Partial Characterization of Exopolysaccride Produced by Anabaena Species

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Abstract

Anabaena species produced moreextracellular carbohydrate lit^{-1} in ASNIII media than in BG11 media. The monosaccride analysis showed the presence of the glucose, galactose, mannose, rhamnose, fructose, xylose and arabinose as observed by HPLC and chemical analysis. The exopolysaccride was found to be extremely thermostable as the TGA analysis showed minimal decomposition upto $200^{o}C$. The intrinsic viscosity and the reduced intrinsic viscosity suggest rheological stability of the exopolysaccride. The infrared spectra of exopolysaccride showed specific absorbance of O-H stretching at $3433cm^{-1}$ and the bending vibration at around $1383cm^{-1}$. The absorbance at $1120cm^{-1}$ probably indicates the presence of sulphur containing functional group.

KEYWORDS :- EPS, HPLC, FTIR, TGA

INTRODUCTIONS

Various microbial systems are known to use various types of metabolic pathways in different environmental conditions. As the nutrients and abiotic conditions changes the microbial systems show different levels of adaptabilities to the changing environment. Cyanobacteria are prokaryotic photoautotrophs and they possess prokaryotic cell structure. The species of cyanobacteria are having a variety of metabolic activities and some of the cyanobacterial species are even able to fix atmospheric nitrogen.

Depending upon the cultural conditions, 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 exterme 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 useful rheological attributes therefore they are of industrial importance. (Belinger et al., 2010, Golaguel et al. 1999, Richert et al. 2005).

The EPS produce by cyanobacteria are more advantageous and technically more useful as compared to other EPS. The cyanobacterial EPS consist of distinctive characteristics that makes them different from other conventional EPS produced by other microbes. The common characteristics of cyanobacterial EPS can be characterised as follows.

1) The number of sugar monomers are generally low and they are usually between 6-10 different monomeric units.

2) Because of 6-10 monomeric units a large number of combinations results in increasingly large number of pausible structural conformations of the polymeric unit.

3) The cyanobacterial EPS show the presence of sulphate groups and two different types of uronic acids. Both these groups impart a strong anionic nature to the resultant polymer.

4) Hydrophobicity of the polymer is a very important criteria and it has been noted that the EPS produced by cyanobacterial species have very high hydrophobicity due to the presence of ester linked acetly groups, deoxy sugars and peptidic mioties. (Dephilippis, Sili Paperi and VincenZini 2001, Shephard, Rockey, Sutherland and Roller 1995)

MATERIAL AND METHODS

Partial characterization of the EPS

Sample processing for analysis

The dried EPS obtained from various experiments done during optimization were pulled together and samples were send to two different centres for partial characterization. The analysis of FTIR and TGA was kindly done by sophisticated test and instrumentation centre, kochi university and HPLC and viscosity measurement were outsourced tosai biosystem private limited, Nagpur.

TGA

A small of portion of the sample is weighed and put in a clean ceramic panthe weight of the pan is nullified initially. The pan with the sample is placed on the TG balance and weighed again. The analysis is done in the temperature range RT to 750 Degree C at a heating rate of 10 deg per minute in N_2 atmosphere using Make /Model Perkin Elmer STA 600.

FTIR (Fourier Transform Infrared Spectroscopy)

A small quantity of the sample is added to KBr in the ratio 1:100 approximately. The matrix is grind for 3-4 minutes using mortar and pestle. The fine powder is transferred into 13 mm diameter die and made into a pellet using a hydraulic press by applying a pressure of 7 tonnes. The fine pellet is subjected to FTIR analysis using universal pellet holder. (a single drop of oil is poured on the KBr pellet in case of liquid samples).

Infrared spectral data were collected on Thermo Avtar 370 FTIR spectrometer.

Spectra are collected over a range of 4000–400 cm−1at 4 cm−1 resolution with an interferogram of 32 scans.

HPLC (High Profile Liquid Chromatography)

HPLC of the sample was done using Shimadzu/spincotechModel CTO- 10AS VP

VICOMETRY :-

The viscometric analysis was done by sai bio system private limited but they did not specify the diluant, methodology employed and the instrument used stating the reason of trade secrets. However, their results are acceptable to us as they match with the values reported by others.

