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REMOVAL OF ORGANIC DYE BY *Chlorella vulgaris* FROM AQUEOUS SOLUTION

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ABSTRACT

The potential of *Chlorella vulgaris* towards the removal of Malachite green and Methylene blue from aqueous solution was examined. Dye wastewater is more difficult to treat in large scale. The biosorption of dye studied in different parameters. The increasing presence of dyes represent a major environmental toxicity hazard, therefore finding and development of new method for dye removal from waste water has generated significant interest. Chemical, physical and electrochemical methods have limited use as they have many disadvantages compared with the biological methods. This paper examine the capacity of *Chlorella vulgaris* Beijerinck to decolorize two model dyes Malachite green and Methylene blue from their aqueous solution. The effect of dye concentration, algal concentration (number of cells/ml culture) and pH on the rate of decolorization was studied. The dye concentration at different times of the decolorization process was measured. The rate of dye decolorization was found to increase with the increase of algal concentration and to certain extent, the increase of dye concentration. The decolorization of dye is also affected by the pH of the media. In all it concluded that *C. vulgaris* showed high efficiency for dye removal of aqueous medium.

KEYWORDS: *Chlorella vulgaris*, Decolorization, *Dunaliella salina*, Malachite green, Methylene blue

A vast amount of dye effluent is continually released into the water posing a significant hazard to the natural environment and human health. The treatment of industrial effluents is a challenging topic in environmental science as control to water pollution has become of increasing importance in recent year.

The estimated number of the synthetic dyes on the market are more than 10,000 with annual production over 700,000 tons worldwide. These dyes are used in paper industry, textile, cosmetics, food and pharmaceutical industries. Furthermore, some dyes are dangerous to cells and living organisms due to their potential mutagenicity, toxicity and carcinogenicity (Rajeswari *et al.*, 2011; Pavko, 2011).

The wastewater from the dye-based industries often consist of toxic dye compounds along with substances such as dispersants, acids, bases, salts, detergents, oxidants, etc (Ho, 2005). Therefore, discharge from textile industries were usually high in color content, biochemical oxygen demand (BOD), chemical oxygen demand (COD) and suspended solids (Akar *et al.*, 2009). The released dye contaminants cause major environmental pollution by affecting photosynthesis process, penetration of light and causes health issues to living organisms (Padmesh *et al.*, 2005). The use of conventional physical and chemical treatment such as ion exchange, flocculation, irradiation, coagulation, precipitation and adsorption or sometimes a combination of these methods for the decolorization of dyes are limited due to limitations such as operational costs, formation of hazardous byproducts and intensive energy requirement (Jarosz-wilkolazka *et al.*, 2002; Soni *et al.*, 2012). Thus the situation demands the development of cost effective and innovative strategies to remove the dye contaminants from the industrial wastewater to minimize the risk of environmental threat.

Methylene blue (MB) and malachite green (MG) are widely used dyes. Both dyes have been reported for their negative impact on living cells and organisms. The oral median lethal dose (LD_{50}) of methylene blue and malachite green in rats has been estimated as 1180 and 275 $mg.kg^{-1}$, respectively (Seif *et al.*, 2004). It was also found that at low and moderate doses of MB arterial blood pressure increased, whereas at high doses it will worsen systemic hypotension, mycocardardial depression and hypertension after endotoxemia. MG has been reported to cause carcinogenesis, mutagenesis, chromosomal fracture, teratogenicity, and respiratory toxicity (Al-Fawwaz and Jacob, 2011).

The chemical structure of MG and MB dyes are illustrated in Figure (1).

