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Enhanced Biomass Production of Cyanobacterium, A Local Isolate from Nalco Captive Power Plant Area, Angul in Heterotrophic Conditions

Swain PK, Panda MK, Behera GS, Mohanta RK and Padhi SB

Centre for Environmental Studies (CES),
Forest & Environment Department, Government of Odisha
Corresponding Author: prasannaswain19@gmail.com
Mobile: +91-7873059381; Fax: 0674-2553182

ABSTRACTS

Enhanced biomass production of a cyanobacterium (*Nostoc* sp.) isolated from NALCO captive power plant area in heterotrophic conditions was presented. The amino acids used as a substrate for heterotrophic growth of *Nostoc* sp.. The amino acids supported the heterotrophic growth of *Nostoc* sp. at different degrees. Under light conditions the specific growth rate of *Nostoc* sp. was maximum with casein hydrolysate (CHL), under dim light the specific growth rate was maximum with hydroxyl proline (HPL) and under dark condition the specific growth rate was maximum with alanine (ALA). The composite amino acid casein hydrolysate (CHL) efficiently supported heterotrophic growth of organism in all the conditions studied. The differential responses of the organism towards different amino acids in support of heterotrophic growth have been reported.

Key words: *Nostoc* sp; heterotrophic growth; CHL (Casein hydrolysate); HPL (Hydroxyproline); ALA (Alanine).

INTRODUCTION

Studies on the heterotrophic growth of blue-green algae have developed much interest now days [1-10]. Padhi et al, (1984) [11] reported the growth and pigment production of four filamentous blue-green algae under heterotrophic conditions. Dash & Padhi, (1988) [12] reported the heterotrophic growth of Scytonema schmidlei. Amino acids which are also naturally occurring organic nutrients can serve as both nitrogen and carbon sources. Stephens et al., (1969)[13] reported the importance of amino acids for heterotrophic growth of blue-green algae. Bottomley & Van Baalen, (1978) [14]

observed appreciable heterotrophic growth of Nostoc sp. in presence of casein hydrolysate, from which the alga efficiently transported and utilized the amino acids. Westiellopsis prolifica liberates ammonia, amides and poly peptides in its active growth phase as extra cellular product [15]. So the effect of exogenous amino acids on growth of blue-green algae is of obvious ecological importance. In this present communication, we report the heterotrophic growth of Nostoc sp. in presence of exogenous amino acids.

MATERIALS AND METHODS

The experimental organism, Nostoc sp is a soil blue-green alga collected from water logged fields of NALCO captive power plant area situated about 139 km.from Bhubaneswar,Odisha,India. The axenic culture of the test organism was being maintained in a culture room at 2,200 Lux light intensity and 24 ±2ºC temperature in nitrate free BG11 medium in the laboratory condition. The stock culture was regularly transferred to fresh medium in 30 days interval and always exposed to a light period of 10 hours daily. The cultures were hand shaken twice daily.

The experiments were done in 100 ml conical flasks with 25 ml basal medium with or without various treatments viz alanine, glycine, aspartic acid, hydroxy proline, arginine, leucine and casein hydrolysate. All glass double distilled water was used throughout. The chemicals used for culture were of AR Grade (BDH) and the aminoacids used for treatment were supplied by Sigma Chemical Company, USA. Stock solutions of the above amino acids were prepared and were aseptically added to the basal medium separately to obtain a desired final concentration.

The flasks without the treatments were served as control. After autoclaving, the pH of all the media were brought to 7.8 using a Systronics Digital pH meter 335 equipped with glass and calomel electrodes. White day fluorescent tubes were utilized as continuous source of illumination for light (2,200 Lux) and dim light (200 Lux) conditions throughout. The experiments in complete darkness were carried out in a dark cabinet.

