

Images of the Project Site



**SITE LOCATED FOR
INSTALLING
RACEWAY POND
MODEL AT NIT
HAMIRPUR STP**

31°42'17.7"N 76°31'19.8"E







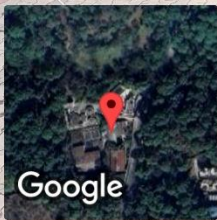
Hamirpur, Himachal Pradesh, India
PG3F+X39, NH 3, Anu, Hamirpur, Himachal Pradesh 177005, India
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Long 76.5227°
06/05/24 10:21 AM GMT +05:30



Hamirpur, Himachal Pradesh, India
PG3F+X39, NH 3, Anu, Hamirpur, Himachal Pradesh 177005, India
Lat 31.704926°
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06/05/24 10:14 AM GMT +05:30



 **GPS Map Camera**



Hamirpur, Himachal Pradesh, India
PG3F+X39, NH 3, Anu, Hamirpur, Himachal Pradesh 177005, India
Lat 31.704855°
Long 76.522543°
06/05/24 10:13 AM GMT +05:30



Fig 3: Visit to Bajuri Zone 3 Sewage Treatment Plant



Fig 4: Visit to Hatli Zone 2 Sewage Treatment Plant



Fig 5: Wastewater, algal sample from Hatli

Visit to NIT Hamirpur's Sewage Treatment Plant for sample collection

A visit to NIT Hamirpur's Sewage Treatment Plant was organized to collect wastewater sample along with algal samples if found in the treatment facility. Algae were found growing in the outlet zone of primary sedimentation tank so it was scrapped, put in the collected wastewater sample.



Fig 6: Algal sample collection from outlet zone of primary sedimentation tank.

The collected sample was brought in the NIT H lab. Algae present in the wastewater sample were left for few weeks to grow. Later it was filtered and after that would be subjected to isolation protocol.



Fig 7: Filtered wastewater sample containing algae.

ANNEXURE 3

Microscopy and Isolation of collected algal samples

Algal samples were collected from three different locations;

1. Primary sedimentation tank of Hatli STP (sample 1)
2. Primary sedimentation tank of NIT Hamirpur STP (sample 2)
3. Outside Mechanical Department [M.D] of NIT Hamirpur (sample 3)

and brought to Centre for Energy Studies, National Institute of Technology Hamirpur for isolation and identification. The samples were kept at $\pm 25^{\circ}\text{C}$ under cool white fluorescent light.



Fig No. 1: Algae growing in Hatli STP



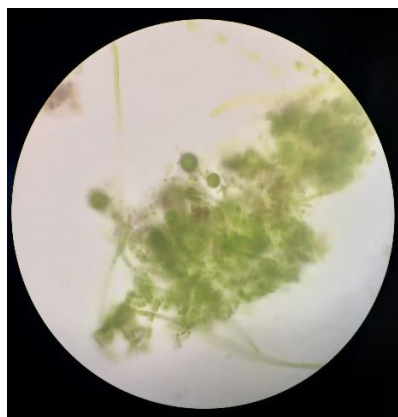
Fig No. 2: Algae growing in NIT H STP



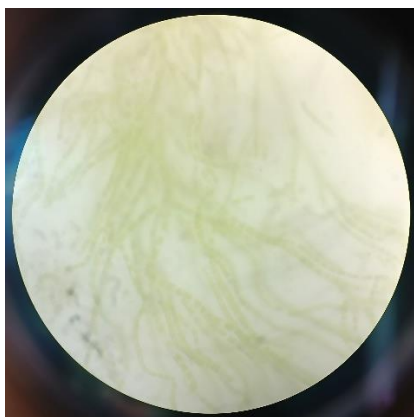
Fig No. 3: Algae growing outside M.D

All the three algal samples were diluted and viewed under microscope for possible morphological identification of type of microalgal species present in sample.