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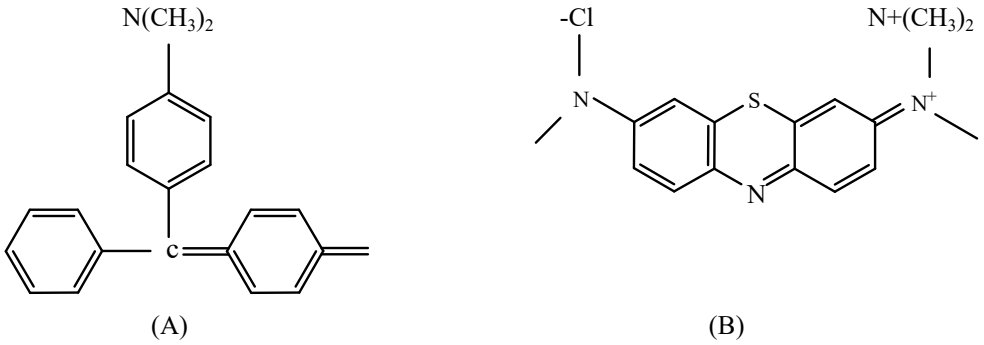

Figure 1: Chemical structure of Malachite Green (a) and Methylene blue (b)

The removal of color from waste waters is often more important than the removal of the soluble colorless organic substances, which usually contribute the major fractions of the biochemical oxygen demand (BOD). Methods for the removal of BOD from most effluents are fairly well established; dyes, however, are more difficult to treat because of their synthetic origin and mainly complex aromatic molecular structures. Such structures are often constructed to resist fading on exposure to sweat, soap, water, light or oxidizing agents.

Color affects the nature of water, inhibits the sunlight penetration into the stream and reduces photosynthetic activity, meanwhile some ofthe dyes are carcinogenic and mutagenic. Removal of color from dye bearing wastewaters is a complex problem because of difficulty in treating such wastewaters by conventional treatment methods (Kumar *et al.*, 2006). Algae have been found to be potential biosorbents because of their availability in both fresh and saltwater. The biosorption capacity of algae is attributed to their relatively high surface area and high binding affinity (Tien, 2002). Cell wall properties of algae play a major role in biosorption; electrostatic attraction and complexation are known to take place during algal biosorption.

In the present study the efficiency of *Chlorella vulgaris* Beijerinck to remove malachite green and methylene blue from their aqueous solution was evaluated. MG and MB were selected for experimental study.

MATERIALS AND METHODS

Algal Biomass and Growth Conditions

The axenic cultures of the *Chlorella vulgaris* Beijerinck, were kindly provided by Botany Department, Faculty of Science, BHU, Varanasi. *Chlorella vulgaris* was grown in Woods Hole MBL medium (Nichols, 1973). Sets of 250ml Erlenmeyer Pyrex-glass flasks were supplied with 100 ml of the appropriate medium specific for each species. Each flask was inoculated with inoculum of pre-grown culture in the exponential phase.

All flasks were grown under controlled laboratory conditions (temperature, 25±3º C; light, 80 µmol m^{-2} s^{-1}) in a controlled culturing chamber under a regime of 16 h light / 8 h dark.

Dyes Used

The two dyes used in this study were the triphenylmethane dye malachite green (MG) and the basic dye methylene blue (MB), obtained from Merck. A stock solution (1000 mg/L) of MG and MB was prepared by dissolving an appropriate amount of each dye in distilled water, and sterilized using a 0.45-µm membrane filter.

Dye Conc. Analysis

The dye concentration in supernatant solution were determined at characteristic wavelength (MG λ_{max} = 617 nm; MB λ_{max} = 663.7 nm) before and after removal by double beam UV–visible spectrophotometer (Perkin–Elmer Lambda 4B). All adsorption experiments were performed in triplicate and the mean values were used in data analysis.

Qualitative

The decolorizing activity was expressed in term of percentage decolorization and was determined by monitoring the decrease in absorbance at absorption maxima (max) of MG dye (617 nm) as well as of MB dye (663.7 nm). The un–inoculated media supplemented with MG or MB dye were used as references. In all experiments, the medium was added with a single dye. The efficiency of dye removal was expressed as the percentage ratio of decolorized dye concentration to that of initial one.

Effects of Algal Biomass, Dye Concentration and pH on Decolorization

To examine the effect of biomass the amount of dye removal the culture flasks were inoculated by 3.0, 6.0, 9.0 and 12.0x10^6 cells /ml for *C. vulgaris*. For the effect of dye concentration on the decolorization efficiency, various concentrations of MG and MB from 1ppm to 10 ppm were prepared from the stock solution and inoculated with stock culture. The effect of pH on rate of decolorization was examined, the pH of the solutions were adjusted to the required value (range from: 2 to 11) by adding either 1N HCl or 1N NaOH solution. After inoculation, the absorbance and decolorization rate were determined at 1, 3, 5 and 7 h for *C. vulgaris* treated with MG and MB the decolorization rate were determined at 1, 3, 5 and 7 days after inoculation. The absorbance and decolorization rate were determined as described above.

