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Removal of arsenic by Cyanobacterial species in static culture condition

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Abstract

Three species of Cyanobacteria namely, *Anabaena, Nostoc* and *Leptolyngbya* were grown in BG11 medium supplemented with arsenic salt (250µg/L). The flasks were kept in a static condition with the exposure of light 30-35 µMole of photon $m^{-2}s^{-1}$and the exposure of period was maintained as light: dark ratio of 14:10 hrs. After seven days incubation, MLSS (mg/L) increased considerably in all three species. The difference in mean arsenic removal between *Anabaena* and *Leptolyngbya* is statistically significant and the removal efficiency of *Leptolyngbya* is much higher in comparison to *Anabaena* and *Nostoc* hence, it can be safely concluded that *Leptolyngbya* is most suitable for removal of arsenic in static culture system.

Keywords: Arsenic | Cyanobacteria | adsorption

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Introduction

Arsenic is a toxic metalloid and the compounds containing arsenic are known to cause toxicity to plants, animals and microbes. The primary aim of the present study is to assess the nature of effect, if any, on Cyanobacterial species and the capacity of these species in the removal of arsenic from the system. In the present study three organisms namely, *Anabaena, Nostoc* and *Leptolyngbya* were used for arsenic exposure in static culture condition. Although for any commercial application the static culture is not suitable yet, the static culture studies definitely provide the basic data on the different subjects, on the basis of which scale up and the other study can be designed. The Cyanobacterial membrane surface is very good adsorbent system for positive charged metal ions. However arsenic is being a metalloid ion can enter into the cells and get accumulated intracellularly using the transporters of other compounds such as phosphate (Akira Takahashi *et al.*, 2001). The report of arsenic tolerance and removal by Cyanobacterial species require a justification for the use of *Cyanobacteria* in the management of arsenic. Conventionally there are different techniques

available for the management of heavy metals including arsenic. The common technologies available include coagulation, adsorption, ion-exchange, electro-coagulation and biological process (Wickramasinghe, S.R.*et al.*, 2004, Zhang, Y.*et al*., 2003, Kim J. and Benjamin, M.M., 2004, Kumar P.R.*et al.*, 2004).

Materials and Methods

Development of static culture system

For effective growth of Cyanobacteria in the static culture system it is necessary to optimize the growth of Cyanobacterial species. Sterile BG11 medium was prepared by adding 500 mg of amoxicillin per liter and the medium was sterilized by membrane filter technique (MFT). 250 ml of this sterile medium was taken in 9 separate sterile 500 ml conical flasks. These flasks were inoculated with 5ml culture of *Anabaena, Nostoc* and *Leptolyngbya* in triplicate. The flasks were kept in the static chamber as shown in photograph 1.1. The light intensity was maintained at 30-35 µMole of photon $m^{-2}s^{-1}$and the exposure of period was maintained as light: dark ratio of 14:10 hrs. All the experiments were conducted in the ambient room temperature approximately 28± 2^0C. The flasks were incubated for 5 days. After each day the flasks were thoroughly mixed and 20 ml culture was taken out aseptically and filtered through GF/C filter papers for determining the MLSS. Each day all the flasks were re-supplemented with 20 ml of sterile BG 11 media to maintain the volume of the flask. All the three samples showed net increase in MLSS mg/L and the increase in MLSS is shown in table 1 and the statistical analysis is shown in table 2, 3 and 4

Estimation of arsenic

Throughout all the experiments mili-Q water was used for carrying out the experiments. The standard solution of the arsenic was prepared by dissolving sodium arsenate in mili-Q water to prepared according to need of the experiment. Whenever the sample was collected, they were filtered through Wattman's filter paper no.42 and the filtrate was maintained at pH 2 by adding nitric acid and all the samples were preserved at 4^oC for further analysis. The arsenic was estimated by Atomic Absorption Spectroscopy (AAS) where standardization was done at the arsenic content of 50 - 250µg/L. Throughout the experiment the arsenic was represented in µg/L.

Results and discussion

Effect of arsenic on growth of Cyanobacterial species

BG 11 medium is prepared in bulk and 500 mg. amoxicillin per liter was added in the medium. 200 ml of such medium was dispersed in 250 ml beakers and beakers were set into three sets of five. In each sets of 5 beakers increasing concentration of arsenic namely 125 µg/L, 250 µg/L, 375 µg/L, 500µg/L, 625µg/L, were added. One set was inoculated with fresh culture of Anabaena with initial MLSS of100mg/L. The second set was inoculated with *Nostoc* with initial MLSS of100mg/L. Similarly, the third set was inoculated with *Leptolyngbya* with initial MLSS of100mg/L. All the flasks were kept in the static chamber with an exposure of light intensity of 30-35 µMole of photon $m^{-2}s^{-1}$and the light: dark ratio of 14:10 hrs. at ambient room temperature of above 30 ± 2^0C.The

flasks were incubated for 7 days. After 7 days of incubation 20 ml of culture sample were taken out and MLSS was determined. The filtrate was stabilized by adding 0.5 ml of concentrated HNO_3 and the residual arsenic in the filtrate was determined by Atomic Absorption Spectroscopy (AAS) method. The net MLSS after 7 days and the residual arsenic are shown in table 5, 6 and 7 respectively and table 1.8 represents the consolidated data of these three tables. The corresponding statistical analysis is shown in table 9, 10 and 11.

