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Factors Affecting Exocellular Polysaccharide Production and Growth by Cyanobacterial Strains

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Abstract
The factors found to affect EPS production are oxygen, pH, temperature, age of culture, medium composition and UV radiation. EPS production was greatest during the stationary phase. Composition analysis of EPS isolated at different growth phases and produced under different conditions (varying carbon source or pH). Various nutritional and environmental factors seem to control which type of exopolysaccharide is formed by cyanobactrial strains.

Key Words: Cyanobacteria, Exocellular Polysaccharides, Diazotrophic

Introduction

The amount and composition of capsular polysaccharide (CPS) of *Gloeocapsa gelatinosa* varied according to its growth phase and culture conditions (Ruangsomboon *et al.*, 2006). Long term exposure to UV-B is also known to induce synthesis of extracellular polysaccharide in *Nostoc commune* DRH1 (Ehling - Schulz *et al.*, 1997). A change in light intensity, temperature and the concentrations of sulfur, iron, phosphate, and potassium affect polysaccharide production (Sutherland, 1982; Myklestad *et al.*, 1995; de Philippis and Vincenzini, 1998). The carbon partitioning of the epipelic diatom *Cylindrotheca closterium* was studied by Alcoverro *et al*. (2000) in the laboratory under varying scenarios of phosphorous and nitrogen limitation. We examined the influence of medium constituents on growth and EPS yield. Our results show that EPS are affected by pH, salt, phosphorus and temperature.

Materials and Methods

Cyanobacterial strains:- Forty three cyanobacterial cultures of which 13 were diazotrophic were obtained from the culture collection at the Department of Microbiology, M.D.S. University, Ajmer.

Culture condition:- Organisms were grown as batch cultures in 200 ml flasks in BG-11 medium. Nitrogen was supplemented in the medium for non diazotrophs only. Cultures were incubated with cool white fluorescent lamps with an irradiance of 1200-1500 lux in 12/12 hr L/D cycle at 30±1 degree centigrade. BG 11 –N medium, for + N medium KNO_3 was added at the rate of mg l^{-1}.

Protein determination (Lowry *et al*., 1951):- In 1 ml of homogenized algal/cyanobacterial suspension proteins were precipitated by suspending the culture in 10 ml of 6% hot (60^0C) TCA for 1 min. TCA was removed by filtering the suspension through a sintered filter by suction and the filtrate discarded. Protein thus precipitated was dissolved in 4.5 ml of hot (55^0C) reagent 'e' and allowed to stand for 3 min. After the reaction, the filtrate was collected in a test tube by suction and volume made up to 5 ml by adding reagent 'e'. Subsequently, 0.5 ml of reagent 'f' was added, mixed rapidly and allowed to stand for 30 min at room temperature. Absorbance was read at 660 nm using a mixture of reagent 'e' and 'f' as blank. Total protein content was estimated from a standard curve prepared by using graded concentration (20-200 µg) of standard bovine serum albumin solution.

Carbohydrate estimation (Roe, 1955):- One ml of homogenized cyanobacterial suspension and standards were mixed with 4ml of anthrone reagent and shaken gently. The test tubes were

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kept in boiling water bath for 15 min. with aluminium foil wrapped on the mouth of each tube to minimise evaporation. The tubes were then cooled in running tap water. Absorbance was read at 620 nm using a mixture of distilled water and anthrone as blank. Total carbohydrate content was estimated from the standard curve, prepared by using graded concentrations (10-100 µg) of standard glucose solution.

Extraction of the biopolymer (Hebber *et al.*, 1992):- After 45 days growth cultures are treated with 10 psi steam for 20 min to loosen the attached polymer, centrifuged and supernatant was collected. To the supernatant chilled isopropyl alcohol was added in the ratio of 1:3 overnight in a freezer. The precipitated biopolymer was separated by centrifugation and dried in an oven at 60º C till constant weight.

Chlorophyll *a* estimation (Porra *et al.*, 1989):- Ten ml of homogenized algal suspension was centrifuged at 5000 rpm for 10 min. The supernatant was removed and the pellet was suspended in 10 ml methanol. The tubes were covered with aluminium foil and kept in water bath at 60°C for 30 min. to ensure complete extraction. The extract was cooled, volume made up to 10 ml with methanol and centrifuged. Absorbance of the supernatant was measured at 652 nm and 665 nm with Systronics 125 UV-visible spectrophotometer using methanol as blank. Chlorophyll contents were calculated using the following formula.

