A COMPARATIVE STUDY ON SELECTIVE BIOSORPTION IN AQUEOUS MIXTURES OF DYES WITH DIFFERENT IONIC CHARACTERS BY CHLORELLA SP. BIOMASS

by

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ABSTRACT

A COMPARATIVE STUDY ON SELECTIVE BIOSORPTION IN AQUEOUS MIXTURES OF DYES WITH DIFFERENT IONIC CHARACTERS BY CHLORELLA SP. BIOMASS

In this study, the adsorption percentages of Chlorella Sp. biomass towards five ionic dyes and their relevant combinations in aqueous media were studied as a model system for wastewater remediation compared to a proper type of activated carbon. Since wastewater effluents often contain a large variety of ionic micropollutants, there are an ongoing need to determine biosorption potential against various structures of single chemical contaminants. To have a more realistic model and understanding of biosorption to remove aqueous chemical contamination, combinations of aqueous contaminants are herein proposed to be monitored.

Five molecules with distinct ionic and structural characteristics, namely a cationic dye Methylene Blue, anionic dyes Isolan Dark Blue and Eriochrome Black T, a zwitterionic dye Methyl Orange, and a larger molecular weight zwitterionic dye Congo Red constituted the targeted set of model adsorbates. Cationic molecules demonstrate a higher tendency to be adsorbed onto Chlorella Sp. than anionic and zwitterionic dyes. Therefore, the cationic dye was selected to investigate its potential to facilitate better biosorption of the selected anionic and zwitterionic dyes, through the possible generation of additional positive charges on the adsorbent surface of microalgae biomass.

Single dyes and selected mixtures of them were employed to determine the removal percentages of Chlorella Sp., by monitoring dye removal through observation of decreasing concentrations using UV absorption spectroscopy, under different aqueous conditions. Reliable measurements of concentration changes in the case of dye mixtures based on light absorption from multiple wavelengths were established.

ÖZET

CHLORELLA SP. MİKROALG BİYOKÜTLESİ KULLANILARAK FARKLI İYONİK KARAKTERLERDEKİ BOYA KARIŞIMLARININ SULU ORTAMDA KARŞILAŞTIRMALI BİYOSORPSİYONU

Chlorella sp. mikroalg biyokütlesinin, suda çözünen beş farklı iyonik boya örneğinin farklı kombinasyonlarına karşı adsorpsiyon kapasitesi, atık su arıtmaya yönelik sistem modeli olarak, uygun bir aktif karbon tipine kıyasla incelenmiştir. Atık sular genellikle çok çeşitli iyonik kirleticiler içerebildiğinden, bu kimyasal kirleticilerin Chlorella sp. mikroalg biyokütlesi ile biyosorpsiyon eğilimlerinin belirlenmesine yönelik ihtiyaç süregelmektedir. Sulardaki kimyasal kontaminasyonun biyosorpsiyon yoluyla giderilmesi konusunda daha gerçekçi bir model ve anlayış geliştirmeye yönelik, adsorbanlar tek tek değil farklı kombinasyonlar halinde araştırılmıştır.

Bu tezde, giderimi sağlanan model adsorbatlar, farklı iyonik ve yapısal özelliklere sahip beş molekül, katyonik boya olarak Metilen Mavisi, anyonik boya olarak Isolan Mavisi ve Eriokrom Siyahı, zwitteriyonik boya olarak Metil Turuncusu ve daha yüksek moleküler ağırlıklı zwitteriyonik boya olan Kongo Kırmızısı'dır. Katyonik moleküller Chlorella sp.'ye anyonik ve zwitteriyonik boyalara göre daha yüksek oranlarda emilim göstermiştir. Katyonik boyanın mikroalgin yüzeyindeki artı yükü arttırma yoluyla ortamda bulunan diğer boyaların biyosorpsiyon oranlarını arttırma potansiyeli araştırılmıştır. UV adsorpsiyon spektroskopisi yoluyla sudaki boya konsantrasyonu değişimleri izlenerek, Chlorella Sp. mikroalg biyokütlesinin farklı kombinasyon ve oranlarda boya karışımlarına karşı, pH ile adsorban ve adsorbatların başlangıç konsantrasyonları gibi farklı koşullar altında giderim kapasiteleri belirlenmiştir. Boya karışımları deneylerinde, her boyanın konsantrasyon değişikliğinin ayrı ayrı güvenilir şekilde ölçülebilmesi için çoklu dalga boylarında ışık adsorpsiyon takibi için gerekli metotlar geliştirilmiştir.

