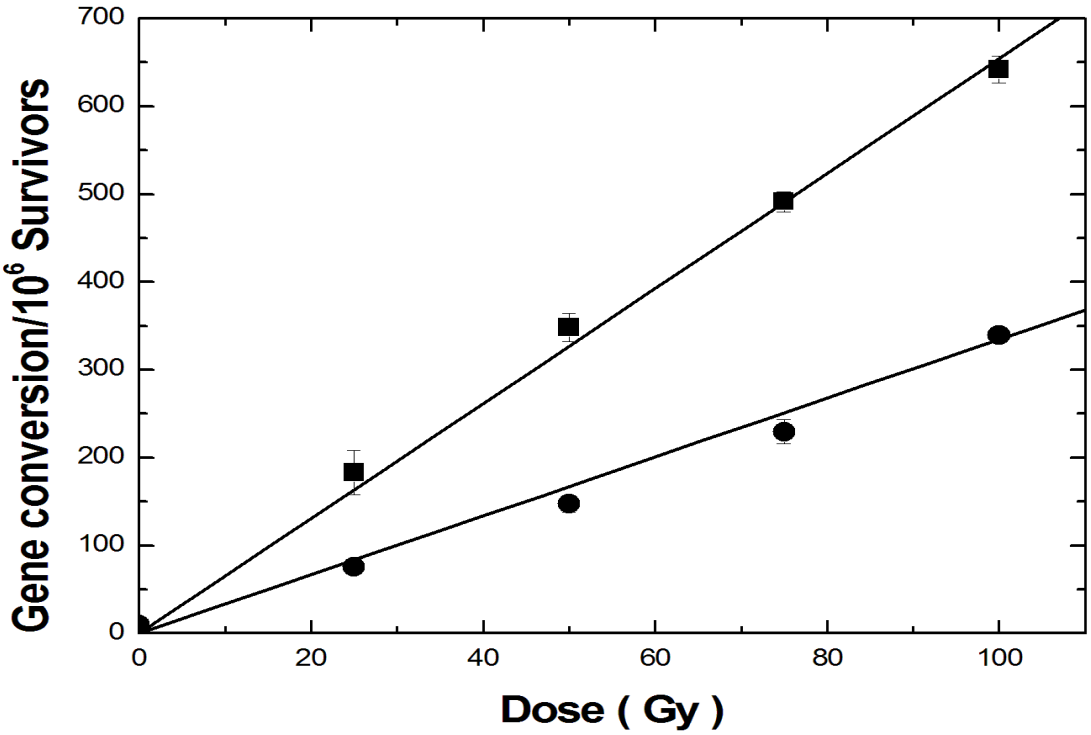


Figure 4: Gene conversion frequency per 10^6 survivor's *vs.* radiation dose under euoxic condition (■) and hypoxic condition (●) after irradiating *Saccharomyces cerevisiae* D7 strain with gamma radiation

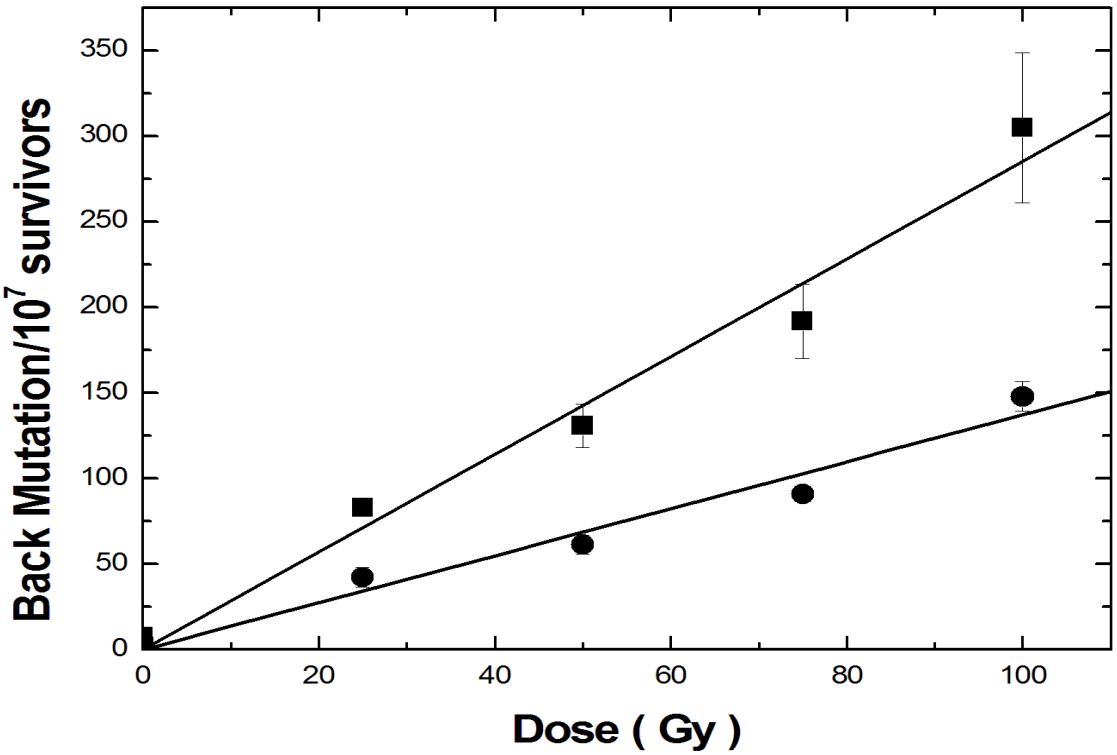


Figure 5: Back mutation frequency per 10^7 survivor's *vs* radiation dose under euoxic

condition (■) and hypoxic condition (●) after irradiating *Saccharomyces cerevisiae* D7 strain with gamma radiation

The gene conversion frequency (G.C.F.) under euoxic and hypoxic conditions can be represented as $G.C.F._{euoxic} = (6.54 \pm 0.10)\ D$ (with $R^2 = 0.99$ and $P < 1\times10^{-4}$) and $G.C.F._{hypoxic} = (3.35 \pm 0.033)\ D$ (with $R^2 = 0.99$ and $P < 1\times10^{-4}$). The back mutation frequency (B.M.F.) under euoxic and hypoxic condition can be represented as $B.M.F._{euoxic} = (2.85 \pm 0.126)\ D$ (with $R^2 = 0.98$ and $P = 2.09 \times 10^{-4} < 0.001$) and $B.M.F._{hypoxic} = (1.37 \pm 0.07)\ D$ (with $R^2 = 0.97$ and $P = 4.23 \times 10^{-4} < 0.001$). The OER value for sublethal doses was calculated using the slopes and was found to be 1.95 from gene conversion and 2.08 from back mutation endpoints.