Chlorophyll *a* ($\mu g.ml^{-1}$) =16.29 x $O.D._{665}$ -8.54 x $O.D._{652}$

Table.1.Screening on Cyanobacteria for growth, carbohydrate content and viscosity in the spent medium

Organism Name	Chl.*a* µg ml^{-1}	Carbohydrate µg ml^{-1}		Protein µg ml^{-1}		Ƞ Cp
(AZAR NO.)		Total	Extracellular	Intracellular	Extracellular	
Aulosira prolifica (2300)	22.48	276.36	71.44	273.82	38.63	2.23
Nostoc commune (1203)	12.50	196.90	46.69	138.74	21.41	2.26
Nostoc cacicola (1205)	11.56	200.77	76.22	204.43	40.48	2.32
Anabaena oryzae (2221)	2.58	214.83	62.30	181.38	50.81	2.51

Screening for Biopolymer Production:- Maximum biopolymer was produced by the organisms after 45 days of incubation in stationary phase. Steam treatment of the cultures for the release of attached slime increased the extracellular carbohydrate contribution of the culture. Eighty nine percent of the total carbohydrate content of the culture of *Aulosira prolifica (2300)* was attributable to the extracellular component followed by *Nostoc commune (1203)* and *Nostoc calcicola* (1205).

Maximum polymer yield of 0.21mg μg^{-1} chl *a* was obtained from *Nostoc calcicola (*1205) and *Anabaena oryzae (2221)* followed by *Nostoc commune (1203)*, *Anabaena* sp. (2224) and *Aulosira prolifica (2300)* that produced 0.1mg ECP μg^{-1} chl a. The biopolymer contained 10.1 to 16.5 % carbohydrate and 4.75- 9.1% protein (Table -2)

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Optimization of biopolymer production

Effect of pH:- pH optimization for growth of the selected forms *viz. Nostoc calcicola* (1205), *Nostoc commune (1203), Aulosira prolifica (2300)* and *Anabaena oryzae (2221)* was done in buffered medium at pH 7,8,9 and 10. Both the *Nostoc calcicola* (1205) and *Nostoc commune* (1203) strains showed better growth at pH 7.0 however viscosity of the medium and polymer production was more at pH 10.0 in *Nostoc calcicola* (1205) (Table 4.4). Total carbohydrate contents of *Nostoc cacicola* (1205) was nearly four times higher at pH 10.0 (409 µg ml^{-1}) than at pH 7.0 (111 µg ml^{-1}) while for *Nostoc commune (1203)* total carbohydrate contents showed a decrease with increase in pH.

Table 2. Effect of different pH on growth and extracellular biopolymer production of test cyanobacteria

Organism	pH		Chl.*a* µg ml^{-1}		Carbohydrate µg ml^{-1}		Protein µg ml^{-1}	Biopolymer µg 100µg^{-1}		η cp	Polymer	polymer chlorophyll^{-1}	DW
	Initial	Final	Intial	Final	Total	Extra cellular	Extra cellular	Carbo hydrate	Protein	Medium	mg ml^{-1}	mg µg^{-1}	mg l^{-1}
Nostoc	7	7	0.15	1.99	111	35.99	50.29	52.6	36.75	0.88	0.2	0.1	7.5
calcicola (1205)	8	7.9	0.15	1.38	202.1	67.65	158.29	20.8	17.52	0.95	0.37	0.26	6.8
	9	9	0.15	0.18	77.88	32.58	129.6	27.56	16.72	0.95	0.22	1.2	12
	10	9.2	0.15	0.79	408.64	258.12	106.86	24.57	18.44	1.17	0.75	0.94	32
Aulosira prolifica (2300)	7	7.1	0.45	0.61	134.39	130.98	95.49	42.35	18.1	0.95	0.25	0.4	18
	8	8	0.45	0.27	74.47	24.3	36.21	16.65	6.43	0.95	0.13	0.46	9
	9	9	0.45	0.33	73.5	49.63	68.42	16.84	13.99	0.88	0.1	0.29	10
	10	9.9	0.45	6.75	776.42	699.45	423.82	33.86	12.44	4.79	2.03	0.3	282
Nostoc commune (1203)	7	7	0.46	1.8	362.36	229.38	84.66	27.33	14.22	1.62	0.82	0.45	67
	8	8	0.46	1.65	229.86	44.75	86.02	16.07	7.74	0.88	0.47	0.28	62
	9	9	0.46	1.45	145.59	114.9	164.51	17.03	16.22	0.95	0.32	0.22	30
	10	9.2	0.46	0.29	137.31	116.36	119.58	15.63	12.7	0.95	0.1	0.33	14
Anabaena oryzae (2221)	7	7.1	0.28	0.45	120.26	111	105.51	36.67	25.12	0.95	0.3	0.66	19
	8	8	0.28	0.04	115.88	42.81	83.04	51.26	7.71	0.88	0.2	4.47	4
	9	8.9	0.28	0.04	148.51	138.28	69.51	63.38	10.08	0.88	0.18	4.24	5
	10	9.8	0.28	6.36	442.74	405.23	548.87	26.93	18.5	3.9	1.49	0.23	240

