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Growth and Biochemical Changes of the Blue-Green Alga, *Anabaena doliolum* in Domestic Wastewater

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Abstract- The use of cyanobacteria (blue-green-algae) in waste treatment systems has proved to be cost-effective and beneficial. In the present research, an attempt has been mead to study the growth efficiency of the BGA, *Anabaena doliolum* in domestic wastewater and whether it could be used as an agent for low cost biological treatment of sewage. Pigment (chlorophyll & carotenoid), protein and biomass of *Anabaena doliolum* a blue-green alga, were measured when grown in different concentrations of domestic wastewater with or without Basal Nutrient Medium (BNM). The maximum chlorophyll (3.421µg/ml), carotenoid (1.047µg/ml) and protein (253.402µg/ml) were recorded on 16th days of growth followed by a declining phase. Highest biomass (1394.567mg/ml) was recorded in 100% wastewater with BNM. A two way analysis of variance for chlorophyll, carotenoid and protein content of the alga showed a significant difference between treatments of different concentrations of wastewater and between different days of growth. The result of the present study indicates that, *A. doliolum* could be used as an agent for low cost biological treatment of domestic wastewater through nutrient manipulation.

Index Terms- biomass, blue green algae, carotenoid, chlorophyll, domestic wastewater; growth rate, protein

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1 INTRODUCTION

Domestic wastewater (sewage) is the primary source of water pollution in India, especially in and around large urban centres. City wastewater is a large water resource to compensate the water shortage to certain extent, if managed properly. Reuse of wastewater is a viable solution in many instances [1], [2]. Untreated domestic wastewater contaminates fresh water bodies with high BOD and COD loading. It also contains a variety of essential plant nutrients. [3] in his experiment on impact of domestic wastewater on seed germination and physiological parameters of rice and wheat has found that, domestic wastewater supports the seed germination and subsequent growth.

Therefore, domestic wastewater must be treated properly to reduce the pollution load before it is allowed to mix up with the nearby water bodies. Biological treatment of wastewater may provide alternatives to costly physico chemical treatment and offers a high potential for waste stabilization [4]. Microalgae exhibit a number of heavy metal uptake process by different metabolism [5]. Algae when grown in wastewater can supply O_2 to heterotrophic aerobes to mineralize pollutants, such as hydrocarbons of petroleum [6], [7], toxic by-products of the industry [8] and heavy metals such as Cr, Cu, Fe, Mn, Ni, and Zn [9] from the environment or for rendering them to harmless species is defined as phytoremediation [10].

Algal systems, more particularly the Blue Green Algae (BGA) are not only useful in treating the wastes but also produce a variety of useful by-products from their biomass [11]. Cyanobacteria can be used as bioindicators of water pollution in different water habitat [12].

BGA are photosynthetic, non-vascular plants which contain chlorophyll *a* and have simple reproductive structure. They are known to have considerable potential in treating wastewater by reducing the pollution load [13], [14], [15], [16], [17], [18], [19], [20], [21] The use of sewage for enhancing cyanobacterial production is also an alternative proposition in view of economic implications and environmental contingencies [11], [22].

In the present research, an attempt has been made to study the growth efficiency of the BGA, *Anabaena doliolum* in domestic wastewater and whether it could be used as an agent for low cost biological treatment of sewage.

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2 MATERIALS AND METHOD

The experiment was conducted for a period of 20 days and the day of inoculation was considered as the 0^{th} day and the subsequent readings were taken on 4^{th}, 8^{th}, 12^{th}, 16^{th} and 20^{th} days of growth after inoculation of the alga into different concentrations of sewage with or without BNM as shown in Table 1. Pigment (chlorophyll *a* and carotenoid), protein and biomass were estimated on every fourth day of incubation.

TABLE 1 Different concentrations of domestic wastewater with or without the basal nutrient medium	
Concentration of Wastewater (%)	**Basal Nutrient Medium**
Control (distilled water)	+
25	+
50	+
75	+
100	+
Pure	_
+ indicates with nutrients; - indicates without nutrients	

2.1 Wastewater sample

For the present study, domestic wastewater sample was collected from the municipality area of Bhubaneswar. Bhubaneswar is the capital city of the state of Odisha, India. The city has a population of 8, 37,737 as per 2001 census. The drainage system in the city is controlled by the river Kuakhai and Daya. The drains in the city cover an area of about 103.43 sq km with the drainage length of 37.18km. Wastewater samples were collected from the city and were brought to the laboratory for analysis of various physico-chemical parameters [23]. Table 2 shows the physico-chemical analysis results of the wastewater. Out of the twenty one different parameters studied, total suspended solid, BOD & COD were found to be higher than the prescribed standards and all other parameters for which standards are available are within the prescribed standard. However, parameters like total nitrogen, sulphate, Zn, Cu are found to be significantly lower than their corresponding standard values. Two parameters like total residual chlorine and selenium are not detected in the wastewater sample thus their contribution is not significant in the sample.

