

# Effects of an Electric Field of Sinusoidal Waves on the Amino Acid Biosynthesis by *Azotobacter*

A. Martin Gonzalez and M. T. Izquierdo

Institute Jaime Ferran of Microbiology, Joaquin Costa 32 Madrid-6, Spain

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Electric fields of sinusoidal waves have been applied in cultures of *Azotobacter vinelandii*, with potentials between 0 V and 10 V, intensities from 0 mA to 16 mA and frequencies between 5 Hz and 200 KHz.

The influence of the electric field of sinusoidal waves on the nitrogen fixation on the post-culture medium composition has a maximum at 5 V, 8 mA and 20 Hz. The rate of synthesis of specific amino acids by *Azotobacter* depends on the frequency and potential of the electric field applied. The concentration of each amino acid present in the post-culture medium is increased according to the electric field employed and the amino acid biosynthesis in culture medium is activated during the first days of incubation.

## Introduction

The process of  $N_2$  fixation involves the consumption of a great amount of energy which is supplied during the metabolism of the carbohydrates by means of the ATP. Nitrogenase is the enzyme involved in  $N_2$  fixation which is constituted by the iron-sulfur proteins.

The transport of electrons through the nitrogenase produces the reduction of the  $N_2$  to  $NH_3$ . The reaction of  $NH_3$  with glutamate in the presence of glutamine synthetase of the cell yields glutamine. All this complex system consists in a reduction of the  $N_2$  by means of electrons from Fe and Mo atoms to  $N_2$ , although the mechanism of such a transference is still unknown.

There are only a few papers dealing with the application of electric fields in microbiology. Van Nostran and collaborators [8] observed small variations in the reproduction rate of yeast cells after the application of different magnetic fields at different temperatures. Sadornichy and colleges [6] and Gerencser and coll [2], observed inhibitions in the growth of *E. coli* with electric fields.

## Methods

### Bacterial strain

The strain *Azotobacter vinelandii* (DSM, 382) German Collection of Microorganisms (Zentrale Göttingen) was employed.

The cultures were incubated at 28 °C during 7.8 and 10 days at 170 rpm.

### Media

The culture medium employed was the medium Burk [5] modified, comprising  $g \cdot l^{-1}$ : sucrose 15;  $K_2HPO_4$  0.8;  $KH_2PO_4$  0.2;  $MgSO_4 \cdot 7 H_2O$  0.2;  $CaSO_4 \cdot 2 H_2O$  0.1;  $Na_2MoO_4$  0.001;  $FeCl_3$  0.003. The pH was 7.3 (pH optimum for the growth of the strain of *Azotobacter vinelandii*).

### Application of electric fields

The electric field was applied through platinum electrodes. They were placed in a glass flask of three mouths of 250 ml of capacity with 100 ml of culture. The surface of the electrodes was the corresponding to a platinum filament of 5 cm long and 0.40 mm of diameter.

The electric waves applied to the cultures of *Azotobacter* were supplied by an oscillator "ATAIO" Model AI-202 of high stability (frequency range between 20 Hz and 200 KHz) and an oscillator "EXACT" (frequency range between 0.2 Hz and 2 MHz).

### Determination of amino acids

The determination of amino acids in the post-culture medium [4] was performed by hydrolysing aliquots of 5 ml in sealed tubes with 6 N HCl at 110 °C during 20 h [1]. The hydrolysate was desiccated in vacuum at 30 °C and the residue was dissolved in 5 ml of bidistilled water acidulated at pH 2.2 with 0.1 N HCl and assayed in the Full-Automatic Amino Acid Analyzer Model JEOL.

Reprint requests to A. M. Gonzalez.

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The total nitrogen was assayed by the method of K. Swaminathan and K. C. Sud [7]. The determination of free amino acids in the post-culture medium was carried out as described by Lahoz, Reyes and Beltra [4].

## Results and Discussion

A maximum protein concentration in the medium of *Azotobacter vinelandii* cultures after growth was obtained through the application of an alternating electric field of 50 Hz at 5 V and 8.5 mA during 7 days. The amino acid content of such medium increases almost 3 times in these conditions, compared with standard cultures (Table I).

After 7 days of incubation, the pH of cultures subjected to an electric field shows significant differences with standard cultures, the former being 5.6 compared with the standard 7.7.

The pH variation is maximum applying a voltage of 5 V. The fixed nitrogen concentration on the medium reaches its peak when applying voltages between 5 V and 6 V (Fig. 1).

