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Removal of Corafix Yellow GD3R using Halimeda macroloba and its impact on Macrotyloma uniflorum

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Abstract

Synthetic aromatic chemicals known as dyes are primarily employed in the textile industry and are fatal if released into aquatic and terrestrial ecosystems without proper treatment. The present study characterizes the biosorption efficiency of Halimeda macroloba for the removal of Corafix Yellow GD3R dye from the aqueous solutions. At different concentrations of dye (100-500mg/L), biosorbent dose (100-500mg/L), pH (4-10), temperature (20°C - 40°C) and incubation period (24-120hrs), the ability of seaweed to remove the Corafix Yellow GD3R dye was evaluated. Maximum removal of Corafix Yellow GD3R (84%) was observed at pH 8 with 200 mg/L of dye exposed to, 300 mg/L biosorbent at 25°C. Desorption using 0.1N NaOH revealed a 60% of recovery on the first day. The UV-Vis and FT-IR analyses were performed to study the interaction between the adsorbate – bioadsorbent. The nontoxic nature of the treated dye solution was verified by the substantial growth of Macrotyloma uniflorum (Horse gram). H. macroloba, under optimal conditions effectively removed the dye and may be used in the exclusion of pollutant from textile sectors, providing a clean and green eco-friendly approach to society.

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Keywords: Halimeda macroloba, Corafix yellow GD3R, Optimization, Decolorization, Characterization, Phytotoxicity.

1. Introduction

The availability of clean drinking water has been under intense strain due to the growing human population, particularly in emerging nations [1]. The textile manufacturing industry in India is the most significant economic sectors in the country, making up around 14% of all industrial production [2]. Synthetic aromatic dyes are often utilized in diverse industries, including cosmetics, plastics, food, textiles, paper, and medicines. More than 10,000 synthetic dyes, or 10-15% of all dyes used in the dyeing process, are detected in coloured wastewater [3].

Throughout the process, enormous amounts of water are consumed, from washing the fibers to bleaching, mercerizing, dyeing, printing and washing the finished products. Every day, enormous amounts of textile dye containing effluents are often dumped into natural water bodies worldwide. Owing to the elevated BOD and COD values, which are extremely harmful to biological life, untreated textile effluent can quickly deplete dissolved oxygen if discharged into surface water sources [4]. Consumption of the dyes present in the water bodies may lead to health problems such as nausea, hemorrhage, skin and mucous membrane ulcers and cause severe damage to the central nervous system, brain, liver, and reproductive systems [5]. Due to stringent government regulations, textile industry has met ever-higher levels of treatment for effluents. Efforts have recently been undertaken to create effective and justifiable solutions designed for the treatment of dyeing wastewater [6].

Azo dyes currently account for the most of dye chemistry production, and their relative significance may rise in the future. They are essential to the management of the dye and printing markets, since more than 60% of all dyes are azo dyes. The chemical groups in the azo dyes allow forming covalent connections with the fabrics, which serve as their defining characteristics [7]. In the present study, Corafix Yellow GD3R, an azo- reactive dye is chosen, as the candidate dye because of its usage as a coloring agent in textile industries. Corafix dyes are cost-effective, their productivity rate is

higher, and the dyeing process is diminutive. Simultaneously, they reduce water transparency, have a poisonous effect, and are genotoxic to aquatic plants and animals [8].

To decolorize textile effluent, several traditional physical and chemical wastewater treatment methods have been proposed, including using ion exchange, activated carbon, flocculation, froth flotation, reverse osmosis, ozonation and membrane filtration [9]. Although all of these methods were highly adaptable and helpful, they all result in secondary waste products that need to be addressed further due to the high cost and disposable issue. The use of biomass originating from biological entities such as plants, microbes, algae and seaweeds proven to be an alluring, alternative, eco-friendly, and economically advantageous method for dye removal from aqueous solutions [10]. Application of seaweeds as a biosorbent to remove remnants of dyes and other substances from diluted aqueous solutions with minimal sludge production may be an attractive technique for the textile industry [11]. Thus in the current study, H. macroloba was selected as the biosorbent owing to the abundance of reactive functional groups on their surface and high affinity for dye removal [12]. The decolorization of Corafix Yellow GD3R using H. macroloba has not yet been recorded in the literature, and the current study is the first report to explore the seaweed in this way. Thus the current study is designed to investigate the decolorization of Corafix Yellow GD3R using H. macroloba and to assess the toxicity of the dye solution treated against horse gram under laboratory conditions.

