Cellular engineering

Athermal physiological effects of microwaves on a cynobacterium Nostoc muscorum: evidence for EM-memory bits in water

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Abstract—*Athermal physiological effects of continuous wave and modulated microwaves were studied on a cynobacterium Nostoc muscorum. The study shows that different microwave frequencies in continuous wave and modulated modes produced significantly different physiological effects on the algae. Water-mediated bioeffects further present additional proof that water has the capability to remember the imposed electromagnetic field characteristics for an extended period of time.*

Keywords—*A663, Average filament length, Carbohydrate, Carotenoid, Chlorophyll A, Cynobacterium Nostoc muscorum, Heterocyst frequency, Microwave, Phycocyanin, Protein*

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1 Introduction

SEVERAL BIOLOGICAL effects of the direct exposure of biosystems to microwaves have been reported (GANDHI, 1987; SMITH and BEST, 1989). Microwaves have been reported to cause thermogenic bioeffects, which were found to vary depending on far-field versus near-field location, power density, duration, frequency, polarisation, modulations, pulses etc. There is, however, little information on athermogenic bioeffects of microwaves. In this paper, we report the athermal physiological effects of different frequencies of microwaves, under continuous wave (CW) and modulated conditions, on the physiology of a cynobacterium Nostoc muscorum.

The electromagnetic (EM) field is reported to cause athermal bioeffects by changing the microstructures of the 'live' water (i.e. water that takes part in the biological activities) (MARKOV, 1984; RAI, 1993). Further, there is also evidence that water exposed to EM fields undergoes structural changes that remember the imposed field for an extended period of time (RAI, 1993; DEL GUIDICE *et al.*, 1988). However, there is no consensus of opinion that water remembers the imposed field after the exposure is terminated. On the basis of physiological data, we have reascertained this capability of water.

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2 Materials and methods

2.1 *Materials*

Nostoc muscorum, a filamentous, heterocystous cynobacterium, was grown and routinely maintained in BG_{11} nutrient solution (RIPPAKA *et al.*, 1979) at 25 ± 1 °C under 2500 lux light intensity. Ten days after incubation, the culture was centrifuged to obtain the inoculum to incubate into the microwave-restructured growth medium.

The microwave restructuring of the growth medium by the CW and modulated microwaves in an S-band was performed by exposing to microwaves every experimental sample set of the sterilised growth medium (20 ml of each set contained in cotton stoppered glass sample tubes) for one hour.

2.2 *Exposure of sterilised BG_{11} in an S-band*

The experimental set up for exposure is shown schematically in Fig. 1. The microwave source was an S-band VHF signal generator*, adjustable from 2–4 GHz. The microwave power was delivered through a coaxial cable to a waveguide attenuator† and then to a (2–20 GHz) power amplifier§. The rest of the equipment and components were obtained from SICO†.

* S470, ECIL, Hyderabad, India; † SICO, Ghaziabad, India; § 8349B, Hewlett Packard Co., USA