We have also investigated the effect of the different electric fields on the rate of cell growth measuring the optical density of the cultures at 640 nm along the 10 days of incubation. This effect on cell growth is more intense during the first few days, decreasing and becoming constant after 7 days (Fig. 2). It was observed that, when applying a voltage gradient of sinusoidal waves to *Azotobacter* cultures, the total amino acid content in the culture medium reached its maximum after subjecting there

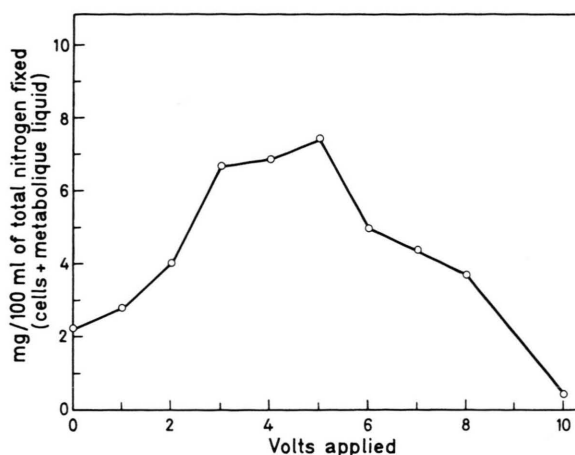


Fig. 1. Total nitrogen fixed in cultures of *Azotobacter vinelandii* (mg/100 ml), with electric field of sinusoidal waves between 0 and 10 V.

cultures to an 5 V electric current. The levels of lysine, arginine, aspartic acid, serine and glycine, appeared increased.

Increased levels of lysine, arginine, aspartic acid, serine and glycine were found in the medium after using potentials of 5 V.

Glycine levels were specially high, being synthesized at a rate of 8:1 in weight compared to the standard culture. Other amino acids, such as glutamic acid, reach their maximum concentration using 2 V.

Maximum concentrations of alanine, valine, leucine, isoleucine and phenylalanine were obtained with potentials of 4 V.

Amino acids	Volts						
	0	2	3	4	5	6	8
Lysine	861	465	946	672	1 225	847	775
Arginine	20	12	14	174	196	194	45
Aspartic acid	990	1 131	1 392	1 540	2 080	1 090	1 052
Threonine	148	817	361	753	659	645	580
Serine	997	1 085	179	1 286	2 195	761	824
Glutamic acid	341	2 417	2 188	864	979	660	801
Glycine	549	643	3 512	3 074	4 662	508	610
Alanine	453	593	171	831	589	585	616
Valine	91	363	63	446	364	386	403
Methionine	28	10	12	10	12	10	10
Isoleucine	65	268	93	332	289	299	305
Leucine	107	440	99	615	489	452	472
Tyrosine	45	110	181	180	188	191	89
Phenylalanine	70	217	110	357	296	245	270
Total	4 765	8 571	9 321	11 134	14 223	6 873	6 852

Table I. Effects of an electric field of sinusoidal waves on the amino acid content in the hydrolisate of the postculture medium by *Azotobacter vinelandii*, seven day old, et different potentials and frequencies of 50 Hz (in µg by 100 ml).

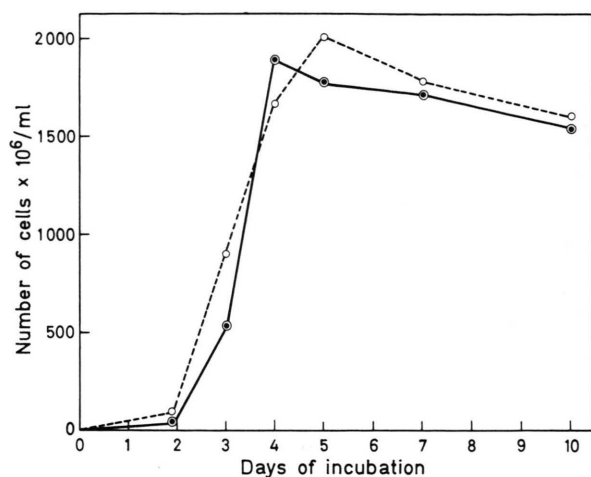


Fig. 2. Number of cells  $\times 10^6/\text{ml}$ , during 10 days of incubation. ●—● No electric field; ○— —○ electric field (5 V, 8 mA, 50 Hz).

The influence of variation of the electric frequency was also studied. Culture mediums after growth were hydrolysed and the amino acid concentration was stimulated. As it can be seen in Table II, some amino acids, such as lysine, aspartic acid, arginine, serine, alanine and glutamic acid reach their highest levels using electric frequencies of 20 Hz; others, such as glycine, isoleucine, leucine, tyroxine and phenylalanine show increased levels with frequencies of 50 Hz. With higher frequencies, increased amounts

of valine have been observed. As Table II shows, the total amino acid content in the medium after applying low frequency waves (20 Hz) is 3.5 times higher than in the standard cultures.