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LIST OF SYMBOLS

A	Absorbance
b	Optical Length
C_0	Initial Concentration
C_{final}	Final Concentration
q	Adsorption capacity
V	Total Volume
ε	Molar Absorptivity Coefficient
λ_{max}	Maximum Wavelength

LIST OF ACRONYMS/ABBREVIATIONS

AC	Activated Carbon
BBM	Bold's Basal Medium
C.I.	Color Index™
CR	Congo Red
EBT	Eriochrome Black T
EDTA	Ethylenediaminetetraacetic acid
EDCs	Endocrine Disrupting Chemicals
IDB	Isolan Dark Blue
ISKI	İstanbul Water and Sewage Administration
LOD	Limit of Detection
MB	Methylene Blue
MO	Methyl Orange
Nm	Nanometer
PVDF	Polyvinylidene difluoride
RT	Room Temperature
UV-VIS	Ultraviolet-Visible
WL	Wavelength
WWTP	Wastewater Treatment Plant

1. INTRODUCTION

As the industrialization and human population is increasing, a large variety of new chemical pollutants emerge and contaminate soil, air, and water in global scale and in our local region. Air pollutants are partially deposited in water and soil, then rain and irrigation waters wash away soil pollutants, including agricultural chemicals into water bodies. Also, large variety of chemical contaminants arising from industrial productions are commonly released to water bodies, hence water systems as whole have been recorded to undergo an increasing contamination of a large diversity of chemical pollution [1–3].

In most part, conventional water treatment techniques are not designed and capable of removing chemical micropollutants. In the document released by TÜBİTAK (The Scientific and Technological Research Council of Turkey) in 2017, there is a list of 136 micropollutants detected in the effluents of WWTPs in Turkey [4]. They all exceeded limits of environmental quality standards and released to water bodies. Additionally, 11 micropollutants were seen in the effluents of the drinking-water-treatment plants at high concentrations. Some examples of these local tap water pollutants are heptachlor epoxide, dichloromethane, and benzene, which are all endocrine-disrupting chemicals (EDCs). Methods used in conventional water treatment to remove these emerging micropollutants have so for been inadequate, and better techniques were most generally considered expensive. Therefore, adequate and inexpensive, preferentially environmentally friendly techniques for water treatment are currently critically needed.

Adsorption process is considered a promising alternative in water treatment due to its eco-friendliness, simplicity of design, and cost effectiveness [2], [5–7]. A large literature of biosorption studies is pointing out to the potential of various bio-based and low-cost materials for water and wastewater remediation to remove micropollutants through adsorption process [5–13]. This process can remove different types of micropollutants through various intermolecular interactions, for example electrostatic interactions that do not require any external energy consumption. In industrial adsorption removal processes for micropollutants, activated carbon, which is a modified and engineered adsorbent, can undoubtedly be useful in removing various pollutants from water [14–27]. However, its

common use in wastewater treatment is somewhat limited due to its relatively high cost, even though it is a sorbent with high potential of micropollutant removal. Different types of chemical functional groups are generated on the surface of the activated carbon, through pyrolysis and chemical activation. The variety of functional groups on activated carbon provide efficient electrostatic interactions with different types of ionic micropollutants [27]. Energy consuming and relatively expensive production of activated carbon is its major drawback. Hence novel green alternatives, such as certain types of microalgae, have been attracted much attention [28].

