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Study on the use of algal biomass in removal of dyes from Aqueous systems

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ABSTRACT

Present study provides direct information regarding the potential of algal biomass in removing dyes from wastewaters and multi-component systems. Algal community growing in natural water streams of nearby area of Jaunpur district will be grown in the laboratory in different culture media such as AAP, BG 11 and CHU 10 will be employed. Different algae will be expected to show varying affinity for the different media as these media have varying compositions. Textile effluent pollution levels might fluctuate over time. Furthermore, heavy metals, which are either used or produced as by-products of various dyeing and sizing processes, are now common and persistent in textile effluents. Textile effluents were collected from industrial sites based on ease of access and availability of the industry. Effluents were collected from discharge outlets of an ETP located outside of a textile dyeing factory in this location for this investigation and were labelled as JTE (Jaunpur Textile Effluent). It should be noted that the effluents utilised in this investigation were taken from the discharge ports of an ETP located directly outside a textile dyeing factory in Jaunpur District. The findings also show that the effluents have an impact on water quality, which has the potential to cause serious ecological as well as other healthcare catastrophes in the future. The isotherm simulation revealed that the Langmuir isotherm equation, rather than the Freundlich equation, might better represent adsorption on nanoalumina. Nanoalumina may be made in a straightforward and cost-effective manner.

Figures : 06 References : 10 Tables : 03
KEY WORDS : Algal Biomass, Industrial effluents, Pollution, Removal of Dyes

Introduction

Sorption of heavy metals by algal materials has been studied frequently considering single metal solution, while reports on multi-component systems are comparatively limited. A number of workers have used fly ash and activated carbon for concurrent sorption of heavy metals and dyes from aqueous solutions. They recommended that solutions containing dyes and metal ions concomitantly are difficult to treat as these two species can influence sorption of each other onto sorbents surfaces. In this regard, some workers have reported inhibition in sorption of both heavy metals and dyes from two-component solutions[1]. Dye wastewaters from the textile and dyestuff industries must be handled because of their influence on water bodies and growing public concern about their toxicity and carcinogenicity. Biosorption methods, which may be used to remove harmful substances from industrial wastewaters, have become increasingly important from environmental standpoint[2]. The hazardous character of industrial effluents dumped into natural water resources is a major source of environmental contamination. We are paying or will pay in the near future is unquestionably too great. Water demand has skyrocketed in the agricultural, industrial, and home sectors. With the rapid rise of the textile industry, water pollution from dye wastewater and sludge began. India has a slew of environmental issues that necessitate industrial wastewater treatment solutions. The Indian government is putting more money and effective pollution control technologies into the creation of environmental infrastructure, such as sewage systems to handle industrial wastewater[3]. Dyeing has been used by humans for thousands of years. All colors were more or less natural until the late nineteenth century, with key sources such as plants, insects, and mollusks, and were normally made on a small scale.[4]

Dyes in History

Mauveine, the first human-made (synthetic) organic dye, was prepared. Hundreds of thousands of synthetic colours have been created. Natural dyes were quickly superseded by synthetic dyes. They were less expensive, offered a wider spectrum of new colours, and gave dyed materials superior characteristics. Dye stuff is a coloured substance that has a specific affinity for the substrate to which it is applied. Three subsystems make up a coloured

TABLE-1: The chemical constituents of the BG-11 medium (Major salts)

S. No.	Chemical	Formula	Concen-- tration (g L^{-1})
1.	Sodium nitrate	$NaNO_3$	1.3
2.	Di-potassium phosphate	K_2HPO_4	0.16
3.	Magnesium sulphate	$MgSO_4.7H_2O$	0.832
4.	Calcium chloride	$CaCl_2.2H_2O$	0.125
5.	Citric acid	$C_6H_8O7.H_2O$	0.015
6.	Ferric ammonium citrate	$C_6H_8FeNO_7$	0.018
7.	EDTA (disodium salt)	$C_{10}H_{16}N_2O_8$	0.023
8.	Trace metals*		1 ml
9.	Agar (if needed)		1%
10.	Distilled water		1000 ml

compound[5]. To begin, there's the chromophore, which is a type of electron-accepting molecule. Carbonyl, ethylene, azo, nitro, nitroso, sulphur, and other carbon-sulphur groups are common chromophore groups of dye compounds. Second, auxochromes are frequently used to describe nucleophiles. Amino, sulfonic, hydroxyl, and carboxylic groups are electron-withdrawing groups. The final coloured chemical is a chromogen, which is made up of a system of conjugated double bonds that connect the chromophore and auxochrome to generate a colour[6] . The fundamental function of the chromophore is to produce colours that improve the dye's solubility and affinity for fibres[7]