Salt requirement:- Organisms were further characterized for salt requirements, its effects on biopolymer production at their pH optima. *N.calcicola* (1205) and *N. commune* (1203) strains were tested at pH 7.0. Both the strains showed decrease in growth and biopolymer production with increase in salt concentration (Table 2).

For *Nostoc commune* (1203) pH 7.0 without salt supplementation appeared to be a better condition at it produced 0.15mg polymer per ml of the medium giving it a viscosity of 1.08 cps while *Nostoc calcicola* (1205) produced 0.21 mg ml^{-1} without any appreciable increase in viscosity of the medium. *Aulosira prolifica* (2300) at pH 10.0 showed good growth both without and with 0.2 M NaCl giving a polymer concentration of 0.55 and 0.70 mg ECP per ml and 1.52 and 1.34 cps viscosity of the extacellular medium respectively. Increasing the salt contents beyond this decreased the growth, carbohydrate and polymer content drastically.

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Table.3. Effect of NaCl concentration on growth and extracellular biopolymer production of test cyanobacteria

Organism	pH		NaCl conc^{n}	Chl*a* µg ml^{-1}		Carbohydrate µg ml^{-1}		Protein µg ml^{-1}	Biopolymer µg 100µg^{-1}		Ƞ medium	Polymer	DW
	Initial	Final	Molar	Intial	Final	Total	Extra cellular	Extra cellular	Carbo hydrate	Protein	cp	mg ml^{-1}	mg l^{-1}
Nostoc	7	7.11	0	0.16	1.48	160.2	115.88	1.57	20.62	21.36	0.94	0.21	70
calcicola (1205)	7	7.14	0.2	0.16	0.61	105.16	75.84	17.81	16.1	16.28	0.95	0.15	30
	7	7.16	0.4	0.16	0.13	31.6	43.29	3.73	0.31	0.37	0.94	0.08	8
	7	7.11	0.6	0.16	0.07	26.73	33.55	2.92	0.26	0.29	0.94	0.05	4
	7	7.11	0.8	0.16	0.08	27.71	34.53	2.11	0.27	0.21	0.96	0.04	4
	7	7.11	1	0.16	0.13	27.22	28.68	4	0.27	0.4	0.95	0.04	7
Aulosira prolifica (2300))	10	9.2	0	0.06	5.12	411.07	267.86	169.65	32.14	13.74	1.52	0.55	246
	10	8.8	0.2	0.06	6.68	385.74	298.06	188.6	24.86	10.22	1.34	0.7	243
	10	8.6	0.4	0.06	2.92	174.82	118.1	85.48	21.82	6.08	1.1	0.54	116
	10	8.22	0.6	0.06	0.17	57.42	31.55	10.23	3.32	1.61	0.96	0.19	20
	10	8.2	0.8	0.06	0.1	33.06	20.83	5.09	0.33	0.5	0.95	0.09	12
	10	8.2	1	0.06	0.13	25.27	16.6	1.84	0.25	0.18	0.96	0.04	16
Nostoc commune (1203)	7	7.15	0	0.15	1.55	141.69	93.95	39.73	14.74	14.33	1.08	0.15	61
	7	7.15	0.2	0.15	0.52	88.51	78.06	26.21	11.46	9.97	0.95	0.08	20
	7	7.15	0.4	0.15	0.16	66.68	63.27	14.56	0.64	1.45	0.95	0.07	6
	7	7.15	0.6	0.15	0.12	51.57	51.57	5.36	0.54	0.53	0.96	0.05	5
	7	7.15	0.8	0.15	0.08	45.24	39.4	1.57	0.55	0.15	0.95	0.03	3
	7	7.15	1	0.15	0.05	35.5	28.68	2.65	0.52	0.26	0.95	0.02	2
Anabaena oryzae (2221)	10	9.06	0	0.23	5.39	531.39	488.53	282.53	25.86	13.71	2.48	1.79	207
	10	8.78	0.2	0.23	6.89	434.45	337.03	302.56	18.04	12.36	2.45	1.74	204
	10	8.75	0.4	0.23	3.62	309.75	282.96	245.71	16.99	11.85	2.34	1.4	120
	10	8.7	0.6	0.23	3.1	241.07	209.89	90.62	16.41	8.57	1.51	1.19	110
	10	8.36	0.8	0.238	0.66	103.21	61.32	18.35	4.77	2.89	1.06	0.39	25
	10	8.14	1	0.239	0.11	29.65	33.06	1.03	1.68	1.56	0.99	0.18	5