2. Materials and Methods

2.1. Chemicals

The stock solution was made by dissolving 1g of Corafix yellow GD3R dye in 1L of water and left overnight to completely dissolve the dye powder. Table 1 and Fig. 1 provide descriptions of the dye's chemical structure and properties.

2.2. Collection and preparation of seaweed

The seaweed was collected from R.K. Algae Research Project Centre, Mandapam, Ramanathapuram, Tamil Nadu, India. With distilled

water, the collected seaweed was surface sterilized and air dried. The dried seaweed was powdered, sieved and stored in an airtight container at room temperature for further studies.

Compound name	***Corafix Yellow GD3R***
CAS NO	171599-84-1
Molecular weight	1160.5071 g/mol
Molecular formula	C_{34}-H_{29}-CI-N_{12}-O_{19}-S_6.x-Na

Table 1: Characteristics of the Dye Corafix Yellow GD3R

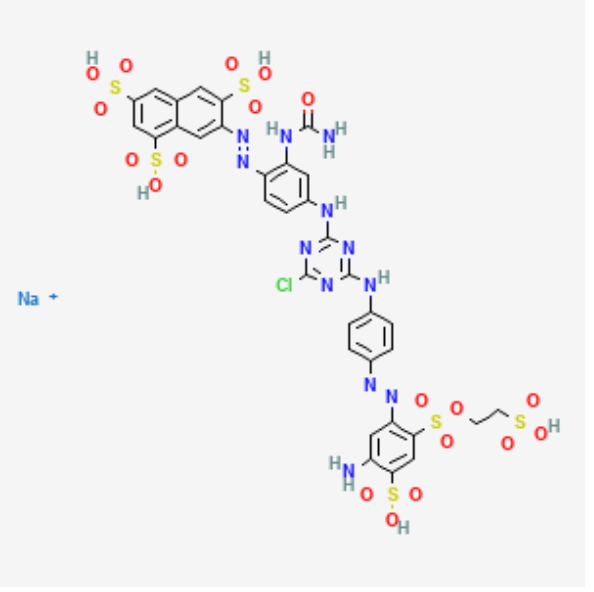

Fig.1: Structure of the Dye Corafix Yellow GD3R

2.3. Optimization of process parameters for Corafix Yellow GD3R decolorization

To determine the optimal conditions for the decolorization of Corafix yellow GD3R, the powdered seaweed was taken into a series of 250 ml Erlenmeyer flasks holding various dye concentrations (100-500mg/l) with diverse biosorbent concentrations (100-

500mg/l). The pH was established at various range (4 to 8) using 1N HCl / 1N KOH and incubated at various temperatures (20°C-40°C) for different time intervals (24-120 hrs). The decolorization efficiency was calculated using the formula,

$$\text{Decolourisation (\%)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100 \quad [13]$$

Under the optimal conditions, maximum decolorization percentage was calculated, and further studies were carried out under the optimized conditions.

2.4 Desorption activity

Desorption experiment was executed to explicate the dye sorption mechanism of the biosorbent under optimized conditions using the eluents, 0.1 N HNO_3, 0.1N NaOH, 0.1N CH_3COOH and 0.1N HCl. The desorption efficiency was calculated using the formula,

$$\text{Desorption efficiency (\%)} = \frac{\text{Amount of dye desorbed}}{\text{Amount of dye resorbed}} \times 100 \quad [14]$$

2.5. Analytical studies

The decolorization efficiency of the seaweed was analyzed in the untreated and treated dye solutions using UV-Visible spectrophotometer (Hitachi U2800, Tokyo, Japan). The adsorption peak was recorded from the wavelength of 300 - 700nm. Fourier Transform Infrared (FT-IR) spectroscopy (Shimadzu 8400S, Japan) was used to investigate the changes in the functional groups that obstruct the dye degradation process at 600 - 4000 cm^{-1}.