Experimental Methodology

Microalgal Species Isolated from an Effluent Disposal Facility

A microalgal species was recovered from the outflow location of a textile effluent treatment plant. The isolation process was carried out in order to get robust and viable algal strains, as well as to use their acclimatization characteristic for the remediation of textile effluent. It is envisaged that textile effluents included enriching nutrients that would be great for microalgal communities to flourish in a way comparable to that of widely viable nutrient medium[8]. It was necessary to dilute the fluidized the microalgal culture many times before they could be cultured in culture room (28ºC, 16:8 h light: dark cycle, 100μmol photons $m^{-2}s^{-1}$). Agar plates treated with Chloramphenicol (an antibacterial agent) and Amphotericin B were used to stripe the bacteria once they had grown to a certain size (antifungal agent). This procedure was done several times until pure algal cells were obtained. To ensure that there was no bacterial or fungal contamination, individual microalgal cells were grown in liquid BG-11 media for additional examination and characterization[9].

The Chemical Constituents of the BG-11 Production Medium

The composition of commercially available nutrient dense BG-11 medium as illustrated in the Tables-1 and 2[10].

TABLE-2: The Concentration of Trace Metals in the BG-11 Substance

S. No.	Chemical	Formula	Concen-tration (g L-1)
1.	Boric acid	H_3BO_3	3.10
2.	Manganese chloride	$MnCl_2.4H_2O$	2.52
3.	Zinc sulphate	$ZnSO_4.7H_2O$	0.331
4.	Sodium Molybdate	$NaMoO_4.2H_2O$	0.41
5.	Copper Sulphate	$CuSO_4.5H_2O$	0.182
6.	Cobalt nitrate	$Co(NO_3)_2.6H_2O$	0.0541
7.	Distilled water		1000 mL

TABLE-3: Characteristics of microalgal species identified from textile wastewater discharge locations

Gathering location	Recognized as	Dye decolonization effectiveness (%) in 150 mins (40 mg L-1)	Electrogenic ability				
			Ipa	Vpa	Ipc	Vpc	(ΔV)
FTE	*Chlamydomonas* sp. TRC-1	40.30	0.72	0.41	-0.53	0.35	0.36
GTE	*Desmodesmus armatus* sp. KB-1	40	0.64	0.45	0.53	0.25	0.34
GTE -	*Nanochloropsis* sp.	19.51	-	-		-	-
GTE	*Scenedesmus* sp.	29.52	0.92	0.82	-0.83	-0.37	1.15

Characterization of Microalgal Species Isolated

Classification based on morphology

Confocal Light Microscopy (CLM)

Underneath the guidance of a light confocal microscope, the morphological properties of microalgal species separated from FTE and GTE were studied (Olympus FluoViewTM FV1000). The microalgal isolates were stimulated at a wavelength of 488 nm and analysed under oil absorption magnifications of 100, 200, and 450 times their original size.

Scanning Electron Microscopy (SEM)

The morphology of the microalgal isolated was investigated using scanning electron microscopy (SEM) (ZEISS EVO Series, EVO 50). Shubert and Wilk-procedures Wozniak's for preparing and fixing samples were followed. A 2.5 percent gluteraldehyde solution in 0.1M potassium phosphate buffer was used to fix microalgal cells, and a 2 percent OsO_4 solution was used to post fix them. Using a graded ethanol series (10 percent, 20 percent, 30 percent, 50 percent, 70 percent, 90 percent, 100 percent), the fixed microalgal material was dehydrated for 45 seconds at 20 mV and gently coated with gold for 45 seconds at 100 percent. It was possible to sustain high vacuum conditions at 7.4×10^{-4} pa.

