Nuclear Science and Techniques **22** (2011) 156–159

Fast neutron radiation inactivation of *Bacillus subtilis*: Absorbed dose determination

SONG Lingli* ZHENG Chun AI Zihui LI Junjie DAI Shaofeng

Institute of Nuclear Physics and Chemistry, CAEP, MianYang 621900, China

Abstract In this paper, fast neutron inactivation effects of *Bacillus subtilis* were investigated with fission fast neutrons from CFBR-II reactor of INPC (Institute of Nuclear Physics and Chemistry) and mono-energetic neutrons from the Van de Graaff accelerator at Peking University. The method for determining the absorbed dose in the *Bacillus subtilis* suspension contained in test tubes is introduced. The absorbed dose, on account of its dependence on the volume and the form of confined state, was determined by combined experiments and Monte Carlo method. Using the calculation results of absorbed dose, the fast neutron inactivation effects on *Bacillus subtilis* were studied. The survival rates and absorbed dose curve was constructed.

Key words Fast neutron, Inactivation, Bacillus Subtilis, Absorbed dose

1 Introduction

The research of biological effects and mechanisms of ionizing radiations on biology cells are important to radiology, radicidation, radiation dosimetry, radiation biology and inactivation of bioterrorism agents[1-5]. Single-celled microorganisms are widely employed in studying radiation damage mechanisms and effects of ionization radiations. Most of the researches use X-rays, γ-rays or charged particles, and only a few researches were done with neutron beams[6-16].

However, the investigations using neutron beams often placed emphasis on survival rate testing method, D10 value and the inactivation curve, instead of the effect of the irradiation conditions, such as the scattered neutrons around the samples, which is an important factor to the absorbed dose. Because of the neutron dosimetry uncertainties, the dose-survival rate curves might be quite different, being far from their application in neutron therapy or medical sterilization.

To evaluate the biological effect caused by neutrons, the absorbed doses in this work were determined by combining the experimental conditions with Monte Carlo simulation on account of the dependence on the volume and the form of confined state. From the simulation, the deposited energies in the test tubes were obtained and converted to absorbed doses.

2 Exposure conditions

2.1 Neutron irradiation facility

The fast neutron sources are at Chinese Fast Burst Reactor II (CFBR-II) of INPC (Institute of Nuclear Physics and Chemistry) and the Van de Graaff accelerator is at Peking University. The neutron energy of CFBR-II is averaged at 1.12 MeV. The Van de Graaff provides mono-energetic neutrons of 4 or 6 MeV.

2.2 Radiation exposure

The *Bacillus subtilis* samples were contained in polythene test tubes of 1-cm inner diameter and 0.075-cm wall. The samples were placed 20 to 30 cm away from the core of CFBR-II reactor, which was operated in pulse mode to irradiate the samples with three fluxes: 1.61×10^{13}, 1.25×10^{13} and 0.72×10^{13} cm^{-2}.

Supported by Science and Technology Foundation of China Academy of Engineering Physics (No.2008A0103002)

* Corresponding author. *E-mail address:* Lingli_Song@163.com

Received date: 2011-02-28

The samples to be irradiated with the 4 or 6 MeV neutrons were placed 1 cm to 20 cm away from the source target of the accelerator. The fluxes at the sample spot were 3.32×10^{12}, 1.87×10^{12}, 1.48×10^{12} and 0.99×10^{12} cm^{-2} for 4 MeV neutrons and 2.33×10^{12}, 1.56×10^{12}, 0.96×10^{12} and 0.78×10^{12} cm^{-2} for 6 MeV neutrons. Temperature of the irradiation room was controlled. The sham group was kept at 16°C. Each treatment had three repetitions.

2.3 Treatment of samples

The *Bacillus subtilis* var. niger spore suspension samples were prepared by standard method[15] and concentration of the suspension was 10^7 cfu·mL^{-1}. The irradiated samples were stored in a refrigerator before counting their survival rate using the plate-count method. The count uncertainty of a sample is within 5%.

3 Results

3.1 Survival rate

Table 1 shows the plate-count results of spores irradiated by fast neutrons to different fluxes.

Table 1 Survival rates of *Bacillus subtilis* spores irradiated by fast neutrons.

Neutron energy /MeV	Neutron flux /$10^{12}$$cm^{-2}$	Survival rate
1.2 (CFBR-II)	16.10	0.130
	12.50	0.179
	7.18	0.235
	3.32	0.379
4 (Van de Graaff)	1.87	0.505
	1.48	0.579
	0.99	0.634
6 (Van de Graaff)	2.33	0.367
	1.56	0.518
	0.96	0.693
	0.78	0.752

3.2 M-C simulation of energy deposited in test tube containing water

For characterizing the sterilization status with the dose-survival rate curve, the neutron energy deposition in the test tubes containing water was simulated with the MCNP code. The Monte Carlo simulation model was a test tube containing water of Φ1 cm×1 cm in volume, which was irradiated by neutron beams of 1.12, 4 or 6 MeV from a neutron source 30 cm away. The neutron beam incidence was perpendicular to the axis of the test tube.

In the experiments the spore suspension in the test tubes was in concentration of 10^7 cfu·mL^{-1}. The average volume of *Bacillus subtilis* spores is about Φ0.2μm×2μm. Then the volume fraction of *Bacillus subtilis* spores in the suspension was about 10^{-7} to 10^{-6}, which means that the simulation can be performed with just water. Strictly, the neutron beams should be in the same solid angle as the experiments, but comparing the source and sample dimension with the source- sample distance, the incidence can be simplified to parallel beams.