2.6 Phytotoxicity assay

The seeds of horse gram, red soil, and sand in equal proportion were selected for the pot culture experiment. Nine pots were set for the present study, and five healthy seeds were sown in each pot. The

seeds were periodically irrigated with tap water (Control), dye untreated and treated solutions. At the end of the 7th day, the seedlings were uprooted and assessed for the biometric parameters, namely germination percentage, vigour index, shoot length and root length. The germination percentage of the seedlings was calculated using the formula,

$$Germination\ percentage = \frac{Number\ of\ seeds\ germinated}{Total\ number\ of\ seeds\ sown}\ x\ 100 \quad [15]$$

The protrusion of radical through seed coat was taken as the criterion for germination.

On the seventh day following seeding, the seedlings were plucked, rinsed under running water to remove soil, and pressed between layers of filter paper to remove water droplets before the shoot and root lengths were measured. The maximum length of each shoot was recorded from the ground level to the tip and the root length was measured upto the tip of the root from the endline of the shoot. The ratio of germination percentage to plant height was used to calculate the vigour index.

3. Results and Discussion

3.1. Characteristic features of *Halimeda macroloba*

The selected seaweed was identified as *Halimeda macroloba*, green macro algae belongs to Chlorophyta (Fig. 2). The systematic classification is depicted in Table 2. *H. macroloba* is found on the sub tidal region of the South East coast of Tamil Nadu, India and also seen on the shallow water where they can use sunlight for their growth. *H. macroloba* have prolific, thick, oval, upright, compressed, mildly calcified thalli which are vivid grayish green when it is dry or fresh and possess a holdfast for the attachment to the substrate. They also possess peripheral utricle on the surface and seem to have rectangular to subcuneate basal segment from which two or more independent segments emerge in a single plane and collectively forms an overall folded fan-shaped base [16].

Division	**Chlorophyta**
Class	Ulvophyceae
Order	Bryopsidales
Family	Halimedaceae
Genus	*Halimeda*
Species	*macroloba*

Table 2: Systematic Classification

Fig. 2 : Dried Seaweed of *H. macroloba*

3.2. Optimization of process parameters for Corafix Yellow GD3R decolorization

A number of variables, including temperature, contact time, initial dye concentration, biosorbent concentration, and pH of the solution, influences the complex process of dye biosorption. Consequently, a batch study was conducted to optimize the aforementioned variables for the effective sorption of the chosen dye. The optimization parameters for the decolorization of Corafix Yellow GD3R using *H.macroloba* was depicted in Fig. 3a – 3e.

Initial dye concentration is an important factor in the resistance of the dye between the solid and aqueous phases thereby increases the uptake. As a result, dye concentration is a key factor in the process of removing colour from an aqueous solution. [17]. The effect of varying dye concentrations (100-500mg/L) on the decolorization of Corafix Yellow GD3R using *H. macroloba* was investigated at pH 8 with 300mg/L biosorbent concentration at 25°C for 96hrs of