The slopes under euoxic condition (6.54 and 2.85) is more than the hypoxic condition (3.35 and 1.37) in both the endpoints, which indicates the radio sensitizing effect at sublethal doses due to the oxygen, which enhances the gene conversion and back mutation frequency. In the case of euoxic irradiation, part of the DNA strand breaks are additionally produced by oxygen reacting free radicals, which is absent under hypoxic condition [Hirayama *et al.,* 2005] and leads to radio-resistance.

4.2 Cell Survival, Back Mutation, and Gene Conversion Analysis under Euoxic and Hypoxic Irradiation using 8 MeV Pulsed Electron Beam

Diploid yeasts *Saccharomyces cerevisiae* X2180, *Saccharomyces cerevisiae* D7, and *Saccharomyces cerevisiae rad52* were exposed to microsecond pulses of 8 MeV electrons under euoxic and hypoxic conditions. The gene conversion and back mutation frequency of *Saccharomyces cerevisiae* D7 strain was studied in the dose range of 0 to 100 Gy. The doses were delivered at the rate of 100 Gy/min. The survival response of yeast cells, exposed to 8 MeV electron beam under euoxic and hypoxic condition is shown in Figures 6 - 8, and the observed parameter values from the theoretical fit are shown in Table 3.

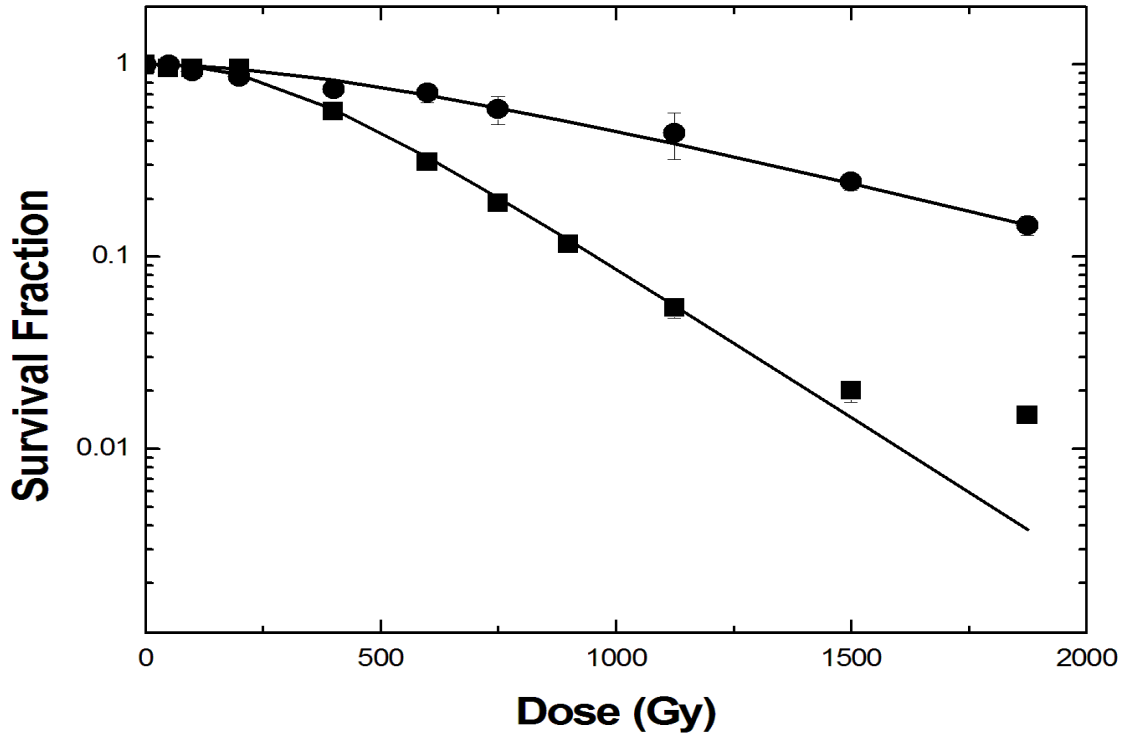


Figure 6: Radiation dose *vs.* Survival response of *Saccharomyces cerevisiae* X2180 strain under euoxic condition (■) and hypoxic condition (●) after irradiating with the 8 MeV pulsed electron beam

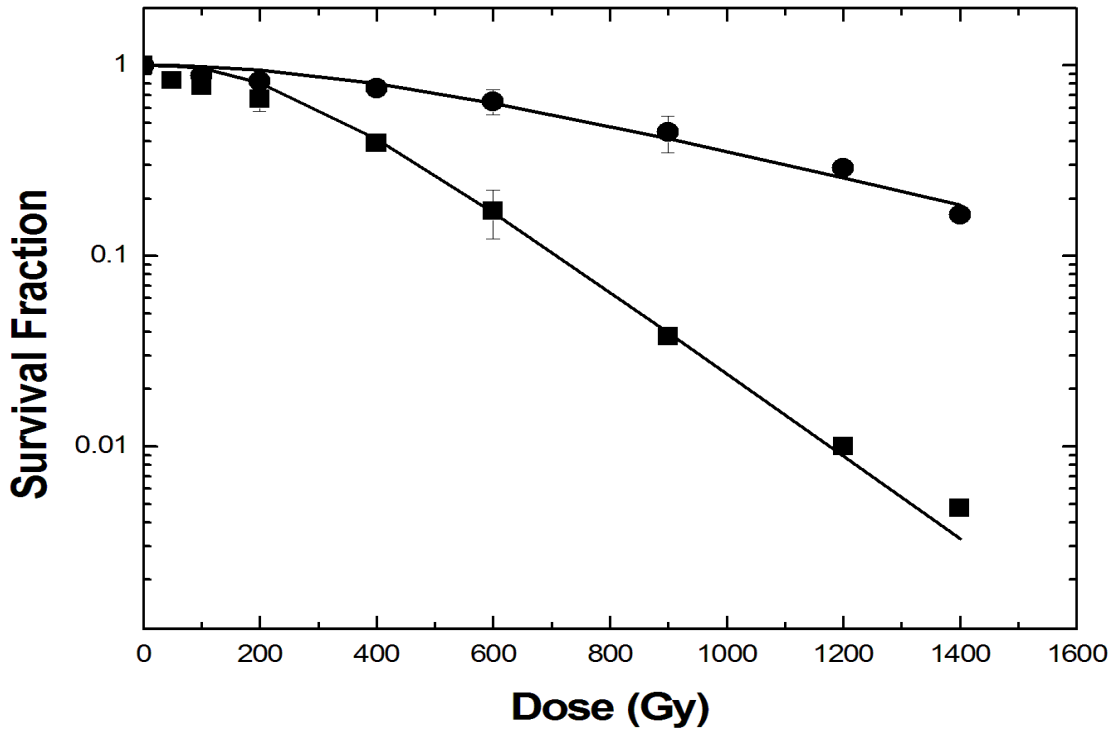


Figure 7: Radiation dose *vs* Survival response of *Saccharomyces cerevisiae* D7 strain under

euoxic condition (■) and hypoxic condition (●) after irradiating with the 8 MeV pulsed electron beam

