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ELECTRIC FIELD - A NOVEL SOURCE FOR ENHANCED BIOMASS AND PROTEIN PRODUCTION IN MICROALGAE.

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A self designed electric field apparatus has been tested on the growth of chlorococcalean alga *Ankistrodesmum fusiforms*. At a voltage of 1 KV, cultures exposed for a period of 1-5h instricated rapid growth leading to the enhanced biomass production. With 1 h treatment, the density of the cultures was enhanced ten folds. Subsequently, number of amino acids, quantity of free amino acids and protein contents were increased. 2% increment in protein contents in contrast to unexposed cultures was significant. Electric field has been suggested as a cheap and best source for algal biomass production and enhanced amount of protein with particular reference to developing countries.

Key Words : Amino acids, *Ankistrodesmus fusiformis*, Biomass, Electric field, protein.

Biological effects of electric field have generated much interest in recent years. The study pertaining to the magnitude of electric field on the human beings, animals and individual cells has been substantial. Initially it was employed it setting the bone disorders, but recent discovery (Polk & Song, 1990) stimulated the interest in the this field that the sinusoidal extremely low frequency magnetic fields affect RNA transcription and protein synthesis.

Earlier report (Bauchinger & at all 1981) showed that the 50Hz alternating electric field in 380 KV switch yard was ineffective for workers over a period of 20 years. (Nordenson and Hansson 1984 & 1988) observed the increased frequency of chromosomal aberrations in peripheral blood cells of workers. DNA damage caused by CO^{60} was repaired (Frazier *et al.*, 1990) by exposure to electric field. Amongst animals, ruminants and non-runinants were equally experimented upon. There was no change either on body weight or reproductive capacity, when male mice were exposed to 50 Hz electric field (Kowalczuk & Saunders, 1989) and rats to chronic exposure of 65 KV & 150 KV/m (Rommereim *et al.*, 1989). But high voltage electric current impact + 500 KV (Angell & Bracken, 1990) on beef cattle production, body weight calving and survival was found to have enhanced.

Studies pertaining to plants with electric field impact are few. High spherical symmetry electric field 50-75 Hz (Asencor *et al.*, 1990) was applied to *Saccharomyces cervisae, Klokecera apiculata* and *Citrobacter fruendii*, similarly root tips of zea mays (Brayman & Morton, 1990) were exposed to 60 Hz. Amongst algae, *Spirulina platensis* and *S. subsalsa* have been subjected to 1 KV and 5 KV of electric field (Srivastava, 1992). Present communication in the first on record with a eucaryotic chlorococcalean genus *Ankistrodesmus fusiformis*, which was subjected to a low voltage of electric field of 1 KV. Its impact was observed on the growth dimensions, Amino acid profile and protein contents.

MATERIALS AND METHODS

Electric field was provided to the cultures by self designed high voltage power supplying apparatus. It supplied stable voltage continuously across the aluminium electrode. One of the electrodes was earthed and other one was held parallel to the first one and were well insulated. The distance between the two electrodes could be adjusted through the screw arrangement. To avoid interaction between the voltage of the electric field and the atmosphere, the electrodes were covered with a wooden cover.

Petri plates of 50 x 17 mm were employed 3 ml of the freshly growing cultures formed a uniform depth of 2mm. The culture samples were exposed to 1 KV for a period of 1-5h with a gap of 1h between the two exposures. A set of 5 test tubes (150 x 15 mm) in duplicate were prepared containing 7 ml sterilized Juller's solution (Manix, 1931) The exposed 2 ml sample was added to these tubes and the final volume was made upto 10ml. One set of these tubes was used for recording optical density and the counter set was

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Table 1 : *A fusiformis* Qualitative analysis of amino acids subjected to electric field at 1 KV