incubation. The Corafix Yellow GD3R at the 200 mg/L exhibited maximum decolorization (84%), and minimum decolorization (47%) was pragmatic with 500mg/L of dye by *H. macroloba* within 96hrs of incubation (Fig. 3a). Hence, the results showed that dye uptake and decolorization increased linearly with increased dye concentration up to a certain extent, and then it levels off. The decrease in dye removal at higher concentrations may be due to the limited ability of the dye to bind to the fixed mass of the biosorbent in the aqueous solution and the rise in intraparticle diffusion or the blockage of active binding sites by the seaweed degrading enzymes [18]. The methylene blue removal by *Euchema spinosum* at lowest concentration showed higher uptake and elimination of the dye [19]. The biosorbent acts as a binding site for the dyes during the biosorption process. As a result, the concentration of the biosorbent significantly impacts the dyes removal from the aqueous solutions. Consequently, the importance of its involvement in treatment experiments is crucial to determine the capacity of biosorbent in the removal of dyes at particular concentrations [20]. While maintaining the other parameters constant, the percent removal of Corafix Yellow GD3R by *H. macroloba* was examined at different biosorbent concentrations (100-500mg/L). Using *H. macroloba*, the percent dye removal ranged from 88-50% (Fig. 3b), where the maximum dye removal (88%) was noted at 300 mg/L biosorbent concentration, indicating that the surface area and binding sites for the attachment of dye molecules was high. Beyond 300mg/L, the dye removal was insignificant, which could be related to the aggregation of the biosorbent at a higher dosage, or blocking of the binding sites on the biosorbent surface [21]. The literature reveals that complex interactions of numerous factors, including solute availability, interference between the binding sites, and electrostatic interactions, may cause of the decrease in sorption capacity with increasing biosorbent concentration [22].

The pH is a crucial factor to be considered throughout the dye sorption process since it influences the biosorption capacity, solubility and the colour of the dye [23]. The effect of pH on Corafix Yellow GD3R removal by *H. macroloba* has been examined at different pH ranges (4–10) while maintaining the same levels of the other variables. The sorption capacity of Corafix Yellow GD3R by *H.macroloba* was low at pH 4 (43%), which increased monotonically

up to pH 8 (82%). Subsequent decrease in pH beyond 8 resulted in the reduction of dye removal (Fig. 3c). The pH, which is the primary factor affecting the sorption process, determines the degree of ionization of the adsorptive molecule and the level of functional group dissociation on the active sites of the biosorbent according to the surface charge of the biosorbent [24]. Polysaccharides, lipids, melanin, proteins and various functional groups found in cell walls can bond with dye molecules and efficiently decolorize the dye. The electrostatic interaction between the positively charged cell surface and the negatively charged dye anions may account for the higher dye uptake observed at lower pH levels [25].

Temperature is a crucial kinetic parameter that alters the biosorbent ability for sorption [25]. At temperature between 20°C - 40°C, the impact of *H. macroloba* on Corafix Yellow GD3R removal was investigated by keeping the other parameters constant. The dye removal capability increased from 76 to 85% when the temperature was increased from 20 to 25°C and a further increase from 30°C to 40°C leads to a gradual decline in dye removal (Fig. 3d). The temperature profile shows that the sorption capacity declines when the temperature raises and thereby indicates that the biosorbent loses its property due to denaturation [26]. The decrease in dye removal with an increase in temperature from 35°C - 60°C may reduce the surface activity of the biosorbent or cause damage to the active binding sites in the biosorbent [27].

Contact time between the dye molecules and the biosorbent is crucial for successful sorption throughout the treatment process. The ideal contact time and dye concentration are significant essential elements in batch sorption studies to determine the adsorbate to bind on the biosorbent sites [28]. The biosorption experiment was carried out at different time intervals (24-120 hrs) to assess the efficiency of *H. macroloba* in dye removal. The percent removal of Corafix Yellow GD3R by the seaweed increased as the incubation period was increased from 24-96 hrs, and thereafter a sharp decline in the dye removal from the aqueous solution was noticed (Fig. 3e).

In general, the decolorization rate is initially rapid due to the abundance of vacant surface sites. Still, later remaining sites become difficult to occupy due to the attraction between the solute molecules and the solid-bulk phases [29]. The accumulation of dye molecules surrounding the biosorbent may impede dye migration since the

active sites are occupied. Also, the development of resistance to diffusion of dye molecules might have occurred at an increased biosorbent concentration which may contribute to the gradual decline in the dye removal [30].

Thus, *H. macroloba,* under optimized conditions degraded Corafix Yellow GD3R to 84%. For effective decolorization process parameters namely the dye concentration (200mg/L), biosorbent concentration (300mg/L), incubation period (96hrs), pH (8) and temperature (25°C) should be an optimized level (Fig. 4).