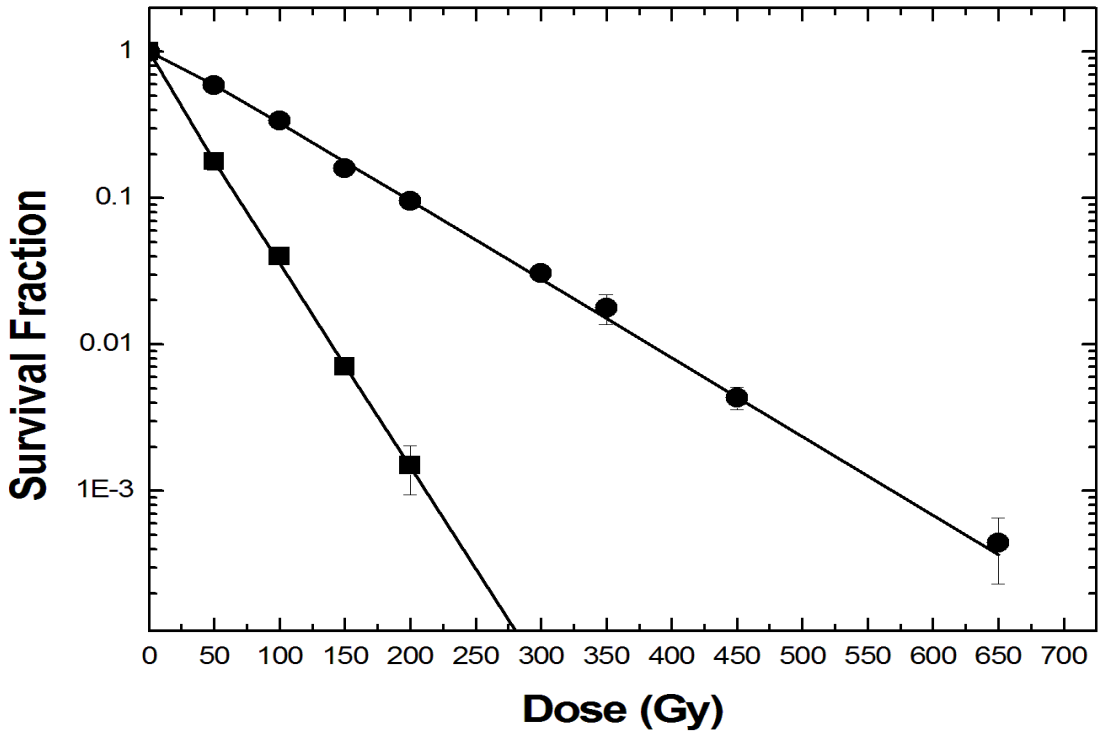


Figure 8: Radiation dose *vs* Survival response of *Saccharomyces cerevisiae rad52* strain under euoxic condition (■) and hypoxic condition (●) after irradiating with the 8 MeV pulsed electron beam

The survival response of D7 and X2180 were found to be sigmoid, and the survival response of *rad52* was purely exponential. The theoretical values for the different parameters were obtained using the multi target single hit model. From the theoretical fit values, the survival response of *Saccharomyces cerevisiae* X2180 under euoxic condition can be represented as $S = 1 – (1- \exp (-4 \times 10^{-3} D)^{3.98}$ ($chi^2 = 6.8 \times 10^{-4}$, $R^2 = 0.99$) and for hypoxic condition as $S = 1 – (1- \exp (-1.3 \times 10^{-3} D)^{2.08}$ ($chi^2 = 2.6 \times 10^{-3}$, $R^2 = 0.97$). The D_0 values, calculated from the equation are 250 Gy and 769 Gy for euoxic and hypoxic irradiation, respectively. From these values, the calculated OER value at 37 % survival is 3.07.

The survival response of *Saccharomyces cerevisiae* D7 under euoxic condition can be represented as $S = 1 – (1- \exp (-5.01 \times 10^{-3} D)^{3.65}$ ($chi^2 = 1.07 \times 10^{-2}$, $R^2 = 0.93$) and for hypoxic condition as $S = 1 – (1- \exp (-1.8 \times 10^{-3} D)^{2.42}$ ($chi^2 = 4.25 \times 10^{-3}$, $R^2 = 0.95$). The D_0 values calculated from the equation are 200 Gy and 556 Gy for euoxic and hypoxic

conditions, respectively. From these values, the calculated OER value at 37 % survival is 2.78.

The survival response of *Saccharomyces cerevisiae rad52* under euoxic condition can be represented as $S = 1 – (1- \exp (- 3.2 \times 10^{-2} D)^1$ ($chi^2 = 3.86 \times 10^{-6}$, $R^2 = 0.99$) and for hypoxic condition as $S = 1 – (1- \exp (-1.24 \times 10^{-2} D)^1$ ($chi^2 = 6.0 \times 10^{-5}$, $R^2 = 0.99$). The D_0 values calculated from the equation are 31 and 81 Gy for euoxic and hypoxic conditions, respectively. From these values, the calculated OER value at 37 % survival is 2.61.