1.1. Microalgae

More than 100.000 microalgae species are estimated to be present on Earth, and they have been existing since the early stages of the planet. They can be grown in fresh or saline water, and they demonstrate high adaptability to various conditions [29]. Microalgae species generally have much higher photosynthesis capacity than plants. Their CO_2 capturing abilities are 20 times higher than that of a moderate climate forest [30]. They also have high growth rates, which renders them relatively inexpensive to grow [31]. Microalgae are rich in a variety of valuable biomolecules, such as fatty acids, proteins, polysaccharides, antioxidants, and vitamins [32,33]. These biological macromolecules contain sulfonates, carboxylates, or amino functional groups, as examples of charge forming functionalities [34]. It is that variety of chemical functionality on cell surfaces that allows for higher adsorption capacities in some microalgae species. The extent of adsorption of an ionic pollutant molecule is considered to be driven by the competition between attractive and repulsive forces, based on charge interactions [35].

In the previous thesis work [36], seven different microalgae species were investigated towards cationic Nile blue dye. Removal yields were shown to be different for different types of microalgae, where Chlorella appeared as an attractive candidate being one of the best of tested biosorbents, as shown in Figure 1.1. Therefore, in the current thesis work Chlorella Sp. was selected as target biosorbent against different type of dyes. Another microalgae, Scenedesmus, was also utilized in some of dye adsorption tests as comparison.

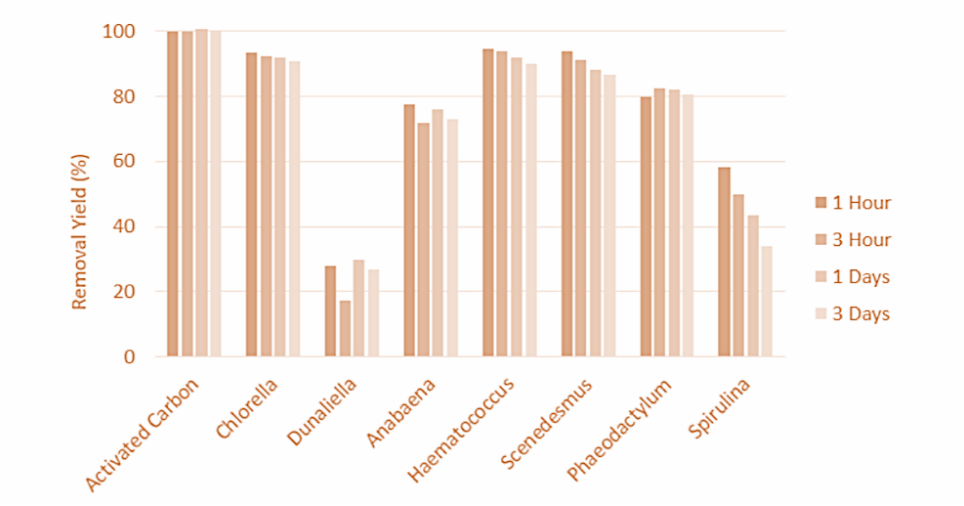


Figure 1.1. Nile blue removal yields (%) by various microalgae and activated carbon [36].

Chlorella is a green microalgae and known with its high nitrogen and phosphorus absorbing capacities and reducing the chemical oxygen demand [37]. It has a single to triple layered rigid cell wall composed of pectin, microfibril and hemicellulose [38]. This robust cell wall constitutes a network with embedded matrix of polymeric substances, such as uronic acid as unit of pectin, rhamnose, arabinose, and xylose as major components of hemicellulose. Microfibrils consist of chitin with N-acetylglucosamine units [39–41]. All these molecules make Chlorella itself rich in chemical functionality which enables the microalgae to interact with various ionic pollutant molecules selectively. On the other hand, the cell wall of Scenedesmus consists of only monosaccharide derivatives, glucosamine is not found in its cell wall composition [42–45].

