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Detection of Positive and Negative Gas Ions in Ultraviolet Irradiated Nitrogen Gas and Their Effect on Nitrogen Fixation in Continuous Cultures of *Azotobacter vinelandii*

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Positive and negative gas ions have been found in ultraviolet irradiated nitrogen gas. An increased nitrogen fixation in continuous cultures of *Azotobacter vinelandii* supplied with positive gas ions has been detected compared to cultures fed with non-irradiated nitrogen gas. Negative gas ions did not have any effect on nitrogen fixation.

An increase in nitrogen fixation due to a decreased glucose consumption in cultures of *Azotobacter* supplied with ultraviolet irradiated nitrogen gas, compared to cultures fed with non-irradiated gas, has been reported previously.[1] A correlation between this increase and an increase in gas ions in irradiated nitrogen, as compared to non-irradiated nitrogen gas, has also been shown.[2]

In this paper a report is given on the effect of negatively and positively charged gas ions in nitrogen gas in continuous cultures of *Azotobacter vinelandii*, strain ATCC 7492.

MATERIALS AND METHODS

The culture vessel with all accessory equipment has been described previously.[1] The stainless steel tube between the ultraviolet lamp and the culture vessel could be connected to one of the poles of a 550 V battery. The other pole was earthed. In this way the gas ions produced by the ultraviolet irradiation of the nitrogen gas could be separated into negative or positive ions, which were in turn fed to the culture. The detection of the gas ions has been described by Zacharias.[2] A Philips' TUV 6W germicidal lamp was used as the irradiation source. The sampling technique and the analysis methods have been reported previously.[2,3] The glucose yield and nitrogen yield (the amount of bacteria propagated from one g glucose and one g nitrogen, respectively) were calculated from the results of the analysis and the dry weight determinations.[1,2] The nitrogen fixation was calculated from the analysis and expressed as mg nitrogen fixed per g glucose consumed. Measurements of the voltage, produced by gas ions induced by ultraviolet irradiation of nitrogen gas, were recalculated in amperes.

The continuous cultivation of the bacteria, *Azotobacter vinelandii*, strain ATCC 7492, was performed as described by Zacharias.[1]

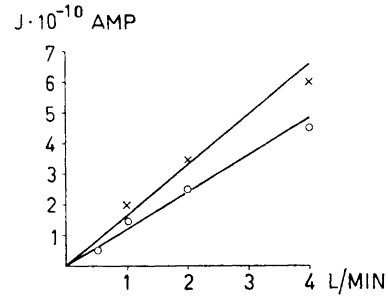

Fig. 1. Production of positive and negative gas ions in ultraviolet irradiated nitrogen gas at different flowrates of the gas. Voltage = 550 V. × = negative ions; O = positive ions. The ultraviolet source used was a Philips' germicidal lamp, marked TUV 6W.

RESULTS

Fig. 1 shows that negative and positive gas ions are produced in nitrogen gas irradiated with ultraviolet light. The amount of negative ions is greater than the amount of positive ions.

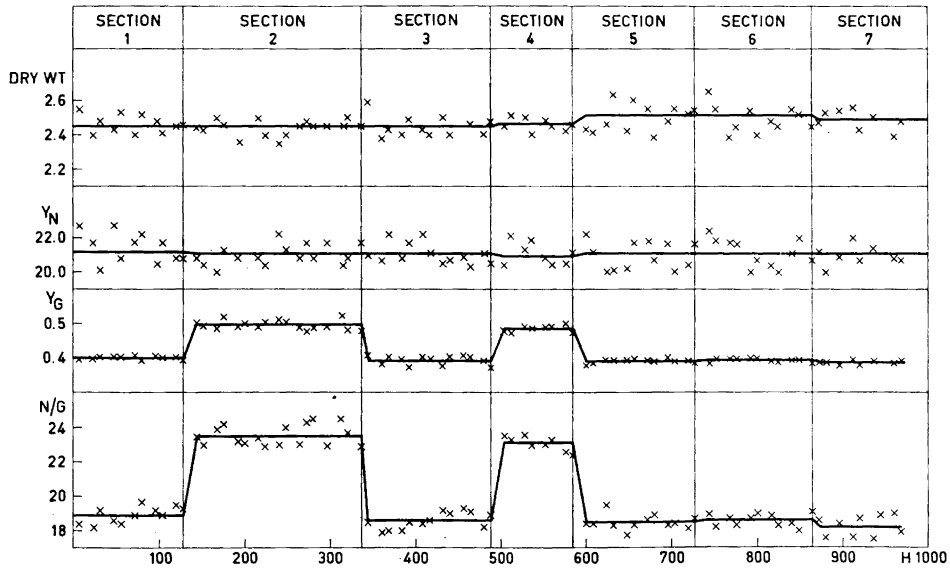

Fig. 2. Effect of ultraviolet irradiated nitrogen gas, Sections 2,4,6, on *Azotobacter vinelandii*, strain ATCC 7492, compared to organisms grown on non-irradiated gas, Sections 1, 3, 5, 7. Each mark is a mean value of four samples taken from two different experiments. Sections 1, 3, 5, 7 = Non-irradiated and non-separated nitrogen gas. Section 2 = Irradiated and non-separated nitrogen gas. Section 4 = Positive nitrogen gas ions. Section 6 = Negative nitrogen gas ions. Dry wt. = Dry weight expressed in g bacteria/litre. Y_N = Nitrogen yield expressed in g bacteria/g nitrogen. Y_G = Glucose yield expressed in g bacteria/g glucose. N/G = Nitrogen fixation expressed in mg nitrogen fixed/g glucose.
Dilution rate = 0.299 ± 0.008 h^{-1}. Air = 300 mM O_2/l/h. N_2 = 0.5 litre/min. The ultraviolet source was a Philips' TUV 6W germicidal lamp.