RESULTS

DETERMINATION OF TOTAL CARBOHYDRATE AND TOTAL PROTEIN CONTENT OF THE EXOPOLYSACCRIDE PRODUCED BY ANABAENA SPECIES

Two sets of five different flask containing 100ml of BG11 medium in one set and 100ml of ASNIII medium in the second set were prepared. All the media were sterilised by membrane filtration after the addition of cyclohexamide and antibiotic ampicillin- streptomycin combination. All the flask were inoculated with 1ml fresh culture of steady density growth of anabaena species. The flask were incubated for 7days in a static chamber at 28 ^{o}C $\pm$ 2^{o}C with light intensity of 35-40 µmol, photon $m^{-2}s^{-1}$ with light:dark period of 14:10 hours. After incubation the contents of the flask were separately centrifuged at 5000 r.p.m for 20 min and the supernatant was concentrated to ¼ volume. The total amount of carbohydrate and protein were measured by Dubios method and Lowry's method and the results are shown in table 1 and 2.

TABLE 1 :- Total carbohydrate and protein content of exopolysaccride produced in BG11 media

Flask number	Total carbohydrate mg/gm	Total protein mg/gm
1.	832.65	41.45
2.	840.24	38.36
3.	826.66	44.24
4.	835.42	46.70
5.	830.78	37.20
Mean	3501.126	178.19

TABLE2 :- Total carbohydrate and protein content of exopolysaccride produced in ASNIIImedia

Flask number	Total carbohydrate mg/gm	Total protein mg/gm
1.	944.63	38.79
2.	951.42	37.08
3.	936.88	46.12
4.	942.44	42.11
5.	946.20	39.20
Mean	3964.61	171.94

CONFORMATION OF MONOSACCRIDE COMPOSITION BY HPLC METHOD

The gross monosaccride analysis of the exopolysaccride produced by anabaena has already being reported determined by the Dubios method, we wanted to conformed the composition of monosaccrides present in the exopolysaccride by HPLC method. The EPS fraction was produced and percipitated and dried and sent for HPLC analysis to Sai biosystem private limited for analysis. The report submitted by the organization is shown in graph 1 and table 3

Table 3: Monosaecharide composition of EPS produced by Anabaena

Name	Retention Time	Area	Area %
Mannose	3.359	390395	20.32
Fructose	4.021	180673	9.40
Galactose	5.053	242681	12.63
Xylose	6.137	205914	10.72
Arabinose	7.352	531388	27.66
Glucose	9.925	224015	11.66
Rhamnose	13.187	146227	7.61
Totals		1921293	100.00

FTIR analysis of EPS

The various types of vibrations resulting in stretching and bending modes of vibration are observed due to interactions between the adjacent functional groups present in the EPS. The EPS produced, was sent to Kochi university sophisticated instrumentation centre for FTIR analysis to conform the nature of stretching and bendings in the exopolysaccride produced by the anabaena. The results are shown in graph 2.

Thermal behaviour of exopolysaccride

The EPS sample was also sent to sophisticated instrumentation centre, Kochi university, Kochi for thermal gravimetric analysis or TGA . The results of temperature vs weight and heat flow as well as the weight percent loss graphs are shown in graph 3,4, 5 and 6.

Viscometeric analysis of exopolysaccride

The EPS produced was sent to Sai biosystem private limited, Nagpur for viscometric and specific gravity analysis. The results are shown in table 4

TABLE 4 :- Viscosity and specific gravity measurement of exopolysaccride produced by anabaena species.

Intrinsic viscosity (CST)	Specific gravity Gm/cc	Reduced viscosity (CST)	Specific gravity Gm/cc
29.1	10019	27.5	10009

DISCUSSION

It can be seen from table 1 and table2 that the total carbohydrate produced by anabaena is slightly more in ASNIIImedium than in the BG11 medium. In our monosaccride analysis by Dubios method we could not trace the presence of fructose however, the HPLC of the same sample shows around 9.40% of fructose present in the sample. A part from this all other fractions are comparable with the chemical analysis. The results of FTIR are clearly suggesting of specific stretching and bending for example: the deformation of stretching at 1635 cm^{-1} suggest the stretching vibration of carboxylic group.