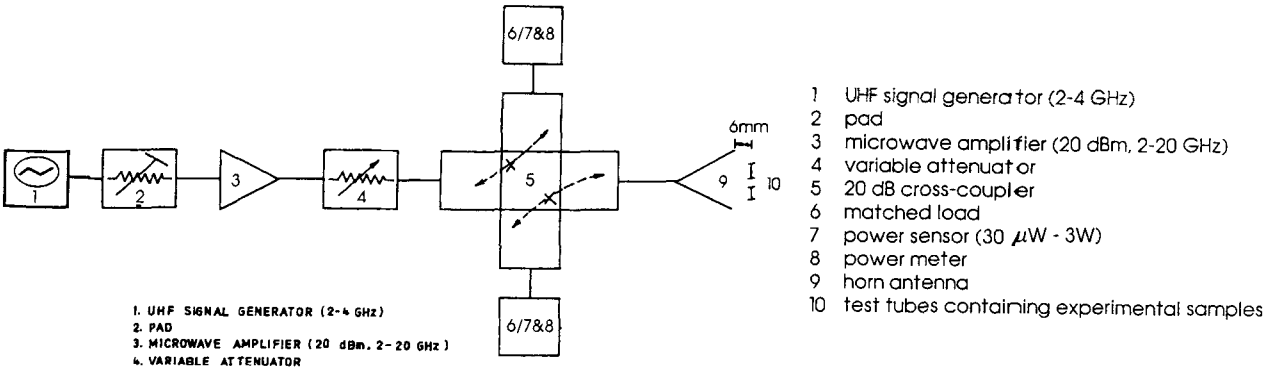

Fig. 1 *Block schematic of experimental set up for microwave exposures*

A 20 dB cross-directional coupler, power sensor and meter were used to measure incident and reflected powers. Incident power level equal to 65·0 mW was maintained throughout the treatment to the samples of sterilised growth medium in all cases. 12 test tubes each containing 10 ml samples were taken in each of the two treatment groups, control and experimental. Six equal sub-groups, each with two test tubes containing BG_{11} samples, were formed in each treatment group. Three experimental subgroups with samples of sterilised BG_{11} were exposed to 2·42, 2·71 and 2·97 GHz CW microwaves at incident power densities of 2·04, 1·98 and 1·92 mW/cm^2, respectively, for 1 hr duration in the near field of an S-band horn antenna, whose aperture area was 44·0 cm^2. The incident microwave power, as measured by the use of the cross-coupler, power sensor and meter at different frequencies, was the same.

Slight differences in the reported incident power density at different frequencies are due to the differences in the

Table 1. Mean physiological response of Nostoc muscorum to microwave exposures for one hour

treatments	A663	heterocyst frequency	average filament length	protein, μg ml^{-1}
control	0.500 ±0.02	5.667 ±0.025	31.467 ±1.10	89.00 ±2.0
CW microwave frequency, GHz				
2.42	0.280 ±0.03	5.367 ±0.20	34.900 ±1.21	61.133 ±1.90
2.71	0.267 ±0.02	5.900 ±0.40	36.667 ±0.61	52.467 ±0.15
2.97	0.293 ±0.01	5.667 ±0.25	33.833 ±1.25	52.500 ±0.1
modulated microwave frequency, GHz				
2.42	0.243 ±0.02	7.367 ±1.02	22.733 ±3.92	36.167 ±4.14
2.71	0.300 ±0.03	6.667 ±1.02	29.333 ±0.75	52.100 ±7.03
2.97	0.457 ±0.04	6.233 ±1.15	31.867 ±5.61	66.067 ±3.57

treatments	chlorophyll A, μg ml^{-1}	phycocyanin. μg ml^{-1}	carotenoid, μg ml^{-1}	carbohydrate, μg ml^{-1}
control	1.231 ±0.03	1.363 ±0.02	43.667 ±1.24	30.667 ±0.38
CW microwave frequency, GHz				
2.42	0.856 ±0.09	0.920 ±0.02	25.333 ±9.45	20.517 ±0.59
2.71	0.884 ±0.01	0.787 ±0.01	23.667 ±1.52	17.500 ±0.01
2.97	0.821 ±0.07	0.787 ±0.01	22.333 ±4.04	17.700 ±0.05
modulated microwave frequency, GHz				
2.42	0.556 ±0.19	0.543 ±0.07	16.333 ±1.52	13.227 ±2.32
2.71	1.041 ±0.08	0.782 ±0.10	26.333 ±1.52	17.427 ±2.34
2.97	0.814 ±0.06	0.993 ±0.05	32.000 ±2.64	22.010 ±1.19

± = standard deviation

characteristics of the measurement systems at these frequencies. The same antenna was used to expose the remaining three subgroups of experimental BG_{11} samples to 1·0 KHz square-wave modulated microwaves at frequencies of 2·42, 2·71 and 2·97 GHz, respectively, at the same respective incident power densities as used for the CW exposures for 1 hr duration. The distance between the antenna aperture and the medium samples was 6 mm in each case. The experimental test tube samples were periodically shaken by hand to ensure a uniform distribution of temperature by the microwaves, if occurring, in each case during treatment. The same number of test tubes containing control samples were kept in the same room as the source, but were isolated from exposure to the microwaves.

A larger aperture horn in the Fresnel region, a power sensor and meter were used to measure the power transmitted through the samples in each experiment. The incident, transmitted and reflected powers (for CW microwave exposures and 1·0 kHz square-wave modulated microwave exposures) were first measured with the power sensor and meter when two empty test tubes of a subgroup were kept in the near field of the antenna. Corresponding powers were again measured when test tubes containing experimental samples of sterilised BG_{11} of that subgroup were kept in the near-zone field of the antenna.