All the three cultures were grown in the similar physical conditions such as light intensity, temperature of incubation and period of L: D ratio and were kept same. When the three cultures were grown for seven days the MLLS mg/L increased considerably in all the three species. *Anabaena* grew from 125± 7 to 1280± 8 mg/L of MLLS and *Nostoc* showed a net growth of 110± 4 mg/L MLLS to 1620± 12 mg/L of MLLS. Similarly *Leptolyngbya* grew from 85±7 mg/l of MLLS to 1460±9 mg/L of MLLS. However, when t-test was performed between the mean growth of *Anabaena* and *Nostoc,* the null hypothesis was not rejected.

Theoretically, the null hypothesis is where the net growth increase is same. The basis for t-test is that the null hypothesis is not rejected if, - t Critical two-tail < t Stat < t Critical two- tail holds good, this indirectly means that the difference between the means is not statistically significant at P(T<=t) two-tail.

The null hypothesis is generally rejected when t stat < - t Critical two-tail or t Stat > - t Critical two- tail. In this case the difference between the means is statistically significant at P(T<=t) two-tail.

As can be seen from table 2, - t Critical two-tail is - 2.446911846 which is less than t Stat = -2.379765934. Hence, - t Critical two tail is < t Stat at P(T<=t) two-tail at 0.054785625. Hence, the difference between the means of the MLLS of *Anabaena* and *Nostoc* is statistically not significant. Although there is apparent difference in the MLLS of the two species yet statistically the difference is untenable and these two species grow at the same extent.

Similarly, when the mean MLLS of *Nostoc* and *Leptolynbya* are compared by t-test, here to we found that–t Critical two-tail < t Stat, therefore the null hypothesis cannot be rejected and the mean difference in MLLS of these two species is statistically is not significant (table 3).

Similarly comparison of *Anabaena* and *Leptolyngbya* for mean MLLS reveals that – t Critical two-tail < t Stat, hence the difference in mean between these two species is statistically not significant. However, the p-value of *Anabaena, Nostoc* and *Leptolyngbya* reveals that the confidence limit of *Anabaena* and *Leptolynbya* is quiet different *i.e.* P(T<=t) two-tail is 0.17928327.

It can be clearly said that as far as the growth in BG11 medium is concerned, all the three species are growing similarly in the BG11 medium at the provided physiochemical conditions.

When arsenic removal studies were performed at five different concentrations of arsenic in µg/L, the % removal of arsenic at different concentration were found to be different of *Anabaena, Nostoc* and *Leptolyngbya* as can be

seen from consolated table 8. The statistical analysis at table 9 shows that t Stat > t Critical two- tail.

S. No.	Time of incubation in hrs.	MLSS (mg/L)		
		Anabaena	*Nostoc*	*Leptolyngbya*
1	24	125±7	110±4	85±7
2	48	240±5	340±7	180±6
3	72	380±10	430±6	380±5
4	96	470±6	490±8	495±7
5	120	640±5	740±12	760±10
6	144	1000±12	1360±15	1280±12
7	168	1280±8	1620±12	1460±9

Table 1: Cultural growth in terms of MLSS (mg/L)

S. No.		***Anabaena***	***Nostoc***
1	Mean	590.7142857	727.1428571
2	Variance	173986.9048	312190.4762
3	Observations	7	7
4	Pearson Correlation	0.99367339	
5	Hypothesized Mean Difference	0	
6	df	6	
7	t Stat	-2.379765934	
8	P(T<=t) one-tail	0.027392812	
9	t Critical one-tail	1.943180274	
10	P(T<=t) two-tail	0.054785625	
11	t Critical two-tail	2.446911846	

Table 2: T-test: paired with *Anabaena* and *Nostoc* for means of MLSS (mg/L)

S. No.		***Nostoc***	***Leptolyngbya***
1	Mean	727.1428571	662.8571429
2	Variance	312190.4762	283498.8095
3	Observations	7	7
4	Pearson Correlation		0.992136337
5	Hypothesized Mean Difference		0
6	df		6
7	t Stat		-2.320955016
8	P(T<=t) one-tail		0.029685643
9	t Critical one-tail		1.943180274
10	P(T<=t) two-tail		0.059371286
11	t Critical two-tail		2.446911846

Table 3: T-test: paired with *Nostoc* and *Leptolyngbya* for means of MLSS (mg/L)

S. No.		***Anabaena***	***Leptolyngbya***
1	Mean	590.7142857	662.8571429
2	Variance	173986.9048	283498.8095
3	Observations	7	7
4	Pearson Correlation	0.994451028	
5	Hypothesized Mean Difference	0	
6	df	6	
7	t Stat	-1.520158288	
8	P(T<=t) one-tail	0.089641635	
9	t Critical one-tail	1.943180274	
10	P(T<=t) two-tail	0.17928327	
11	t Critical two-tail	2.446911846	