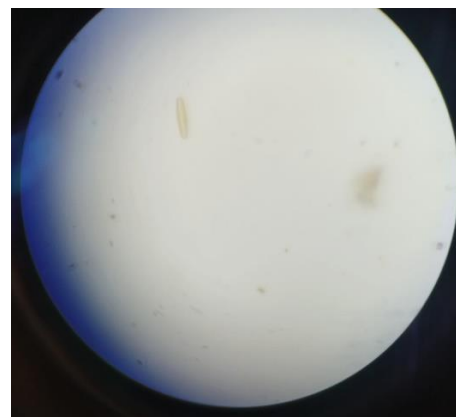
1. Microscopy of Hatli STP sample



(4A)



(4B)



(4C)

Fig No. 4: 4A-cluster of spherical and filamentous microalgae; 4B-group of filamentous microalgae; 4C- presence of diatoms

2. Microscopy of NIT H STP sample

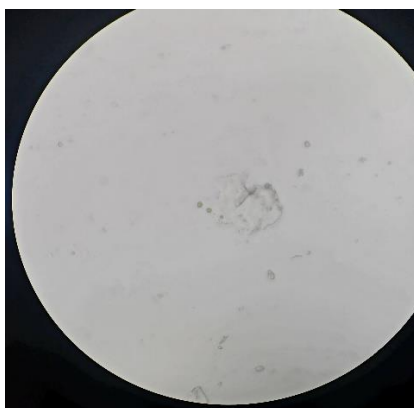


Fig No. 5: Isolated spherical microalgal cells.

The algal sample collected from NIT Hamirpur's STP contained mostly unialgal spherical cells.

3. Microscopy of Mechanical Department sample

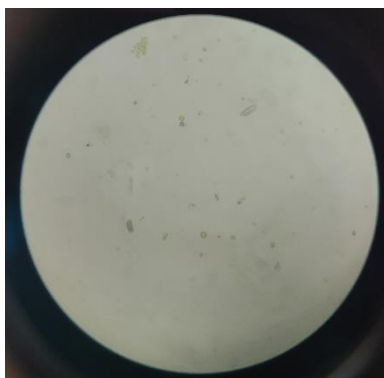


Fig No. 6: Single and clumped spherical microalgal cells.

After microscopy of the three algal samples, 0.2µl all the three serially diluted samples were spreaded on each sterile wastewater plates and BG-11 plates for isolation. After spreading, the plates were kept at $\pm 25^{\circ}\text{C}$ under cool white fluorescent light for cell growth.

	Wastewater plate	BG-11 plate
Sample 1 (Hatli STP)	A petri dish labeled 'SHIVNGI' containing a wastewater plate. It shows a dense, irregular greenish growth of microalgae spread across the surface of the agar.	A petri dish labeled 'STANZIN' containing a BG-11 plate. It shows a dense, irregular greenish growth of microalgae spread across the surface of the agar.
Sample 2 (NIT H STP)	A petri dish labeled 'RANCHAL' containing a wastewater plate. It shows a dense, irregular greenish growth of microalgae spread across the surface of the agar.	A petri dish labeled 'OLYMPIA' containing a BG-11 plate. It shows a dense, irregular greenish growth of microalgae spread across the surface of the agar.
Sample 3 (M.D)	A petri dish labeled 'Siddhesh 54' containing a wastewater plate. It shows a dense, irregular greenish growth of microalgae spread across the surface of the agar.	A petri dish containing a BG-11 plate. It shows a dense, irregular greenish growth of microalgae spread across the surface of the agar.

All the three collected algal samples showed the ability to grow on sterile wastewater and BG-11 plates indicating that the species are able to grow on both mediums. Each plate will further be subjected to streaking and sub-culturing until isolated microalgal colonies are obtained for growing in sterile wastewater and sterile BG-11 broth for further isolation.