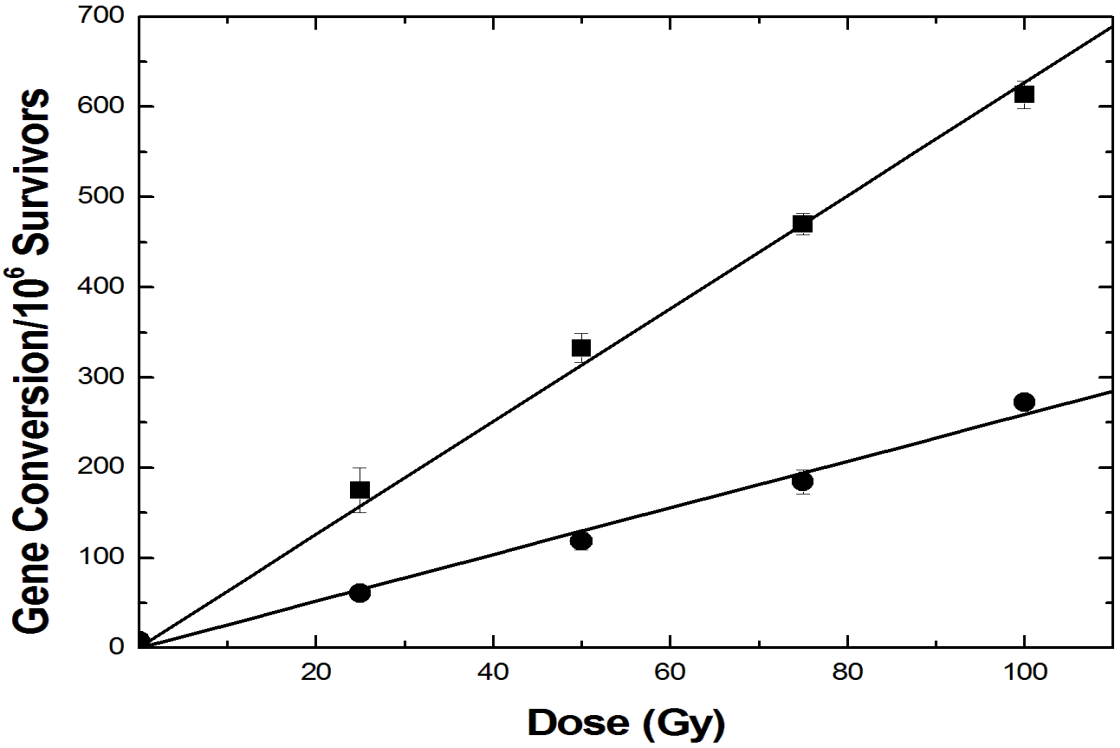


Figure 9: Gene conversion frequency per 10^6 survivor's *vs* radiation dose under euoxic condition (■) and hypoxic condition (●) after irradiating *Saccharomyces cerevisiae* D7 cells with 8 MeV pulsed electron beam

Figure 9 and Figure 10 show the gene conversion and back mutation response of D7 exposed to various doses of 8 MeV electrons under euoxic and hypoxic conditions. The G.C.F. per Gy and B.M.F. per Gy was calculated from the slope of the response curves. The G.C.F. was found to be 6.27 ± 0.11 under euoxic

Parameter	**Yeast Strains**					
	***Saccharomyces cerevisiae* X2180**		***Saccharomyces cerevisiae* D7**		***Saccharomyces cerevisiae rad52***	
	Euoxic	**Hypoxic**	**Euoxic**	**Hypoxic**	**Euoxic**	**Hypoxic**
n	3.98 ± 0.19	2.08 ± 0.06	3.65 ± 0.22	2.42 ± 0.40	1	1
k	$4\times10^{-3} \pm 3\times10^{-4}$	$1.3\times10^{-3} \pm 7\times10^{-4}$	$5\times10^{-3} \pm 5.6\times10^{-3}$	$1.85\times10^{-3} \pm 1.1\times10^{-3}$	$3.2\times10^{-2} \pm 4\times10^{-4}$	$1.24\times10^{-2} \pm 2\times10^{-4}$
Chi^2 (χ^2)	6.8×10^{-4}	2.6×10^{-3}	1.07×10^{-2}	4.25×10^{-3}	3.86×10^{-6}	6×10^{-5}
R^2	0.99	0.97	0.93	0.95	0.99	0.99

Table 3: Parameter values from the theoretical fit for *Saccharomyces cerevisiae* D7, X2180, and *rad52* after euoxic and hypoxic irradiation using 8 MeV pulsed electron beam

irradiation and 2.58 ± 0.08 under hypoxic irradiation of the million survived cells per Gy, and the B.M.F. was 2.63 ± 0.12 under euoxic irradiation and 1.07 ± 0.06 under hypoxic irradiation of the ten million survived cells per Gy of the absorbed dose. It is clear that the gene conversion decreased by a factor of 2.43 and back mutation decreased by a factor of 2.45 in the absence of oxygen.

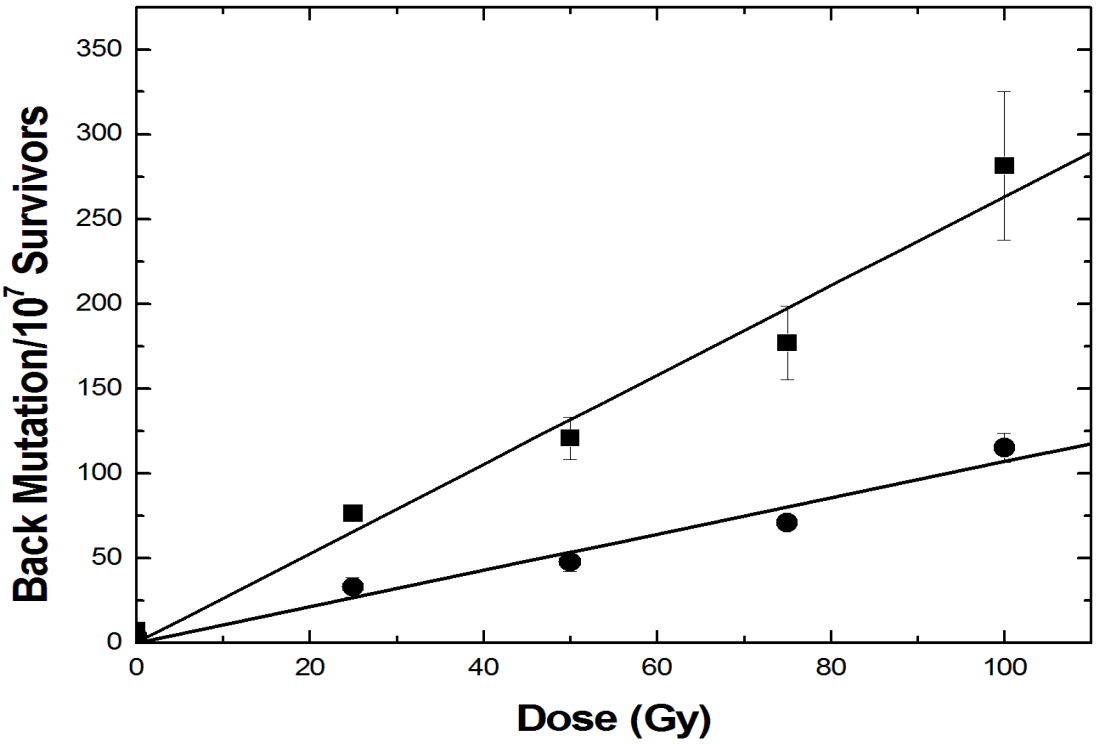


Figure 10: Back mutation frequency per 10^7 survivor's *vs.* radiation dose under euoxic condition (■) and hypoxic condition (●) after irradiating *Saccharomyces cerevisiae* D7 cells with 8 MeV pulsed electron beam

Oxygen is one of the best known modifiers of radiation sensitivity. The sensitizing effect of oxygen has been attributed to the production of more radiolytic products, which react with the DNA and increase the amount of biological damage. From Figures 1 –10, it is clear that the radiation is more effective to inactivate the cell in the presence of oxygen than in its absence.