CALCULATION

Decolorization activity (%) was calculated according to the formula: % removal = 100 $(C_0–C_e)/C_0$ where C_0 and C_e are the initial and equilibrium concentrations (mg L^{-1}) of the dye in solution, respectively.

RESULTS AND DISCUSSION

Algae have been found to be potential biosorbents because of their availability in both fresh and saltwater. The biosorption capacity of algae is attributed to their relatively high surface area and high binding affinity. Table 1&2 showed the direct relationship between the algal concentration and the percent of dye removal by algal species. Approximately, complete removal of malachite green from its aqueous solution was achieved by using the highest concentration of algal species, and after 3 hours in the case of *Chlorella vulgaris*. The cell wall of *Chlorella vulgaris*, may be the reason that accelerates the dye removal. Cell wall properties of algae play a major role in biosorption; electrostatic attraction and complexation are known to take place during algal biosorption (Satiroglu *et al.*, 2002).

Table 1: Effect of algal Conc. on % removal of malchite green by *Chlorella vulgaris*. MG = 5ppm, pH = 7.0, *C. vulgaris* concentration : cells ×10^6/ml

Time (hours)	% of removal			
	3 cell/ml	6 cell/ml	9 cell/ml	12 cell/ml
1	66.2	73.4	74.5	80.2
3	85.4	86.7	87.3	90.5
5	86.3	88.4	90.1	91.6
7	99.7	89.6	92.4	93.5

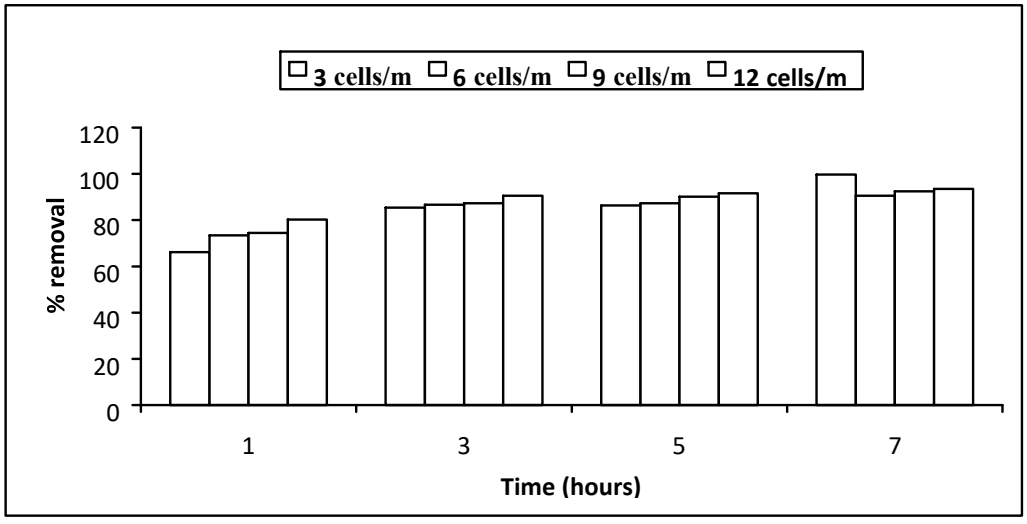

Figure 2: Effect of algal Conc. on % removal malachite green by *Chlorella vulgaris*, MG=5ppm, pH = 7.0, *C. vulgaris* = cells×10^6/ml

Table 2: Effect of algal Conc. on % removal of methyline blue by *Chlorella vulgaris*. MB = 5ppm, pH = 7.0, *C. vulgaris* concentration : cells ×10^6/ml

Time (hours)	% of removal			
	3 cell/ml	6 cell/ml	9 cell/ml	12 cell/ml
1	16.4	30.3	45.2	53.5
3	20.1	37.9	51.4	63.4
5	20.4	44.3	58.6	64.6
7	38.3	58.6	65.7	76.7

