Optimization of Exopolysaccride Production by Anabaena Species

[a]Deepti Bhabhra, [b]K.Roychoudhry

[a]S.K.Porwal College,Kamptee, India

[b]Ex. Officiating Principal, S.K.Porwal College, Kamptee, India

Abstract

Anabaena species grown in BG11 produced exopoysaccride in a static culture maintained at 28°C ±2°C at a light intensity of 35-40 µmole photon $m^{-2}s^{-1}$ with a light : dark period of 14:10 hours. The increase in citric acid and ferric ammonium citrate to 0.24 gm/lit produced 49.83% of EPS in comparisons to total cell mass. The decreased divalent cation to 0.005gm /lit enhanced the EPS production to around 30.40% of the total cell mass. Ammonium sulphate when substituted as nitrogen source reduced total cell mass while extensively produced exopolysaccride to almost 126.3% of the total cell mass. Similarly increase in salinity gave 39.12% of EPS of the total cell mas. Change in oxygen tension did not affect the EPS production and the pH optima was found to be pH 7.

KEYWORDS : anabaena, EPS, optimization, nutrient modification.

INTRODUCTION :-

Certain cyanobacterial species are capable of producing extracellular polysaccrrides which are variously called extrapolysaccrides, extracellular polysaccrides, exopolysaccride or simple 'EPS'. The cyanobacterial EPS are of extreme importance in the present day as they have many biotechnological applications. In general microbial EPS possess novel functionalities and better physiochemical properties. At times microbial EPS are highly cost effective (Selbmann et al.,). The algal cells and cyanobacteria are able to produce EPS that possess extremely usely rheological attributes therefore they are industrial important.(Belinger et al., 2010, Golaguel et al., 1999, Richard et al., 2005).

The production of EPS by cyanobacteria species are dependent on various factors. These factors not only influences the quantity of EPS production but also affects the quality of EPS produced. The conditions such as the presence of growth stimulants, age of the culture, nitrogen, and phosphorous contents, salinity etc.affects the quality of EPS production.(Chakrobaty et al., 2012). In view of this thorough studies on EPS productions using a specific bioreactor under controlled conditions is immediately called for.

For optimising the production of EPS different approaches are involved including variations in media compositions, pH, salinity and oxygen tension. We selected BG11 medium for the optimization of EPS production using anabaena species. The rationale behind media alteration was to minimally change selected ingredients of BG11 media without disturbing the gross composition of this medium. The physical parameters such as pH, salinity and oxygen tension where distinctively varied to optimize the EPS production.

MATERIALS AND METHOD :-

Purification and stabilization of stock culture

150ml of sterile BG11 media was despensed in 250ml Erlenmeyer flask. The medium was made free of bacteria and other algal matters by adding cyclohexamide to the tune of 0.16Mm of cyclohexamide as final concentration. The bacterial growth were inhibited by achieving a final concentration of 40µgm/ml of ampicillin and 100µgm/ml of streptomycin. (Allen 1968, Parikh and Madamwar 2005). Different flask were inoculated with the cultures of anabaena in triplicate. Similarly these same cultures were grown in ASNIII medium in the same fashion as that of BG11. The flask were incubated in a static chamber of size 2ft×1 ft maintained at $28^{o}C\pm 2^{o}C$ with the exposure of light intensity of 40-45 µm photons/m^{-2}/s^{-1}. The light source was fitted in the hood of the chamber and the exposure of light:dark period was kept constant at 14:10 hours. The intensity of light was standardize using Hanna's lux meter. The cultures were incubated for 7-10 days and then the purity of the cultures were confirmed by stricking the cultures on BG11 agar plates. The final conformation of the purity of the species was done microscopically.

Partial purification of supernatant

After the centrifugation of the culture contents the supernatant were concentrated to ¼ of the volume on the magnetic stirrer maintained at 60℃ for 6-8 hours. From these concentrated supernatant the EPS was precipitated by adding equal volume of cold acetone very gradually. The mixture of concentrated supernatant and the acetone were kept at $4^{o}C$ overnight. The EPS were dried finally in a desiccator.