The three biological endpoints, cell survival, back mutation, and gene conversion were studied in different yeast strains using 8 MeV electrons and gamma radiation under euoxic and hypoxic conditions, suggest that the presence of oxygen during radiation exposure is one of the main factors

to modify the final biological response. The obtained OER value and the differences in the survival curve shoulders under euoxic and hypoxic condition indicates the contribution of hydroxyl radicals in cell inactivation. The observed OER values in the present study, at a dose which gives 37 % of cell survival (exponential region) are comparable to previously published reports on different cell lines after irradiation with low LET radiations [Kiefer and Ebert, 1970, Kiefer and Bettina, 1979].

4.3 Variation of OER with radiation dose studies using ^{60}Co-gamma radiation and 8 MeV pulsed electrons.

In the present study, efforts were made to formulate an equation, which would relate OER and absorbed dose. Presently, OER is calculated on the basis of D_0 doses, which gives the OER value in the exponential region. The multi-target single hit model was used to develop a relation, which connects OER and dose. Generally, OER is represented by taking the ratio between the hypoxic and euoxic doses required to produce the same biological effects.

$$OER = \frac{D_H}{D_E} \; At\ same\ biological\ effect \tag{9}$$

Where, D_H is a hypoxic dose and D_E is a euoxic dose. Considering the multi-target single hit model, the survival fraction S, can be represented as (from Equation 2.17)

$$S = \left\{1-\left[1-\exp(-kD)\right]^{n}\right\} \tag{10}$$

Where, S represents survival fraction, k is inactivation constant, D is dose, and n gives the number of targets. To calculate the OER value, we are considering the survival level is same then using equation (10), we can write

$$S_H = S_E$$

$$\left\{1-\left[1-\exp(-k_H D_H)\right]^{n_H}\right\} = \left\{1-\left[1-\exp(-k_E D_E)\right]^{n_E}\right\} \tag{11}$$

Simplifying (11); considering dose (D) is very high,

$$\{1-n_H \exp(-k_H D_H)\} = \{1-n_E \exp(-k_E D_E)\}$$

$$\frac{n_H}{n_E} = \left\{ \frac{\exp(-k_E D_E)}{\exp(-k_H D_H)} \right\}$$

$$\Rightarrow \frac{n_H}{n_E} = \exp(-k_E D_E + k_H D_H)$$

$$\ln(\frac{n_H}{n_E}) = (-k_E D_E + k_H D_H)$$

$$\frac{1}{D_E} \times \ln(\frac{n_H}{n_E}) = \left\{ -k_E + \frac{k_H D_H}{D_E} \right\}$$

$$\left\{ (\frac{1}{D_E} \ln(\frac{n_H}{n_E}) + k_E) = \frac{(k_H \times D_H)}{D_E} \right\}$$

$$OER = \frac{D_H}{D_E} = \left\{ \left[\frac{1}{(D_E \times k_H)} \times \ln(\frac{n_H}{n_E}) \right] + \frac{k_E}{k_H} \right\} \text{ [Nairy } et\ al.,\text{ 2014]} \qquad (12)$$

Equation 4.3 gives the relation between OER and dose. In Equation 4.3, D_E, n_E, and k_E represent dose, number of targets, and inactivation constant under euoxic condition, respectively, and n_H and k_H represent the number of targets and inactivation constant under hypoxic condition, respectively. The variance in an OER can be calculated using the following equations, considering equation (12) the k_H, n_H, k_E and n_E are variables

$$(\sigma y)^2 = \left\{ \left[(\frac{\partial y}{\partial k_H})^2 \times (\sigma_{k_H})^2 \right] + \left[(\frac{\partial y}{\partial k_E})^2 \times (\sigma_{k_E})^2 \right] + \left[(\frac{\partial y}{\partial n_H})^2 \times (\sigma_{n_H})^2 \right] + \left[(\frac{\partial y}{\partial n_E})^2 \times (\sigma_{n_E})^2 \right] \right\}$$

[Nairy *et al.,* 2014] (13)

Where, y represents the OER value.

$$\left(\frac{\partial y}{\partial k_H}\right)^2 = \left\{\left[\frac{-1}{(k_H{}^2 \times D_E)} \times \ln\left(\frac{n_H}{n_E}\right)\right] - \frac{k_E}{k_H{}^2}\right\}^2$$

$$\left(\frac{\partial y}{\partial k_E}\right)^2 = \left(\frac{1}{k_H}\right)^2$$

$$\left(\frac{\partial y}{\partial n_H}\right)^2 = \left(\frac{1}{n_H \times k_H \times D_E}\right)^2$$

$$\left(\frac{\partial y}{\partial n_E}\right)^2 = \left(\frac{1}{n_E \times D_E \times k_H}\right)^2$$

Accordingly, the standard deviation of the mean (at 95% confidence level) was calculated. The Figures 11- 13 represent the variation of OER with gamma radiation dose, and the Figures 14 - 16 represent the variation of OER with 8 MeV pulsed electron dose for *Saccharomyces cerevisiae* D7, *Saccharomyces cerevisiae* X2180 and *Saccharomyces cerevisiae rad52*, calculated using Equation 12. For both the radiations, the OER value was found to increase with the dose for *Saccharomyces cerevisiae* X2180 and *Saccharomyces cerevisiae* D7 strains, whereas for *Saccharomyces cerevisiae rad52*, it was constant. The OER values varied from 1.51 to 2.53 for D7, 2.02 to 2.98 for X2180, 2.58 for *rad52* in gamma radiation, and in electron radiation, the OER values varied from 2.20 to 2.67 for D7, 2.06 to 3.02 for X2180, 2.58 for *rad52*.

Studies reported in the literature indicate that for sparsely ionizing radiation the contribution of OH radical to cell killing is about 55% under euoxic condition and about 20% under hypoxia [Roots *et al.,* 1985]. In the case of euoxic irradiation, part of the DNA strand breaks would additionally be produced by the oxygen reacting free radicals, which are absent under hypoxic condition [Hirayama *et al.,* 2005].