Table 3: Effect of algal Conc. on rate of dye removal (mg/l/h) of malachite green by *Chlorella vulgaris*. MG = 5ppm, pH = 7.0, *C. vulgaris* concentration : cells ×10^6/ml

Time (hours)	Rate of dye removal (mg/l/h)			
	2.5 cell/ml	5.0 cell/ml	7.5 cell/ml	10.0 cell/ml
1	3.12	3.31	3.32	4.16
3	1.22	1.23	1.23	1.23
5	0.92	0.92	0.92	0.92
7	0.62	0.62	0.63	0.63

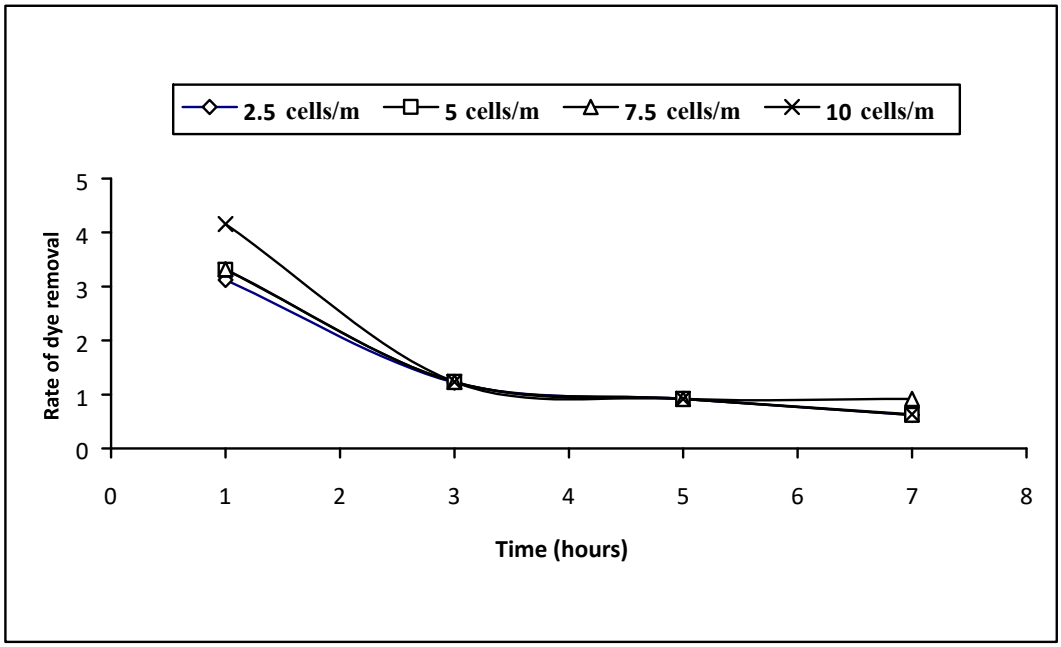

Figure 3: Effect of algal Conc. on rate of dye removal (mg/l/h) of malachite green by *Chlorella vulgaris* , MG=5ppm, pH = 7.0, *C. vulgaris* = cell×10^6/ml

The same conclusion was believed for methylene blue, although with less removal capacity. Functional groups such as hydroxyl, carboxylate, amino and phosphate found on the algal cell surface are considered to be responsible for sequestration of contaminants from wastewater. The dye removal, especially by using algae, may be attributed to the accumulation of dye ions on the surface of algal biopolymers and further to the diffusion of the dye molecules from aqueous phase onto the solid phase of the biopolymer (Ozer *et al.*, 2006). Thus, the dye removal using this alga may be due to extracellular polymers which consist of surface functional groups that enhance sorption of the dye molecules onto the surface of the polymer during dye removal process (Shukla *et al.*, 2002). The released metabolic intermediates (long chain biopolymers) which have excellent coagulation capacity along with the dye remaining in the aqueous phase tend to adsorb onto the surface of the polymers and settle (biocoagulation) (Mohan *et al.*, 2002).