OPTIMIZATION BY MEDIA ALTERATION

Three fundamental changes were brought about in the basic BG11 medium for optimizing the EPS production. They include variation in citric acid and ferric ammonium citrate, variations in magnesium and calcium salt and the change in nitrogen source from sodium nitrate to potassium nitrate and ammonium sulphate.

ALTERATION IN CITRIC ACID CONCENTRATION

Conventionally BG11 contains 0.0056gm of citric acid and 0.016gm ferric ammonium citrate per litre of the medium. The modified medium contained citric acid + ferric ammonium citrate to around 0.024gms of citrate and ferric ammonium citrate.

500ml of modified BG11 medium containing 0.016gm of citric acid and ferric ammonium citrate, 0.024gm of citric acid + ferric ammonium citrate per liter were despensed in three separate flask. The flask were treated with ampicillin-streptomycin combination in the concentration as described earlier. The flask were inoculated with 1ml of freshly grown anabaena species from a steady density culture. The flask were incubated in static chamber at 28℃ ± 2℃ with the exposure of light intensity ranging from 35-40 µmol photon m^{-2} s^{-1} with a light : dark ratio of 14 :10 hours for 7 days. After incubation the cells were centrifuged at 5000 r.p.m for 20 min at 20℃ in C24 Remi centrifuge. The supernatant was concentrated to ¼ of its volume using a magnetic stirrer at 60℃ for 6-8 hours. The concentrated supernatant was gradually mixed with equal volume of cold acetone and kept for overnight at 4℃. The EPS was

dried in a desiccator and the dried EPS was weighed. The centrifuged cells from the pellet were recovered and dried at 103℃ for one hour and total dry cell mass was calculated. The dried EPS are shown in photograph 1 in net change in total dry cell mass and total dry weight of EPS are shown in table 1.

TABLE 1

EFFECT OF CITRATE CONTENT ON EPS PRODUCTION BY ANABAENA SPECIES

Citric acid + ferric ammonium citrate Gm/lit	Total cell mass weight Gm/lit	Total dry weight of EPS Gm/lit	% EPS of total cell mass
0.016	7.006	2.837	40.49
0.020	9.986	1.95	19.52
0.024	7.324	3.65	49.83

ALTERATION IN CALCIUM AND MAGNESIUM CONTENT

The conventional BG11 medium contains 0.036 gm of magnesium sulphate and 0.0367 gm of calcium chloride. 500 ml of BG11 medium were taken in 3 flask of 1 lit size and the total calcium and magnesium in terms of magnesium sulphate and calcium chloride was modified to 0.05gm, 0.06gm and 0.07gm/lit while depensing in three different flask. The flask were treated with ampicillin-streptomycin combination as described earlier. Each of the flask was inoculated with 1ml of freshly culture anabaena species from steady density state. The flask were incubated in the static chamber at 28℃ ± 2℃withthe exposure of light intensity of 35-40µmolphoton $m^{2-}s^{-1}$withlight : dark ratioof14-10 hoursfor 7 days . after incubation the flask werecentrifugeat 5000 r.p.m for 20 min at 20℃in a RemiC24 centrifuge and the supernatant was concentratedto ¼ and the dry weight of EPS was calculated as described above. similarly total dry cell mass and total dry EPS were also calculated.Theresults are shown in table2

TABLE 2

EFFECT OF CALCIUM + MAGNESIUM CONTENT ON EPS PRODUCTION BYANABAENA SPECIES

Magnesium sulphate + calcium chloride Gm/lit	Total cell mass weightGm/lit	Total dry weight of EPSGm/lit	% EPS of total cell mass
0.05	5.22	1.587	30.40
0.06	5.716	1.65	28.86
0.07	6.248	1.025	16.40

EFFECT OF NITROGEN CONTENT ON EPS PRODUCTION

Conventional BG11 medium contains 1.50gm of sodium nitrate per litre as the principle nitrogen source . We change the nitrogen source to a separately of either

potassium nitrate or ammonium sulphate keeping the final content in both the cases as 1.5gm/lit. We prepared two separate medium of BG11 one containing 1.5gm of potassium nitrate and the other containing 1.5gm ammonium sulphate per litre. 500 ml of each media were taken in 1 litre elernmyer flask and the experiment was conducted exactly in the same fashion as described earlier. Finally the total dry cell mass and total dry weight of EPS were calculated and the results are shown in table 3.