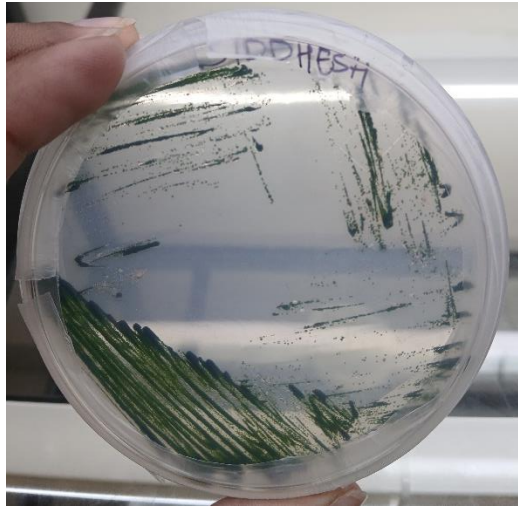


Fig No. 6: Streaking of isolated colony on plate.

ANNEXURE 4

Wastewater collection

SITE: Sewage treatment Plant, Jalandhar

LOCATION: longitude: 31.276658547644704; Latitude: 75.59105942308554

SAMPLE: water sample from the nearby canal of STP at Jalandhar

For the collection of the wastewater sample, some Sewage treatment plants (STP) were located in Jalandhar and a visit to an STP located in Mithapur, Jalandhar (**Fig 1**) which comes under **Satluj Action Plan**, was planned.