Table 4: Effect of algal Conc. on rate of removal (mg/l/h) of methyline blue by *Chlorella vulgaris*. MB = 5ppm, pH = 7.0, *C. vulgaris* concentration : cells ×10^6/ml

Time (hours)	Rate of removal (mg/l/h)			
	3 cell/ml	6 cell/ml	9 cell/ml	12 cell/ml
1	0.83	1.61	2.31	2.47
3	0.32	0.79	0.86	0.91
5	0.30	0.41	0.48	0.49
7	0.31	0.42	0.43	0.43

Table 5: Effect of different dye Conc. on %age of removal of malachite green by *Chlorella vulgaris*. pH = 7.0, *C. vulgaris* concentration : 6.0 ×10^6 cells/ml

Time (hours)	% age of MG dye removal					
	1 ppm	3 ppm	5 ppm	7 ppm	9 ppm	10 ppm
1	5.4	68.2	79.3	82.4	83.4	90.2
3	81.2	94.3	94.1	94.2	94.4	94.4
5	84.6	99.3	99.2	98.6	99.1	99.3

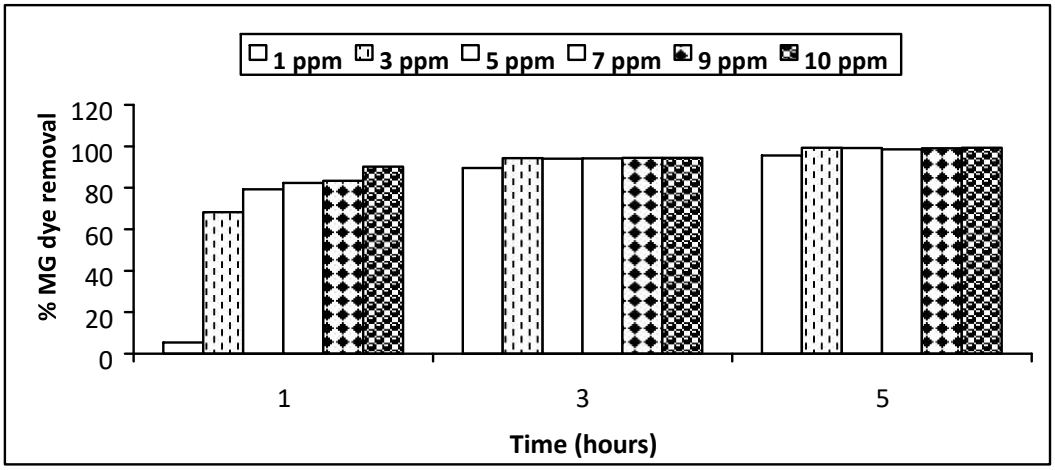

Figure 4: Effect of different dye Conc. on % of removal of malachite green by *Chlorella vulgaris*, pH = 7.0, *C. vulgaris* = 6.0×10^6 cell/ml

Table 6: Effect of different dye Conc. on %age of removal of methyline blue by *Chlorella vulgaris*. pH = 7.0, *C. vulgaris* concentration : 6.0 ×10^6 cells/ml

Time (hours)	% age of MB dye removal					
	1 ppm	3 ppm	5 ppm	7 ppm	9 ppm	10 ppm
1	49.1	63.4	63.9	72.4	72.7	72.3
3	53.7	64.9	65.1	66.3	66.1	66.7
5	55.9	64.4	64.5	68.7	70.2	70.4
7	71.2	76.7	76.8	78.6	79.4	79.9

The rate of dye decolorization was found to increase with the increase of algal concentration. At the same time maximum rate of dye decolorization was recorded during the first hour in all algal concentrations. This means that the algal tendency of dye removal decreased by time, mostly due to saturation or physiological disturbance of the stressed algal cells. Although the rate of dye decolorization was found to increase, to certain extent, with the increase of dye concentration the *Chlorella vulgaris* showed less efficiency to remove high dye concentrations, especially 7 and 10 ppm. This was mostly due to the decrease of light penetration into the culture that resulted in some function disorder of the algal cells.

