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INFLUENCE OF GAMMA RADIATION ON ETHANOL PRODUCTION FROM YEAST

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The effect of up to 6000 Gray (Gy; 1 Gy = 1 J/k) ^{60}Co gamma irradiation on the fermentative capacity of two strains of yeast cells is reported. Ethanol production by the irradiated cells was unchanged for both strains at 3000 Gy and reduced 43% for only one strain at 6000 Gy in spite of a marked decrease in viability at higher doses (2-8% at 3000 Gy and 0.01% at 6000 Gy). These results suggest that the yeast fermentation system for converting sugar to alcohol is a relatively radioresistant process and not inhibited by the stable by-products produced during irradiation. Furthermore, these data indicate that radiation polymerization for immobilizing these cells should not interfere with their fermentation capacity.

Key words: ethanol production, gamma irradiation, yeast cells, radioresistance.

Among the advantages of using immobilized whole cells in continuous fermentation is that of higher production yields, with a complementary reduction in cost. Immobilized cell technology has the disadvantage that the bound microorganisms are unable to carry out fermentation processes involving coenzymes in multiple pathways (1).

The immobilization of enzymes or microbial cells by radiation polymerization has been the subject of recent studies (2,3), but the effect(s) of the conditions used for each step of the immobilization process, such as irradiation, on the enzyme activities of immobilized cells has not been analyzed in detail.

Precultured yeast cells can be immobilized on an acrylic polymeric matrix by irradiation with 500 Gy of ^{60}Co at a dose rate of 21.6 Gy/min. At this dose, neither enzyme activity nor cell viability seems to be affected by radiation (4).

The objective of the present study was to evaluate the modifications of the fermentative capacity when the viability of yeast cells declines as a function of irradiation dose. An overnight culture of *S. cerevisiae* cells (two strains selected from the collection of Escola Superior de Agricultura Luiz de Queiroz, São Paulo) grown in 1% bacto-yeast extract, 2% bacto-peptone, 2% dextrose (denoted YPD) was washed twice and resuspended in saline. Cell suspensions (2 x 10^8 cells/ml) were divided into 3-ml samples and irradiated in glass tubes in a Gammacell 220 Irradiation Unit (Atomic Energy of Canada Ltd.) at a dose rate of 20 Gy/min. Direct cell counts were made with a Neubauer camera mounted on a Zeiss

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microscope. For the evaluation of viability, ten-fold serial dilutions were made in sterile saline. From appropriate dilutions, 0.19 ml was added to the surface of 20 ml of the solidified YPD agar plating medium in each of two petri dishes and incubated at room temperature until colonies appeared (about 48 h). Viability was considered as the ability of microorganisms to proliferate. For ethanol production assays, cells kept at 5°C for 1 week after irradiation were centrifuged at 3000 rpm for 10 min and resuspended in 3 ml nutrient medium consisting of 11.5% glucose, 1% bacto-yeast extract, and 2% bacto-peptone. After a 1-h fermentation period at about 23°C, the cells were centrifuged and 0.05 ml of the supernatant was assayed for the presence of ethanol by the alcohol dehydrogenase (ADH)/nicotinamide dinucleotide method. The absorbance at 340 nm was recorded and the ethanol production reported as g EtOH l^{-1} h^{-1}.

Figure 1 shows the dose-effect curves for ^{60}Co gamma-radiation on two strains of yeast cells designated I and II, as determined by immediate plating. Strain II seemed to be more radiosensitive than strain I under the conditions used. A dose rate of 20 Gy/min was too high to allow repair of damage during irradiation. At a total dose of 1500 Gy, survival for strains I and II was 20.4 and 11.2%, respectively. We assume that the inactivation of cells occurred due to the accumulation of both reparable and irreparable damage. No dose rate effect has been reported by others above the high dose rate value (20 Gy/min) used here (5).

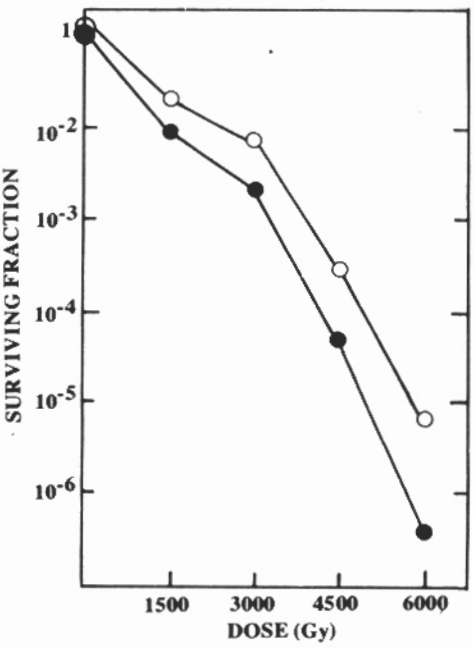

Figure 1 - ^{60}Co gamma radiation survival curves for yeast cells. The dose rate was 20 Gy/min to cells suspended in saline. ●—●, Strain I; ○—○, strain II.

Table 1 - *Effect of gamma radiation on yeast ethanol production.*

Cells were irradiated at 20 Gy/min and stored at 5°C for 1 week before measurements. Results are reported as EtOH l^{-1} h^{-1} produced by cells incubated at 23°C without shaking. Data are the mean of two different irradiation samples.

Cumulative dose (Gy)	Strain I	Strain II
0	1.29 ± 0.05	1.24 ± 0.08
1500	1.17 ± 0.04	1.14 ± 0.06
3000	1.15 ± 0.01	1.12 ± 0.12
4500	1.18 ± 0.03	0.95 ± 0.14
6000	1.18 ± 0.03	0.72 ± 0.04

Table 1 shows the ethanol yield of the irradiated cells. The amount of ethanol produced for the different doses was unchanged in strain I, thus showing no influence of radiation on the fermentative capacity of the cells. Only a slight decrease occurred in the radiosensitive strain.

The present results show that the ethanol production by fermenting yeast is not

significantly affected by irradiation. No inhibition by relatively stable by-products formed during irradiation was demonstrable. Therefore, radiation polymerization appears to be an effective and useful technique for inducing immobilization of yeast cells.

References

1. McGhee, E.J., Julian, G. St., Detroy, R.W. and Bothast, R.J. (1982). *Biotechnology and Bioengineering*, 24: 1155-1163. **2.** Fujimura, T. and Kaetsu, I. (1983). *International Journal of Applied Radiation and Isotopes*, 34: 929-931. **3.** Higa, O.Z., del Mastro, N.L. and Castagnet, A.C. (1986). *Radiation and Physical Chemistry*, 27: 311-316. **4.** del Mastro, N.L. and Higa, O.Z. (1986). *Arquivos de Biologia e Tecnologia*, 29: 71. **5.** Frankenberg, D. (1979). *International Journal of Radiation and Biology*, 36: 317-324.

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