❑ Electrochemical Function in Contrasting Perspective

Cyclic voltametric (CV) tests were carried out on the separated microalgal species to properly appreciate their electrochemical activity. A three-electrode setup was used in the experiments. Using an Auto lab PGSTAT-10, a 3 electrochemical laboratory with Indium Tin Oxide (ITO) or Fluorine Tin Oxide (FTO) as the working electrode, Ag/AgCl as the potentially facilitate, and platinum wire as the counter electrode, CV investigations were carried out. With an electrolyte source of KCl solution (PH 3.2; 0.1M; containing 5 mM $Fe(CN)_6^{3,4-}$) as the electrolyte, CV scans were seen within -1.0V and 1.0V potential at a scan rate of 100 mV s^{-1} in the range of -1.0V to 1.0V prospective.

Making a Working Electrode

The separated microalgal species was cultured on the working electrode for 15 days under visible light irradiation to determine their viability. For the production of an algal biofilm on the working electrode, working electrodes (ITO/FTO) were put in 0.4 OD of exponential phase culture (100 mL) in an incubator at 24 °C, lit with a 1500 lx light source to allow for the growth of an algal biofilm on the working electrode. An anaerobic environment was created in order to determine if extracellular electron transfer happened through a direct or indirect process. For the measurement, nitrogen gas was introduced into the electrolyte buffer for 35 minutes before and throughout the procedure.

Culture Growth Conditions Optimized

Bioremediation is seen as a huge difficulty since microorganisms are not well adapted or acclimatized to the highly contaminated and poisonous textile effluents, and as a result, it was improved before it could be used on a broad scale in the textile industry. Once the most suitable microalgal species had been identified, the circumstances for their cell proliferation were optimized.

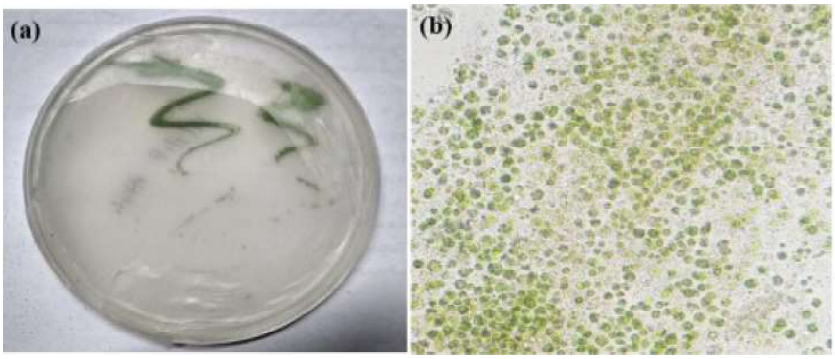

Fig. 1: (a) Agar plate development of pure algal colonies and (b) Green unicellular colonies observed underneath a light microscope (40x magnification) are shown

The pH, temperature, and salt concentration of the textile effluents that were collected were all high. Furthermore, because the effluents included a high concentration of textile colors, the penetration of light becomes critical for algae development. As a result, cells were grown in BG-11 medium at various temperatures (15°C, 25°C, and 40°C) in order to determine the best temperature, pH, salinity, and photoperiod for the isolated microalgae species under study. Growth was also examined in an orbital shaker for 8 days at varying pH (2-10) with a pH range of 2 to 10. With the help of HCl (0.1N) and NaOH (0.1N) solutions, the pH of the medium was raised to 7. For the purpose of determining the influence of salinity on the development of algal cells, various amounts of NaCl salt were utilized (0-300 mM L^{-1}). The impact of changing the photoperiod (light: dark cycle, 100mol photons m^{-2} s^{-1}: 0:24; 12:12; 16:8; 24:0) on the development of algal cells was studied to see whether it had any influence.

- **Studies on Growth Figures**

The dry cell weight (DCW) and growth rate of algae were used to assess the amount of growth occurring. The growth rate of the bacteria in BG-11 media was tracked by measuring the optical density (OD) at 680 nm until a reduction in growth was noted. Following a previously published approach for converting OD_{680} measurements to DCW (gL-1), the following procedure was used:

Biomassconcentration (gL^{-1}) = α x OD680 +β

Analysis and Results

Microalgal Species Isolated using FTE

The FTE discharge location was effectively colonized by a unicellular microalga (formerly designated as FTE-1), which was effectively separated. Green colonies of cells developed when cells were cultured on nutritional agar media (Fig. 1).

