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Thermo-Tolerant Microalgal Diversity in the Chromium Metal Polluted Sites of Sukinda Mining Area

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A B S T R A C T

Sukinda valley of Jajpur district in Odisha is one of the most important chromite hubs of India. The water logged places of this area show the growth of a few numbers of chromium resistant microalgae. Here an experiment was carried out to find out the thermo-tolerant microalgal diversity in the chromium metal polluted sites of Sukinda mining area. Microscopic study and SEM analysis were done for the morphological identification of the algae. Four different thermo- tolerant micro algal strains were identified from the sampling sites of this region among which one green micro alga (*Chlorella* sp.) and three cyanobacteria (namely *Oscillatoria* sp., *Microcystis* sp. and *Fischerella* sp.) were found. The temperature and hexavalent chromium tolerance properties of these algae were studied taking a temperature range of 25 to 55˚C and Cr (VI) concentration of 10 to 100 PPM. Maximum temperature and Cr (VI) tolerance were observed in *Fischerella* sp. and *Chlorella sp.* respectively. Hence, these organisms can be used for the phycoremediation of warm industrial effluents rich in Cr (VI).

Introduction

The discharge of untreated toxic metal containing industrial effluents in to the water bodies is a matter of serious concern with regards to environmental pollution. Chromium is considered as one of the most hazardous toxic metals due to its deleterious health effects such as nephrotoxic (Abdia and Kazemia, 2015, Mishra *et al.*, 2010), mutagenic (Nigam *et al.*, 2015) and carcinogenic activities (Thompson *et al.*, 2002, Shen *et al.*, 2013).

It is found as a metal contaminant in the waste discharge of many industries such as electro plating, tanning, dye, paper mills, aluminium and electroplating industries. Although it is present in different forms in the environment, Cr (III) and Cr (VI) are found to be most stable (Michailides *et al.*, 2013).

It is also regarded as the 16th most hazardous metal in the earth according to the Agency for Toxic Substances and Diseases Registry (ATSDR), (Pavlović *et al.*, 2019, Grandjean, 2016). Cr (III) has both beneficial and

detrimental behaviour (Mohan and Pittman Jr., 2006), mostly it is required in a trace amount for the reduction of blood cholesterol level, diabetes (Mohan and Pittman Jr., 2006) and normal maintenance of carbohydrate metabolism (Focardi *et al.,* 2013). On the other hand, Cr(VI) is highly water soluble and permeable through cell membranes (Wuana and Okieimen, 2011; Sultan and Hasnain, 2005) and affects the protein and nucleic acid synthesis.

The interim exposure to Cr(VI) causes skin and stomach irritation or ulceration (levels above minimum limits) while the long-term exposure (levels above maximum limits) can cause damage to liver and kidney circulation, dermatitis, nerve tissue damage, and even death (Enterline, 1974; Kotas and Stasicka, 2000). Moreover, Cr (VI) is mutagenic and carcinogenic in nature.

Therefore, the treatment of the chromium contaminated industrial effluents is highly essential before their discharge in to the water bodies. Some conventional methods like coagulation, chemical reduction, precipitation, foam flotation, electrolysis, reverse osmosis, ion-exchange, use of activated charcoal, filtration, membrane process, liquid extraction, electro-dialysis and evaporation (Owlad, 2009, Ali, 2012; Ali and Gupta, 2006, Argun *et al.*, 2007) are followed for chromium removal. But all these methods appear to be unsafe, expensive and unreliable (Camargo *et al.*, 2005, Gandhi *et al.*, 2013). Therefore, the exploration of a suitable, promising and alternative biological method for the removal of Cr (VI) is the need of the hour.