Table 3 : EFFECT OF NITROGEN SOURCE ON EPS PRODUCTION

	KNo_3	**$(NH_4)_2SO_4$**
Cell mass weight	5.83 gm/lit	4.022 gm/lit
Dry weight of EPS	3.76 gm/lit	5.08 gm/lit
% EPS of total dry weight of cell	64.49%	126.3%

EFFECT OF pH ON EPS PRODUCTION BY ANABAENA SPECIES.

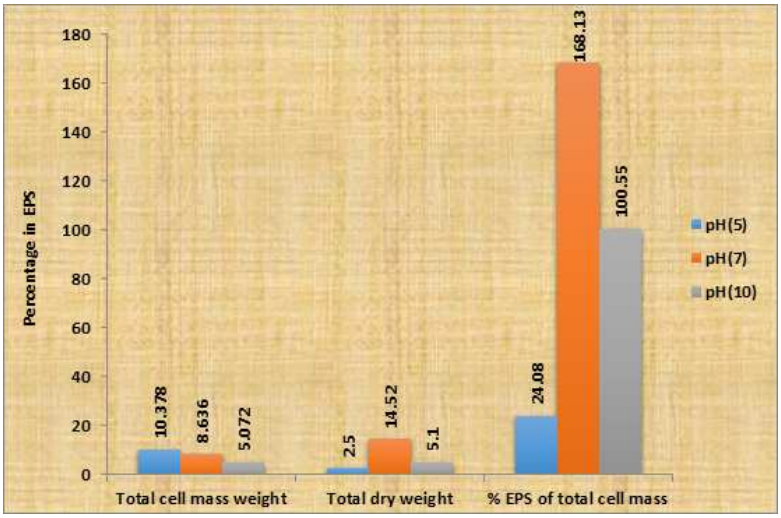
The fermentative processes and the growth of microbes show distint pH dependency depending upon the nature of the microbial species. Most of the cyanobacterial species prefer moderately neutral pH however, certain cyanobacterial species are partial aciduric and some are partial alkaliduric. The exopolysaccride production may differ extensively on the pH conditions because of the vary nature of exopolysaccrides of cyanobacteria. Presence of high level of negative charge on the exopolysaccrides therefore suggests that slight alkaline conditions may be favorable while that of acidic conditions may hinder the production of exopolysaccrides. We studied the production of exopolysaccride by anabaena species in BG11 medium at three pH conditions namely: pH 5, pH 7, pH 10.

The conventional BG11 medium was prepared with the change of pH to 5 and 10 by either concentrated HCl or concentrated H_2SO_4. The conventional BG11 was prepared at pH 7. These studies on the effect of pH was carried out by conducting experiments in the same line,as described earlier. The total cell mass and the total dry weight of the EPS were measured and the results are shown in table 4 and pH optimum graph is shown in graph 1.

Table 4: THE EFFECT OF pH ON EPS PRODUCTION BY ANABAENA SPECIES

	Total cell mass weight Gm/lit	**Total dry weight of EPS Gm/lit**	**% EPS of total cell mass**
pH(5)	10.378	2.5	24.08
pH(7)	8.636	14.52	168.13
pH(10)	5.072	5.1	100.55

Graph 1 :- The effect of pH on EPS production by anabaena species.