Ten day old cultures of the organism were washed with sterilized distilled water and homogenized in a micro tissue homogenizer. An aliquot of cell suspension equivalent to 1 mg dry weight was used as inocula throughout the experiments. The experimental cultures were harvested in two day interval up to 20 days of incubation. Triplicates were setup to each set of experiments. Growth was routinely measured by homogenizing the culture and estimating the optical density at 530 nm in a Spectrophotometer. The corresponding dry weight values were obtained from the standard plot of dry weight vs optical density [16]. The growth vs. time plot was divided into exponential and death phase. The specific growth rate (K_e) and death rate (K_s) were calculated by least squares analysis of the equation of Kartz & Myers (1955)[17]

Selection of Amino acid Concentration

The optimum concentration of a particular amino acid selected on the basis of its growth support. The growth rate was linearly dependent on the amino acid concentration

employed, but after an optimum concentration (≈ 10 μg ml^{-1} for all the amino acids mentioned), there was a fall in growth rate with increase of amino acid concentration. So a concentration of 10 μg ml^{-1} of each substrate was selected for treatment.

RESULTS

In order to visualize the differential heterotrophic growth of the blue-green alga Nostoc sp, experiments were conducted in these conditions i.e. light (2,200 Lux), dim light (200 Lux) and complete darkness in presence of exogenous amino acids. The results are represented in table 1 in terms of specific growth rate constant (K_e) and death rate constant (K_s).

Effect of amino acids in light

The control showed a continuous growth phase upto 12^{th} day of incubation after which the death phase started. The photoheterotrophic growth enhancement as seen from the specific growth rate (K_e) **(Fig. 1)** followed the order of: Caseinhydrolysate>control>alanine≈ hydroxyproline ≈ aspartic acid > glycine ≈ arginine > leucine. Though the treatments did not support efficient photoheterotrophic growth in comparison with the control value, their nature of effect in the period of study can be classified as,(a) showing same pattern as that of control (alanine, hydroxyproline and casein hydrolysate),(b) optimum stagnancy of growth rate (aspartic acid and arginine) and (c) continuous growth support (leucine and glycine). The treatment of Caesin hydrolysate (composite amino acid source) supported the photoheterotrophic growth efficiently.

Effect of amino acids in dim light

In dim light condition, the control showed a time lag of ≈ 4 days, then a continuous growth and no death phase was observed in the period of study. The photoheterotrophic growth support is given in table 1. The order of support being: Hydroxyproline >arginine>aspartic acid ≈ leucine>casein hydrolysate>alanine>glysine >control.The results obtained in dim light showed two different types of effect in the period of incubation i.e. (a) continuous support of growth rate (K_e) after an initial lag of ≈ 4 days like that of control **(Fig. 2A)** (alanine, glycine, casein hydrolysate) with almost same growth rate constant, (b) significant photo heterotrophy after a time lag of 6-8 days **(Fig. 2B)** (except arginine) showing death phase after 16th day of incubation (hydroxyproline, aspartic acid and leucine). The K_e values were also comparatively higher than that of control.

Table1:-Effect of amino acids on specific growth rate, specific death rate and overall growth rate constants* of Nostoc sp in different conditions of growth.

Conditions	Treatment	Lag phase		Exponential Phase		Death phase		Overall growth phase(20 days)
		$K_1 \times 10^2$ $days^{-1}$	Period, days	$K_e \times 10^2$ $days^{-1}$	Period, days	$K_s \times 10^2$ $days^{-1}$	Period, days	$K_e \times 10^2$ $days^{-1}$
Light	Control			4.4	0~12	1.9	<12	2.3
	Alanine			4.0		1.9		2.8
	Casein hydrolysate			5.3		1.6		3.5
	Glycine			3.0		-1.1[a]		2.3
	Hydroxyproline			4.0		1.9		3.5
	Aspartic acid[b]			4.0		0.0	<11	2.6
	Leucine[c]			2.4		-2.4[a]	-	2.4
	Arginine[b]			3.0		-0.2[a]	<12	2.4
Dim light	Control[e]	-3.7[d]	0~4	1.4	<4			0.5
	Alanine[e]	-2.5		1.7				1.0
	Casein hydrolysate[e]	-2.0		2.2				1.7
	Glycine[e]	-2.5		1.5				0.8
	Hydroxyproline	-1.6	0~8	4.75	8 ~16		<16	0.7
	Aspartic acid[b]	-1.9	0~6	2.4	6~ 16		<16	0.8
	Leucine[c]	-1.9	0~6	2.4	6 ~16	0.85	<16	0.6
	Arginine[f]	-5.8	0~12	3.75	<12			-2.7
Dark	Control	-8.3	0~4	~0[g]	4 ~17	8.4	<17	-2.0
	Alanine	-8.3	0~5		5~ 15	8.4	<15	-1.9
	Casein hydrolysate	-8.3	0~5		5 ~12	5.5	<12	0.042
	Glycine	-11.5	0~4		4 ~16	7.6	<16	-0.2