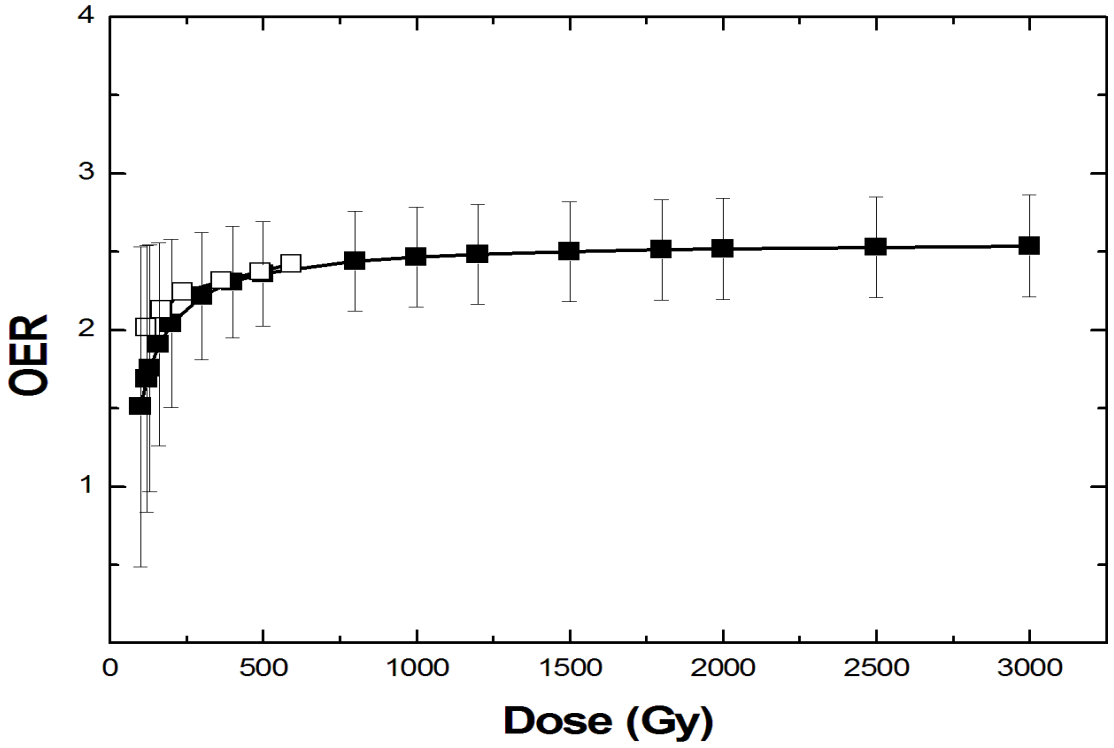


Figure 11: Variation of OER with gamma radiation dose for *Saccharomyces cerevisiae* D7 strain, the experimental (□) and theoretical (■) values

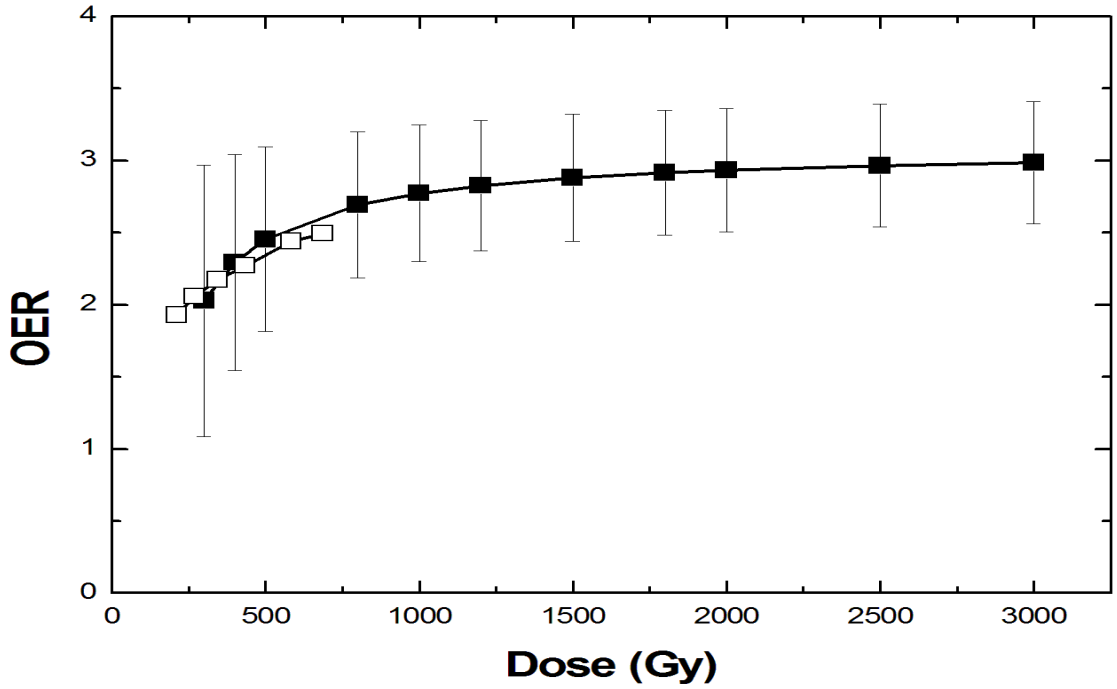


Figure 12: Variation of OER with gamma radiation dose for *Saccharomyces cerevisiae* X2180 strain, the experimental (□) and theoretical (■) values