EFFECT OF SALINITY ON EPS PRODUCTION BY ANABAENA SPECIES

Although cyanobacteria are ubiquitous in nature yet a large number of strains of cyanobacteria can exist in hypersaline conditions and even in these halophilic conditions they are able to produce exopolysaccrides. Many species of cyanothece group has been isolated from hypersaline conditions and their exopolysaccride have been characterisied (Materassi R etal., 1998). In view of this we decided to observe the effect of salinity on EPS production. the change in salinity may result in concomitant change in pH and conductivity of growth medium.

We decided to keep the pH in the alkaline range so that a net change affected by the growth of cyanobacteria may come down to neutrality. With this view a BG11 medium was designed with pH 8.5 and conductivity of 3.12 ms/cm. Three saline conditions were generated by adding Nacl in the range of 5%, 7% and 10% weight/volume. These media were inoculated with anabaena species and incubated in the static chamber as described earlier. Apart from the measurement of total cell mass and total dry weight of EPS, the pH and conductivity were also measured after the incubation for accessing the effect of salinity using a Hanna's pH/conductimeter and the results are shown in table 5.

Table 5 :- EFFECT OF SALINITY ON EPS PRODUCTION BY ANABAENA SPECIES.

Salinity	pH		Conductivity		Total cell mass weight Gm/lit	Total dry weight of EPS Gm/lit
	Initial	final	Initial	final		
5%	8.3	7.9	10.45	9.11	3.712	13.74
7%	8.3	8.0	10.59	9.52	11.1	20.04
10%	8.2	8.0	10.62	9.88	7.08	39.12

EFFECT OF AVAILABLE OXYGEN ON EPS PRODUCTION BY ANABAENA SPECIES.

Cyanobacterial species are autotrophic in nature and carry out oxygenic photophosphoration. There are certain cyanobacteria which are simultaneously

nitrogen fixers and therefore such species are sensitive to hyper oxygen as higher oxygen levels inhibits the nitrogenase activity. However for most of the cyanobacteria low to moderate levels of oxygen are well tolerated.

250 ml of BG11 medium were despensed in three different 500ml elernmyer flask each flask was fitted with the silicon tubing and an aerator dip in the medium. The aeration was supplied with three aerating pumps. The assemblies were placed in front of illuminating fluorescent lamps. The flask were incubated with 1ml of steady density culture of anabaena species, except temperature all other parameters were maintained at room temperature. Since the assemblies were placed in the open room, therefore the incubation was at room temperature. The bubbling of air was done at three levels namely low, medium and high. The number of bubbles of air was so adjusted in the three flask such that the low bubbling flask contain approximately 3-4 mg of oxygen, moderate flow contain 5-6 mg of oxygen and that of high flow contain 7-8 mg of oxygen lit^{-1} respectively. After 7 days of incubation the dry weight of EPS were measured as described in earlier. The results are shown in table6 and the assembly is shown Photograph 3.

TABLE 6 :- EFFECT OF OXYGEN LEVEL ON EPS PRODUCTION BY ANABAENA SPECIES.

	Total cell mass weight Gm/lit	**Total dry weight of EPS Gm/lit**	**% EPS of total cell mass**
O_2 (low)	5.05	2.94	58.21
O_2 (medium)	5.854	3.32	56.71
O_2 (high)	8.506	4.5	52.90

DISCUSSION

The EPS production by anabaena not only depends upon nutrient contents but also on the physical conditions provided for the cultivation can be observed from the results obtained in the experiments performed here. Citric acid and ferric ammonium modification and change of nitrogen source resulted in different levels of EPS production. If we measure the percent of EPS produced with rwspect to the total cell mass calculated as :

$$\%\,\text{EPS production} = \frac{\text{Dry weight fo EPS gm/lit}}{\text{Dry weight of cell mass gm/lit}} \text{x}100$$

Then this can act as a good parameter for comparison of EPS production with respect to total growth, as can be seen from table 1 to 6. Addition of additional citrate+ ferric ammonium citrate at double strength provided around 9-10% increase in EPS production in comparison to cell mass.