*Deviations in K values are within ± 5%

a: Negative value in K_s represents growth in the death phase.

b: No growth or death observed upto 20 days after the exponential phase

c: continuous growth without showing death phase over the 20 days of study in light

d: Negative values in growth rate in the lag phase (K_1) indicates decrease of the organism

e: Continuous growth support without showing death phase over 20 days in dim light

f: No death phase was observed

g: Growth stagnancy in the exponential phase in dark

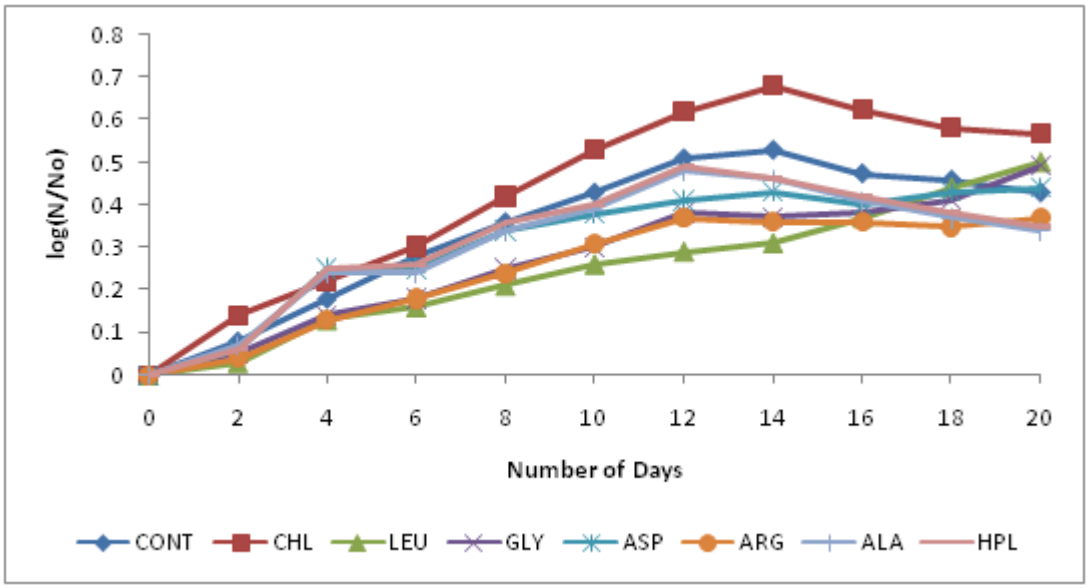

Figure-1:-Effect of aminoacids on the growth of *Nostoc* sp. in continuous light(CONT, Control; CHL, Casein hydrolysate; LEU, Leucin; GLY, Glycine; ASP, Aspartic acid; ARG, Arginine; ALA, Alanine; HPL, Hydroxyproline)