Utilization of some potent microorganisms is the on-going trends for the detoxification of toxic chromium metal. Microbes like bacteria, fungi, algae, yeast, aquatic plants, moss, etc are being used for the above-said purposes (Benazir *et al.*, 2010, Katircioglu *et al.*, 2012, Ozdemir *et al.*, 2004, Mohanty *et al.*, 2006, Ergene *et al.*, 2006). Among them, microalgae have drawn the attention of many scientists because of their ability for the adsorption, absorption or reduction of hexavalent chromium (Rajeswari *et al.*, 2011). The microalgae are able to grow in adverse environment; they have a short doubling time (Lv *et al.*, 2017), after their growth the biomass can be easily harvested for the extraction of chromium from them for further use.

Sukinda mining area is well known for its chromium deposits. Opencast and underground mining are the major cause of chromium pollution in this locality. The flora, fauna and the human population of this mining area is negatively affected due to Cr (VI) pollution (Mishra *et al.*, 2010). Moreover, this will lead to the soil texture transformation with its biomagnifications.

The water logged places and the sewage water of this area encourage the growth of a few number of Cr (VI) resistant microalgae. Moreover, the industrial effluents contaminated with Cr (VI) show some variations in temperature during their discharge. Therefore, the exploration of thermo-tolerant chromium resistant microalgae for the bioremediation of hexavalent chromium of industrial effluents is a great challenge. Hence, in the present investigation, emphasis has been given to the study of thermo-tolerant microalgal diversity in the chromium metal polluted sites of Sukinda mining area.

Materials and Methods

Collection of sample

Sample 1 and 2 were collected from OMC, Sample 3 and 4 were from Damsala Nala and

Sample 5 and 6 were from Kaliapani mining regions which are some water logged regions within the Sukinda mining area.

Those algal samples were collected during the winter season of 2017. This study site was located between latitude 21^0 1' to 21^0 4' N and longitude 85^0 45' to 85^0 48' that covers about 2 to 3 kilometre. The sample collection sites were morphologically different from one another.

Water and algal samples were kept in zipper bags and then taken to laboratory. All the algal samples were thoroughly washed with running tap water, then in distilled water and morphologically studied under the microscope for further culture. Some physico-chemical properties (such as pH, EC, TDS, DO, temperature and Cr (VI) concentration) of the collected water samples were observed *in vitro*.

Isolation

The algal samples were studied under the inverted microscope and depending upon their morphology the micro algae are isolated. Then the algae were spotted on different plates containing different culture medium (BG 11, Chu's medium no. 10, Allen and Arnon media and F/2 media).

Then After 10 to 15 days of incubation some algal strains were found to grow on the plates and after subsequent repetition the well-grown cultures were picked out from the plates separately and transferred to flasks containing the specific medium for their better growth.

Maintenance and growth optimization of the algal isolates

Only four strains were able to grow *in vitro* and were named as SM1, SM2, SM3 and SM4. Maximum biomass of each strain was obtained after growth optimization by considering one of the growth factor at a time and other factors as constant. Various growth factors such as different nutrient media, incubation period, pH, temperature and light and dark period were considered during this study.

Cr (VI) tolerance by the isolated algal strains

The isolated algal strains were allowed to grow in 100ml of respective medium containing varying concentrations of Cr (VI) ranging from 0 to 100mg/L. The growth was observed on the dry weight basis. The cultures were centrifuged by taking the cultures in pre-weighed centrifuge tubes at 16000 rpm for 10 minutes.

Then the supernatant was discarded or filtered out and the pellets were placed in a hot air oven at 60^0C for 24 hours. Now the dried pellets along with the tube were weighed and the final weight was calculated by subtracting the initial weight of the centrifuge tube from the final one containing the cell pellets (Bottomley and Van Baalen, 1978, Razi S., 2009).

Study on thermo-tolerant property

To study the effect of temperature variation on the biomass production, the isolated algal strains were inoculated in 250 ml Erlenmeyer flask containing 100 ml of the respective medium and the flasks were placed in the illuminated shaker incubator (REMI, RIS-24Plus) at temperature 25ºC, 30ºC, 35ºC, 40ºC, 45 ºC, 50 ºC and 55 ºC under an illumination of 2400 lux with 16:8 hr light and dark period for 15 days. The biomass was interpreted in terms of dry weight per 100 ml of respective medium.