Calcium and magnesium are divalent cations and when their levels are decreased below the level of conventional media the relative EPS production with respect to cell mass increased in table 2. Since the EPS contains high level of anionic charges therefore, higher levels of divalent cations present in the medium might have inhibited the production of EPS. This effect needs to be investigated at molecular level to ascertain the etiology of the effect. Similarly the change in the nitrogen source from conventional sodium nitrate to potassium nitrate or ammonium sulphate resulted in relative high EPS production. As can be seen from table 3. Supplementation of ammonium sulphate in place of sodium nitrate may not be supportive of growth but

promotes EPS production. Since sodium nitrate or potassium nitrate are techniqually similar salts with almost same nitrogen content therefore, they promote cell growth more in comparison to ammonium sulphate.

The changes in the pH and the concomitant change in the EPS distinctly reflects that anabaena prefers neutral pH as has been reported by most of the workers. The production of EPS is highest in neutral pH in comparison to acidic or alkaline pH.(table 4)

Salinity plays significant role in exopolysaccride production. It can be seen from table 5 that there is significant drop in pH and concomitant increase in conductivity with increase in salinity which is also reflected as comparatively more EPS production under hypersaline conditions.

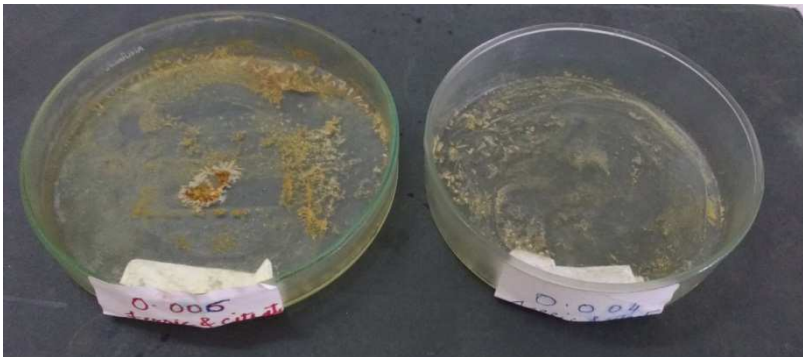
As per as anabaena is concerned the variation in the oxygen tension did not have much difference either on the cell growth or on the EPS production, only slightly it can be observed that high oxygen tension however, the concomitant EPS production reduced (table 6)

CONCLUSION

It can be concluded that increase citrate content and decreased divalent cations of the medium enhances the EPS production with a optimum pHat 7. The increase in salinity similarly increases EPS production whereas oxygen tension does not effect the overall production of EPS.

REFERENCES

- Allen, M, 1968 Simple conditions for growth of unicellular blue green algae, J. Phycol 4, 1-3.
- Belinger B.J. et al. 2010 Composition of extracellular polymeric substances from periphytor assemblages in the florida everglades. J. Phycol. 46: 484-496
- Chakarborty et al 2012 : Chemical Characterization and the stress induced changes of the extracellular polysaccride of the marine cyanobacterium, pharmidium tenue. J. Algal biomass utln. 2012, 3 (1) : 11-20
- Gloaguen, V. et al. 1999 Capsular Polyaccharide produced by the thermophillic cyanobacterium Masligocladus laminosus. Eur. J. Biochem. 266:762-770
- Parikh A, Madamwar D (2005) Partial Characterization of Extracellular polysaccharides from cyanobacteria. Biorsour Technol. 97 : 1822-1827
- Richert, L & Golubic, S et al 2005 Characterivation of Exopolysaccharides produced by cyanobacteria isolated from polynesian microbial mast. Current Microbiology 51: 379-284
- Selbmannl L S. Fenice, M., Fedenci, F.. & Petruccioli M (2002). Production and structural characterization of the exopoiysaccharide of the Antarctic fungus Phomaherbarurn CCFEE 5080. Research in Microbiology 153, 585-592



Photograph 1: Dried EPS produced by alteration in citric acid concentration.

Photograph 2 : Dried EPS produced by changing salinity concentration

Photograph 3 : Assembly of photo reactor at different oxygen level.