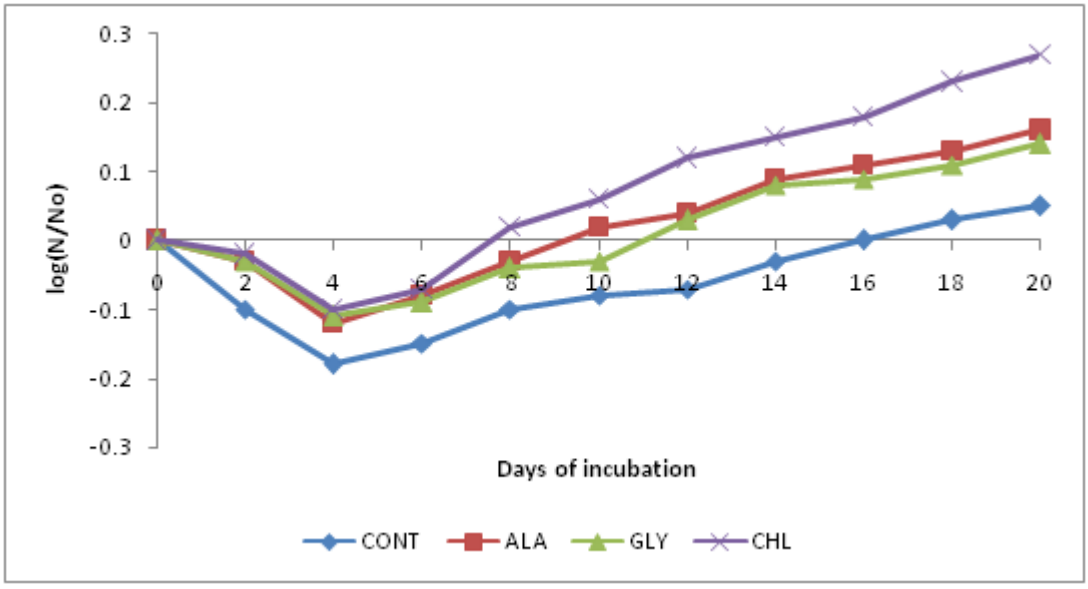

Figure-2A:-Effect of aminoacids on the growth of *Nostoc* sp. in dim light(CONT, Control; ALA, Alanine; GLY, Glycine; CHL, Casein hydrolysate)

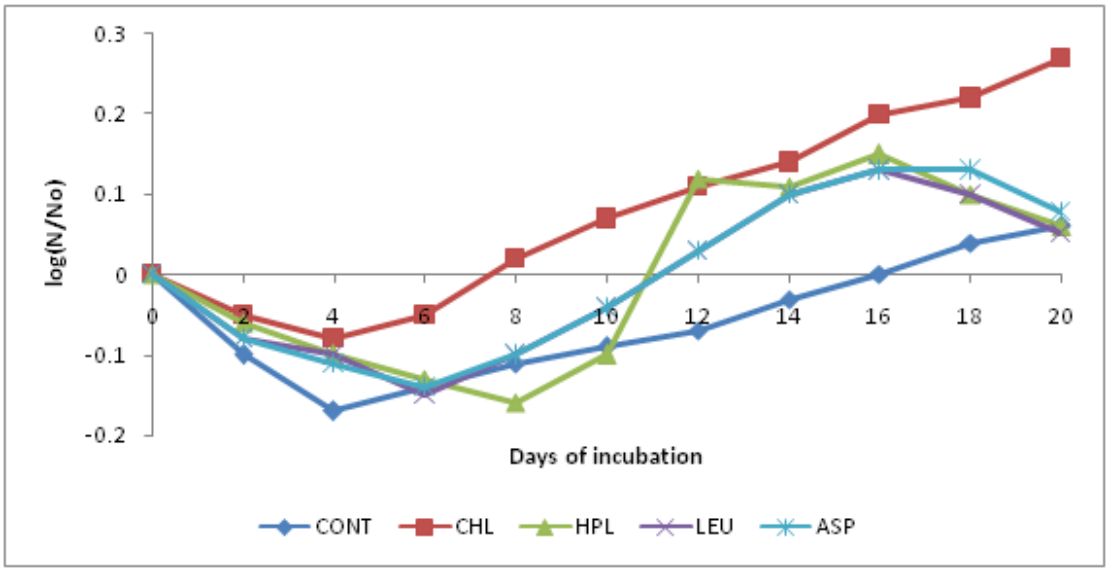

Figure-2B:-Effect of aminoacids on the growth of *Nostoc* sp. in dim light(CONT, Control; CHL, Casein hydrolysate; HPL, Hydroxyproline ;LEU, Leucin; ASP, Aspartic acid)