Table 1. Mean values calculated from the determinations shown in Fig. 2.

The ± sign indicates sample standard error.
Dry wt. = dry weight expressed in g/litre.
Y_G = glucose yield expressed in g bacteria/g glucose
Y_N = nitrogen yield expressed in g bacteria/g nitrogen
N/G = nitrogen fixation expressed in mg N/g glucose

Section	Dry weight	Y_G	Y_N	N/G
1	2.45 ± 0.02	0.400 ± 0.001	21.2 ± 0.3	18.9 ± 0.2
2	2.45 ± 0.02	0.496 ± 0.003	21.1 ± 0.2	23.5 ± 0.2
3	2.45 ± 0.07	0.392 ± 0.004	21.1 ± 0.2	18.6 ± 0.1
4	2.48 ± 0.05	0.483 ± 0.004	20.9 ± 0.1	23.1 ± 0.1
5	2.51 ± 0.07	0.388 ± 0.002	21.1 ± 0.3	18.5 ± 0.1
6	2.51 ± 0.04	0.392 ± 0.002	21.1 ± 0.3	18.6 ± 0.1
7	2.49 ± 0.06	0.388 ± 0.002	21.1 ± 0.3	18.2 ± 0.2

The dry weight, the glucose yield, the nitrogen yield and the nitrogen fixation in continuous cultures of *Azotobacter vinelandii*, strain ATCC 7492 supplied with non-irradiated nitrogen gas are recorded in Fig. 2, (Sections 1, 3, 5, 7). When the cultures are fed with irradiated but non-separated gas (Fig. 2, Section 2), the glucose yield and nitrogen fixation are increased. The same effect was also recorded for cultures supplied with positive gas ions (Fig. 2, Section 4). Negative gas ions did not have any effect (Fig. 2, Section 6). No significant change could be detected in the dry weight or the nitrogen yield throughout the experiments.

Each point is the mean value of four samples collected from two experiments. Table 1 shows the mean values and the sample standard errors of the different sections recorded in Fig. 2.

DISCUSSION

The first steps in nitrogen fixation are unknown. Some information has been gained from cell free systems with the help of N—15.[6–8] The results of these experiments support the theory that the nitrogen molecule is fixed by an enzyme and reduced by hydrogen to ammonia.[9] Wilson and Burris assumed that only nitrogen molecules with an energy higher than some unknown critical value could be fixed.[10] Virtanen introduced the concept of an ionized nitrogen molecule.[11] An increase in nitrogen fixation in *Azotobacter* cultures supplied with ultraviolet irradiated nitrogen gas compared to cultures fed with non-irradiated gas has been shown previously.[1–3] Using continuous culture technique it could be shown that this increase was due to a decrease in glucose consumption.[1] It has also been shown that ultraviolet irradiated nitrogen gas contains an increased amount of gas ions.[3] The increase in nitrogen fixation due to a decrease in glucose consumption by *Azotobacter* has been correlated to the amount of gas ions produced by irradiation of the nitrogen gas.[3]

In this paper it is shown that both negative and positive gas ions are produced in ultraviolet irradiated nitrogen gas. A direct ionization of the

nitrogen gas is impossible.[4] Photoelectrons can, however, be released from the glass walls of the lamp housing.[4] These electrons are known to combine with gas molecules or condensation bodies and in this way produce negative gas ions, so called cluster ions and Langevins' ions.[4,5]

The production of positive gas ions can be explained by a photoeffect in liquid condensation bodies.[4] A release of an electron leaves the particle positively charged. As the negative ions are produced in two ways and the positive in only one way, the amount of negative ions ought to be greater than the amount of positive ions. Experimental data support this theory.[4]

The gas ions reaching the culture can either react with some component in the media or be absorbed directly by the bacteria. Assuming the former possibility, a new chemical substance is produced containing more nitrogen than before. This substance is then absorbed and metabolised by the organisms. In the latter possibility, the ions might be stabilized, *e.g.* by gathering a protective shell of water molecules, and in this way react directly with the cells.

The bacterial cell surface is assumed to be negatively charged.[12] This might provide an explanation for the effectivity of positive gas ions. On the other hand, enzymes controlling the permeability or transport through the cytoplasmic membrane might allow only the positive ions to pass. A third possibility is that the cell is permeable to both negative and positive ions but that only the positive ions can react with enzymes involved in nitrogen fixation.

It seems reasonable to assume that the energy-rich nitrogen gas ions react more readily with the nitrogen fixing system than do nitrogen gas molecules. This might imply that less energy is needed for nitrogen fixation when gas ions are used, with a lower glucose consumption as a result. Experimental data support this theory.

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