FTE-1 Classification

❑ **Classification based on morphology**

CLM

Figs. 2 (a) and (b) depict the CLM observation of the algal isolate, which confirms the existence of both

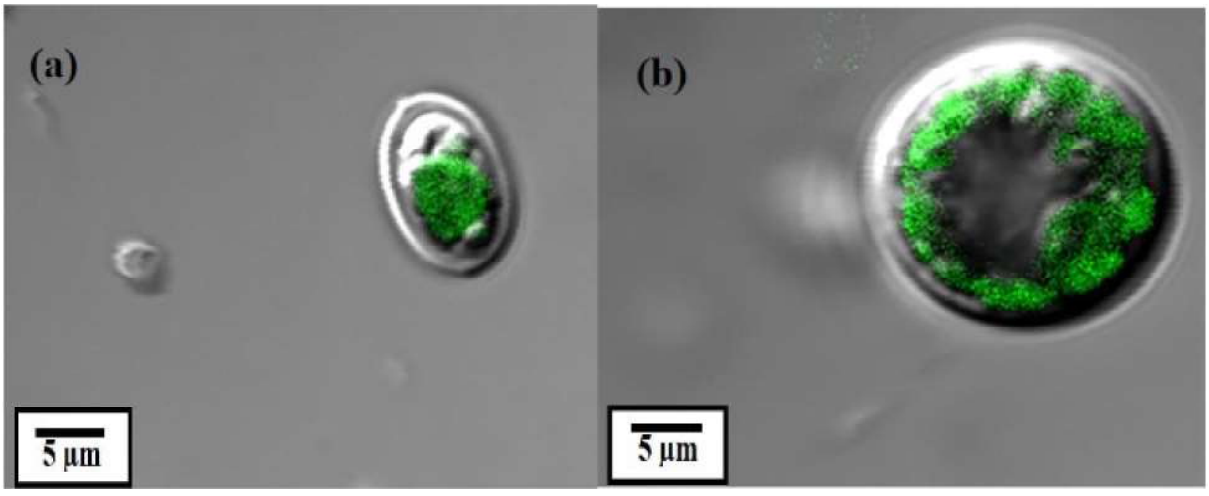

Fig. 2: Photograph of FTE-1 cells taken under confocal light microscopy, showing (a) Ovoid (400 amplification) versus (b) Spherical (400 maximum aperture) cells, respectively.

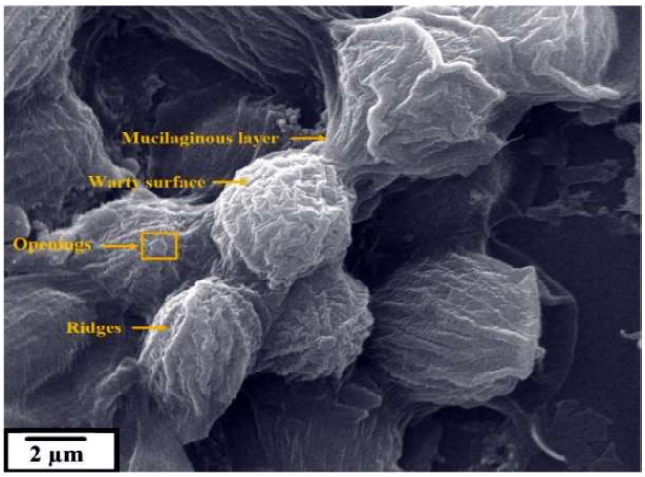

Fig. 3: At a resolution of 5K, scanning electron micrographs of separated microalgae FTE-1 were taken

ovoid and spherical shaped cells, indicating that FTE-1 utilized this physical change as a survival tactic in reply to the multiple toxic environmental pressures existing in its environs FTE-1 cells ranged in size from 6 to 10 microns.

SEM

The SEM analysis indicates that the base of the microalga isolation was decorated with ultra-structural characteristics including a warty surface and grooves, which were seen in the wild (Fig.3). The cells ranged in size from 6 to 10 microns. It was also discovered that the cells were surrounded by a mucilaginous layer. The bursting at the cell's apex was also captured on video.