The thermal gravimetric analysis suggests that the EPS is thermal stable and does not show sharp decomposition at lower temperature. The thermal decomposition is normally seen at much higher temperature except for physical decomposition at around 200℃.

The minor decreased in reduced viscosity and specific gravity from the intrinsic values suggests rheological stability of the exopolysaccride.

The production of extracellular polysaccride by different cyanobacterial species under different conditions of physical and chemical environment around it is suppose to be a survival strategy of these species. (Sahlan Ozturk BA 2008, Priester JH et al., 2006, Federico Rossi and Roberto Dephilippis 2015). Cyanobacterial EPS show definate chemical and physical characteristics with respect to both capsular and released polysaccride. The CPS and RPS are generally produced by different biosynthetic pathways (Micheletti E et al 2008) their gross composition are distinctly different (Vincenzini M et al 1990).

In our experiments with these species of cyanobacteria namely anabaena. The amount of carbohydrate and total protein produced by anabaena species fairly corroborate with the amount of total neutral sugars and protein produced by anabaena species BTA992 as reported by Romi Khangembam etal., 2016. It is a well know fact that the abundant presence of arabinose and xylose among the cyanobacterial species particularly anabaena. So far there are 13 different monosaccrides that are reported amongst cyanobacterial EPS (Rossi F and Dephilippis R 2015). These monosaccharides include the common occurence of rhamnose, arabinose, galactose, glucose, mannose, xylose, fucose, galactoronic acid and guluronic acid. The carbohydrate analysis of our study fairly matches with the contemporary work reported (Rossi etal graph of HPLC)

The FTIR analysis shows distinct stretching and bending as can be seen from the FTIR spectrum . A specific absorbance of O-H stretching can be observed around 3433 cm^{-1} . A very mild C-H stretching can be observed at 2097cm^{-1}, bending vibration of C-H can be observed at 1383 cm^{-1}. Interestingly absorbance at 1120cm^{-1} suggest the presence of sulphur containing functional groups. Similarly studies have been reported by other workers (Lamia Trabelsi et al., 2009, Parikh and Madamwar 2005)

The thermal gravemetric analysis suggest that the exopolysaccride produced by anabaena is extremely thermostable upto around 200℃. Similar observation have

also been observed by many workers (Parikh and Madamwar 2005, Shah V et al., 2000).

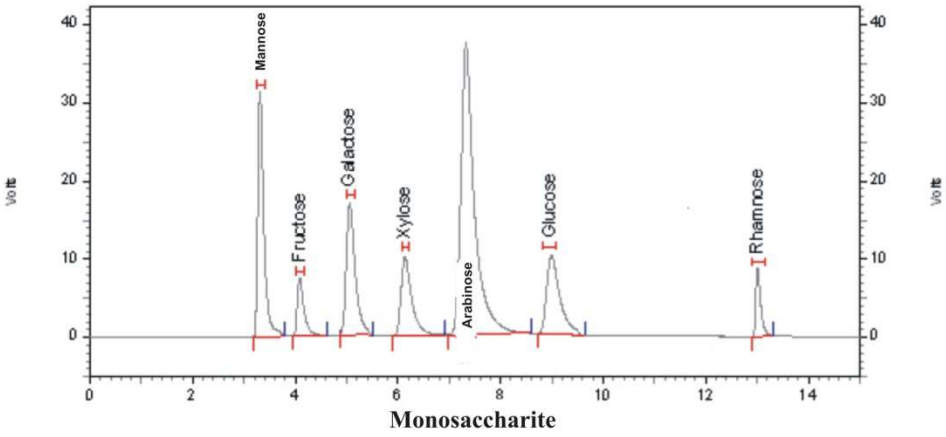
CONCLUSION :-

The mean EPS produced by anabaena species is more in ASNIII media than in BG11 media. The HPLC shows the presence of glucose, mannose, galactose, rhamnose, arabinose, xylose and fructose. The intrinsic viscosity of the EPS was found to 29.1 CST and the EPS was found to be highly thermostable and did not show decomposition upto 200^{o}C. The FTIR analysis showed specific stretching and bending vibration involving specific functional groups.

