

## Effect of Nitrogen Gas, Irradiated with a Beta-Ray Source, on Nitrogen Fixation in Continuous Cultures of *Azotobacter vinelandii*

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Negative and positive gas ions have been found in nitrogen gas, irradiated with a  $^{90}\text{Sr}$  source. An increased nitrogen fixation in *Azotobacter vinelandii* supplied with positive gas ions has been detected, compared to cells fed with non-irradiated gas or negative gas ions. The effect was found to be one of a decreased glucose consumption.

An increase in nitrogen fixation in batch cultures and in continuous cultures of *Azotobacter* supplied with ultraviolet irradiated nitrogen gas, compared to cultures fed with non-irradiated gas, has been shown previously.<sup>1,2</sup> This increase was due to a decrease in glucose consumption.<sup>2</sup> The ultraviolet irradiated nitrogen gas could be shown to contain positive and negative gas ions.<sup>3,4</sup> The increase in bacterial nitrogen fixation was found to be caused by positive gas ions.<sup>4</sup> In this paper some experiments are reported where the ultraviolet irradiated nitrogen gas was substituted for nitrogen irradiated by a beta-emitting isotope.

### MATERIALS AND METHODS

A cultivation vessel with all accessory equipment, similar to the one described by Zacharias,<sup>2</sup> was used. The ultraviolet lamp and lamphousing was, however, replaced by the equipment shown in Fig. 1. It consists of a two-way stopcock, E, an irradiation source, A, and a stainless steel tube, B. At C (Fig. 1) the equipment was connected to the nitrogen supply and at D to the capillary tube of the culture vessel. When the stop-cock is turned, as shown in Fig. 1, the gas bypasses the irradiation source, A. If the stop-cock is turned 180 degrees, the gas will pass the irradiation source, A, and flow through the stainless steel tube, B, into the culture vessel.

The stainless steel tube could be electrically charged positive or negative as described previously.<sup>2</sup> In this manner the culture could be supplied with non-irradiated or irradiated gas and with positive or negative gas ions.

The irradiation source was shielded by a steel tube with a wall thickness of 0.8 mm (not shown in Fig. 1).

Sterilization and the aseptical assembly of the equipment, the inoculation and cultivation of the bacteria has been described by Zacharias.<sup>2</sup> The equipment shown in Fig. 1

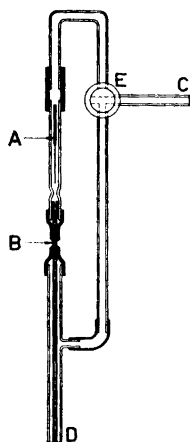


Fig. 1. The equipment used for irradiation of nitrogen gas.

- A = Irradiation source ( $^{90}\text{Sr}$ ).
- B = Stainless steel tube.
- C = Entrance of nitrogen gas.
- D = Exit of nitrogen gas.
- E = Two-way stopcock.

was, however, sterilized separately as one unit and aseptically connected to the nitrogen gas supply and the culture vessel. The organism used was *Azotobacter vinelandii* strain ATCC 7492. Measurements of gas ions were performed as described previously.<sup>3</sup> The beta-ray source used was a strontium-90-foil, delivered in strips enclosed in glass vials with a wall thickness of less than 0.5 mm (The Radiochemical Centre, Amersham, Buckinghamshire, England). The medium, the sampling technique, the analysis methods used and the calculations have all been described previously.<sup>3</sup>

## RESULTS

The production of gas ions in nitrogen gas irradiated with a  $200\ \mu\text{C}\ ^{90}\text{Sr}$  source at different gas flows is shown in Fig. 2. Both negative and positive ions are produced, the latter being in greater quantities than the former. No gas ions could be detected using a  $20\ \mu\text{C}\ ^{90}\text{Sr}$  source.

Fig. 3 shows the effect of beta-irradiated and non-separated nitrogen gas (Section 2), positive gas ions (Section 4), and negative gas ions (Section 6), on

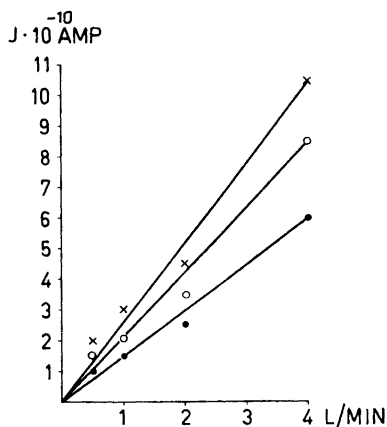


Fig. 2. Production of gas ions in nitrogen gas irradiated with beta-rays at different flowrates of the gas.