Microscopic identification of the algal isolates

The algal isolates were morphologically identified using light microscope, compound microscope and scanning electron microscope (SEM). Identification of those cultures was done by using the available taxonomic literatures by Rippka *et al.*, 1979 and Desikachary, 1959.

Results and Discussion

Sample collection sites

Fig.1. indicated the different sampling sites such as S1, S2, S3, S4, S5 and S6 of Sukinda mining area. The physico-chemical properties of the water samples were displayed in the Table No. 1. pH of the water samples of different sites was observed within 6.9 to 7.8, while the electrical conductivity was between 170 to 240 mS/cm. Highest pH (7.78) was studied in S3 region while lowest pH (6.98) was in S4. Temperature of the sites was varied from 29 to 32ºC. The range of total dissolved solid was found to be in a range of 130 to 160 PPM. TDS was highest in S3 (157±1.3 PPM). The concentration of hexavalent chromium was varied from 29 to 50 PPM. Higher range of Cr (VI) was observed in S2 region of OMC
that is about 46.29±1.34.

Isolation, maintenance and growth optimization of algal strains

Four different kinds of algal strains were isolated and named as SM1, SM2, SM3 and SM4. Figure 2. Revealed the growth optimization of the algal isolates. BG11 media was found to be suitable for the growth of SM1, SM2 and SM3 while SM4 was found to grow more in Allen and Arnon medium. With increase in the inoculum size, the growth was increased. The growth of the algal isolates were studied under different pH keeping all other parameters constant. Maximum growth was observed in SM1 and SM3 at pH 6.5, SM2 in 7.0 and SM4 in 7.5 pH. SM1, SM2 and SM3 indicated the maximum biomass production on 12th day of inoculation but in SM4, on 20th day of inoculation. 18:6 hours of photoperiod was the optimum condition for the growth of the algal strains. The growth optimization of the algal strains under different concentrations of Cr (VI) indicated the maximum growth of SM1 and SM3 at 60 PPM, SM2 at 80 PPM and SM4 at 40 PPM.

Study on thermo-tolerant property

All the four strains were able to grow well up to 40 ºC temperature. Beyond 45ºC the growth of SM1 and SM4 were reduced. The finding of temperature tolerance was graphically presented in Fig.3.

Identification of the algal strains

The isolated strains were morphologically identified by using the light microscope and scanning electron microscope (SEM). Fig. 4 and 5 depicted the pictures of the algae. Four different micro algae were isolated *in vitro*. Among them, three were blue green algae and one green alga.

The light microscopic and scanning electron microscopic study revealed that SM1 was a single celled, spherical or oval, light green coloured motile strain along with a mucilaginous cell envelope. The size of the cell was varied from 1.5- 2.5 µm in diameter. It was identified as *Chlorella sp.* which came under the Trebouxiophyceae class of Chlorophyta (Fott and Novakova, 1969). The SM2 strain was found to be un-branched, motile, filamentous, non heterocystous and the size varies from 30-40 µm, the end cell were flat and rounded and dark blue-green in

colour. The filaments were uniseriate trichomes. It was identified as *Oscillatoria* sp. SM3 was unicellular, spherical, colonial, 4-7 µm in diameter, freely floating and bluish green in colour. The organism was identified as *Microcystis* sp. SM4 was beaded filamentous in structure, 4-6 µm in diameter, blue green in colour with heterocysts. From its morphological appearance it was identified as *Fischerella* sp.

Discussion

In the present investigation, the physico-chemical parameters such as pH, temperature, TDS, EC, DO and Cr (VI) concentration of the water samples collected from different study sites of Sukinda mining area were carried out. Neutral pH was noticed in all most all the water samples, similar findings were observed by Pattnaik *et al.,* 2017 and Samuel *et al.,* 2012. According to Mishra *et al.,* 2010, 6.5 to 9 pH indicated the increase in concentration of Cr (VI). EC and TDS were found very high, which was previously marked by Dhakate *et al.,* 2008 and Pattnaik *et al.,* 2017, the presence of heavy metals and other ionized elements were the major cause of high EC. These hazardous wastes, landfills and the dissolved metals were the cause of moderate amount of TDS (Dutta, 2015).