1.2. Dyes as a Pollution Model

Synthetic dyestuffs, an important group of synthetic organic compounds and common aqueous pollutants, are used extensively in textile, paper, and printing industries, and dyehouses. Over 100,000 commercially available dyes are registered, with a production of over 700.000 metric tons per year. Dyeing industry effluents constitute one of the most problematic wastewaters to be treated in part due to their high chemical and biological oxygen demands. Due to a reduction in light penetration, dyes may have a major impact on

aquatic life's photosynthetic activity. On the other hand, majority of dyes are also toxic to aquatic life, or other species [46–51].

Textile industry in Turkey holds a relatively important position in the world and thus plays a major role in country's exports [52]. The total monthly consumption of textile dyes is about 20.000 tons for one of the major textile factories in Turkey [53] Therefore, the waste of textile factories are common pollutants found in soil and water depending on the region. According to İstanbul Water and Sewage Administration (ISKI) Annual Report of 2020, pollutants coming from the textile sector in the European side of İstanbul make majority of waste products found in the wastewater effluents, with a total average flow rate of 13.704 m^3/day [54]. That is in part why ionic dyes, that are also commonly employed in textile industry, but also ionic, nonbiodegradable contaminants, were chosen as model adsorbates for the adsorption tests involving Chlorella. With their high solubility and stability, they tend to accumulate in aqueous environments [55].

Since dyes are colored molecules, apart from visual detection, accurate quantifications can be done through inexpensive and convenient techniques based on UV-VIS spectroscopy. Great majority of dye molecules have ionic character, providing them relatively high-water solubility [55]. Ionic dyes can be classified into three groups as cationic, anionic, and zwitterionic, latter bearing different chemical functionalities that can lead to both cationic and anionic charge formation in aqueous media [56–58]. For non-modified and non-specific adsorbents such as biosorbents, the type of charge on the adsorbate result in a certain level of selectivity [9].

In this thesis, Methylene Blue was selected as a cationic dye, Congo Red and Methyl Orange as zwitterionic dyes, and Eriochrome Black T and Isolan Dark Blue as anionic dyes. As seen in Figure 1.2, four of these dyes are azo dyes and nevertheless they are all conjugated molecules which makes them colored. Ionic moieties on the dye structures bring specific properties. Methylene Blue (MB) has cationic sites based on amine groups. Congo Red (CR) and Methyl Orange (MO) have both cationic and anionic sites with sulphonate and amine groups. Eriochrome Black T (EBT) and Isolan Dark Blue (IDB) have only anionic sites rising from sulfonate groups. IDB is different than other dyes, it is a metal complex dye coordinated with Cr (III) metal. This type of dyes also named as mordant dyes where dye

molecules chelated with a cation [59]. This complexation gives the dye to have superior characteristics in light and wet fastness on wool and polyamide [60]. This chromium (III) ion tends to accumulate in nature and have drastic environmental damages [61]. Due to their ionic character, ultimate structures of selected dye molecules highly depend on pH of the solution.

