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Detection of Gas Ions in Ultraviolet Irradiated Nitrogen Gas and their Effect on Nitrogen Fixation in Batch Cultures of Different Species and Strains of the Genus *Azotobacter*

BENGT ZACHARIAS

Department of Bacteriology, Karolinska Institutet, Stockholm 60, Sweden

An increase of gas ions in UV-irradiated commercial nitrogen gas, as compared to non-irradiated gas, has been noted. This increase could be correlated to an increased nitrogen fixation in different species and strains of the genus *Azotobacter*.

Some authors have proposed that the nitrogen gas molecules fixed by *Azotobacter* are in a state of activation [1]. Other authors have considered this state of activation to consist of ionized nitrogen gas molecules [2–4]. It has been shown previously that, in an *Azotobacter* culture, the ratio, mg nitrogen fixed per g glucose consumed, is increased when the culture is fed with ultraviolet irradiated nitrogen gas, as compared to cultures fed with non-irradiated gas [3,4].

In this paper, some experiments are reported designed to show an increase of gas ions in ultraviolet irradiated nitrogen gas compared to non-irradiated gas. It is also shown that the effect of ultraviolet irradiated nitrogen gas on nitrogen fixation is a general effect among most of the *Azotobacter* species.

EXPERIMENTAL

Organisms. The organisms used were *Azotobacter vinelandii* strains ATCC 7492; ATCC 9046 and 0; *Azotobacter agilis*, strain ATCC 9040; *Azotobacter chroococcum* strain ATCC 9049; *Azotobacter macrocytogenes*, strain NCIB 8700.

Methods. The bacteria were cultivated as described elsewhere [4]. Separate sparger tubes were used for air and nitrogen. Each strain was run in two different experiments. A portion of the culture was centrifuged in a refrigerated centrifuge (Servall) at 4000 *g* for 20 min. The supernatant was analysed for glucose, using a method described by Hultman, Redei and Nagy [5,6]. The cell mass was washed twice with distilled water and dry weight determinations were carried out by drying the cells at 105°C.

The dried cell mass was then analysed for nitrogen by the Dumas method using a Coleman Nitrogen Analyzer, model 29. The gas ions in the nitrogen gas were collected in an ionization chamber described by Karmen *et al.*[7] The ions collected were measured

according to Krueger *et al.*[8] with the help of a Vibrating Reed Electrometer, type N 616 B (Ekco Electronics Ltd., England). The chamber was connected with one of the UV lamps at a time. The distance between the chamber and the lamp, and all the other arrangements, were identical with those of earlier cultivation experiments [4].

Gas analysis. The commercial nitrogen gas used contains no less than 99.7 % N_2 and probably no less than 99.9 % N_2 according to the information provided by the supplier (Svenska AB Gasaccumulator). The contaminants included the gases oxygen (0.03–0.04 %) and carbon dioxide (< 0.01 % and probably < 0.005 %), and water (0.05 mg/l gas).

Calculations. The amount of glucose consumed by the bacteria, the amount of nitrogen fixed and the ratio mg nitrogen fixed per mg glucose consumed, were calculated as described previously [4]. The Vibrating Reed Electrometer readings in volts were recalculated to amperes. The difference between the values in amperes when the lamp used was switched on and off was recorded.

RESULTS

In order to calibrate the ionization chamber, four liters of nitrogen gas per minute were flushed through the lamp housing and the chamber. Different potentials were applied between the chamber and earth. The differences in the measured current with different potentials are plotted in Fig. 1. The amount of current measured showed increases when the potential of the chamber was increased up to about 475 V. This means that, as the potential increases, more and more of the charges in the gas are collected in the chamber. From the potential of 475 V up to 725 V, there is no increase in the measured current, which shows that all charges in the gas are trapped and measured in the chamber. On these grounds, a potential of 550 V was chosen for further work. Fig. 2 shows the differences between the 6 W, the 15 W and the 30 W lamps when used at different flow-rates of nitrogen gas. At a flow-rate of 0.5 l nitrogen per minute, the 6 W lamp gives about 2.8 times as much current as the 15 W lamp, and the 30 W lamp gives about twice as much.