TABLE 2
PHYSICOCHEMICAL CHARACTERISTICS OF DOMESTIC WASTEWATER

Sl. No.	**Parameters**	**Values**	**Standard for disposal into inland surface water**
1	Colour	Light Black	-
2	Odour	Unpleasant	-
3	Ph	6.85	5.5-8.0
4	Turbidity (NTU)	94	-
5	Total Suspended Solid mg/l	250	100
6	Total Dissolved Solid mg/l	630	2100
7	Total Solids mg/l	880	-
8	Oil & Grease mg/l	4.0	10
9	Total Residual Chlorine mg/l	ND	-
10	Total Kjeldahl Nitrogen(as N) mg/l	8.2	100
11	Free Ammonia(as NH_3) mg/l	1.8	-
12	Biochemical Oxygen Demand (3 days at 27^0C) mg/l	130	30
13	Chemical Oxygen Demand mg/l	280	250
14	Copper (as Cu) mg/l	0.019	3.0
15	Zinc (as Zn) mg/l	0.057	1.0
16	Selenium (as Se) mg/l	ND	-
17	Dissolved Phosphate (as P) mg/l	2.5	5.0
18	Sulphide (as S) mg/l	1.1	2.0
19	Sodium (as Na) mg/l	95	-
20	Potassium(K) mg/l	30	-
21	Sulphate(SO_3) mg/l	240	1000

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2.2 Test organism and culture condition

The nitrogen-fixing cyanobacterium, *Anabaena doliolum* was employed as the test organism in the present study. Experiments were conducted using axenic culture of *A. doliolum*. The alga was grown in the recommended BG11 nitrogen free medium in the laboratory. The culture was maintained at appropriate environmental conditions. It was hand shaken thrice daily to get a uniform suspension and exponential growth phase of the alga was maintained.

2.3 Estimation of pigments (chlorophyll *a* & carotenoide) and protein

Algal suspension (10ml) was centrifuged for 10 minutes at 5,000 rpm. Residues containing the homogenised algal filaments were extracted with 80% methanol. The pigment content (µg/ml of algal suspension) was determined spectrophotometrically and was calculated by using the formula of [24]. Protein content (µg/ml of algal suspension) was estimated following the method of [25].

2.4 Biomass measurement

For biomass study, a known amount of culture was harvested on the last day (20^{th} day) of the study and oven dried at $105^{0}C$ until constant weight was obtained and was expressed in mg/ml.

2.5 Statistical analysis

The data obtained from the experiments were from triplicates and were represented as mean ± standard deviation. Further, the variations in the results obtained with respect to different concentrations of treatment of wastewater as well as different days of growth were statistically analysed using 2-way Analysis of Variance (ANOVA) test. Minitab software (version 14.0) was used for the statistical analysis.

3 RESULTS AND DISCUSSION

3.1 Chlorophyll content

Fig. 1 shows the changes in chlorophyll content over days of growth of *A. doliolum* during 20 days incubation period at various concentrations of sewage. It was observed that, with increase in days of incubation the total chlorophyll content increases giving a peak value on 16^{th} day of growth followed by a decline in all the concentrations except in pure wastewater where the peak value was reached on 12^{th} day of incubation. Further, the highest chlorophyll was recorded in 100% wastewater with BNM (peak value 3.421 on 16^{th} day), followed by 75% wastewater (peak value 3.310 on 16^{th} day), 50% wastewater (peak value 2.968 on 16^{th} day), 25% wastewater (peak value 2.519 on 16^{th} day), control (peak value 2.386 on 16^{th} day). The lowest chlorophyll was recorded in pure wastewater without BNM (peak value 1.022 on 12^{th} day).