Table 7: Effect of different dye Conc. on rate of decolarization (mg/l/h) of malachite green by *Chlorella vulgaris*. pH = 7.0, *C. vulgaris* conc. : 6.0 ×10^6 cells/ml

Time (hours)	Rate of decolorization					
	1 ppm	3 ppm	5 ppm	7 ppm	9 ppm	10 ppm
1	0.0	2.1	4.1	6.54	7.52	8.82
3	0.27	0.94	1.76	2.13	3.11	3.21
5	0.16	0.21	1.05	1.36	1.39	1.46

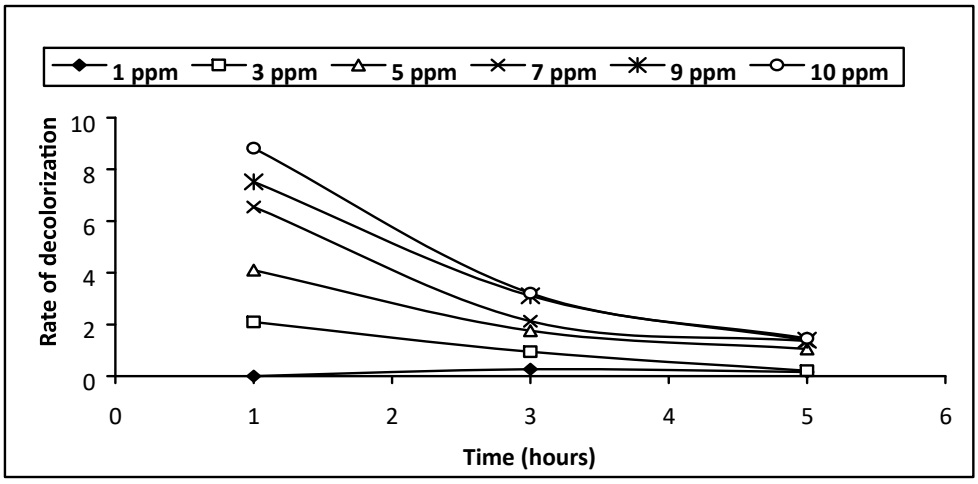

Figure 5: Effect of different dye Conc. on rate of decolorization (mg/l/h) of malachite green by *Chlorella vulgaris*, pH = 7.0, *C. vulgaris* = 6.0×10^6 cells/ml

Table-8: Effect of different dye Conc. on rate of decolarization (mg/l/h) of methyline blue by *Chlorella vulgaris*. pH = 7.0, *C. vulgaris* concentration : 6.0 ×10^6 cells/ml

Time (hours)	% age of MB dye removal					
	1 ppm	3 ppm	5 ppm	7 ppm	9 ppm	10 ppm
1	0.32	2.11	3.51	5.11	5.87	5.94
3	0.14	0.89	1.53	2.01	2.22	2.28
5	0.06	0.53	0.56	0.58	0.63	0.68
7	0.10	0.47	0.48	0.49	0.53	0.57

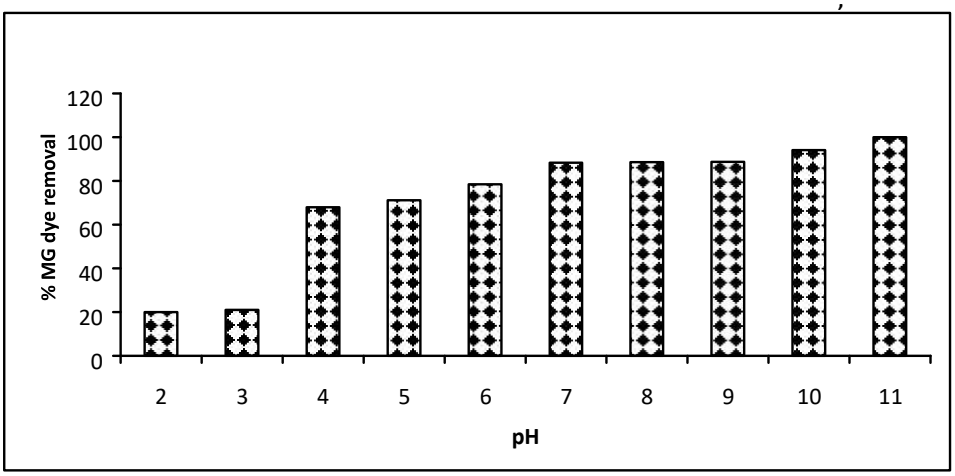

Figure 6: Effect of different pH on % removal of malachite green by *Chlorella vulgaris* MG=5ppm, *C. vulgaris* = 6.0×10^6 cells/ml