❑ **Molecular Classification**

As a result of the fact that different microorganisms are capable might have morphology that is identical to one another, further molecular recognition was achieved on FTE-1. It was determined if the samples were human or not by employing 18S ribosomal DNA sequencing of the ITS-1 and ITS-4 regions. Using the BLAST programme, the acquired sequence was matched to the GenBank database to determine its identity. In the GenBank database, this sequence was assigned the accession number MF162271 when it was submitted for inclusion. A strain of Chlamydomonas sp. TRC-1 microalgae was discovered and identified.

❑ **Phylogenetic Research**

The MEGA X programme was used to create multiple phylogenetic trees, which were then used to conduct a molecular phylogenetic study of the FTE-1 gene. In order to create phylogenetic trees, the methods Neighbor Joining (NJ), Maximum Likelihood (ML), and Minimum Evolution (ME) were employed (Fig. 4). A total of 14 nucleotide sequences were included in the analysis. All positions with gaps and incomplete data were deleted from consideration. 147 locations were included in the final dataset, for a total of 147[16]

Fig. 3 depicts the ideal NJ tree with a total branch length of 48.57 and a sum of branch length of 48.57. In order to ensure that the phylogenetic tree was displayed to scale, the branch widths (located adjacent to the branches) were scaled to match the units used it to calculate the evolutionary distances required to estimate the phylogenetic tree.

The life cycle was also estimated using the ML approach based on the Jukes-Cantor model, which was used to infer the evolutionary history. The tree with the highest log probability (-508.81) is depicted in Figure -4 as the most likely tree. A matrix of pairwise distances computed using the Maximum Composite Likelihood (MCL) technique was fed into the NJ and Bio NJ algorithms, and the topology with the highest log likelihood value was selected as the starting point for the heuristic search. The tree was drawn to scale, and the lengths of the branches were measured in terms of the number of replacements per site (next to the branches).

Fig. 3 depicts the ideal ME tree with a total branch length of 48.26 and a sum of branch length of 48.26. (c). In order to ensure that the phylogenetic tree was displayed to scale, the branch lengths (located adjacent to the branches) were scaled to match the units used to calculate the broader development required to estimate the phylogenetic tree. The evolutionary distances were calculated using the number of differences technique, and the units of the evolutionary distances are the number of base differences between each sequence of DNA. Using the Close-Neighbor-Interchange (CNI) technique with a searching threshold of 1, the ME tree was explored for possible matches. The first tree was created using the Neighbor-joining technique, which was implemented in C++. All positions with gaps and incomplete data were deleted from consideration[13].

Identification of the Most Beneficial Microalgal Species

As a result, microalgal isolates FTE-1 (*Chlamydomonas* sp. TRC-1) and GTE-1 (*Desmodesmusarmatus* sp. KB-1) were chosen for future experimental tests based on their greatest dye decolonization efficiency of 40 mg L^{-1} of dye and comparable electrogenic capacity with smallest potential difference (V). Table -3 shows the tabulated results of the selection parameters, which are shown graphically.

***Chlamydomonas* sp. TRC-1 growth parameters were optimized using a genetic algorithm.**

pH, temperature range, and salinity conditions are only a few factors that influence the growth of algae in