Fig1: Sewage Treatment Plant- Mithapur, Jalandhar

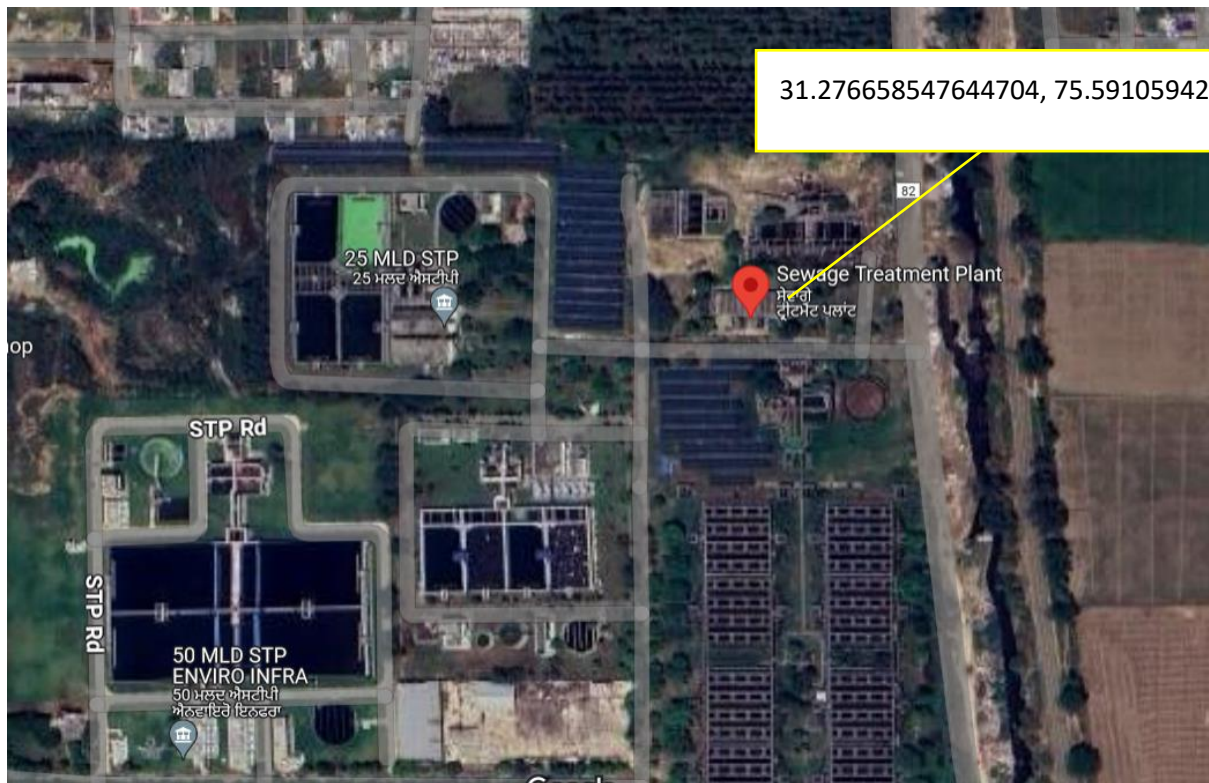


Fig2: STP Site on Google Maps

Due to the unavailability of permission required to collect the sample from the inside of the STP, it was decided to collect the water sample from the nearby canal of the STP. **(Fig3)** The Wastewater Sample was collected in a 5-litre transparent container with proper precautions. **(Fig4)** and the pH of the sample was checked onsite with litmus paper. **(Fig 5)**



Fig3: sample collection site



Fig4: Collection of wastewater sample

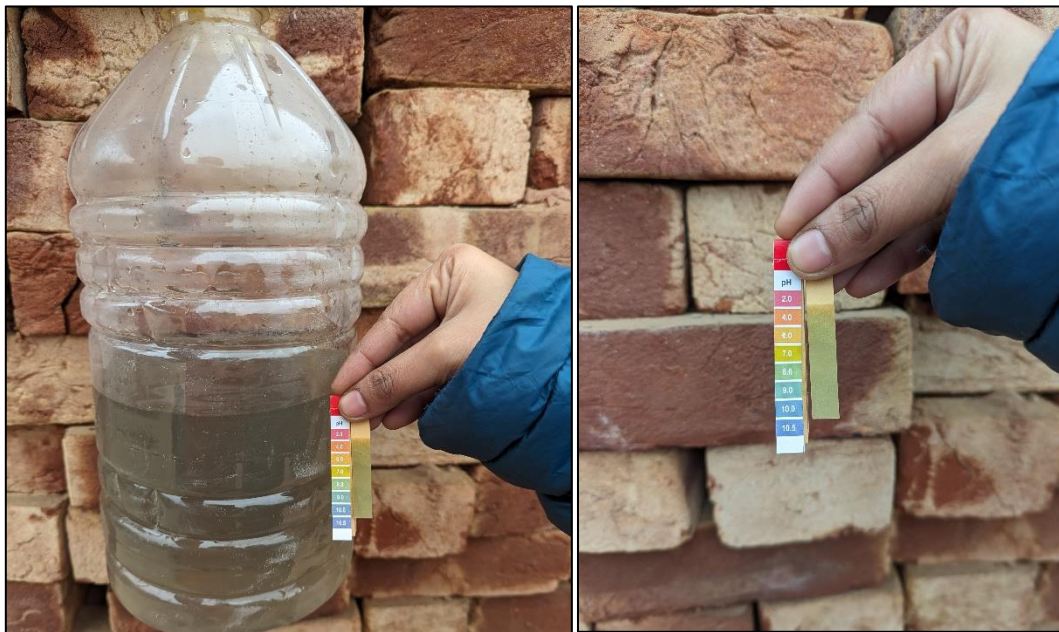


Fig5: onsite check of pH of wastewater with Litmus Paper

The sample was brought to the SSS-NIBE (**Fig 5**) where pH of the collected sample was measured in the lab with pH meter, which was found to be 7.42.