Power absorbed by the experimental samples was determined by calculating the differences in transmitted and reflected powers for empty and filled test tubes. The absorbed power densities for the first three sub-groups of experimental BG_{11} samples to which CW microwave exposures were given are 1·376, 1·290 and 1·253 mW/cm^3, respectively. The absorbed densities for the last three subgroups of experimental BG_{11} samples to which modulated microwave exposures were given are 1·387, 1·289 and 1·201 mW/cm^3, respectively.

2.3 *Measurement of physiological parameters*

The sham- and the microwave-exposed BG_{11} samples were left for ten days at room temperature (20 °C) and then inoculated. The algae was grown under standard conditions. The growth parameters were recorded after ten days of incubation. The experiments were run thrice.

Direct absorbancy (A663) of the samples was recorded at 663 nm in a spectrocolorimeter. Protein content was estimated by the Lowry method, using bovine serum albumin as standard (LOWRY *et al.*, 1975). Chlorophyll A contents of the samples were estimated in 80% acetone and measured as described by Arnon (ARNON, 1949). Carotenoid and phycocyanin content of the algae samples were estimated as described by Myers and Kratz (MYERS and KRATZ, 1955). Carbohydrates were measured using an anthrone reagent (DUBROIS *et al.*, 1956). The average filament length of the algae was calculated by adding up the number of cells in individual counted filaments and dividing it by the total number of filaments. Heterocyst frequency of the algae was calculated as described by Rai (RAI, 1976).

3 Results and discussion

Table 1 presents the frequency-dependent athermal physiological effects of CW and modulated microwaves mediated through the long-term field memory in the restructured medium. The data reveal that the restructured medium caused different physiological effects. The F-statistic of the physiological data of the sham-exposed algae and those exposed to different frequencies of CW microwaves (Table 2, Fig. 2) shows that there is a significant difference in the group means of their average filament length, A663, chlorophyll A, phycocyanin, protein, carotenoid and carbohydrate contents at 0·01 level. There is also a non-significant difference in the group means of their heterocyst frequencies.

The F-statistic of the sham-exposed algae and those exposed to 1 kHz square-wave modulated microwaves at corresponding frequencies (Table 3, Fig. 3) reveals that there

Table 2 ANOVA for sham-exposed and CW microwave-exposed algae

source	sum of squares	d.f.	mean square	F. ratio
(*a*) average filament length				
between	42.537	3	14.179	11.840*
within	9.580	8	1.198	
total	52.117	11		
(*b*) protein				
between	2694.849	3	898.283	469.895*
within	15.293	8	1.912	
total	2710.143	11		
(*c*) A663				
between	0.110	3	0.037	78.548*
within	3.7333E-03	8	4.6667E-04	
total	0.114	11		
(*d*) heterocyst frequency				
between	0.430	3	0.143	1.737
within	0.660	8	0.083	
total	1.090	11		
(*e*) chlorophyll A				
between	0.326	3	0.109	27.950*
within	0.031	8	3.8928E-03	
total	0.358	11		
(*f*) phycocyanin				
between	0.671	3	0.224	743.804*
within	2.4072E-03	8	3.0090E-04	
total	0.674	11		
(*g*) carotenoid				
between	903.583	3	301.194	10.919*
within	220.667	8	27.583	
total	1124.250	11		
(*h*) carbohydrate				
between	346.194	3	115.398	903.193*
within	1.022	8	0.128	
total	347.216	11		