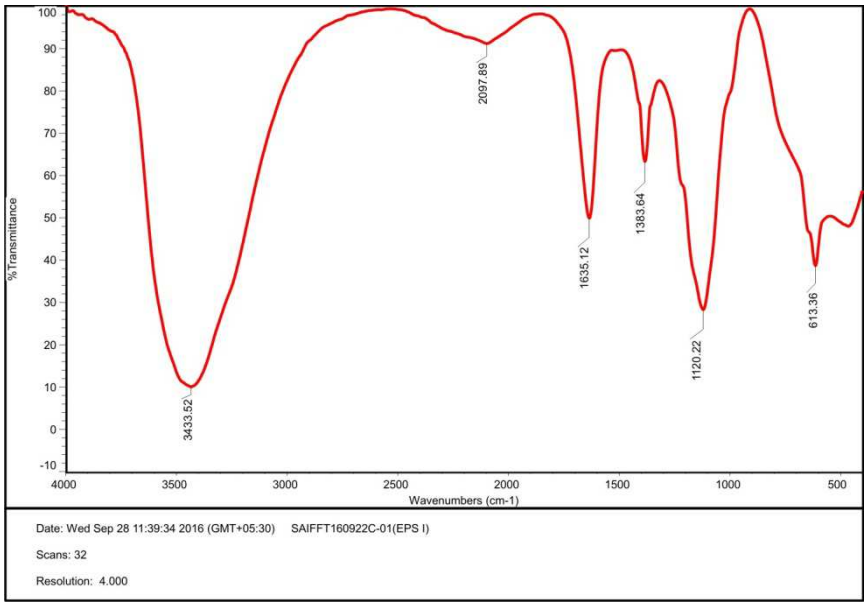
Area % Report

Sample ID: Monosacharides

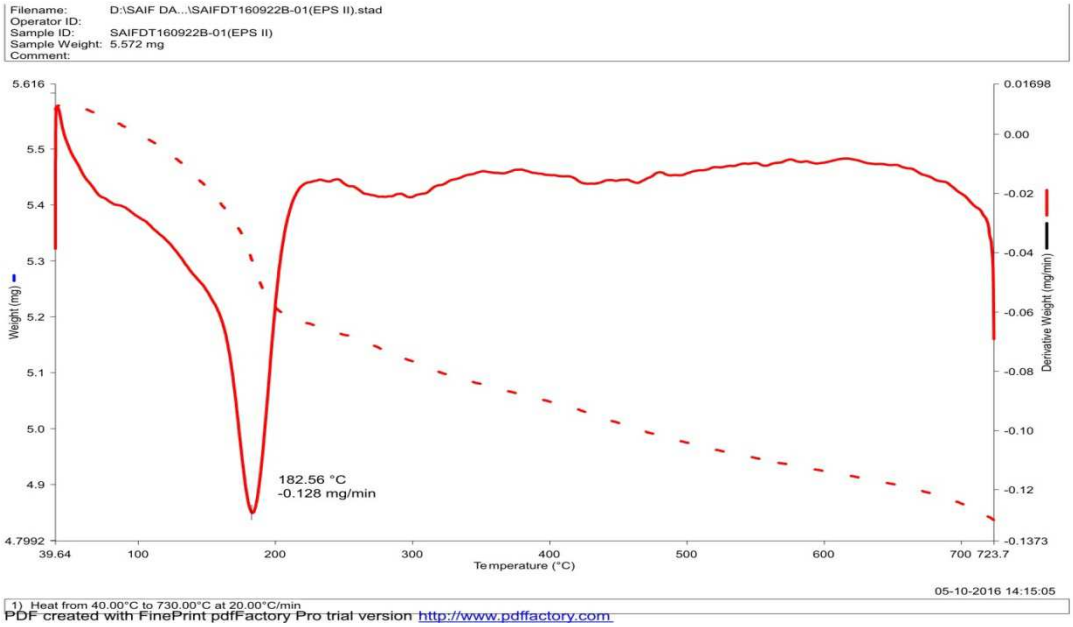
Graph 1: HPLC profile of EPS produced by Anabaena