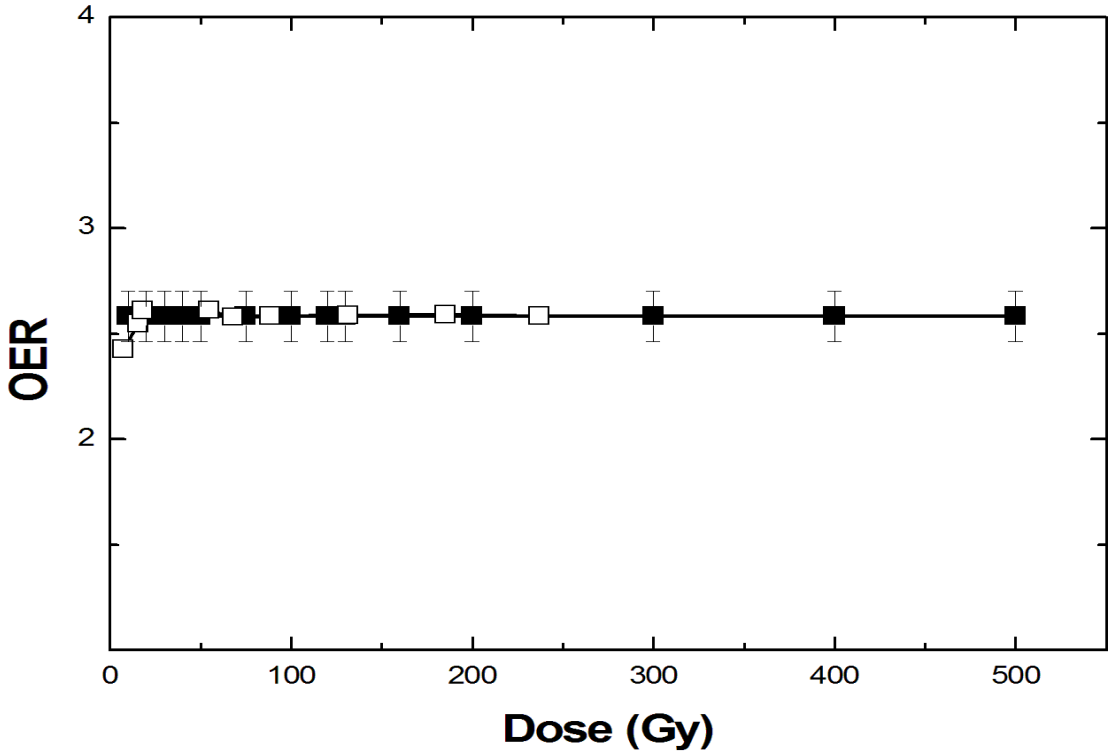


Figure 13: Variation of OER with gamma radiation dose for *Saccharomyces cerevisiae rad52* strain, the experimental (□) and theoretical (■) values

In the case of X2180 and D7 strains, at lower doses the OER value is small, indicating that the initial DNA strand breaks were mostly due to the direct action of the radiation as the radicals due to oxygen is negligible [Nairy *et al.,* 2014]. Most of the damages produced due to the secondary ionization, was repaired through the HR repair mechanism. At higher doses, the damage due to peroxy radicals and multi-ionizing events lead to lethal damage in euoxic condition, whereas in hypoxic condition, the damage is mainly due to direct ionization and part of which may be repaired efficiently, which leads to increase in OER value [Nairy *et al.,* 2014].

In the case of *Saccharomyces cerevisiae rad52* strain, the constant OER value is mainly due to the absence of the repair mechanism, which increases the radio-sensitivity of the strain. In euoxic and hypoxic conditions, throughout the dose range, the produced damages lead to cell death.

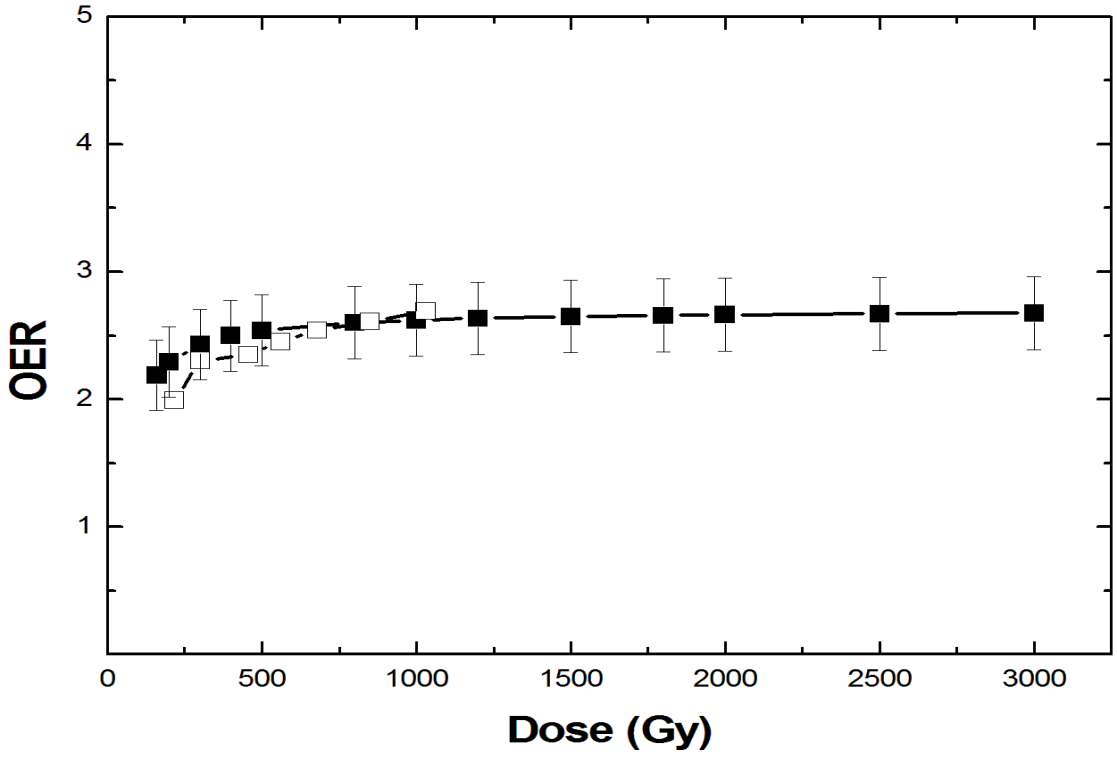


Figure 14: Variation of OER with 8 MeV electron dose for *Saccharomyces cerevisiae* D7 strain, the experimental (□) and theoretical (■) values

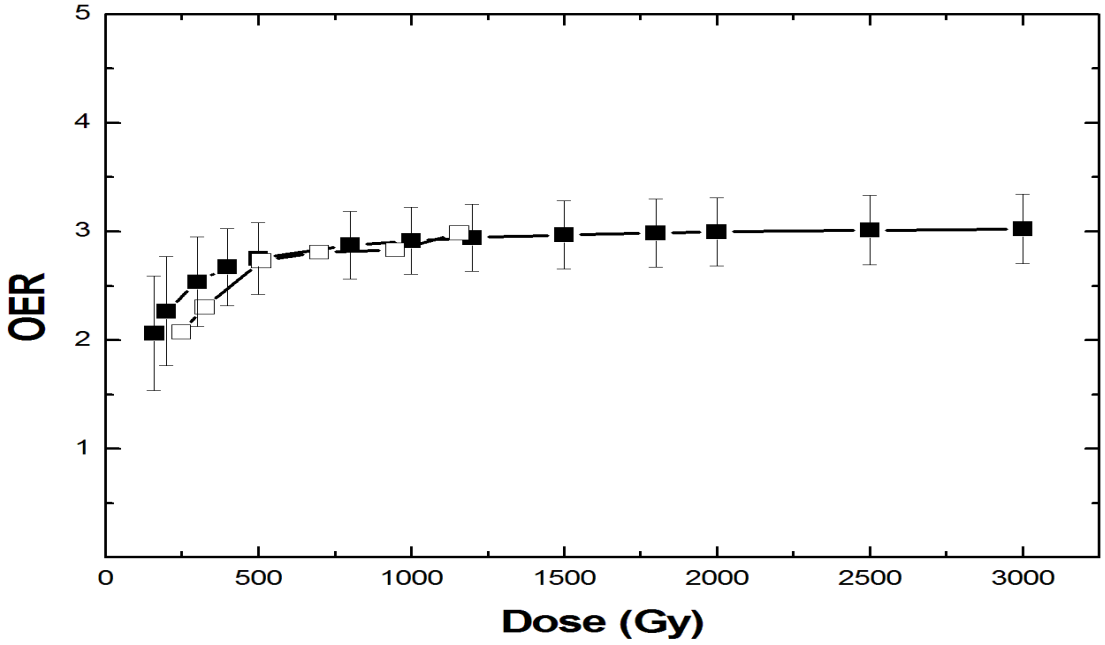


Figure 15: Variation of OER with 8 MeV electron dose for *Saccharomyces cerevisiae* X2180 strain, the experimental (□) and theoretical (■) values