In 7 days-old cultures no free amino acids were found. From time to time, culture samples were observed under light microscope, no broken cells being observed at this stage. The influence of an electrical field of sinusoidal waves (5 V, 12 mA and frequen-

Table II. Effects of an electric field of sinusoidal waves on the amino acid content in the hydrolysate of the post-culture medium by *Azotobacter vinelandii*, seven days old, at different frequency and 5 V (in  $\mu\text{g}$  by 100 ml).

Amino acids	Frequency			
	0 Hz	20 Hz	50 Hz	200 KHz
Lysine	930	2 818	1 224	932
Arginine	28	327	195	14
Aspartic acid	1 080	3 628	2 082	998
Threonine	138	658	658	302
Serine	997	4 150	2 194	1 547
Glutamic acid	360	2 015	978	421
Glycine	589	1 872	4 665	607
Alanine	463	1 172	589	402
Valine	95	311	362	960
Methionine	37	12	12	22
Isoleucine	89	200	287	100
Leucine	111	460	488	12
Tyrosine	40	125	176	82
Phenyl alanine	73	218	295	146
Total	5 010	17 966	14 205	6 545

Table III. Effects of an electric field of sinusoidal waves (5 V, 12 mA and frequencies of 5 Hz and 50 Hz) on the amino acids content in the hydrolysis of the medley of cell and post-culture medium by *Azotobacter vinelandii* (in mg/100 ml).

Days	2			3			5			8		
	0	5	50	0	5	50	0	5	50	0	5	50
Lys	1.4	4.2	1.8	3.1	5.5	5.2	4.2	6.1	6.0	4.4	7.1	8.6
His	0.0	0.8	0.0	0.0	0.8	0.0	1.3	1.0	1.0	1.5	1.8	2.6
Arg	0.0	3.0	1.7	1.6	3.2	2.5	3.3	2.8	3.0	4.3	5.3	6.5
Asp	2.1	5.2	2.4	3.0	7.7	6.5	6.4	8.3	7.3	6.2	9.7	9.3
Thr	0.0	1.4	0.7	0.0	2.2	1.7	1.7	2.1	2.0	2.4	4.1	4.1
Ser	2.8	3.7	1.4	2.1	5.5	4.8	4.0	6.5	5.5	3.1	6.8	6.1
Glu	1.9	6.4	3.5	5.3	9.0	6.4	10.6	9.9	8.7	10.5	12.5	15.9
Pro	0.0	1.7	0.0	0.0	2.0	0.0	1.2	2.7	1.0	2.9	3.0	3.0
Gly	1.2	2.4	1.3	1.7	4.8	4.2	3.5	4.0	4.1	3.2	3.6	5.1
Ala	0.9	3.6	1.8	2.4	3.6	3.3	2.0	5.1	4.7	6.7	7.1	8.7
Val	0.0	1.8	1.0	1.2	2.4	2.1	3.4	2.5	2.5	3.6	4.9	5.6
Met	0.0	0.6	0.3	0.0	0.9	0.5	0.3	1.0	1.0	1.0	1.6	1.5
Ile	0.4	1.6	0.9	0.8	2.1	1.7	2.7	2.2	2.5	3.0	3.3	4.0
Leu	1.2	3.5	2.3	1.4	4.3	3.9	5.2	4.2	4.4	6.0	7.3	7.8
Tyr	0.0	0.6	0.0	0.0	0.7	0.8	0.8	0.9	0.9	1.2	1.9	1.5
Phe	0.0	1.1	0.6	0.0	1.6	1.3	2.1	1.7	3.0	1.9	3.1	3.6
Total	11.9	41.6	19.7	22.6	56.3	44.9	53.7	61.0	57.6	61.9	86.5	90.5

cies of 5 Hz and 50 Hz) on amino acid production by *Azotobacter vinelandii* is more intense during the first few days of incubation (Table III).

Arginine, histidine, threonine, proline, valine, methionine, tyroxine and phenylalanine are synthesised after two days concentration using frequencies of 5 Hz. The concentration of amino acids in the

protein synthesized by *Azotobacter* depends of the potential and frequency of the electric fields. Potential and frequencies about 5 V and 5 Hz in sinusoidal waves, have the stingest influence on the production of amino acids by *Azotobacter vinelandii* (DSM, 382).

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