Dhal *et al.,* 2011 has observed a similar trend of very low DO in the water sample which collected from Baula Nua Sahi nearer to Sukinda and this lower DO indicates the high water contamination of those specified regions (Dhal *et al.,* 2011). High concentration of hexavalent Cr (29-47 PPM) was noticed.

Similar result was depicted by Dutta and Ghosh, 2016 where they found this concentration in a range of 0 to 35 PPM in Sukinda mining area. The accumulation of the soil run off of the mining zone in these downstream areas (sampling sites) are the major cause of high hexavalent chromium concentration.

The fluctuations in physico-chemical parameters influenced the occurrence and distribution of algae in a particular area (Senapati *et al.,* 2011). Our findings in the Sukinda valley are in agreement with the above result. Microalgae belong to Chlorophyta and Cyanophyta are mostly observed in the study sites. Dutta and Ghosh, 2016 has also reported similar kind of result. Three cyanobacterial strains and one green alga were well documented in our study such as *Oscillatoria* sp., *Microcystis* sp., *Fischerella sp.* and *Chlorella sp.*, respectively.

The appearance of more numbers of cyanobacteria may be due to the mechanisms of occurrence under adverse environmental conditions. Cyanobacteria have a tremendous capacity to acclimatize and grow in a variety of adverse environments may be the cause of their dominance in the study sites (Mannan and Pakrasi, 1993). Previously it was reported that *Chlorella sorokiniana* strain can grow in presence of 100 PPM of hexavalent chromium (Husiena *et al.,* 2019). In our present study, the *Chlorella* sp. has shown optimum growth at 80 PPM of Cr (VI) which was in accordance with the above said findings.

This resistance was higher than that of the previously reported *Chlorella* sp. (30 PPM) that was isolated from the effluents of the paper-pulp and electro-plating industries (Yewalkar *et al.,* 2007). Mutation, presence of metallothiones, other specific proteins or involvement of a particular gene were the major cause of chromium resistance (Ruttkay-Nedecky *et al.,* 2013). Temperature is an important factor to regulate the growth of algae. The thermo-tolerant property was noticed in all the four algal strains.

Table.1 Physico-chemical parameters of the sampling sites
(Experiments were carried out in triplicate)

Name of the sites	pH	EC (mS/cm^2)	DO	TDS (PPM)	Temperature (°C)	Cr(VI) conc.(PPM)
S1	7.45	185	6.5±0.78	135±1.5	31.16	33.06±1.92
S2	7.36	190	5.8±0.96	130 ± 1.7	31.29	46.29±1.34
S3	7.78	209	5.2±0.54	157±1.3	30.37	32.93±1.29
S4	6.98	179	6.7±0.64	142±1.9	29.45	29.67±1.75
S5	7.08	233	5.9±0.92	133±1.5	31.24	34.98±1.45
S6	7.13	198	5.8±0.72	145±1.8	31.55	35.49±1.39