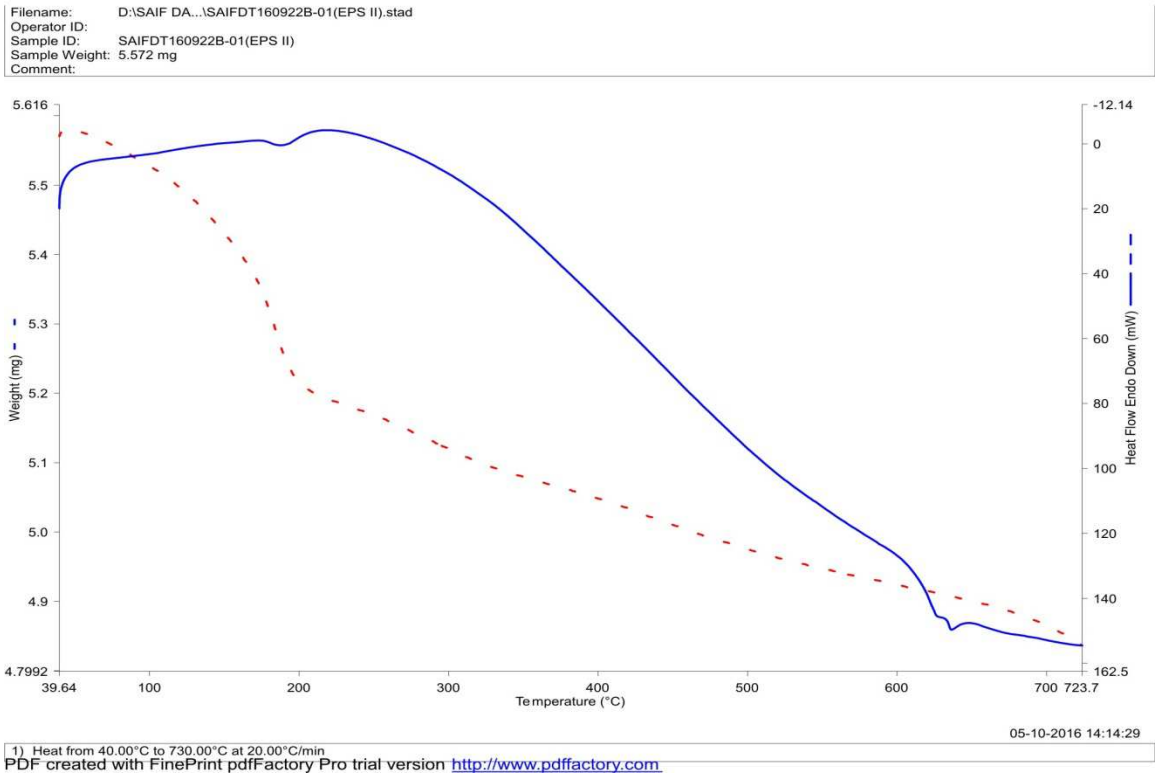
Graph 2 : FTIR analysis of EPS produced by Anabaena



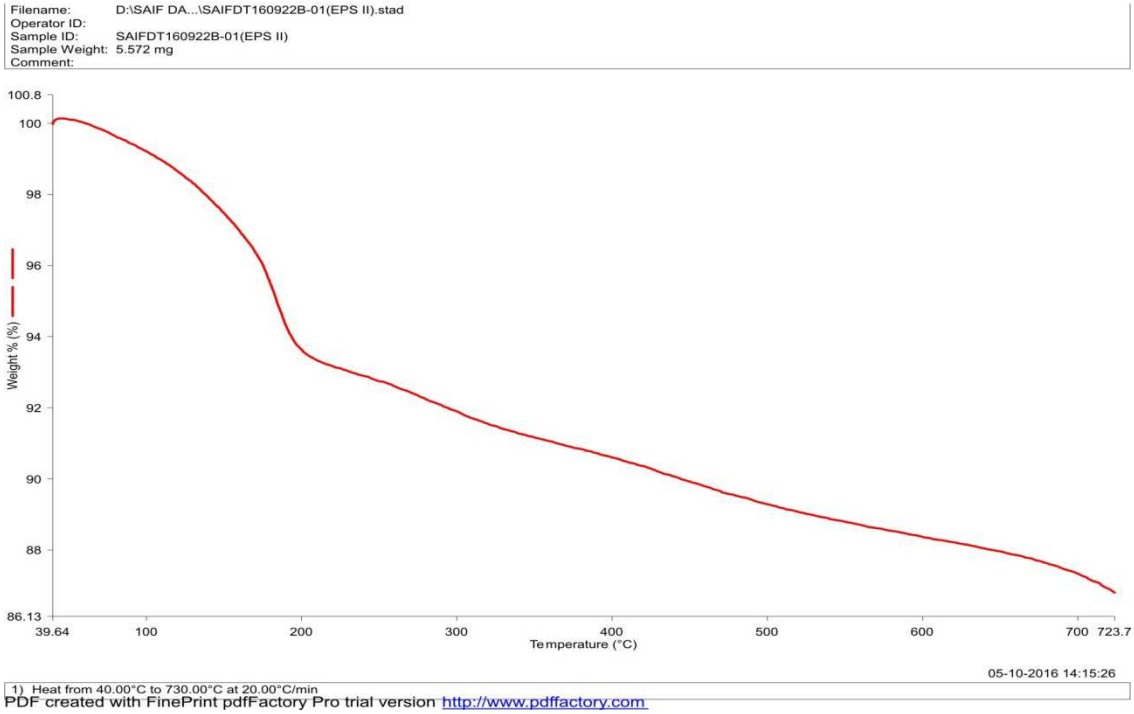
Graph 3 : Thermogravimetric analysis of EPS produced by Anabaena