* significant at 0.01 level

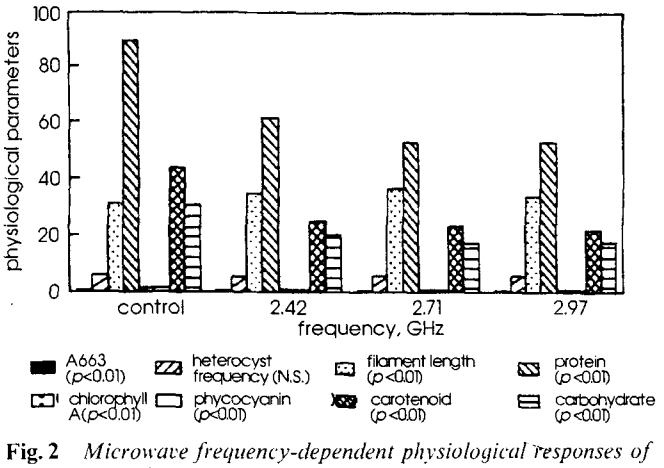

Fig. 2 *Microwave frequency-dependent physiological responses of algae for CW microwave exposures*

Table 3 ANOVA for sham-exposed and modulated microwave-exposed algae

source	sum of squares	d.f.	mean square	F. ratio
(*a*) average filament length				
between	160.783	3	53.594	4.385**
within	97.767	8	12.221	
total	258.550	11		
(*b*) protein				
between	4516.393	3	1505.464	72.173*
within	166.873	8	20.859	
total	4683.267	11		
(*c*) A663				
between	0.136	3	0.045	43.445*
within	8.3333E-03	8	1.0417E-03	
total	0.144	11		
(*d*) heterocyst frequency				
between	4.630	3	1.543	1.772
within	6.967	8	0.871	
total	11.597	11		
(*e*) chlorophyll A				
between	0.776	3	0.255	20.262*
within	0.101	8	0.013	
total	0.867	11		
(*f*) phycocyanin				
between	1.090	3	0.363	69.767*
within	0.042	8	5.2086E-03	
total	1.132	11		
(*g*) carotenoid				
between	1170.917	3	390.306	111.516*
within	28.000	8	3.500	
total	1198.917	11		
(*h*) carbohydrates				
between	502.637	3	167.546	53.492*
within	25.057	8	3.132	
total	527.694	11		

* significant at 0.01 level
** significant at 0.05 level

Table 4 ANOVA for algae samples exposed to CW and modulated microwaves

source	sum of squares	d.f.	mean square	F. ratio
(*a*) average filament length				
between	376.091	5	75.218	8.858*
within	101.893	12	8.491	
total	477.984	17		
(*b*) protein				
between	1561.823	5	312.365	22.558*
within	166.167	12	13.847	
total	1727.989	17		
(*c*) A663				
between	0.087	5	0.017	19.980*
within	0.010	12	8.7222E-04	
total	0.098	17		
(*d*) heterocyst frequency				
between	7.947	5	1.589	2.587
within	7.373	12	0.614	
total	15.320	17		
(*e*) chlorophyll A				
between	0.372	5	0.074	7.096*
within	0.126	12	0.010	
total	0.498	17		
(*f*) phycocyanin				
between	0.356	5	0.071	20.312*
within	0.042	12	3.5035E-03	
total	0.398	17		
(*g*) carotenoid				
between	396.667	5	79.333	3.978**
within	239.333	12	19.944	
total	636.000	17		
(*h*) carbohydrate				
between	137.529	5	27.506	12.965*
within	25.458	12	2.122	
total	162.988	17		

* significant at 0.01 level
** significant at 0.05 level

is a significant difference in the group means of their A663, protein, carotenoid, carbohydrate, chlorophyll-A, phycocyanin contents at 0·01 level. There is also a significant difference at 0·05 level in the group means of their average filament length and a non-significant difference in the group means of their heterocyst frequencies.

The ANOVA (Analysis of Variance) of physiological data pertaining to different frequencies of CW and modulated microwaves (Table 4, Figs. 4*a* and *b*) indicates that there is a significant variance in the group means of A663, average filament length, protein, chlorophyll A, phycocyanin and carbohydrate contents at 0·01 level, and in the carotenoid content at 0·05 level. There is a non-significant variance in the group means of their heterocyst frequencies.