S. No.	Amino acids	Control		1 h		2 h		3 h		4 h		5 h	
		Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound	Free	Bound
1.	L. Proline	-	-	+	+	+	+	+	+	+	+	+	+
2.	L-Tyrosine	-	+	-	-	-	-	-	-	-	-	-	-
3.	L-Leucine	-	+	+	-	-	-	+	-	-	-	-	-
4.	D L-Alanine	-	-	-	+	-	+	-	+	-	+	-	+
5.	D L-Tryptophane	-	-	-	-	-	-	-	-	-	-	-	-
6.	D L-B Phenylalanine	-	-	-	+	-	-	-	-	-	-	-	-
7.	Glycine	-	+	-	-	-	-	-	-	-	-	-	-
8.	Arginine	+	+	-	+	-	-	+	-	+	-	+	+
9.	Cysteine	-	-	-	+	-	+	-	-	-	+	+	+
10.	Cystine	-	-	-	-	-	-	-	-	-	-	-	-
11.	DL-Isoleucine	-	-	+	+	+	+	+	+	+	+	+	+
12.	L-Glutamic Acid	-	-	-	-	-	-	-	-	-	-	-	-
13.	DL-Aspartic Acid	+	+	-	-	-	-	-	-	-	-	-	-
14.	D L-2-Amino n-butynic acid	+	-	-	-	-	-	-	-	-	-	-	-
15.	D L-Methionine	-	+	+	+	+	+	+	+	+	+	+	+
16.	D L-Threonine	-	+	+	-	+	-	+	-	-	-	-	-
17.	D L-ornithine	+	+	+	-	+	-	+	-	-	-	-	-
18.	D L-Serine	-	-	-	-	-	-	-	-	-	-	-	-
19.	Histidine	+	-	-	-	-	-	-	-	-	-	-	-
20.	Hydroxy Proline	-	-	-	-	-	-	-	-	-	-	-	-
21.	L-Lysine	-	+	-	+	-	+	-	+	-	+	-	-
22.	Unidentified	-	-	-	+	-	-	-	-	+	-	-	+
	Total	11		12		9		9		8		7	

Table 2: A fusiformis-Quantitatine analysis of free amino acids and Protein Contents subjected to electric field at 1 kv

	Control	1h	2h	3h	4h	5h
Free amino acids (µg/gFw)	760±1.85	770±1.41	737±1.85	591±1.41	502±1.85	485±1.85
Protein (%)	45.5±0.14	47.5±0.18	45.0±0.23	41.7±0.23	40.6±0.19	40.0±0.14

exclusively employed for cell counts and cell measurements. The results were statistically analysed using standerd method (Bancroft, 1957). Simultaneously, a set of 10 flasks of 500 ml capacity with 300 ml minimal medium added with 10ml of exposed cultures were prepared. These samples were utilized for qualitative analysis (Block, 1951) and quantitative analysis (Lee & Takahashi 1966) of amino acids and protein estimation (Lowry *et al.*, 1951). Parallel set of test tubes and flasks with equal volume of unexposed cultures were maintained as controls. Observations were carried out over a period of five weeks.

RESULTS

With 1h electric field treatment, the optical density record stood a witness to the increased density of the cultures, which was much higher than the unexposed cultures. It was 49 times the initial record after a period of five weeks, while it was hardly 40 times in unexposed cultures. The percentage of healthy cells Fig. 1. a and B also support this observation. They were 91.9% in exposed cultures, against 85% in controlled sample. With 2h treatment, the growth of the exposed cultures had an edge over the controlled sample with slightly, higher values. The density being 43:42 and percentage of healthy cells being 87.7:86.4%. Even 3h treatment did not hamper the growth of *A. fusiformis* but in the subsequent exposures it declined lineraly Fig. IA & B. After 5h treatment healthy cells were reduced to 80% from initial 91%

Cells were healthy brilliant green with intact chloroplast in the samples exposed upto 3h. With further enhanced exposures of 4 and 5h, cultures turned faint green with cells having broken and vacuolated chloroplast. The average length of the cells was more

in exposed cultures than the unexposed ones. After a period of 5 weeks, 1h treated cells measured 45.04µm against 42.3µm of untreated cells. Under subsequent exposures the average length reduced marginally (Fig. 2). However, the breadth of the cells remained unaffected under electric field impact and on an average remained 3.3µm.