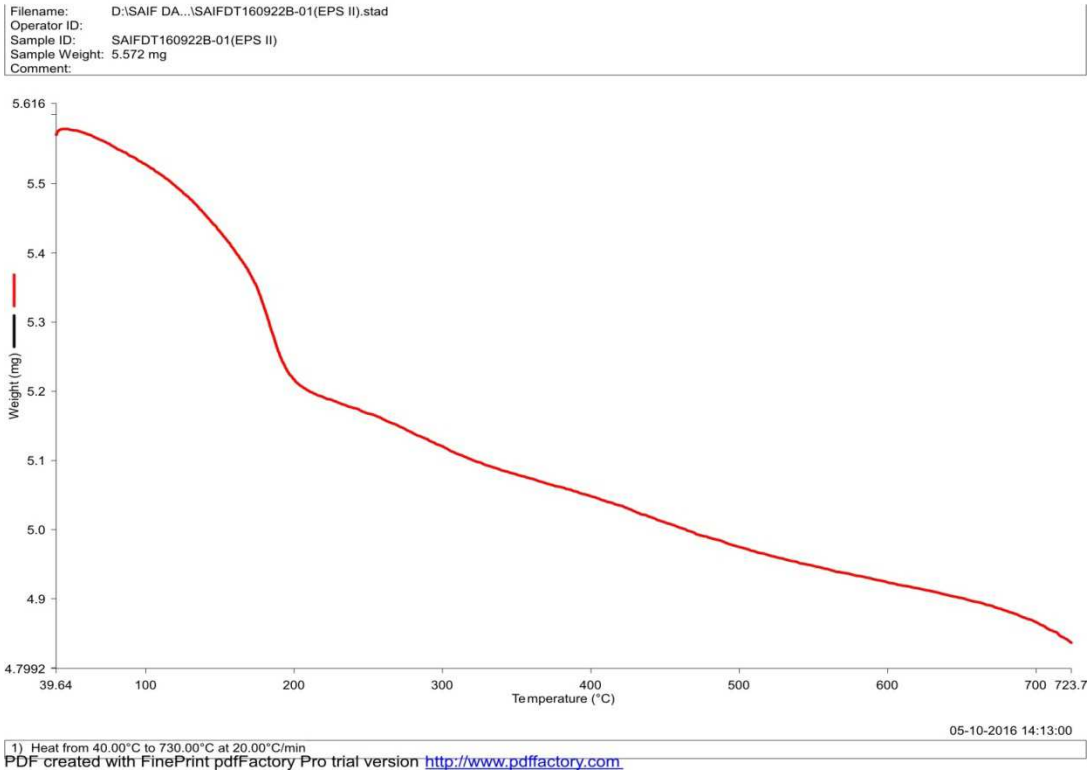
Graph 4 : Thermogravimetric analysis of EPS produced by Anabaena



Graph 5 : Thermogravimetric analysis of EPS produced by Anabaena



Graph 6 : Thermogravimetric analysis of EPS produced by Anabaena



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