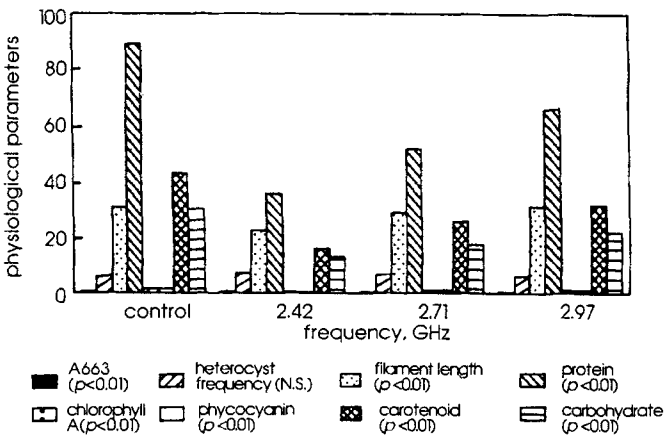

Fig. 3 *Microwave frequency-dependent physiological responses of algae for modulated microwave exposures*

These variances in physiological data suggest that CW and modulated microwaves produce significantly different frequency-varying physiological effects. These field characteristic athermal physiological effects additionally support the idea that water has the capability to remember the imposed electromagnetic fields for an extended period of time. They also suggest that microwaves cause field characteristic bioeffects not by heating, but by producing long-term microstructural changes to the chemistry of 'live' water (water that takes part in biological activities) (RAI, 1993; DEL GUIDICE *et al.*, 1988).

There are many studies (WIGGINS, 1990) that suggest that transient changes in the structural chemistry of cellular water regulate most of the biological processes, including synthesis of biomolecules (BROWN and WOLKEN, 1979). Structural changes of water are also reported to affect the chemistry of aqueous solutions and to control the partitioning of ions corresponding to the Hofmeister series between two aqueous regions of different densities (WIGGINS and VAN RYN, 1986; HOFMEISTER, 1988). In light of these studies, it may be that the restructured medium caused the present physiological effects involving the partitioning of ions between the restructured medium and the algal cells

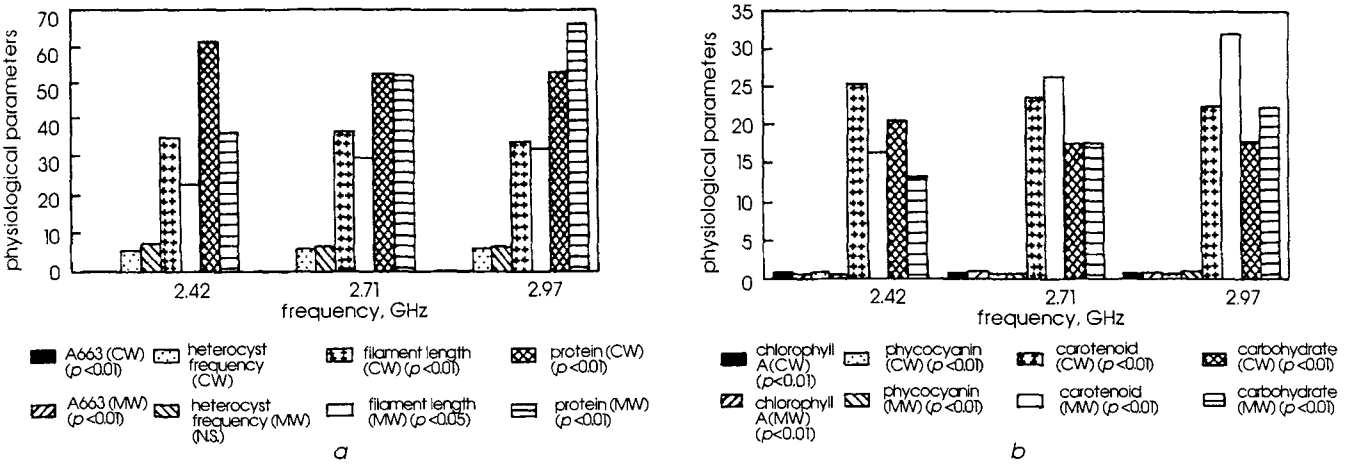

Fig. 4 *Microwave frequency-dependent physiological responses of algae for CW and modulated microwave exposures*

which might have resulted into nutrient deficiency, and also modifying the structure of cellular water of the algae (HEALEY and STEWART, 1973; WIGGINS, 1990).