Fig 5: Collected water sample

Wastewater Characterization

The collected wastewater sample was analyzed for the parameters as shown in the **Table1**

Sample: water sample from nearby canal of STP

Table 1: Wastewater characterization

PARAMETER	UNIT	VALUE	REFERENCE METHOD OF ANALYSIS
pH	-	7.42	pH Meter (APHA 2023 APHA 2023 rd edition)
Sodium	mgL ⁻¹	396.027	Ion Chromatography Technique
Potassium	mgL ⁻¹	15.092	Ion Chromatography Technique
Magnesium	mgL ⁻¹	78.720	Ion Chromatography Technique
Calcium	mgL ⁻¹	181.477	Ion Chromatography Technique
TDS	mgL ⁻¹	753	Gravimetric Method (APHA 2023 rd edition)

Isolation Study of Microalgae

The wastewater sample was observed under the microscope to see the presence of microalgae in the sample. Under 400X magnification, (**Fig 7**) some filamentous microalgae were visualized. 1 ml of the collected sample was inoculated in two different mediums (BG-11 and Zarrouks medium) (**Fig 8**) and kept on the algae culture rack (**Fig 9**) for the growth of microalgae.

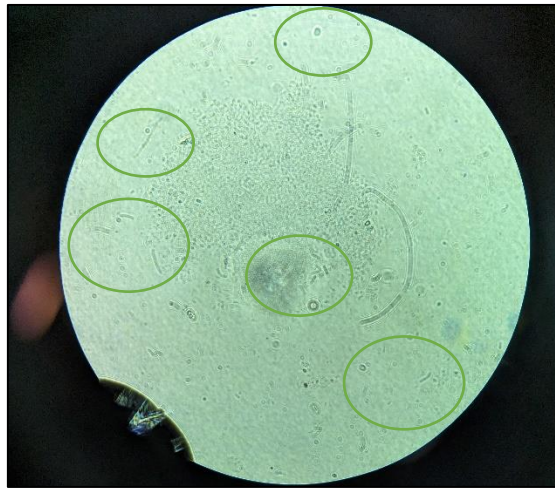


Fig 7: visualization of wastewater (effluent) sample under a microscope (10X)



Fig 8: Inoculation of WW sample in BG-11 and Zarrouks Medium

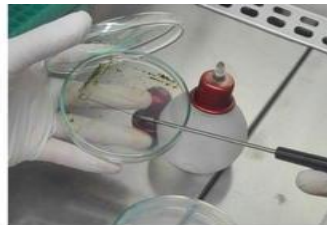


Fig 9: Algae culture rack set up at SSS-1 NIBE

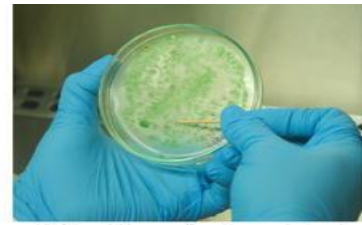
The inoculated effluent sample containing microalgae was subjected to microbiological techniques of spread plating and streaking for the isolation (**Fig 9.1, 9.2**) and purification of microalgal strain. Three microalgae strains were isolated as visualized under the microscope (**Fig 10,11,12**)



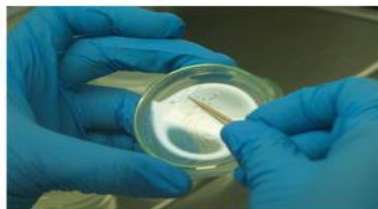
medium distribution with agar



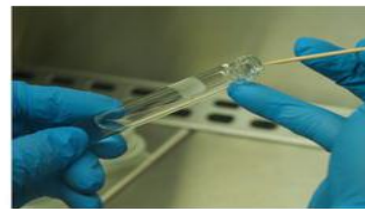
Planting of enriched culture in medium with agar



Selection of microalgal colonies



Planting of selected microalgal colonies in medium with agar



Transfer of unialgal colonies to liquid medium

Fig. 9.1 Isolation Techniques (Source: M.Cobos.et.al.(2019)



Spread plate technique to isolate microalgal colonies



Streaking of an isolated colony to get a pure microalgal colony



Cultivation of isolated pure colonies in 150ml flask containing 100ml BG-11 medium (**growth condition:** culture rack with artificial light of intensity 6000-7000Lux, 16:8 day and night cycle)