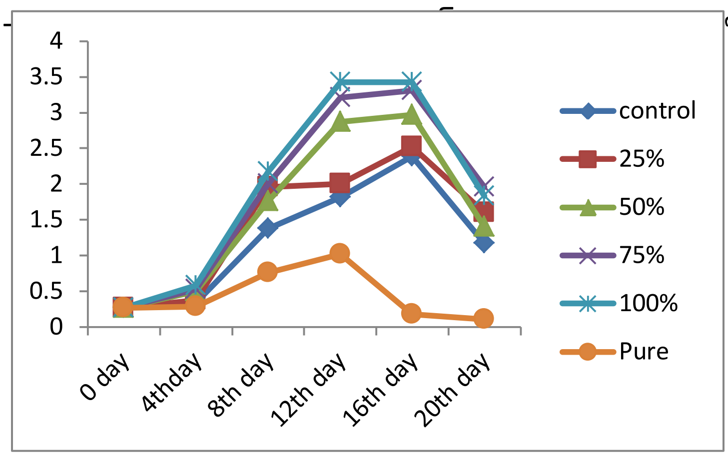

Fig. 1: Changes in chlorophyll content (µg/ml) over days of growth of *A. doliolum* in different concentrations of domestic wastewater.

A two way analysis of variance for chlorophyll content of the alga showed a significant difference between treatment of different concentrations of wastewater ($F_1 = 11.64$ at $P \leq 0.001$) and between different days of growth ($F_2 = 23.07$ at $P \leq 0.001$).

3.2 Carotenoid content

Fig. 2 shows the changes in carotenoid content over days of growth of *A. doliolum* during 20 days incubation period at various concentrations of sewage. It was observed that, with increase in days of incubation the carotenoid content also increases giving a peak value on 16th day of growth followed by a decline in all the concentrations except in pure wastewater where the peak value was reached on 8th day of incubation. Further, the highest carotenoid content was recorded in 100% wastewater with BNM (peak value 1.047 on 16th day), followed by 75% wastewater (peak value 0.943 on 16th day), 50% wastewater (peak value 0.767 on 16th day), 25% wastewater (peak value 0.603 on 16th day), control (peak value 0.513 on 16th day). The lowest carotenoid was recorded in pure wastewater without BNM (peak value 0.321 on 8th day).

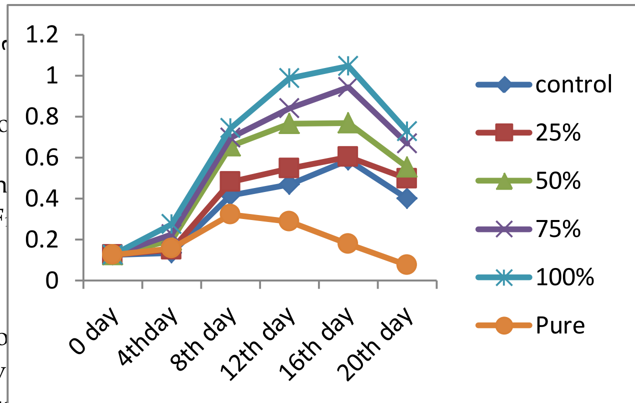

Fig. 2: Changes in carotenoid co ... owth ... rent concentrations of domestic wastewater.

A two way analysis of varian ... ficant difference between treatment of different concentrations of wastewater (F ... th (F_2= 19.05 at P≤ 0.001).

3.3 Protein content

Fig. 3 shows the changes in pro ... of *A. doliolum* during 20 day ... concentrations of sewage. Protein content of the alga also showed a similar trend like pigment content. It was observed that, with increase in days of incubation the protein content increases giving a peak value on 16th day of growth in all the ... except in 50% dilution where the pick value was ... day. The highest protein was recorded in 100% ... th BNM (peak value 253.402 on 16th day), followed ... water (peak value 247.586 on 16th day), 25% wastewater (peak value 239.789 on 16th day), control (peak value 223.586 on 16th day), 50% (peak value 221.839 on 12th day). The lowest protein content was recorded in pure wastewater without BNM (peak value 211.136 on 16th day).