Fig.1 Different sample collection sites of the Sukinda mining area

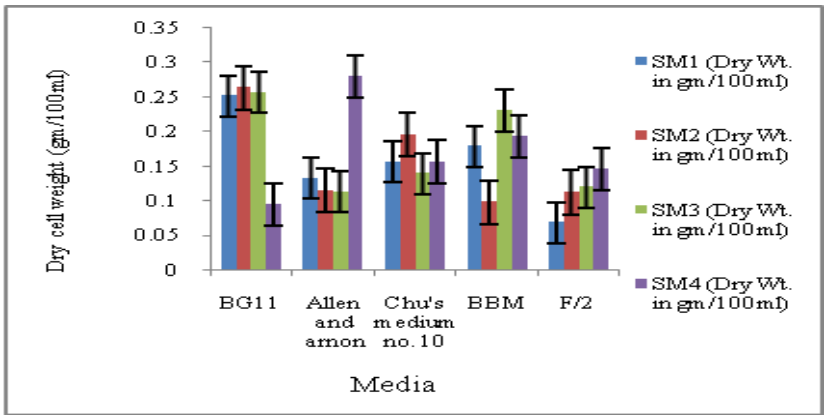

Fig.2.a Optimization of growth using different growth media

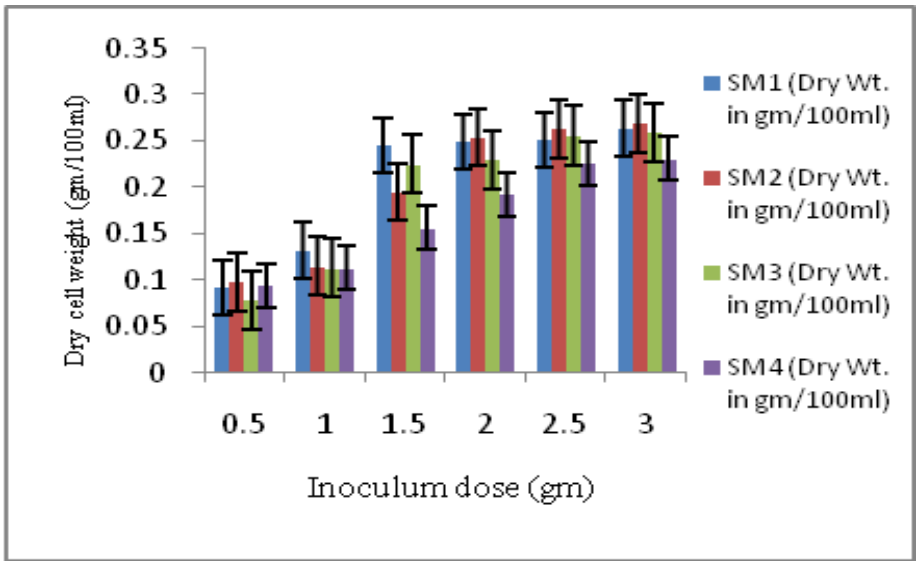

Fig.2.b Optimization of growth using different inoculum dose