To elucidate the mechanism of physiological effects, the correlation among different variables was analysed (Tables 5 and 6). Table 5 shows that the protein, phycocyanin, and carbohydrate contents of the algae are negatively correlated with the frequencies of the CW microwaves at 0·05 level, and other physiological variables are non-significantly correlated with frequencies. Correlation among different physiological variables shows that A663 of the algae is negatively correlated with chlorophyll A; protein is positively correlated with the phycocyanin and carbohydrate contents; chlorophyll A is positively correlated with the carbohydrate contents, each at 0·05 levels; and other variables are non-significantly correlated among themselves.

Table 6 depicts that the A663, average filament length,

Table 5 Correlation matrix for CW *microwave exposures*

		I frequency	II A663	III heterocyst frequency	IV average filament length	V protein	VI chlorophyll A	VII phycocyanin	VIII carotenoid	IX carbohydrate
I	frequency	1.00000								
II	A663	0.24399	1.00000							
III	heterocyst frequency	0.39178	−0.51580	1.00000						
IV	average filament length	−0.27506	−0.31081	0.12272	1.00000					
V	protein	−0.85907*	−0.13757	−0.52786	−0.20253	1.00000				
VI	chlorophyll A	−0.22414	−0.59606*	0.40086	−0.00782	0.17922	1.00000			
VII	phycocyanin	−0.86447	−0.13019	−0.53519	−0.18730	0.99954*	0.16898	1.00000		
VIII	carotenoid	−0.24288	−0.50092	0.24821	−0.39426	0.39110	0.74484*	0.37096	1.00000	
IX	carbohydrate	−0.83456*	−0.09935	−0.55329	−0.23450	0.99696*	0.14681	0.99769*	0.35439	1.00000

* significant at 0.05 level

Table 6 Correlation matrix for modulated microwave exposures

		I frequency	II A663	III heterocyst frequency	IV average filament length	V protein	VI chlorophyll A	VII phycocyanin	VIII carotenoid	IX carbohydrate
I	frequency	1.00000								
II	A663	0.91089*	1.00000							
III	heterocyst frequency	−0.46962	−0.24387	1.00000						
IV	average filament length	0.74573*	0.56530	−0.77315*	1.00000					
V	protein	0.94561*	0.86010*	−0.55014	0.67914*	1.00000				
VI	chlorophyll A	0.49352	0.22998	−0.60043*	0.69929*	0.55261	1.00000			
VII	phyocyanin	0.93932*	0.85780*	−0.58127*	0.68860*	0.99572*	0.56937*	1.00000		
VIII	carotenoid	0.96266*	0.83167*	−0.55291	0.87298*	0.87797*	0.61949*	0.87783*	1.00000	
IX	carbohydrate	0.90624*	0.85107*	−0.59178*	0.67953*	0.98887*	0.54416	0.98998*	0.84198	1.00000

* significant at 0.05 level

protein, phycocyanin, carotenoid and carbohydrate contents of the algae are positively correlated with the 1 kHz square-wave modulated microwave frequencies at 0·05 level. The heterocyst frequency and chlorophyll A content of the algae are non-significantly correlated with the modulated microwave frequencies. Physiological parameters show that the A663 of the algae is positively correlated with the phycocyanin, carotenoid and carbohydrate contents; the heterocyst frequency is negatively correlated with the average filament length, chlorophyll A, phycocyanin and carbohydrate contents; the filament length is positively correlated with the protein, chlorophyll A, phycocyanin, carotenoid and carbohydrate contents; the protein content is positively correlated with the phycocyanin, carotenoid and carbohydrate contents; chlorophyll A is positively correlated with the carotenoid content; phycocyanin is positively correlated with the carotenoid and carbohydrate contents; the carotenoid is positively correlated with the carbohydrate contents, each at 0·05 level; and other variables are non-significantly correlated among themselves.

These correlation differences further reveal that the mechanism of the bioeffects of CW and modulated microwaves are also different, and differently restructured water caused the bioeffects by differently affecting the relationship among the physiological correlations of the algae. Similar effect mechanisms of varying 'live' water structures are reported to occur inside the living cells (WIGGINS, 1990).

4 Conclusion

This study reveals that different frequencies of CW and modulated microwaves produced significantly different athermal physiological effects on algae. These athermal bioeffects indicate that water has the capability to remember the imposed EM fields for an extended period of time. Variously restructured media produced these bioeffects by causing nutrient deficiency and modifying the structural chemistry of algal 'live' water.

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