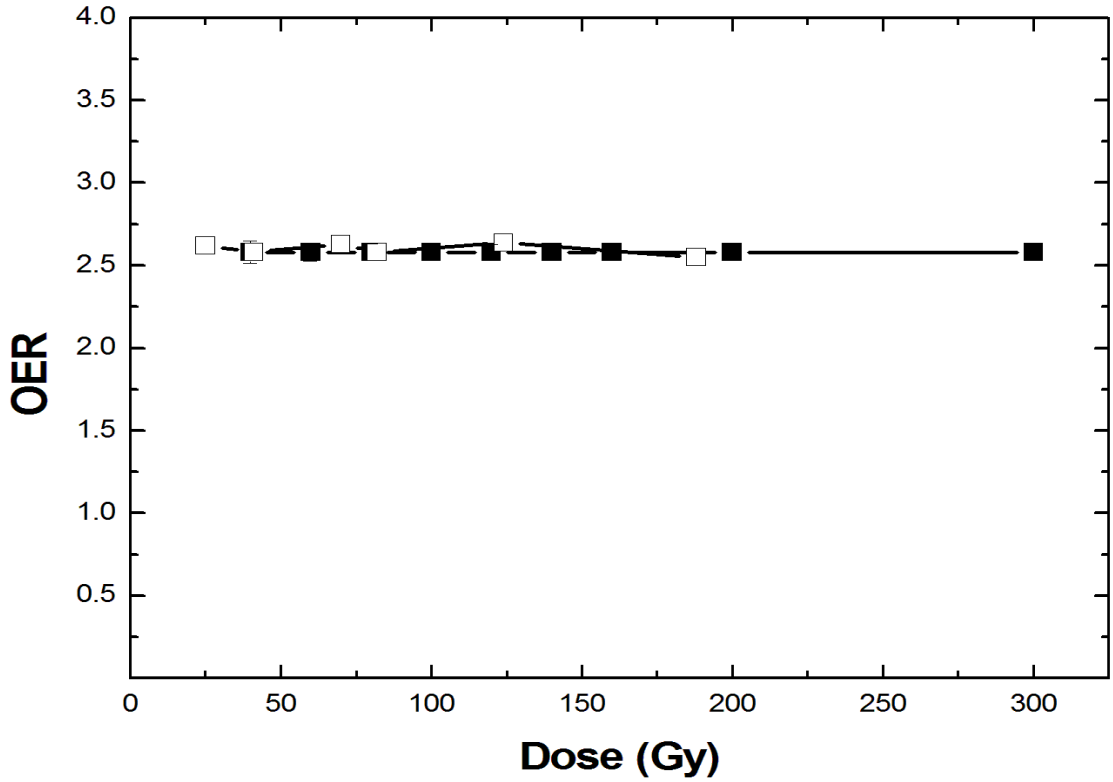


Figure 16: Variation of OER with 8 MeV electron dose for *Saccharomyces cerevisiae rad52* strain, the experimental (□) and theoretical (■) values

The experimental and theoretical OER values were compared. The experimental OER values were extracted from Figures 1- 3 for gamma radiation and Figures 6- 8 for electron radiation. In Figures 11 –16, it is clear that the theoretical estimated values and experimental values are well agreeing, and the experimentally measured values are within the theoretical range [Nairy *et al.,* 2014]. The model gives accurate measurements at higher doses, increasing the application of the model to micro-organisms study.

The difference in the OER values between *Saccharomyces cerevisiae* X2180, D7, and *rad52* strains was observed. The possible reason may be the absence of the repair mechanism. The peroxy molecules interact with the DNA and produce SSBs, DSBs, Base damages, and DNA – DNA Cross links and DNA-Protein Cross links [Hagen and Wellstein, 1965, Meyn *et al.,* 1987, Cecchini *et al.,* 2005]. Under hypoxic condition, the formation of DNA–protein cross-links are greater in number than under euoxic irradiation condition [Meyn *et al.,* 1987]. The radio-resistance of the hypoxic cells mainly depends on the ability to repair by such cross-links, and is greater

under hypoxic condition. Most of the DNA damages were repaired either by HR or NHEJ. NHEJ is an important repair mechanism for radiation induced DSBs in mammalian cells [Polo and Jackson, 2011]. In lower eukaryotes such as yeast cells, the HR mechanism plays a major role. Oxidative lesions are mainly removed by Base Excision Repair (BER), whereas DNA-protein cross links are repaired by the Nucleotide Excision Repair (NER) mechanism. Most of the proteins involved in NER, namely, ERCC1 and XPF form a dimer, stimulating activity capable of incising DNA, 5'side of a lesion such as a bulky adduct or thymine dimer [Brookman *et al.,* 1996, Sijbers *et al*., 1996]. In addition, the HR appears to play a link between cross-link repair and hypoxic resistance, and deficiency in HR leads to a reduction in the OER [Sprong *et al.,* 2006].

In the present study, the repair deficient *rad52* and mutant D7 had lesser OER value compared to the repair proficient X2180 yeast strain. The mechanism of reduction of OER is explained by Sprong *et al.* [2006] using hamster mutants and human fibroblasts cells. In the present study, the reduction in OER was mainly due to the absence of the HR mechanism in *rad52*. The absence of the repair mechanism in *rad52* is previously explained [Resnick and Martin, 1976, Reddy *et al.,* 1981]. Since D7 is a mutant strain, reduced OER value was observed in comparison to X2180. The DNA interstrand cross-links were induced at greater numbers after hypoxic irradiation than euoxic irradiation. In X2180, the hypoxic cells were capable of efficiently removing these cross-links, while *rad52* and D7 cells failed to repair these damages, leading to increased sensitivity of the hypoxic cells. As a result, a decline in the OER value was observed as compared to *X2180*.

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