Fig. 9.2 Agar Plates with Microalgal Colony

Microscopy of Isolated strain

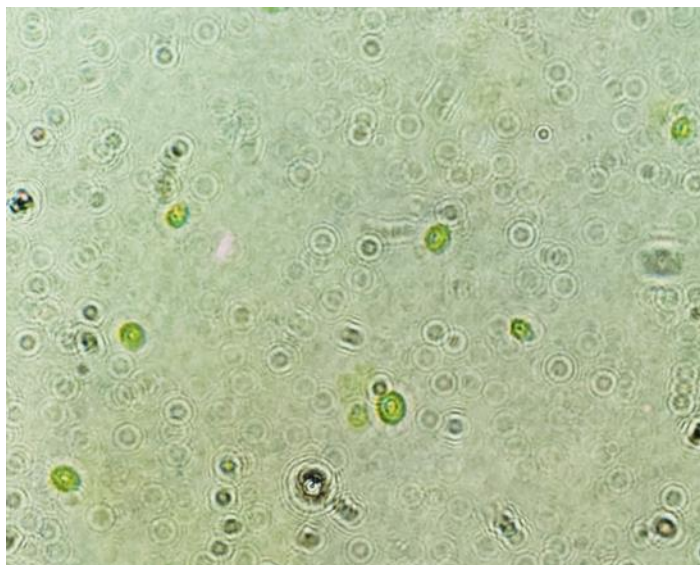


Fig 10: WWEJ1 (100X)

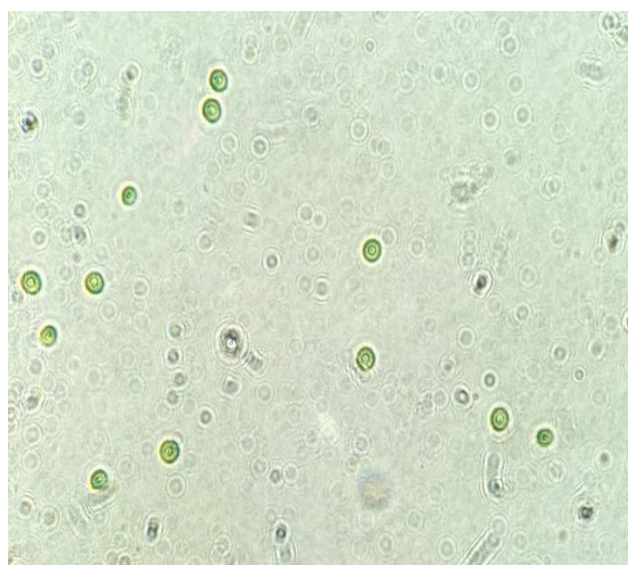


Fig 11: WWEJ2 (100x)