The energies deposited in unit mass of water for unit flux of incident neutrons (MeV·g^{-1}·n^{-1}·cm^{-2}) were calculated as follows: 1.12 MeV, 0.126; 4 MeV, 0.274; and 6 MeV, 0.318.

3.3 Dose effect on the spore survival rate

Using the simulation results, the energies deposited in the test tubes by neutrons of 1.12, 4 and 6 MeV in different fluxes were calculated and converted to absorbed dose (Table 2). And the relationship of the survival rate of *Bacillus subtilis* spores (in logarithm) and neutron dose is illustrated in Fig.1. Converting the absorbed dose into Gy, using the method in Refs.[15,16] ,the relation between Log survivors (y value) and absorbed dose (x value, Gy) is fitted by the linear function

$$\log y = -0.07693 - 0.00266x \quad (R^2=0.9365)$$

From the fitted result, the absorbed dose D10 value is 376 Gy.

Table 2 Simulated dose and spores survival rate

Neutron energy /MeV	Energy deposited /10^{12} MeV·g^{-1}	Absorbed dose / Gy	Survival rate of spores
1.12	2.0286	324.8	0.130
	1.575	252.8	0.179
	0.90468	144.8	0.235
4	0.90968	145.5	0.379
	0.51238	81.98	0.505
	0.40552	64.88	0.579
	0.27098	43.36	0.634
6	0.74094	118.6	0.367
	0.49608	79.38	0.518
	0.30496	48.80	0.693
	0.24709	39.54	0.752

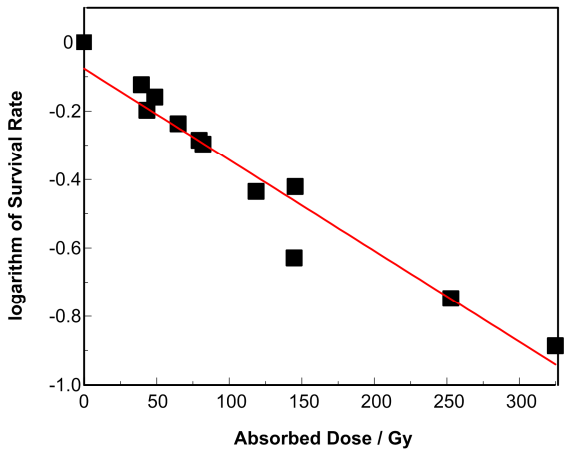

Fig.1 Survival rate and absorbed dose curve.

4 Discussion

Neutrons interact with materials *via* elastic scattering, inelastic scattering, absorption etc., depending on both neutron energy and nucleus mass of the target. A cell contains mainly water and proteins (approximately 90% of the total weight), and the elements are mostly carbon, hydrogen, oxygen and nitrogen. Although characteristics of neutron interaction with different atoms are quite clear, the neutron behaviors in cells are more complicated than in a compound of the same composition as the cell, because the deposited energy is related not only to the elements but also to their distribution and form of existence.

In the literatures of neutron inactivation, the irradiation conditions differ in the energy spectrum, the dosimetry and the sample state (e.g. dry or wet, and nature of the solution). It is hard to decide which factor is the most important one in calculating the absorbed dose, and the uncertainty of survival test method may lead to different dose-survival curves. In this study, we were trying to determine the absorbed dose in small volume samples by combined experiment and M-C simulation, as the first step to understand the interaction mechanisms of neutron irradiating cells.

Acknowledgments

We thank the teams of CFBR-II and the Van de Graaff accelerator at Peking University for their support. We thank also Dr. Chen Xiaoming of Southwest University of Science and Technology for her help in sample preparation and survival rate testing of microbes.

References

1 Lloyd D C, Purrott R J, Dolphin G W, *et al.* Int J Radiat Biol Relat Stud Phys Chem Med, 1976, **29:** 169−182.

2 Nakamura S, Watanabe H, Miura M, *et al.* Exp Cell Res, 1997, **235:** 287–294.

3 Maughan R L, Yudelev M, Kota C, *et al.* Nucl Phys A, 1999, **654:** 1068c−1071c.

4 Weinmann M, Hoffmann W, Todeferdts E, *et al.* J Cancer Res Clin Oncol, 2000, **126:** 584−588.

5 MKr¨amer1, Scholz M. Phys Med Biol, 2006, **51:** 1959–1970.

6 Yonezawa M, Misonoh J, Hosokawa Y. Mutation Res, 1996, **358:** 237−243.

7 Pang D, Popescu G, Rodgers J, *et al.* Scanning Microsc, 1996, **10:** 1105−1110.

8 Murakami M, Hirokawa H, Hayata I. J Biochem. Biophys Methods, 2000, **44:** 31−40.

9 Tanaka K, Gajendiran N, Endo S, *et al.* J Radiat Res, 1999, **40:** 36−44.

10 Pang D, Berman B L, Chasovskikh S, *et al.* Radiat Res, 1998, **150:** 612−618.

11 Hardell L, Sage C. Biomed Pharmacotherapy, 2008, **62:** 104−109.

12 Manti L, D'Arco A. Mutation Res, 2010, **704:** 115–122.

13 Lowy R J, Vavrina G A, Labarre D D. Antiviral Res, 2001, **52:** 261−273.

14 Gazsó L G, Ponta C C. Radiation inactivation of bioterrorism agents. Netherlands: IOS Press, 2005, 153−160.

15 Chen X M, Tan B S, Zhang J G, *et al.* J Radiat Radiat Process, 2007, **25:** 166−170.

16 Chen X M, Ren Z L, Zhang J G, *et al.* Plasma Sci Technol, 2009, **11:** 368−373.