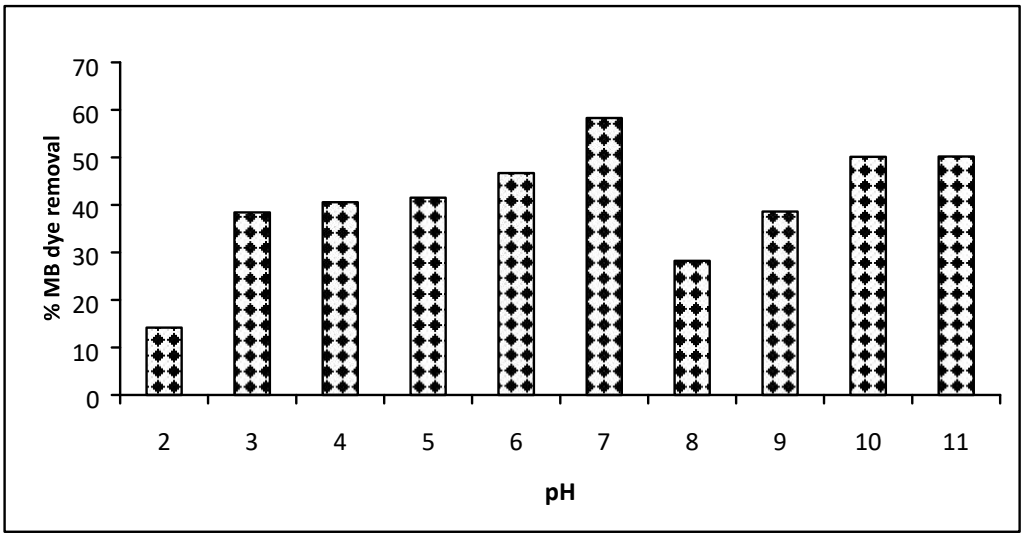

Figure 7: Effect of different pH on % removal of methylene blue by *Chlorella vulgaris*, MB=5ppm, *C. vulgaris* = 6.0×10^6 cells/ml

The percent removal of malachite green increased gradually with the increase of the pH of the media *Chlorella vulgaris*. Meanwhile, the highest percent removal of methylene blue was recorded at pH 7 for algal species. Research workers (Dhaneshwar *et al.*, 2007; Nadeen *et al.*, 2020; Meiwen *et al.*, 2021; Yong *et al.*, 2021; Khalil *et al.*, 2022; Peige *et al.*, 2022) observed that for microalga *Cosmarium* Sp. an increase in pH from 4.0 to 6.0 led to a threefold increase in decolorization rate of Malachite Green, which reached a maximum value of 92.4% at the pH of 9.0. Aravindhan *et al.* (2007) observed that uptake of Basic Yellow dye by *Caulerpa scalpelliformis* increased from 17 to 27 mg/g for an increase in pH from 3.0 to 8.0. At lower pH, the H ions compete effectively with dye cations, causing a decrease in color removal efficiency. At higher pH, the surface of biomass gets negatively charged, which enhances the positively charged dye cations through electrostatic force of attraction. Higher uptakes obtained at lower pH values may be due to the electrostatic attractions between these negatively charged dye anions and positively charged cell surface. Hydrogen ion also acts as a bridging ligand between the algal cell wall and the dye molecule.

In conclusion, the results obtained in this paper were very promising since the single examined species could able to decolorize high percentages of the used dyes. Thus, the use of biomaterials as sorbents for the treatment of dye wastewaters will provide as a potential alternate to the conventional treatment. However, use of biosorbents to remove color in a dye wastewater is still in the research stage. Efforts are needed to commercialize this research through selection of suitable biosorbents based on economic and market analysis, pilot-scale studies with actual wastewaters and full-scale demonstration systems.

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