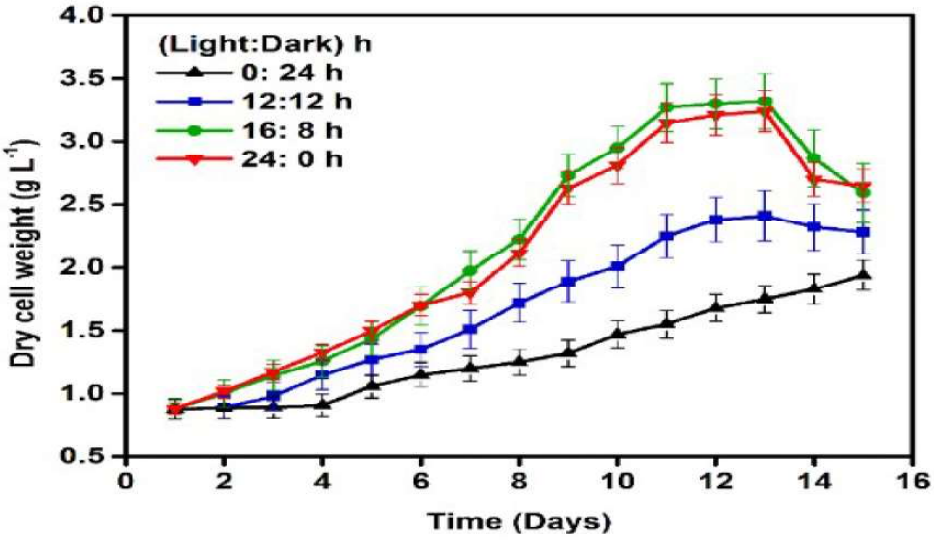

Fig. 4: In a mono-batch study performed under continuous light intensity of 100 mol photons m^{-2} s^{-1}, the impact of photoperiod on the development of C. TRC-1 cells cultured in BG-11 media was investigated

different environments. Because the collected textile effluents had high pH and temperature values, as well as high levels of salt and change in the photoperiod, it was necessary to adjust these factors in order to achieve optimal biomass production from the waste. By employing a totally randomized experimental design, the influence of the factors listed above on the growth rate of C. TRC-1 cells was investigated. In these tests, just one parameter was changed, while the other values remained fixed throughout the experiment.

❑ The Influence of Photoperiod

It is generally assumed that the growth rate of microalgae is related to the length of time that they are exposed to effective light. Given that photosynthesis requires both light and dark phases to function properly, photoperiod is regarded to be a critical characteristic for microalgal development. In a constant light intensity of 100 mol photons m^{-2} s^{-1}, C. TRC-1 cells were grown under a light: dark photoperiod of 24 hours, 12 hours, 16 hours, and 0 hours and 24 hours under the following conditions: 24 hours, 12 hours, 16 hours, and 0 hours and 24 hours (Fig. 4). Under the conditions of a 12:12 h (light: dark) photoperiod, C. TRC-1 cells produced 2.28 x 0.17 g L^{-1} in biomass production. The amount of energy that algae cultures get is directly proportional to their development. The duration of light is proportional to the length of time that the cells divide. The photoperiod of 16:8 h (light: dark) produced the most biomass increase, 2.59 0.23 g L^{-1}, which was the highest reported. However, after 16 hours of irradiation, the variance in growth became less and less noticeable, demonstrating that the generation time varied with the amount of radiation received. When C. TRC-1 cells were cultured in total darkness, just the smallest amount of algal growth was seen. The cells appeared to amass enough energy for cell division when exposed to a 16:8-hour photoperiod cycle of light and darkness. Furthermore, it demonstrates that 8 hours of darkness was adequate for all of the occurrences that occured when there was no light.

❑ The Influence of Temperature

When it comes to practical use in microalgal cultivation, temperature is the most straight forward parameter to manage and control. However, temperature has an impact on the rates of chemical reactions that are relevant to algal various metabolic processes. In order to determine the optimal temperature for effective biomass

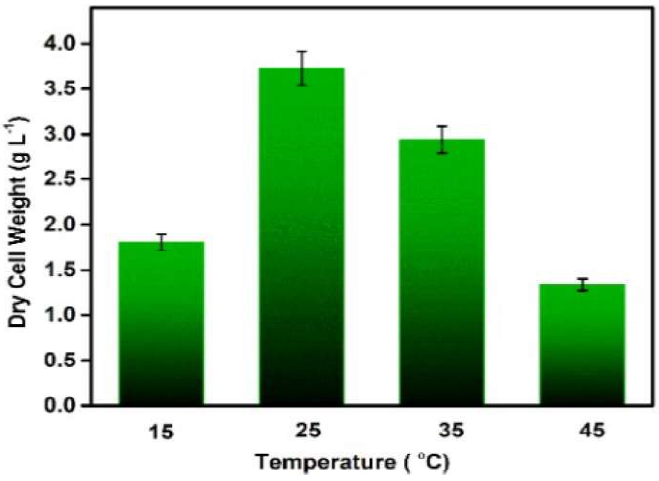

Fig. 5: In a mono-batch experiment conducted beneath continuous light intensity of 100mol photons m^{-2} s^{-1}, the influence of temperature on the proliferation of C. TRC-1 cells cultured in BG-11 media was investigated.