Anabaena oryzae (2221) also showed the same trend as *Aulosira prolifica (2300*) however the ECP produced was appreciable without salt (2.48mg ml^{-1}) with a viscosity of 2.48 cps. Viscosity decreased with increase in the salt content.

Effect of Phosphorous limitation

Aulosira prolifica (2300) and *Anabaena oryzae (2221*) were tested for effect of P limitation both in absence and presence of 0.2 M salt at pH 10 while *Nostoc calcicola* (1205), *Nostoc commune (1203)* were tested only in the absence of salt at pH 7.0.

When the same organism was grown at 0.2M salt concentration, pH 10.0 then the growth as chlorophyll a was doubled from 3.68 µg ml^{-1} to 6.3-6.5 µg ml^{-1} with 0.2 M salt. Although total carbohydrate was more (663 µg ml^{-1}) at 0.23 mM P, however extracellular carbohydrate was more at 20 mg K_2HPO_4 L^{-1} equivalent to 0.11 mM P. The total polymer content also increased to 2.80 mg ml^{-1} but the viscosity was 2.21 cps only *Nostoc commune* (1203, 0M salt) showed best growth and total carbohydrate when supplemented with 0.15 mM

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P. Although the growth reduced by half at 0.05 mM P, however polymer content and its viscosity was higher (0.21 mg ml^{-1} and 0.945 cps respectively).

Table:-4Effect of different concentration Phosphorus on growth and extracellular biopolymer production