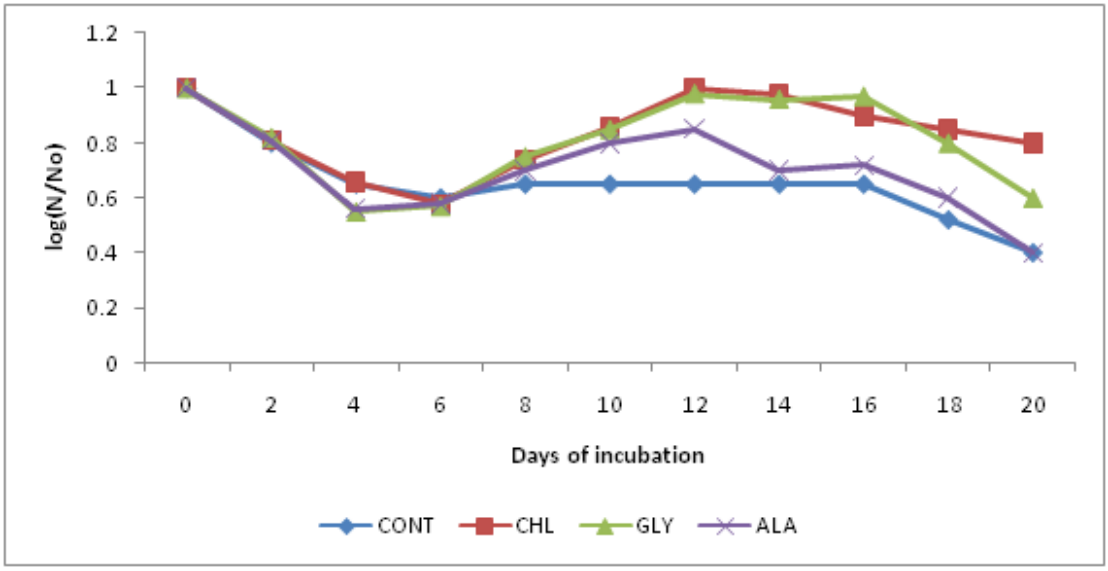

Figure-3:-Effect of aminoacids on the growth of *Nostoc* sp. in darkness (CONT, Control; CHL, Casein hydrolysate; GLY, Glycine; ALA, Alanine)

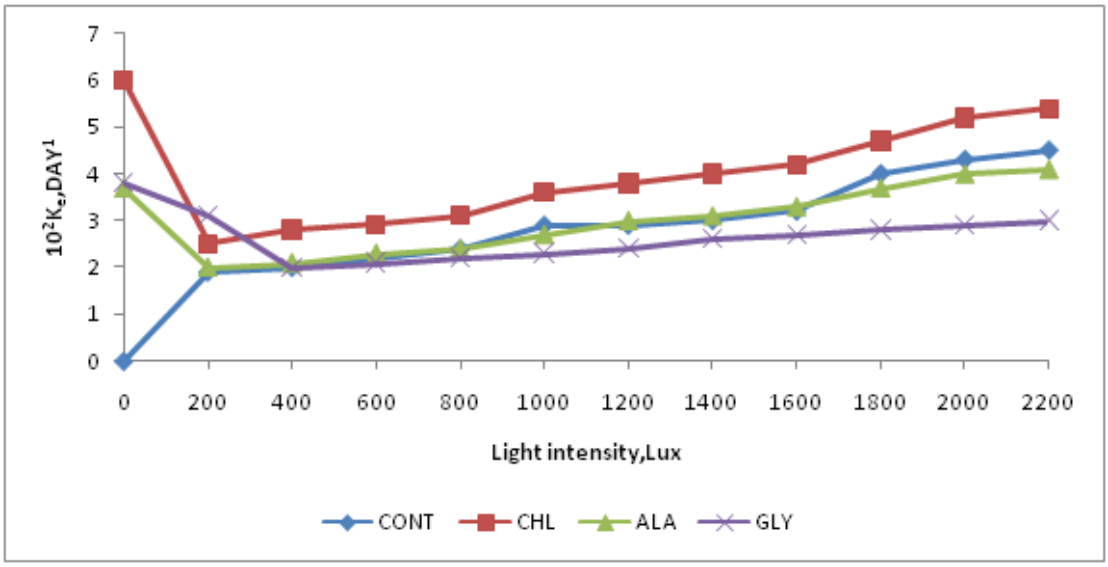

Figure-1:-Effect of different light intensities on the specific growth rate of *Nostoc* sp. in presence of exogenous amino acids (CONT, Control; CHL, Casein hydrolysate; ALA, Alanine; GLY, Glycine)