- Voltage = 550 V.
- × = Non-separated gas ions.
- = Negative gas ions.
- = Positive gas ions.
- The beta ray source used was silver foils, plated with  $200\ \mu\text{C}\ ^{90}\text{Sr}$ .

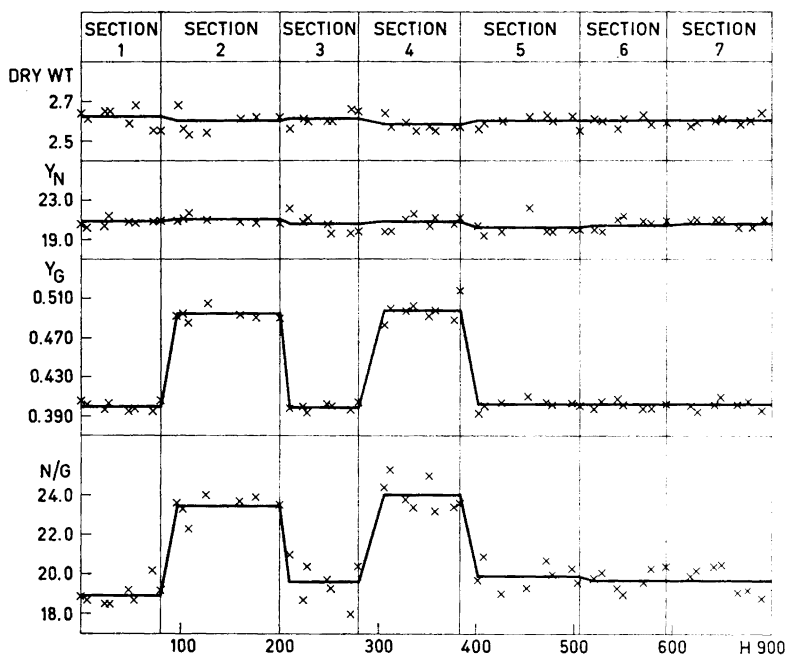


Fig. 3. Effect of beta-irradiated nitrogen gas, Sections 2, 4, 6, on *Azotobacter vinelandii*, strain ATCC 7492, compared to organisms fed with non-irradiated gas, Sections 1, 3, 5, 7. Each mark is a mean value of four samples collected from two experiments.

Sections 1, 3, 5, 7 = Non-irradiated and non-separated nitrogen gas.

Section 2 = Irradiated and non-separated nitrogen gas.

Section 4 = Positive gas ions.

Section 6 = Negative gas ions.

Dry wt. = dry weight expressed in g/litre.

$Y_N$  = Nitrogen yield expressed in g bacteria/g nitrogen fixed.

$Y_G$  = Glucose yield expressed in g bacteria/g glucose consumed.

N/G = Nitrogen fixation expressed in mg nitrogen fixed/g glucose consumed.

Dilution rate =  $0.275 \pm 0.006 \text{ h}^{-1}$ .

Air = 300 mM  $\text{O}_2$ /litre/h.

Nitrogen gas = 0.5 litre/min.

The irradiation source was  $200 \mu\text{C } ^{90}\text{Sr}$  plated on silver strips.

dry weight, glucose yield, nitrogen yield and nitrogen fixation in *Azotobacter vinelandii*, strain ATCC 7492, compared to bacteria supplied with non-irradiated and non-separated nitrogen gas (Sections 1, 3, 5, 7). The glucose yield and nitrogen fixation increased when the bacteria were supplied with irradiated but non-separated nitrogen gas and with positive gas ions. The dry weight and the nitrogen yield did not change throughout the experiment.

When the  $20 \mu\text{C } ^{90}\text{Sr}$  source was used no changes in any of the determined entities could be detected, compared to conditions when no irradiation source was used. The measured values were as follows: 2.5 g bacteria per litre for dry weight, 0.405 g bacteria per g glucose consumed for glucose yield, 20.3 g bacteria

Table 1. Mean values of the results presented in Fig. 3.