Organism	pH		K_2HPO_4	Chl.*a*		Carbohydrate		Protein	Biopolymer		Polymer	Ƞ	DW
				µg ml^{-1}		µg ml^{-1}		µg ml^{-1}	µg $100µg^{-1}$			medium	
	Initial	Final	mg l^{-1}	Intial	Final	Total	Extra cellular	Extra cellular	Carbo hydrate	Protein	mg ml^{-1}	cp	mg l^{-1}
Nostoc calcicola (1205) (no salt)	7	7.21	0	0.19	1.29	49.63	34.01	66.8	14.26	13.29	0.16	0.934	61
	7	7.19	10	0.19	1.44	63.27	44.73	84.39	19.74	10.11	0.16	0.928	70
	7	7.17	20	0.19	1.79	91.42	85.19	86.56	19.83	15.38	0.21	0.932	89
	7	7.13	30	0.19	3.08	91.52	89.08	89.81	19.91	13.69	0.21	0.957	20
	7	7.12	40	0.19	3.24	110.03	92.01	91.97	24.24	18.23	0.23	1.033	21
Aulosira prolifica (2300)(no salt)	10	9.02	0	0.31	2.01	357.98	340.93	202.95	41.3	11.09	0.83	2.07	96
	10	9.18	10	0.31	3.34	399.87	307.04	313.92	32.39	13.79	1.16	2.66	161
	10	9.29	20	0.31	3.84	435.43	421.07	313.38	31.07	15.82	0.95	2.19	185
	10	9.25	30	0.31	3.44	426.66	344.82	328.54	35.48	14.55	1.3	2.3	160
	10	9.11	40	0.31	3.67	226.45	197.22	175.34	25.27	10.95	0.59	1.66	171
Aulosira prolifica (2300) (0.2M salt)	10	9.1	0	0.31	3.12	425.2	281.01	201.87	26.61	10.06	1.06	2.07	111.2
	10	9.07	10	0.31	5.46	487.55	314.13	222.17	18.7	10.76	1.63	2.08	202
	10	9.13	20	0.31	6.28	512.39	383.31	244.09	19.67	8.41	2.8	2.21	230
	10	9.03	30	0.31	6.53	585.95	358.95	261.95	24.37	9.08	0.94	1.67	234
	10	9.04	40	0.31	6.36	663.4	332.16	280.09	18.02	10.49	0.93	1.63	212
Nostoc commune (1203)(no salt)	7	7.16	0	0.14	0.93	35.27	27.91	54.89	30.26	11.36	0.21	0.924	40
	7	7.16	10	0.14	0.9	51.12	38.88	67.61	20.05	14.95	0.21	0.945	40
	7	7.17	20	0.14	1.35	62.78	59.86	78.17	45.23	17.04	0.18	0.913	67
	7	7.13	30	0.14	1.84	67.19	54.73	81.14	14.21	13.96	0.2	0.917	81
	7	7.17	40	0.14	1.42	121.37	87.23	85.75	10.57	6.78	0.17	0.904	70
Anabaena oryzae (2221) (no salt)	10	9.17	0	0.37	2.21	310.24	219.15	297.68	29.37	16.31	0.91	2.41	88
	10	9.27	10	0.37	3.06	397.43	322.9	303.91	24.62	14.66	1.31	2.48	120
	10	9.37	20	0.37	3.5	413.02	384.28	391.34	48.73	19.15	1.47	2.78	124
	10	9.22	30	0.37	4.15	357	328.75	405.68	32.34	20.53	1.41	2.77	161
	10	9.24	40	0.37	3.63	324.36	262.5	414.89	28	20.24	1.14	2.19	122
Anabaena oryzae (2221) (0.2M salt)	10	8.9	0	0.37	2.79	259.58	238.63	183.19	32.78	12.79	1.12	1.92	90
	10	8.92	10	0.37	2.92	410.59	354.57	218.92	24.91	14.28	1.35	2.17	108
	10	9.01	20	0.37	4.68	375.51	330.21	231.91	17.55	8.33	0.9	1.66	170
	10	9.08	30	0.37	4.88	356.03	250.32	300.12	15.99	11.36	1.04	1.79	178
	10	9.04	40	0.37	5.36	337.03	235.71	315.82	18.24	13.14	1.1	1.95	190

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Effect of temperature

Effect of temperature shocks on growth and polymer production was determined for *Aulosira prolifica (2300)* at 0M salt, 0.05mM phosphorus and 0.2M salt supplemented with 0.11mM phosphorus *Nostoc commune (1203*) without salt and 0.05mM phosphorus and *Anabaena oryzae (2221)* without salt and 0.11mM phosphorus (Table 4).

Temperature shocks of 20-50^{o}C did not produce any significant effect on the growth, polymer production or in viscosity parameters. Therefore the organisms were mass cultured without any temperature shocks.

After optimization *Aulosira prolifica (2300), Anabaena oryzae (2221)* and *Nostoc commune* (1203) were selected for mass cultivation on the basis of the growth, polymer production and viscosity. The following conditions were used for mass cultivation (Table 5).

Biopolymer yield

On the optimal conditions for growth and product quality, maximum yields were given by *Aulosira prolifica (2300),* at pH 10.00, 0.2M salt and 50% P limitation (0.11 mM P) producing 12.17 mg biopolymer mg^{-1} dry weight while the same organism at 75% P limitation (0.05Mm P) produced 7.2 mg in the absence of salt. *Nostoc commune (1203)* and *Anabaena oryzae (2221)* produced 5.25 and 11.9 mg ECP mg^{-1} dry weig

Table :5 Conditions used for mass cultivation

Organism	pH	Salt	Phosphorus	Temp ° C	mg biopolymer mg^{-1} dry wt
Aulosira prolifica (2300)	10	0.0M	0.05 mM	30	7.20
Aulosira prolifica (2300), **)**	10	0.2M	0.11 mM	30	12.17
Anabaena oryzae (2221)**)**	10	0.0M	0.11 mM	30	11.8
Nostoc commune (1203)	7	0.0M	0.05 mM	30	5.25

Discussion :

pH :- Physical and chemical parameters are known to stimulate the synthesis of carbohydrates in cyanobacteria (de Philippis *et al.*, 1991; Kroen and Rayburn, 1984 ; Thepenier and Gudin, 1985 ; Tease and Walker, 1987). pH is the master variable that has multipronged effects on the physiology of an organism. Biopolymer production was found to be sensitive to the pH of the medium in all the four test organisms. . *Anabaena oryzae (2221), Aulosira prolifica (* (2300) showed better growth and polymer production when the pH was increased to 10.0. On the other hand, *Nostoc commune* (1203) showed reduction in growth and polymer production with increase in pH.