Fig. 3a - 3e : Optimization parameters for the decolorization of Corafix Yellow GD3R using *H.macroloba*

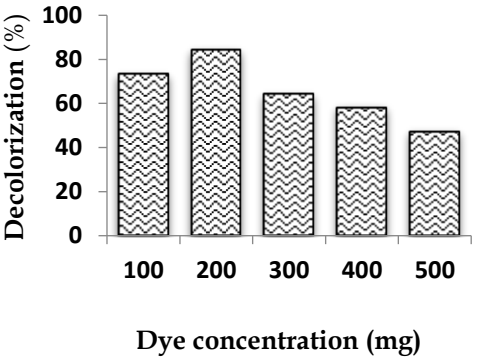

Fig 3a. Dye Concentration

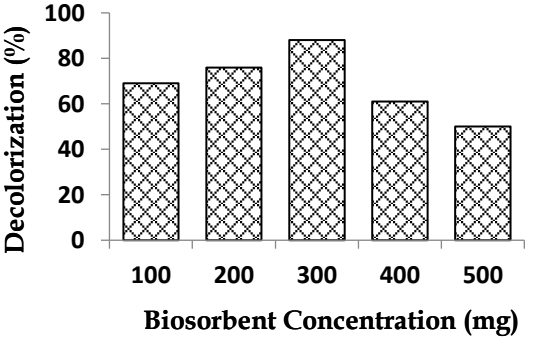

Fig 3b. Biosorbent Concentration

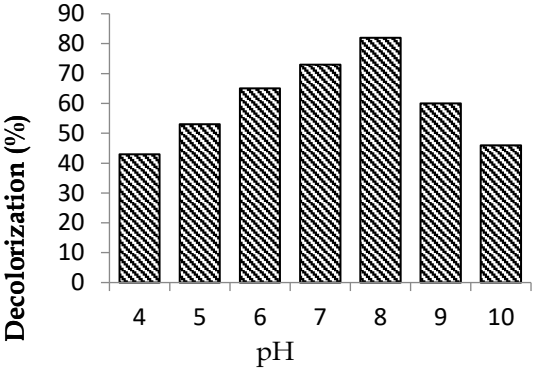

Fig. 3c: pH

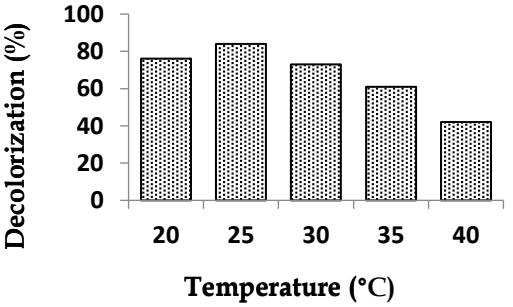

Fig. 3d: Temperature

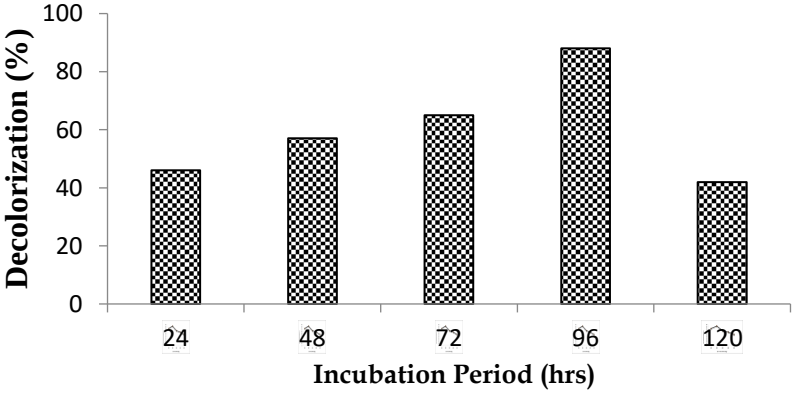

Fig. 3e: Incubation Period

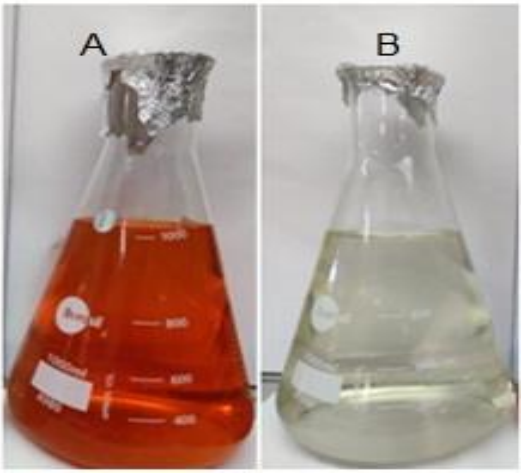

Fig. 4: Decolorization of Corafix Yellow GD3R dye using *H. macroloba* under optimal conditions

A- Untreated dye solution, B- Treated dye solution

3.3. Desorption of Corafix Yellow GD3R under optimized condition

To make the biosorption process economically viable, the biosorbent must be regenerated. Desorption was used to recycle the dye-loaded biosorbent, and it is a very effective chemical process that does not harm the structure of the biosorbent [31, 32]. Experiments involving three successive cycles of adsorption and desorption was carried out with dye-loaded biosorbent inoculated separately into the eluents, namely 0.1N HNO_3, 0.1N HCl, 0.1N NaOH, 0.1N CH_3COOH under optimized circumstances. Maximum recovery of Corafix Yellow GD3R was observed when 0.1N NaOH solution (60%) was used as an eluent on the first cycle when compared with 0.1N HNO_3 (45%), CH_3COOH (42%) and 0.1N HCl (50%) respectively. Further increase in the incubation period did not favour higher desorption efficiency (Fig. 5). The pH of the medium increases when NaOH is used as an eluent for desorption experiments, which in turn increases the negative charges on the biosorbent [33]. As a result, the anionic dye molecules and the negatively charged biosorbent experience an electrostatic repulsive force, which leads to desorption of the dye molecules from the seaweed. This might be due to the action of the alkaline solution, which has broken down or damaged the

adsorption sites or functional groups on the biosorbent's surface. A similar study using alkaline NaOH as an eluent recovered methyl orange from the biosorbent [34, 35].

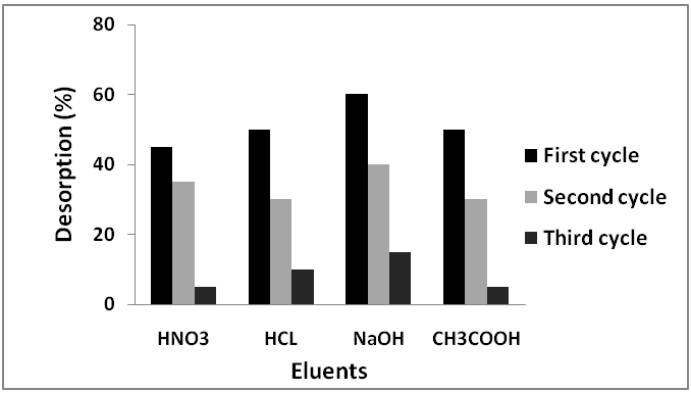

Fig. 5: Desorption of Corafix Yellow GD3R using different eluents

3.4. Analytical study

To confirm dye decolorization process caused either by degradation or biosorption, UV-Vis spectrophotometer was analyzed [36]. As seen from Fig. 6, the spectrum of the Corafix Yellow GD3R dye solution featured prominent peak at 427 nm, whereas the peak was reduced or vanished in the absorption spectrum of the dye treated with *H. macroloba*, thereby signifying the process of biodegradation. Literature reveals that the absorbance peak would either totally vanish or a new peak would develop if dye loss is attributed to biodegradation [37].