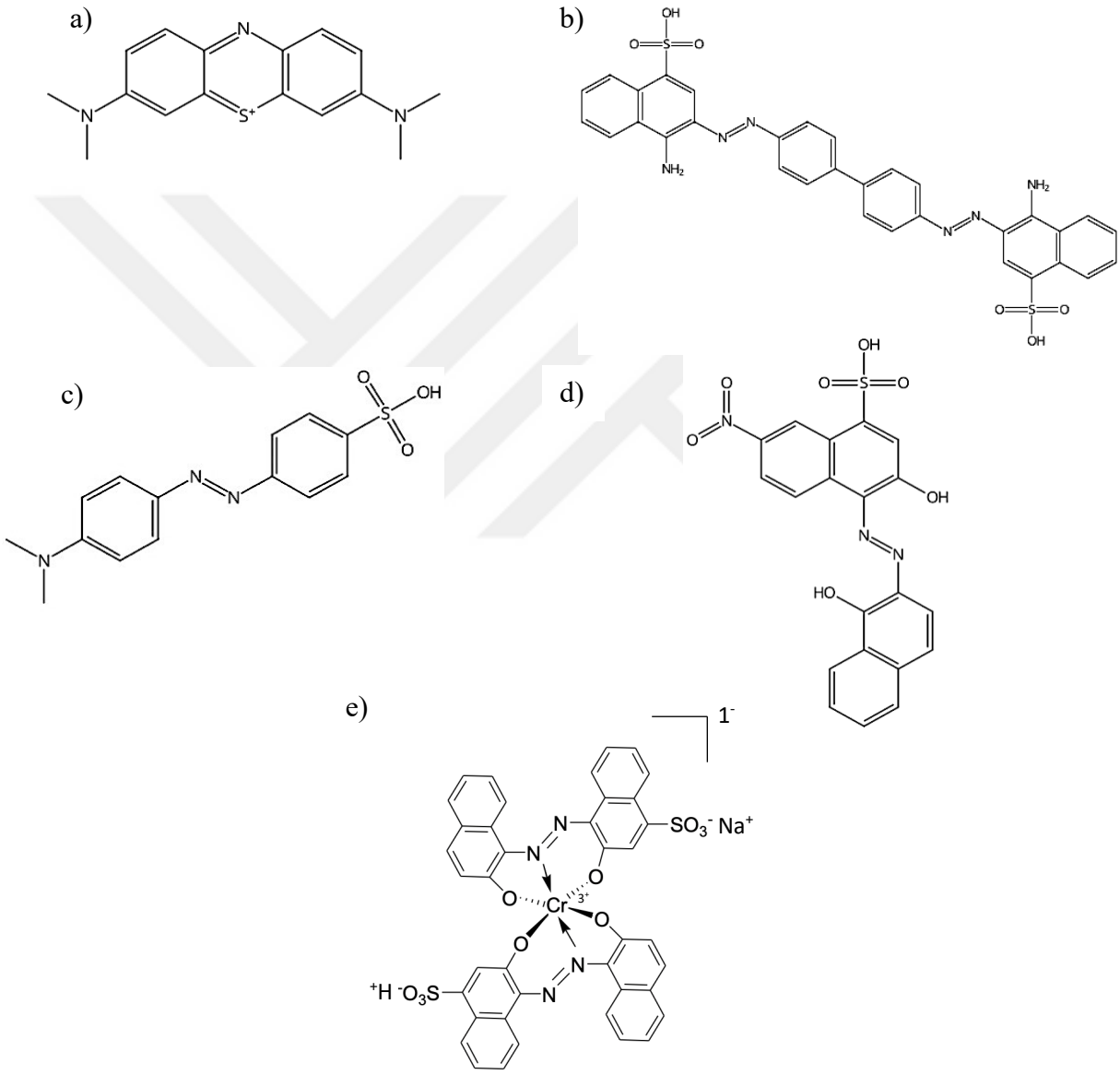


Figure 1.2. Chemical structure of a) Methylene Blue b) Congo Red c) Methyl Orange d) Eriochrome Black T e) Isolan Dark Blue.

1.3. Adsorption of Dyes by Microalgae

Adsorption is a physico-chemical process which does not necessarily require external energy for an effective interaction of the adsorbent with the adsorbate [62]. When water containing soluble contaminants meets a solid adsorbent, solute–solid intermolecular forces of attraction cause certain fraction of the solute molecules to be concentrated or deposited at the solid surface. This mechanism is highly dependent on the chemical nature of the adsorbent's surface, and solutes. Solute retained on the solid surface in adsorption process is called adsorbate and the solid on which it is retained is called adsorbent. Adsorbent can be mechanically separated, for example by settling or filtration, hence allowing for the removal of adsorbed micropollutants from water [7], [12], [26,27].