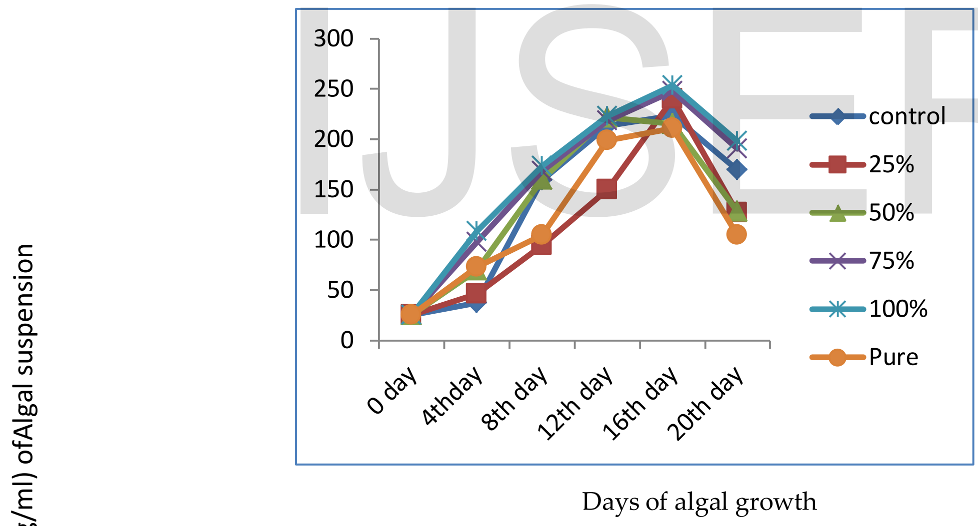

Fig. 3: Changes in protein content (µg/ml) over days of growth of *A. doliolum* in different concentrations of domestic wastewater.

A two way analysis of variance for protein content of the alga showed a significant difference between treatment of different concentrations of wastewater (F_1 = 6.85 at P≤ 0.001) and between different days of growth (F_2= 54.77 at P≤ 0.001).

3.4 Biomass content

The biomass was harvested on the last day of study (20th day). Highest biomass was recorded in 100% wastewater with BNM (1394.567) followed by 75% (1324.175), 50% (1307.274), 25% (1287. 224), control (1223.217) and lowest biomass was recorded in pure wastewater without BNM (689.666) as shown in Table 3.

TABLE 3
BIOMASS OF A. DOLIOLUM GROWN IN DIFFERENT CONCENTRATIONS OF DOMESTIC WASTEWATER

Sl. No.	Concentration of wastewater	Biomass (mg/ml)
1	Control	1223.217
2	25%	1287.224
3	50%	1307.274
4	75%	1324.175
5	100% with BNM	1394.567
6	Pure wastewater	689.666

The increase in pigment (chlorophyll & carotenoid) and protein content with increase in different concentration of sewage and decrease in pigment (chlorophyll & carotenoid) and protein content in pure wastewater indicates that, the pure wastewater lacks few essential nutrients to support the algal growth. During the growth, cyanobacteria uptake nutrients from the medium and thereby reduces the pollution loads. [18], in their study have found out that, *Westiellopsis prolifica* has the capacity to reduce pollution loads when grown in different concentration of paper mill wastewater. Dash and Mishra have also found that, 100% paper mill wastewater with BNM favours the growth of *W. prolifica.* [26] in their study of the effect of sugar factory effluent observed that, higher pigment content in *W. prolific* in pure wastewater supplemented with nutrient medium. In another study, [5] have found out that, *S. bijuga* grown in sewage wastewater contained higher chlorophyll *a,* carotenoid and protein content corresponding to standard medium.

The result of the present study shows that, higher concentration of sewage with BNM favours the growth of *W. prolifica.* However, pure sewage without BNM inhibits the growth of the alga. This shows that, sewage lacks few appropriate nutrients to sustain algal growth. Cyanobacteria are efficient in the removal of nutrients from wastewater. Thus, many cyanobacterial species proliferate in wastewater due to the abundance of carbon, nitrogen and phosphorus which acts as nutrients for algae. [5] in their study on growth and heavy metal accumulation potential of microalgae grown in sewage wastewater and petrochemical effluent have observed that, microalgae cultivated in sewage wastewater with high metal content also accumulated higher metal contents. [20] have also reported the adsorption capacity, quantitative uptake and accumulation of Zn, Pb, Mn and Fe by *Microspora quasdrata.* Therefore, from the present study it is evident that, with proper nutrient manipulation, cyanobacteria can be used as a low cost biological treatment for domestic wastewater.

4 CONCLUSION

From the present investigation, it is understood that, growth response of *A. doliolum* in domestic wastewater is encouraging. Higher growth rate in wastewater with BNM and lowest growth in wastewater without BNM indicates that, pure wastewater lacks the appropriate nutrient to support the growth of the cyanobacteria. Thus, with proper nutrient manipulation, sewage could be utilized as a supplement to inorganic nutrient media for the growth of blue-green algae during biological treatment of wastewater, thereby reducing the cost.

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