The possible interactions between the functional groups on the seaweed cell surface and dye were revealed by FTIR analysis. Fig. 7a and 7b reveal the FT–IR spectra of the dye unloaded and loaded *H. macroloba.* The shifting and increasing of peaks' suggests that *H. macroloba* was involved in the dye-removing process. The peak at 1527.62 cm^{-1} was shifted to 1519.91 cm^{-1} indicating the involvement of N-O asymmetric stretching vibration and nitro compounds

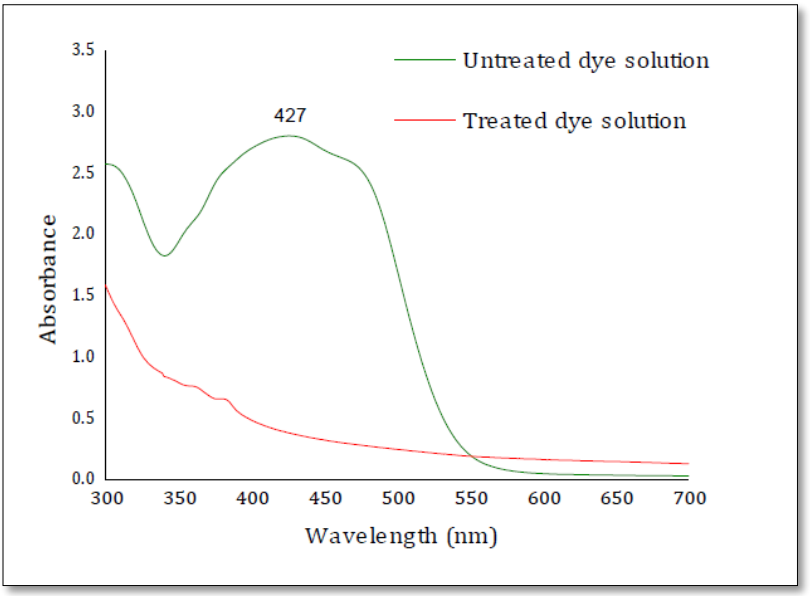

Fig. 6: UV-visible absorption spectra of untreated and treated dye solutions

presence in the binding of dye to the seaweed surface. Also the shift from 871.82 cm^{-1} to 864.11 cm^{-1} indicates =C-H bending and the involvement of alkenes in dye removal. The presence of peaks below 600 cm^{-1} indicates the participation of alkyl halides in dye removal. The presence of board peak at 3834.49 cm^{-1} in the dye unloaded spectrum indicates the presence of hydroxyl groups. Formation of new peak (925.83 cm^{-1}) and the disappearance of peaks in the FT -IR spectrum of dye-loaded seaweed imply the biotransformation of dyes into distinctive metabolites. The treatment of marine algal biomass and *Enteromorpha* with malachite green revealed alterations in the functional groups of the biosorbent and established these groups in dye removal respectively [38]. The amount of adsorption sites, their arrangement, chemical state, and their affinity to interact with the dye affect the presence of functional groups on the cell surface. Thus the results of the FT-IR analysis suggest the presence of hydroxyl, nitro, and aromatic groups on the biosorbent, which may have caused dye adsorption [39].