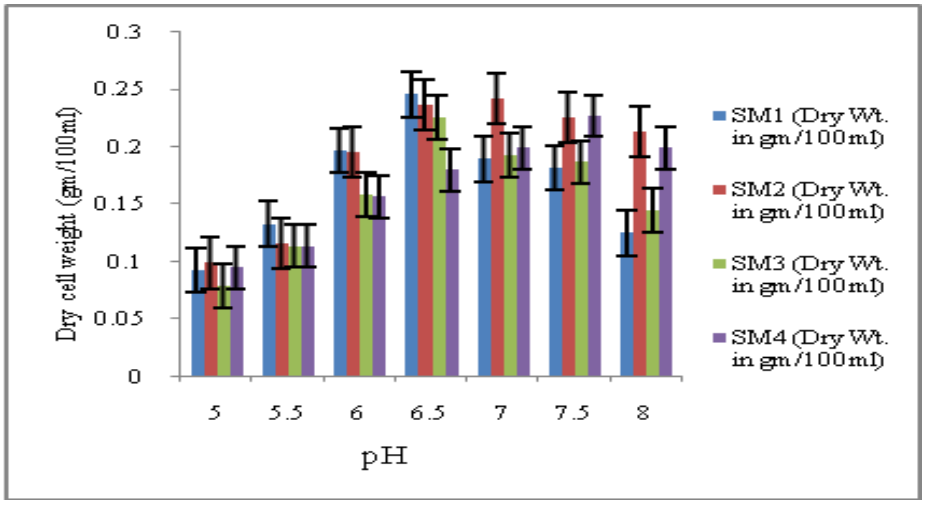

Fig.2.c Optimization of growth using different pH

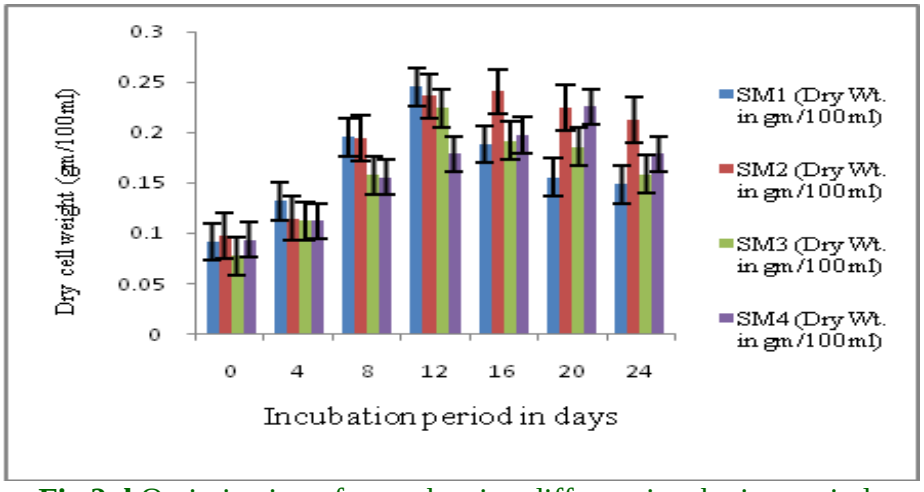

Fig.2.d Optimization of growth using different incubation periods

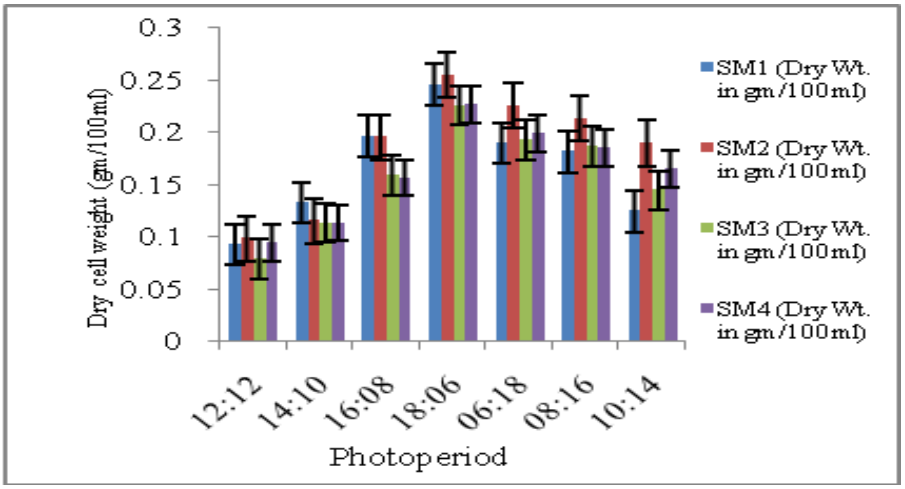

Fig.2.e Optimization of growth using different photoperiods