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Salt:- Increasing the salt concentration above 0.2 M had a negative effect on growth and polymer production in all the forms under study. However the *Aulosira prolifica (* (2300) and *Anabaena oryzae (2221)* showed growth (6.89 and 6.68 µg chl *a* ml^{-1}) and polymer production (17.4 and 0.70 $mg.ml^{-1}$) with 0.2M salt supplement that was comparable to growth (5.39 and 5.12 µg chl *a* ml^{-1}) and polymer production (1.79 and 0.55 mg ml^{-1}) of control.

Phosphorus:- Nutrients are necessary components for the growth of any organism. By optimizing the nutrient ratio ECP can be controlled (Hoa *et al.*, 2003). *Nostoc calcicola* (1205) showed an overall decrease in growth on P limitation. An increase in extracellular and total carbohydrate contents was observed in *Aulosira prolifica* (2300), *Anabaena oryzae* (2221) and *Nostoc commune* (1203). This could be attributed to shifts in carbohydrate metabolism. P limitation leads to depletion of the pool of phosphorylated intermediates in the pentose phosphate cycle, which results in reduced carbon dioxide fixation (Brooks, 1996; Jacob and Lawlor, 1993). Nutrients have been reported as one of the factor in disproportionate production of mucilaginous material (Myklestad, 1995). Under condition of N and P limitation diatoms slow down the cellular division while photo assimilation continues. Increase in photo assimilated carbon may lead to reallocation of central carbon stores to production of extracellular polysaccharides (Kuhl, 1968; Myklestad and Haug, 1972; Palmisano and Sullivan, 1985; Myklestad *et al*., 1989; Monti *et al*., 1992; Myklestad, 1995). Decrease in cell number and increase in tendencies to produce more extracellular carbohydrate under P limitation has also been reported for *Cylindrotheca closterium* by Alcoverro *et al*. (2000). A pronounced effect of P limitation on carbohydrate and EPS production in sludge has been reported by Hoa *et al*. (2003) where it was inversely proportional to P concentration. Production of EPS in response to phosphate limitation has been reported in marine microbes also (Sutherland, 1982).

Biopolymer yield:- Under optimal conditions for growth and polymer viscosity the cyanobacteria produced 5.25 to 12.17 mg ECP per mg dry weight with maximum being produced by *Aulosira prolifica* (2300) with 0.2 M salt and 0.11 mM P. *Westiellopsis prolifica* is reported to produce 3.5 mg biopolymer per mg dry weight (Saxena and Kaushik, 1992) while *Calothrix marchica* produces 26.9 mg EPS (Ruangsomboon *et al*., 2007). *Spirulina, Nostoc, Oscillatoria, Phormidium, Anabaena, Scytonema, Tolypothrix, Fischerella* and *Chlorogloea* studied by Nicolaus *et al.* (1999) produced 1-55.2 mg L^{-1} EPS with the maximum being produced by *Anabaena* strain WSAF sp. A polymer producing strain of *Synechococcus* sp. has been reported to produce 1 gm polymer L^{-1} $month^{-1}$ (Phlips *et al.*, 1989) while *Aulosira prolifica (2300)* used produces 2.80 g L^{-1} $month^{-1}$ in batch conditions.

The extracellular carbohydrate components were 4.3 to 40.5% with maximum being in *Lyngbya infixa* (1109). Since there was no correlation observed between the extracellular carbohydrate content, staining properties and viscosity of the medium, thus five forms producing appreciable quantities of extracellular carbohydrate and a high viscosity in the culture medium *viz. Anabaena oryzae* (2221), *Nostoc calcicola* (1205), *Nostoc commune* (1203), *Aulosira prolifica* (2300) and *Anabaena* sp. (2224) were selected for further studies.

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