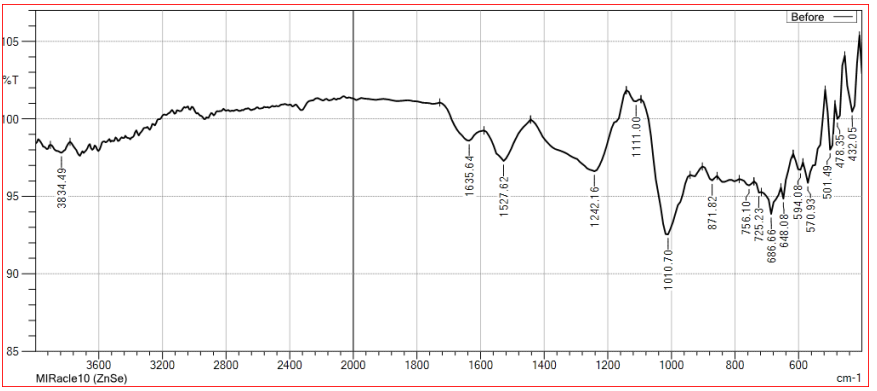

Fig. 7a: FT-IR spectra of *H. macroloba* unloaded with Corafix Yellow G3R

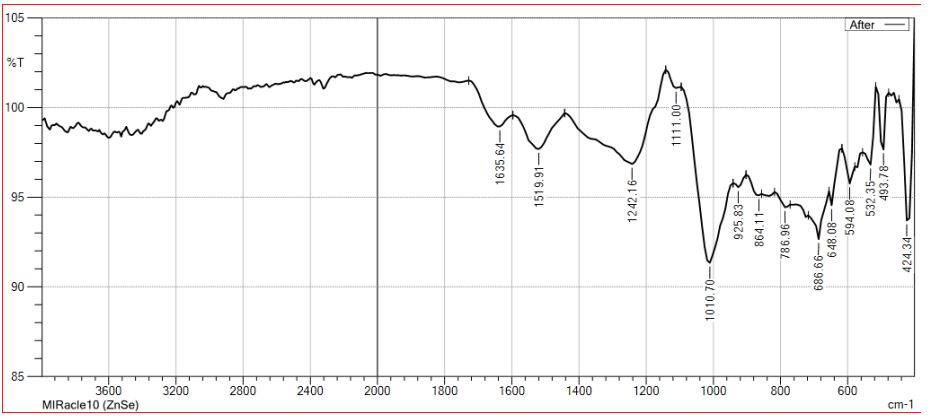

Fig. 7b: FT-IR spectra of *H.macroloba* loaded with Corafix Yellow G3R

3.5. Phytotoxicity study

The discharge of untreated dye solution has a direct impact on soil fertility and also on agricultural fields [40]. The results of the horse gram seedlings exposed to tap water, untreated and treated dye solutions on the seventh day favoured the growth of the experimental plant and the results are shown in Table. 3. A maximum of 100% germination was recorded in horse gram seeds grown with tap water (Control), followed by 90% in the treated dye solution and a minimum of 60 percent germination was recorded in seeds grown with untreated dye solution. The shoot length (9.92,

4.84 and 8.85 cm) and root length (1.25, 0.66 and 1.25 cm) were maximum in seeds exposed to tap water, followed by treated dye solution and untreated dye solution exhibited minimum growth. The vigour index peaked in tap water (1144) followed by treated dye solution (956) while falls to a minimum in untreated dye solution (330). The stages of seed germination and seedling development are crucial for plant growth and development. Radicle and plumule emergence results from the metabolic machinery of the seeds being reactivated. A crucial physiological process known as germination is used to measure the level of contamination [41]. Methylene blue exposed to rice seedlings reduced the rate of plant development, which in turn affects the rate of transpiration [42]. The decline in the vigour of seeds exposed to untreated dye solution could be explained by the interaction of several contaminants with the developing radical. Physiological and chemical characteristics of axes may also be related to seed vigour in addition to those of entire seeds [43].

Table-3 : Biometric parameters of *Macrotyloma uniflorum* seedlings on 7th day

Treatment	***Germination Percentage***	***Shoot Length (cm)***	***Root Length (cm)***	***Vigour Index***
Tap water	100	9.92±0.077	1.52±0.641	1144
Untreated dye solution	60	4.84±0.091	0.66±0.213	330
Treated dye solution	90	8.85±0.430	1.25±0.015	956

The values are mean of triplicates

4. Conclusion

The present study revealed *Halimeda macroloba* ability to decolorize of Corafix Yellow GD3R from aqueous solutions due to its high biosorption capability, sustainability, good reusability, economic benefit, ease of availability, and renewable nature. Also, physicochemical monitoring, together with bioassay studies, demonstrated the success of seaweed in wastewater treatment and can be explored for commercial purposes.

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Author contribution statement

P.M.Yuvanthi : Investigation, Methodology, Writing – Original draft

M. Poonkothai : Conceptualization, Supervision, Validation

Declaration of Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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