Qualitative analysis of free and protein bound amino acids revealed presence of eleven amino acids in controlled culture sample, while 1h exposed cultures contained twelve them (Table 1). Thereafter, under the following exposures, the number of amino acids continued to reduce. Finally with 5h exposure only seven amino acids were recorded. The amino acid profile varied in exposed cultures L-proline and DL-isoleucine have not been observed in controlled cultures, while their presence has been recorded under all exposure periods in free as well as bound form. Similarly L-tyrosine and glycine present in bound state and DL-2-amino-n-butyric acid and histidine in free state and DL-aspartic acid in both condition have totally been suppressed by impact of electric field. DL-methionine present only in protein bound condition in controlled sample has been found in free as well as bound state (Table 1). This variance has further been seen during quantitative analysis of free amino acids and protein contents (Table 2). The free amino acid contents were 760µg/gram of fresh weight (gfw) which were enhanced to 770 µg after 1h exposure to electric field. This amount however, declined when exposed for longer durations upto 5h. Similarly protein contents were increased to 47.5% from 45.5% in unexposed cultures.

DISCUSSION

Electric field, a novel source, has been experimented upon a Chlorococcalean genus *Ankistrodesmus* for the first time. Both the parameters for evaluation of growth pattern i.e. density and percentage of healthy cells proved that growth was enchanced under the impact of electric field. At 1 KV, growth was maximum at 1h exposure time, moderate at 2 and 3h but was suppressed under subsequent exposures upto 5h. These results are in confirmity with earlier work on *Spirulina piatensis* and *S. subsalsa* (Srivastava, 1992) and further draw their support on cows (Angell & Bracken, 1990). It deserves a mention that the high voltage of 25kv proved determental to this species of *Ankistrodesmus* (Singh, 1992). This observation did not agree (Kowalczuk & Saunders; Rommereim, 1989) on mice and rats respectively, where no lethal impacts or change in body weight or in reproductive capacity was observed under a voltage, as high as 65-150 KV. Similarly chromosomal alterations were not observed amongst workers under 380 KV switch yard even after a period of 20 years (Bauchinger *et al.*, 1981). However increased frequency of chromosome aberrations (Nordenson *et al.*, 1984, Nordenson *et al.*, 1988) was found in peripheral blood cells of workers under high voltage switch yard. Direct effect of 50 Hz on chromosomes would be unexpected as subcellular components are shielded by membrane.

The cell length was enlarged, more so at 1h treatment of 1KV voltage. A brief survey of literature did not suggest such a cell enlargement under low voltage electric field. However high voltage 500KV for 30 months increased the body weight of cows by 58 kg of calf gain was 0.93 kg/head/day. The voltage of 1KV for a period of 3h could not influence the chloroplast complex and cell contours. Further exposures inflicted determental impact on cell contents.

The exposed cultures had increased number of amino acids and varried profiles. There were 12 amino acids in 1h treated cultures, in contrast to 11 in controlled ones. This variance was further expressed in the free amino acid contents, which was enhanced after exposure for 1h. Significant impact was expressed in the increase of protein percentage by 2% at the same exposure. This observation supported the earlier contribution that the low frequency electric field affected RNA transcription and protein synthesis.

Furthermore the earlier study (Frazier *et al.*, 1990) demonstrated that 50-75% DNA damage caused by CO^{60} in isolated lymphocytes was repaired by 60 Hz electric field in 20 min and complete repair required 180 min exposure.

The totality of evidences lead to the conclusion that low voltage electric field stimulated growth of *A. fusiformis* in number and in density and in the dimensions as well, while retaining the healthy cellular organisation with normal cells contours. It further increased the quality and quantity of amino acids & protein percentage. This preliminary report is the suggestive of the fact that low voltage electric field may be used as a cheap and best source of procure high yield of biomas and higher contents of proteins

in commerical algae like *Chlorella, Scenedesmus* and of course species of *Spirulina.*

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