Control experiments with no flow of gas but with the lamps switched on and off, gave no recordable differences in the measured current. Table 1 shows that the percentage increase in the ratio, mg nitrogen fixed per g glucose

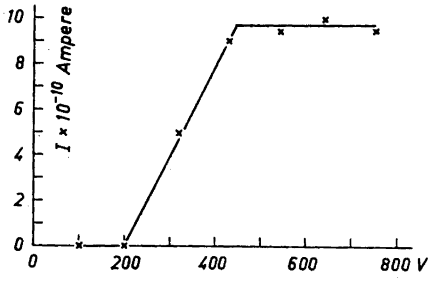

Fig. 1. Variations in measured current produced by gas ions collected in an ionization chamber, with voltage applied to the chamber. The flow of nitrogen gas = 4 l/min.

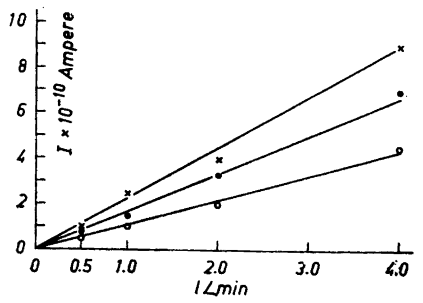

Fig. 2. Current variations as produced by gas ions generated by different UV-lamps at different flow-rates of nitrogen gas. Voltage = 550 V. × = 6 W-lamp; ● = 30 W-lamp; O = 15 W-lamp.

consumed, is about the same for *Azotobacter vinelandii*, strain 0; strain ATCC 7492; strain ATCC 9046; *Azotobacter agilis*, strain ATCC 9040; and *Azotobacter chroococcum*, strain ATCC 9049. The mean increase for these five organisms is 21.31 % for the 6 W lamp, 7.92 % for the 15 W lamp and 17.7 % for the 30 W lamp. The standard deviations for these values are ± 0.76, ± 0.68, and ± 0.69, respectively. *Azotobacter macrocytogenes*, strain NCIB 8700, shows an

Table 1. The effect of irradiated nitrogen gas on the fixation of nitrogen in cultures of different species and strains of the genus Azotobacter.

In each experiment eight cultures were run simultaneously under identical conditions except for the variance in the irradiation of the nitrogen gas. All the cultures were gassed with four liters of nitrogen gas per min and an amount of air corresponding to an oxygen transfer rate of 300 mM O_2/l/h. The nitrogen fixed by the bacteria is expressed as mg. nitrogen fixed per g glucose consumed. This ratio is here called N/G. The increase in N/G obtained in the cultures gassed with irradiated nitrogen gas is expressed in per cent of the mean value of the controls.

Species, strains	Control		Lamps								
			6 W			15 W			30 W		
	N/G	Mean value	N/G	Mean value	% increase of N/G	N/G	Mean value	% increase of N/G	N/G	Mean value	% increase of N/G
	10.3		12.8			11.4			11.9		
Azotobacter	10.4	10.35	12.3	12.55	21.26	11.0	11.20	8.21	12.5	12.20	17.87
vinelandii,	9.1		10.8			9.6			10.4		
strain O	8.9	9.00	11.0	10.90	21.11	9.8	9.70	7.78	10.6	10.50	16.67
	10.3		12.6			12.0			12.5		
Azotobacter	10.7	10.50	13.0	12.80	21.90	10.6	11.30	7.62	12.3	12.40	18.10
vinelandii,	10.2		12.4			10.8			11.9		
ATCC 7492	10.6	10.40	12.8	12.60	21.15	11.6	11.20	7.69	12.8	12.35	18.75
	13.2		15.9			14.4			15.7		
Azotobacter	13.2	13.2	16.3	16.10	21.97	14.2	14.30	8.33	15.1	15.40	16.67
vinelandii,	11.2		13.5			12.2			13.1		
ATCC 9046	11.0	11.10	13.3	13.40	20.72	11.7	11.95	7.66	12.9	13.00	17.12
	13.6		16.4			14.8			16.1		
Azotobacter	13.6	13.60	16.7	16.55	21.69	14.4	14.60	7.35	16.0	16.05	18.01
agilis,	13.2		16.4			14.1			15.8		
ATCC 9040	13.4	13.30	15.7	16.05	20.68	14.4	14.25	7.14	15.6	15.70	18.05
	11.8		14.2			12.4			13.4		
Azotobacter	11.2	11.50	14.0	14.10	22.61	12.8	12.60	9.57	13.8	13.60	18.26
chroococcum,	16.2		21.1			17.5			19.6		
ATCC 9049	16.8	16.50	18.5	19.80	20.00	18.1	17.80	7.88	19.2	19.40	17.58
	7.3		9.5			8.0			8.6		
Azotobacter	5.6	6.45	9.3	9.40	45.74	7.1	7.55	17.05	8.8	8.70	34.88
macrocytogenes,	5.0		7.0			6.1			7.0		
NCIB 8700	5.2	5.10	7.8	7.40	45.10	6.0	6.05	18.63	6.6	6.80	33.33