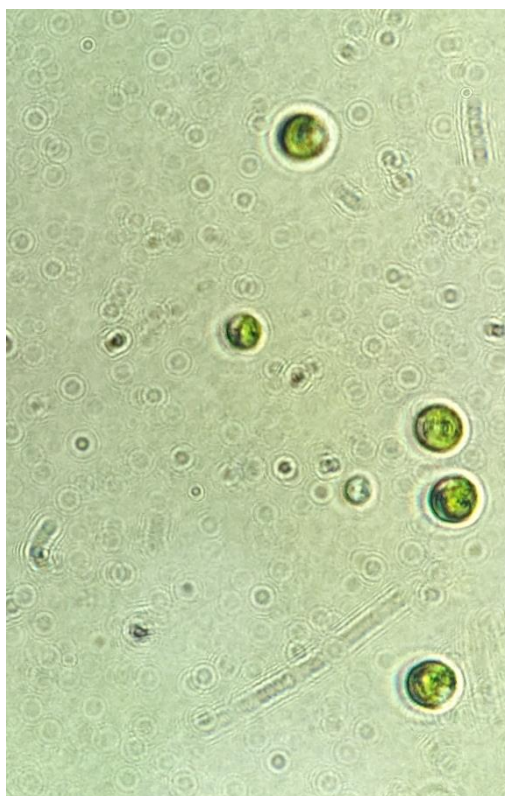
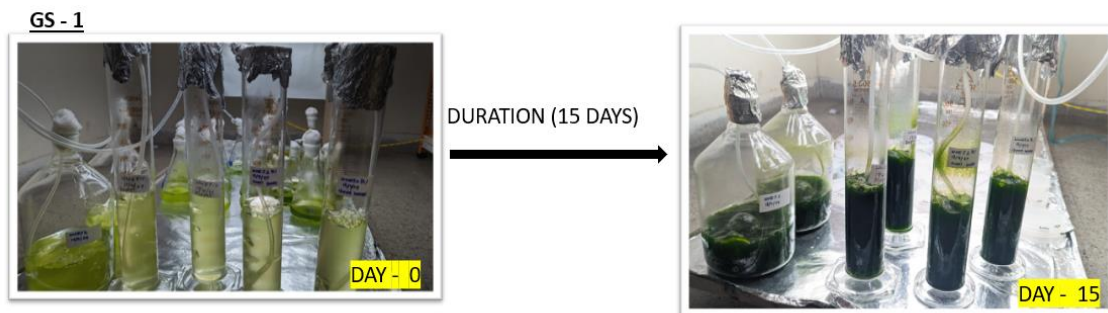


Fig 12: WWEJ3 (40x)

The growth study for the isolated strains was done initially in the conventional media (**fig. 13**) to understand the growth pattern (**Fig. 14**) of the organism and a nutrient removal study will also be done.



- Temperature: $25^{\circ}\text{C} \pm 2^{\circ}\text{C}$
- pH: 7.00 – 7.75
- Light intensity: 7500-8000 Lux
- Growth Media: BG-11
- CO_2 supply: every day (depending upon the cell density)

Fig.13: Tubular PBR setup for Growth study

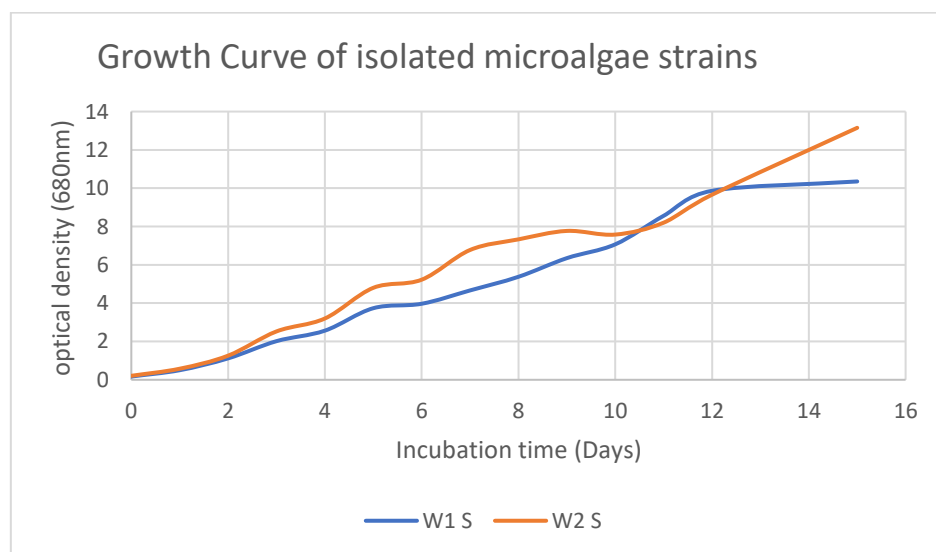


Fig.14: Growth Curve of isolated strain