The  $\pm$  sign indicates sample standard error.

Dry weight is expressed in g bacteria/litre.

$Y_G$  = glucose yield and is expressed in g bacteria/g glucose consumed.

$Y_N$  = nitrogen yield and is expressed in g bacteria/g nitrogen fixed.

N/G = nitrogen fixation expressed in mg nitrogen fixed /g glucose consumed.

Section	Dry weight	$Y_G$	$Y_N$	N/G
1	2.62 $\pm$ 0.02	0.401 $\pm$ 0.002	20.1 $\pm$ 0.1	18.9 $\pm$ 0.2
2	2.60 $\pm$ 0.02	0.492 $\pm$ 0.002	21.0 $\pm$ 0.1	23.5 $\pm$ 0.2
3	2.61 $\pm$ 0.01	0.400 $\pm$ 0.001	20.5 $\pm$ 0.4	19.6 $\pm$ 0.4
4	2.58 $\pm$ 0.01	0.498 $\pm$ 0.003	20.7 $\pm$ 0.2	24.0 $\pm$ 0.3
5	2.60 $\pm$ 0.01	0.402 $\pm$ 0.002	20.2 $\pm$ 0.3	19.9 $\pm$ 0.3
6	2.60 $\pm$ 0.01	0.402 $\pm$ 0.002	20.3 $\pm$ 0.3	19.8 $\pm$ 0.2
7	2.60 $\pm$ 0.01	0.402 $\pm$ 0.002	20.5 $\pm$ 0.3	19.8 $\pm$ 0.2

per g nitrogen fixed for nitrogen yield, 19.9 g nitrogen fixed per g glucose consumed for nitrogen fixation. Table 1 shows the mean values and sample standard errors for the results presented in Fig. 3.

## DISCUSSION

An increase in nitrogen fixation due to a decrease in glucose consumption by *Azotobacter vinelandii* supplied with ultraviolet irradiated nitrogen gas, compared to organisms fed with non-irradiated gas, has been reported.<sup>2</sup> This effect could be correlated to an increase of gas ions in irradiated nitrogen gas as compared to non-irradiated gas.<sup>3,4</sup>

The production of air ions with rays from a radioactive source is well documented.<sup>5</sup> The common beta-ray source used today is <sup>3</sup>H with an energy of 18 keV and a half-life of 12.5 years. The source used here was <sup>90</sup>Sr with an energy of 61 keV and a half-life of 25 years. The <sup>90</sup>Sr source was chosen partly because of the higher energy output and partly because of the longer half-life. The effect of the <sup>90</sup>Sr source on the nitrogen gas gives principally the same result as the one found for ultraviolet light. The possible mechanism or mechanisms for the biological effect of nitrogen gas ions on the nitrogen fixation by *Azotobacter* cells has been discussed previously.<sup>4</sup> Some preliminary experiments indicate a decreased hydrogenase activity in cells supplied with beta-ray irradiated nitrogen, compared to cells fed with non-irradiated gas.<sup>6</sup> No change in malic dehydrogenase and succinic dehydrogenase could be detected in these experiments.<sup>6</sup>

Wilson and Burris have pointed out that the energy used to fix nitrogen is so small that it would probably be undetectable in ordinary experiments.<sup>7</sup> Roberts, on the other hand, is of the opinion that a rather large amount of

energy is needed in nitrogen fixation.<sup>8</sup> His opinion is supported by McNary and Burris who claim "an apparent high energy phosphate requirement for N<sub>2</sub> fixation in the *C. pasteurianum* system".<sup>9</sup> Senez has stated that a mechanism which would enable the cell to control the energy production by its utilization has not yet been found in growing bacteria.<sup>10</sup> Such a mechanism might be at work in nitrogen fixation. When the bacteria are supplied with non-irradiated nitrogen, the energy needed for fixation is larger than when they are fed with positive nitrogen gas ions. The hydrogen needed for this energy production might be produced by a special mechanism involving hydrogenase, as the tri-carboxylic cycle seems to be indifferent to this energy demand.

Further experiments must be performed in order to provide all the details for this theory.

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