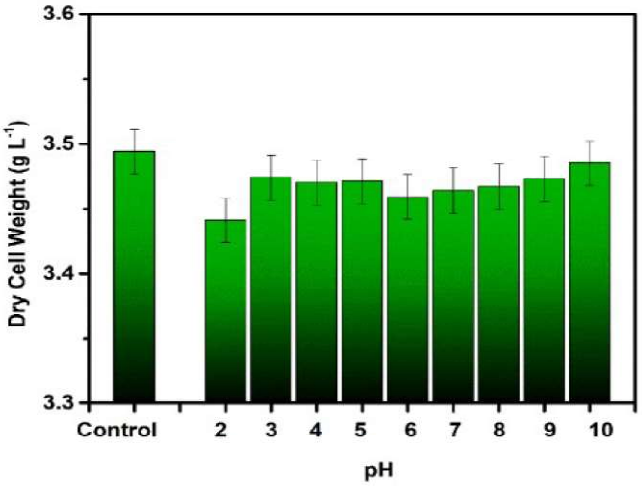

Fig. 6: In a mono-batch study performed under continuous light intensity of 100 mol photons m^{-2} s^{-1}, the effect of pH on the proliferation of C. TRC-1 cells cultured in BG-11 media was investigated.

generation, C. TRC-1 cells were cultivated at several temperature settings (Fig. 5). The optimum temperature range was chosen at 15 degrees Celsius, and the growth rate was reported to be 1.8 0.09 g L^{-1} at this temperature. At 25°C, the highest biomass density of C. TRC-1 cells was found (3.73 0.18 g L^{-1}), trailed by a biomass concentration of 2.94 0.14 g L^{-1} at 35°C. The smallest amount of growth was seen at 45 °C (1.340.067 g L^{-1}), demonstrating that increasing temperatures result in a decrease of cell viability.

❑ **The Influence of pH**

pH is the third most important component in algal development, after temperature and light, it is essential for algal growth. Cells use their energy to keep the pH of their internal environment, which is necessary for the cell to operate at high or low pH levels, depending on the situation. When C. TRC-1 cells were grown under varied pH conditions (2-10), they revealed a substantial difference in their proliferation (Figure -6). Since the pH of the effluent (FTE) was 10.9, the maximum growth of C. TRC-1 cells was seen at pH 10 (3.4850.17 g L^{-1}), which may explain in part its survival in the extremely alkaline FTE effluent. Significant growth was also found at pH 3 (3.474 0.17 g L^{-1}), suggesting the ability of C. TRC-1 to thrive in a variety of environments, including extremely acidic environments. It is also worth noting that the C. TRC-1 was able to modify the pH of the solutions to between 9.8 and 10.2 after 3 days of incubation, demonstrating that it had the ability to swiftly adapt to the ambient pH without significantly impairing cell development.

Because C. TRC-1 produced a variety of metabolites as part of its survival strategy in a hazardous environment, the pH was rising, which might be ascribed to this?

Conclusions

Access to clean water is becoming increasingly difficult to come by, and the necessity for a stable source of energy is becoming increasingly critical in the contemporary age. In response to this concern, scholars have concentrated their efforts on the development of ecologically friendly, non-toxic replacements. One of the most significant issues faced by the textile mills is the development of large quantities of brightly coloured effluents in large quantities. Effluents from these factories contain numerous litres of untreated wastewater, which is discharged into public sewers, which finally empties into a neighbouring aquatic body. The effluents created contain hydrolysed unfixed dyes, surfactants, salts, sulphides, metals, and acidic or alkaline media, among other things. It should be noted that the effluents utilized in this investigation were taken from the discharge ports of an ETP located directly outside a textile dyeing factory in Jaunpur District.

The results of this investigation reveal that nanoalumina is very effective at removing RR120 dye from aqueous solution. The influence of contact time, beginning concentration, initial pH value, and adsorbent dosage on dye removal efficiency was investigated. The optimum pH for RR120 adsorption onto nanoalumina was determined to be no more than 3. The pseudo second order model was shown to be a good fit for the kinetics of RR120 adsorption over nano alumina in the experiments. The isotherm simulation revealed that the Langmuir isotherm equation, rather than the Freundlich equation, might better represent adsorption on nanoalumina. Nanoalumina may be made in a straightforward and cost-effective manner.

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