Effect of amino acids in darkness

The cell yield of the organism in the dark was less in comparison with other conditions of growth, but the results were more interesting. In case of control, there was an initial lag phase of ≈ 4 days, then a growth / death stagnancy upto ≈18 days followed by the death phase (Fig.3).All the treatments showed a time lag of 4 5 days and their trend of growth rate (K_e) being: Casein hydrolysate>alanine ≈ glysine >control. All the treatments showed death phase during the period of study **(Fig.3 and table 1)**.The differential support of growth rate by the treatments, alanine, glycine and casein hydrolysate was more prominent in the extreme conditions of growth **(Fig. 4)**. The above amino acids supported best in the dark condition.

DISCUSSION

Almost all the amino acids treated supported the heterotrophic growth of Nostoc sp at different degrees. The nature of support varied from amino acid to amino acid in the same conditions of growth and for same amino acids in different light conditions. The composite amino acid casein hydrolysate, efficiently supported heterotrophic growth of the organism in all the conditions studied. Casein hydrolysate was treated as a bulk nitrogen source from which the alga efficiently transported and utilized the amino acids and its effects were suggested to be related specifically to fructose metabolism [14]. In casein hydrolysate with addition of various by products, glutamic acid is 1.6-2.3 times higher than normal concentration [18]. Fructose was found to be the best organic source for heterotrophic growth of Nostoc sp. [16].

The low specific growth rate constant (K_e) in presence of exogenous amino acids in comparison with that of control in light may be attributed to the chemical inhibition depending upon the nature of substrate. The efficient support of photoheterotrophic growth by glycine and casein hydrolysate has been reported earlier [13-14]. The effect of leucine, aspartic acid and arginine can be explained on similar grounds. The effect of alanine and hydroxyl praline was almost same with that of control.

In dim light condition, a time lag of ≥ 4 days was observed for all the treatments. This may be due to the adverse light conditions. The greater lag phase was observed in case of hydroxyproline, leucine and aspartic acid; may be due to the additional factor of chemical inhibition. In the latter case, comparatively high K_e and K_s values were observed. Probably these amino acids fulfill the physiological requirement of the organism at this condition of growth. The few selective treatments studied in darkness efficiently supported heterotrophic growth. Comparatively very high K_e and K_s values were observed with an early start of death phase in case of casein hydrolysate treatment.The heterotrophic growth was best supported by the amino acids in complete darkness **(Fig 4)**, may be due to the utilization of the substrates by the organism[13].

Amino acids were known to increase nitrogenase activity, heterocyst frequency [19], but the differential growth support could not be explained on the basis of nitrogen content of the substrates. No definite correlation between specific growth rate constant and molecular weight or charge per molecule was observed which could have explained the permease properties. The growth showed no definite trend with the carbon/carboxyl group content of the substrates. The effect of glycine could not be compared on the basis of its carbon skeleton. No heterotrophic growth was supported in presence of exogenous acetic acid but observed significant growth in presence of Na-acetate in the same pH condition of the culture medium [16]. The support of the latter may be due to extra Na^+ or low acetate concentration employed. However, the effect of aspartic acid was comparable with that of succinic acid though there is little variation in carbon residue. Differential responses of amino acids in terms of uptake and assimilation and a role of the intracellular level of amino acid pool was assigned as one of the probabilities[20].

It was interesting that the organism showed a high time lag of ≈ 12 days in presence of arginine (high nitrogen containing amino acid) in dim light condition and there was no observable mutation [21].

CONCLUSION

All the amino acids treated supported heterotrophic growth of Nostoc sp. The amino acids not only served as simple organic nutrient sources, but also showed specific amino acid effects depending on the physiological requirement of the organism such as carbohydrate metabolism and protein synthesis etc which will support its enhance biomass production. The knowledge of the rates of deamination and transamination of different amino acids would be helpful to understand the growth kinetics of the organism. More probes are necessary to substantiate the differential support of exogenous amino acids in blue-green algae.

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