Table 4: T-test: paired with *Anabaena* and *Leptolyngbya* for means of MLSS (mg/L)

The difference of means of arsenic removal between *Anabaena* and *Nostoc* is statistically significant at P(T<=t) two-tail of 0.002157122. In case of comparison between *Nostoc* and *Leptolyngbya* t Stat < - t Critical two tail at P(T<=t) two-tail is 0.008539247 as can be seen 1.10. Thus, the difference in the mean arsenic removal between *Nostoc* and *Leptolyngbya* is statistically significant. Similarly comparison between *Anabaena* and *Leptolyngbya* also reveals that t Stat < - t Critical two- tail at P(T<=t) two-tail of 0.031909674 (Refer table 1.11). Hence, the difference in mean arsenic removal between *Anabaena* and *Leptolyngbya* is statistically significant. As can be seen from table 8 the removal efficiency of *Leptolyngbya* is much higher in all the five concentration in comparison to *Anabaena* and *Nostoc* hence, it can be safely concluded from the above observation that *Leptolyngbya* is most suitable for removal of arsenic in static culture system.

S. No.	Initial arsenic (µg/L)	Initial MLSS (mg/L)	Residual Arsenic (µg/L)	MLSS after 7 days (mg/L)	% removal of arsenic	% decrease in MLSS
1	125	100	12.6	1050	89.92	17.96
2	250	100	36.9	997	85.24	22.10
3	375	100	126.6	1080	66.24	15.63
4	500	100	382.2	1020	23.56	20.31
5	625	100	446.8	735	28.51	42.58

Growth in terms of MLSS (mg/L) after 7 days = 1280

Table 5: Effect of arsenic on *Anabaena*

S. No.	Initial arsenic (µg/L)	Initial MLSS (mg/L)	Residual Arsenic (µg/L)	MLSS after 7 days (mg/L)	% removal of arsenic	% decrease in MLSS
1	125	100	18.40	1220	85.28	24.69
2	250	100	42.80	1320	82.88	18.52
3	375	100	142.6	1200	61.97	25.92
4	500	100	410.2	1010	17.96	37.65
5	625	100	485.4	945	22.34	41.67

Growth in terms of MLSS (mg/L) after 7 days = 1620

Table 6: Effect of arsenic on *Nostoc*

S. No.	Initial arsenic (µg/L)	Initial MLSS (mg/L)	Residual Arsenic (µg/L)	MLSS after 7 days (mg/L)	% removal of arsenic	% decrease in MLSS
1	125	100	10.6	1200	91.52	17.80
2	250	100	28.4	1240	88.64	15.06
3	375	100	110.3	1050	70.59	28.08
4	500	100	326.2	980	34.76	32.88
5	625	100	410.4	960	34.34	34.25

Growth in terms of MLSS (mg/L) after 7 days = 1460

Table 7: Effect of arsenic on *Leptolyngbya*

S. No.	% removal of arsenic		
	Anabaena	***Nostoc***	***Leptolyngbya***
1	89.92	85.28	91.52
2	85.24	82.88	88.64
3	66.24	61.97	70.59
4	23.56	17.96	34.76
5	28.51	22.34	34.34

Table 8: Percent removal of arsenic by Cyanobacterial sp.

S. No.		***Anabaena***	***Nostoc***
1	Mean	58.694	54.086
2	Variance	970.54178	1044.30348
3	Observations	5	5
4	Pearson Correlation	0.999603902	
5	Hypothesized Mean Difference	0	
6	df	4	
7	t Stat	7.030126649	
8	P(T<=t) one-tail	0.001078561	
9	t Critical one-tail	2.131846782	
10	P(T<=t) two-tail	0.002157122	
11	t Critical two-tail	2.776445105	

Table 9: T-test: paired with *Anabaena* and *Nostoc* for means of arsenic removal

S. No.		***Nostoc***	***Leptolyngbya***
1	Mean	54.086	63.97
2	Variance	1044.30348	785.6492
3	Observations	5	5
4	Pearson Correlation	0.99852276	
5	Hypothesized Mean Difference	0	
6	df	4	
7	t Stat	-4.817386553	
8	P(T<=t) one-tail	0.004269624	
9	t Critical one-tail	2.131846782	
10	P(T<=t) two-tail	0.008539247	
11	t Critical two-tail	2.776445105	

Table 10: T-test: paired with *Nostoc* and *Leptolyngbya* for means of arsenic removal

S. No.		***Anabaena***	***Leptolyngbya***
1	Mean	58.694	63.97
2	Variance	970.54178	785.6492
3	Observations	5	5
4	Pearson Correlation	0.997959998	
5	Hypothesized Mean Difference	0	
6	df	4	
7	t Stat	-3.232174545	
8	P(T<=t) one-tail	0.015954837	
9	t Critical one-tail	2.131846782	
10	P(T<=t) two-tail	0.031909674	
11	t Critical two-tail	2.776445105	

Table 11:T-test: paired with *Anabaena* and *Leptolyngbya* for means of arsenic removal

Photograph 1 Static Photo-Bioreactor

Conclusion

It can be safely concluded from the observations that *Leptolyngbya* is most suitable for removal of arsenic in static culture system.

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