increase which is about double that of the other five strains. The total amount of nitrogen fixed per g glucose consumed is similar for the first five strains mentioned (Table 1), but is about half this amount for *Azotobacter macrocytogenes*, strain NCIB 8700. When the capillary tube between the 6 W lamp and the culture vessel was exchanged for a three times longer tube of the same inner diameter the per cent increase in nitrogen fixation was only 3.9.

DISCUSSION

The results reported here show that an irradiation of commercial nitrogen gas with ultraviolet light from ordinary germicidal lamps produces gas ions.

The wavelengths of the light from the lamps used are not lower than about 2200 Å and the maximum effect lies at about 2537 Å. Ionization of O_2 and N_2 cannot take place with light of longer wavelengths than 1000 Å and 800Å, respectively [9]. This means that a direct ionization of the gas is impossible. The irradiation of the gas, however, takes place in glass mantled UV-lamps where photoelectrons can easily be produced by the interaction of UV-light with the glass walls. These electrons can react with the molecules of the gas and in this manner produce gas ions. The lifetime of such ions is measured in microseconds [10]. It is therefore hardly possible that such ions can be responsible for the biological effect reported.

However, these gas ions often adsorb so-called condensation bodies on their surfaces [9]. Condensation bodies are interpreted as being small particles with dimensions greater than those of molecules. These bodies abound in air and other gases, and function as condensation sites for water vapour. When the gas ions and condensation bodies collide, they unite to form a charged particle with a mass many times greater than that of ordinary gas ions and with a lifetime of about two minutes [10]. These ions are called Langevins ions. It has been shown that nitrogen oxides are important for the production of condensation bodies[9], and ozone is important since it takes part in the production of nitrogen oxides. The lamps used in the reported experiments however are guaranteed by the manufacturer not to produce ozone.

Still another possibility for the gas ions produced to react with constituents in the gas is known [9,10]. The ions can collect a shell of neutral gas molecules and hereby form another type of gas ion, called cluster ions, with a lifetime of some minutes [10].

On these grounds, the gas ions which are produced in the UV-irradiated nitrogen gas and which are sufficiently stable to have a chance of reaching the culture fluid in the biological experiments can be assumed to be nitrogen cluster ions. As the nitrogen gas used is not entirely free of oxygen[4], there is still a slight possibility that the ions can be nitrogen oxide ions or some kind of oxygen ions. It has not been possible, however, to measure any production of nitrogen oxide by measuring pH changes or by titration experiments, nor has it been possible to detect ozone formation by sulphite oxidation experiments. In the biological experiments, the gas ions together with the rest of the gas are forced through the liquid culture containing all the nutrient chemicals and the bacteria population. The ions might react either

with the chemicals and produce some substance that enhances the nitrogen fixation by the bacteria, or they might react direct with the bacteria.

The increase of gas ions in the irradiated nitrogen gas for the different lamps is quantitatively correlated to the increase in nitrogen fixation by the different species and strains of the genus *Azotobacter* (Fig. 2 and Table 1). It has not been possible to account for the extremely high effect of UV-irradiated nitrogen gas upon nitrogen fixation by *Azotobacter macrocytogenes*, strain NCIB 8700, compared to the other species and strains of *Azotobacter* used. The low value of nitrogen fixation for this bacterium has also been found by Nilsson *et al.*[11], especially at a lower oxygen transfer rate.

The results in this report establish a production of gas ions, probably nitrogen cluster ions, in commercial nitrogen gas irradiated by ultraviolet light. This production is also correlated to the increase in nitrogen fixation among some of the species and strains of the genus *Azotobacter*.

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