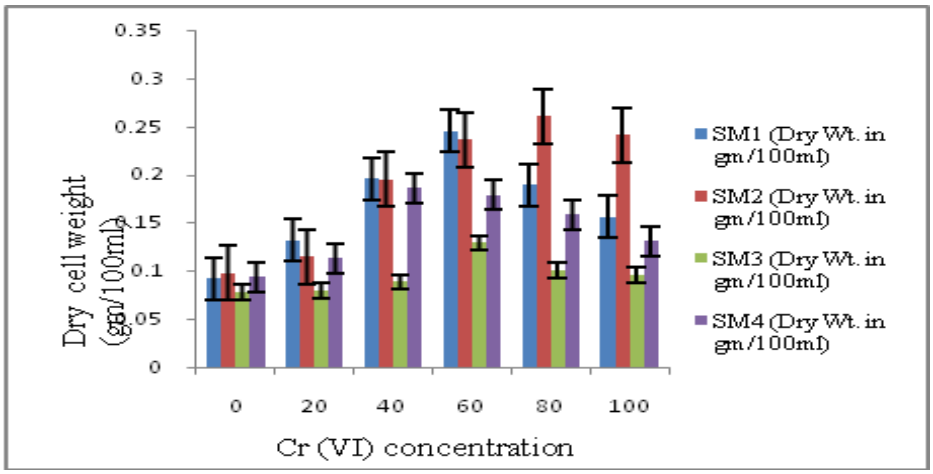

Fig.2.f Optimization of growth using different Cr (VI) concentrations

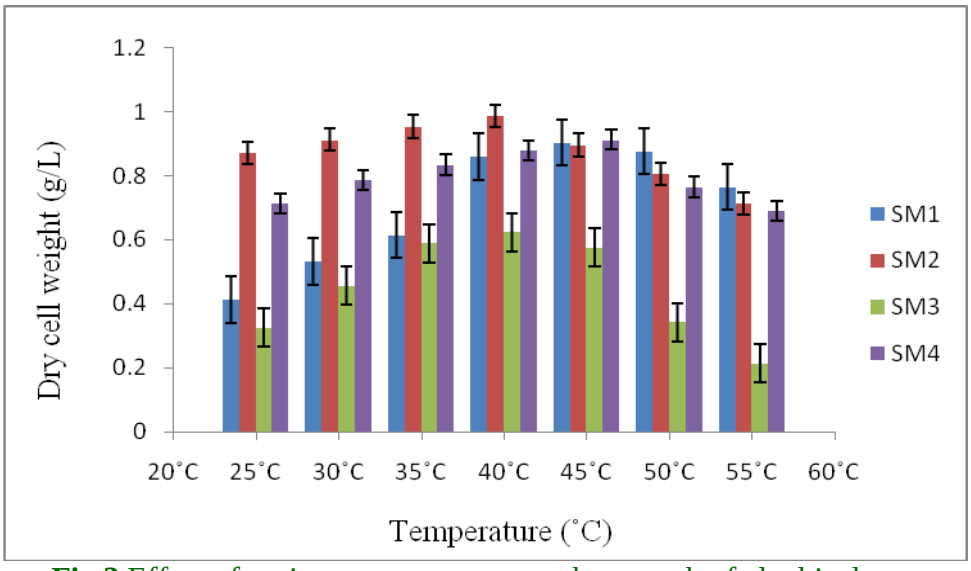

Fig.3 Effect of various temperatures on the growth of algal isolates. Experiments were carried out in triplicate

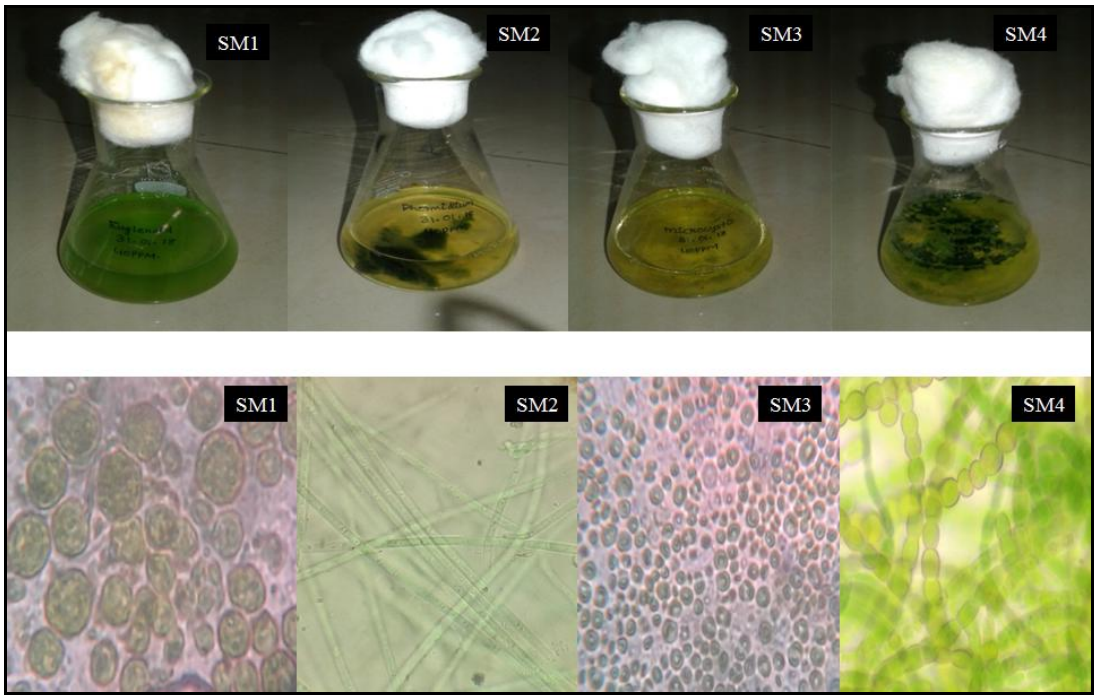

Fig.4 Flask culture and light microscopic photographs of the isolated strains

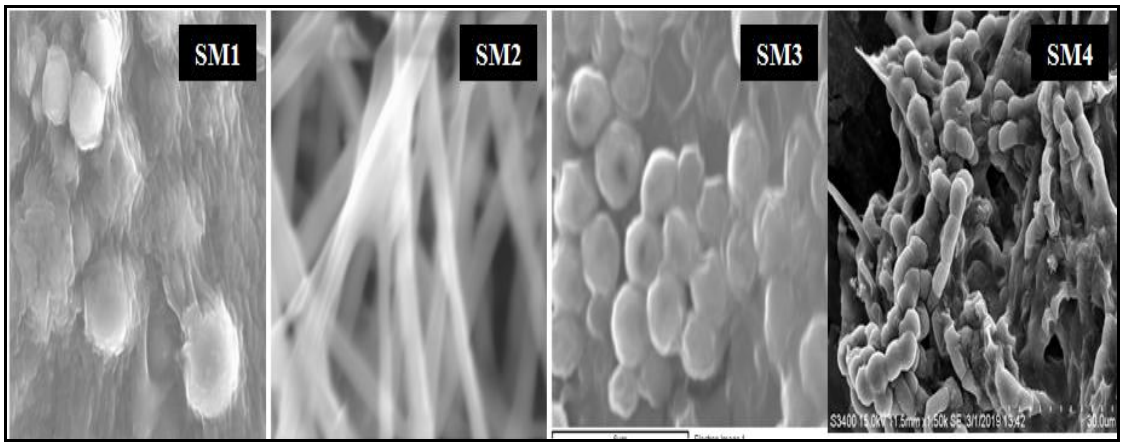

Fig.5 SEM photographs of the algal isolates; SM1, SM2, SM3 and SM4

Chlorella sp. was exhibited maximum growth among the algae at 45˚C temperature. This finding is corroborated with the result of (Bleeke *et al.*, 2014). The genetic modification, mutation or the common hardening mechanism enable them to withstand at high temperature (Adar *et al.*, 2016).

Four thermotolerant and hexavalent chromium resistant microalgal strains were isolated and morphologically identified from different study sites of Sukinda mining area. The physico-chemical parameters for their optimum growth were optimized. The chromium resistance and temperature tolerance properties of these algal strains make the way for their use in the bioremediation of warm chromium contaminated industrial effluents. Hence, further attempts should be taken by utilizing those strains as the potential source of effective bioremediation as well as biofertization.

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