In this thesis, several types of water-soluble ionic dyes are used as model adsorbates, Chlorella sp. microalgae is used as adsorbent/biosorbent, and for comparison a reference adsorbent, activated carbon is employed. Ionic functionalities of dyes are expected to provide basis for Chlorella to interact with these dyes selectively and differently. As the adsorbent is of biological origin, this study is expected to be a contribution to biosorption literature, which includes a large body of research efforts demonstrating the contaminant removal profiles of a large selection of biomaterials.

Encouraged by these past research findings, Chlorella sp. is chosen as the type of microalgae to be used in screening adsorption behavior against mixtures of dyes, as an improved model in comparison to single dye adsorption experiments. Another advantage of Chlorella sp. is commercial availability, globally there is a large number of small or large scale production facilities and companies have been providing this valuable biomaterial for various applications.

Chlorella Vulgaris has been used in several biosorption studies as one of the commercially available well adsorbing microalgae. This feature of microalgae in general was first anticipated by Oswald and Golueke in early 1960s [63]. However overwhelming majority of related studies were based on single dye adsorption experiments [64–73]. One study involved in the comparison of adsorption by living or dead microalgae have shown that for selected adsorbates dead cells may show higher or comparable adsorption capacities

as living cells [74]. Dead microalgal biomass has more porous structure, which can be the reason why dead microalgae tend to have higher adsorption capacities [75]. In one study that is based on SEM analysis for visualization of the adsorption, they concluded that cationic dye adsorption on *Chlorella Vulgaris* is governed by homogenous monolayer coverage [76]. On the other hand, experiments based on the use of living cells bring additional complexity considering possible metabolic removal activities that can interfere with the adsorption removal of dyes. Living cells need appropriate nutrients and bioreactor systems which bring additional parameters for the operation [77,78]. Biosorption processes are a result of surface chemistry and hence isotherm and kinetic models are commonly applied in related studies. Most commonly used isotherms are based on Langmuir and Freundlich models for electrostatically driven removal experiments. Langmuir model states that the adsorption process occurs through monolayer coverage of adsorbent's surface, while Freundlich isotherm is fitted with multilayer coverage [79]. These mathematical models shed light on surface chemistry about how biosorption proceeds between biosorbate and biosorbent. Kinetic studies allow for the assessment of the speed of the process, providing the time necessary to reach equilibrium [80].

In the assessment of the necessary conditions for the best removal yields for dye-microalgae biosorption, critical factors include initial dye concentrations, pH, amount of biosorbent and temperature. In a relevant literature example, they have reported the adsorption profiles of three vinyl sulphone type reactive dyes (Remazol Black B (RB), Remazol Red RR (RR) and Remazol Golden Yellow RNL (RGY)) onto *Chlorella Vulgaris* biomass [81]. The maximum adsorption capacity was reported as 419.5 mg. g^{-1} at 800 mg/L initial dye concentration for RB and RR, and 200 mg/L for RGY dye. These dyes are anionic in the aqueous solution and microalgae cell membrane is expected to get protonated at lower pH values, which was given as the possible reason for the higher adsorption capacities at pH 2, considering electrostatic interactions between the positively charged cell membrane and negatively charged dye molecules.

As the cell wall of Chlorella sp. is prone to be negatively charged, in neutral pH, it shows higher adsorption capacities against dyes having positively charged functional groups. For example, removal yield of a cationic dye, Methylene Blue, by *Chlorella Vulgaris* has been reported to be 83.04 ± 2.94%, while against an anionic dye, Acid Orange 7,

extremely poor removal was reported [80]. Screening through the relevant biosorption literature it becomes obvious that not every dye can be effectively adsorbed by microalgae species. In that sense in order to examine the possibility of better adsorptions of such poor adsorbates by Chlorella sp., combining positively and negatively charged dyes, and zwitterionic dyes is the basic idea in the currently proposed study. To the best of our knowledge such approach is not found in the related literature, especially with Chlorella sp. on the scope.

In literature, mixtures of metallic ions that are common water pollutants have been examined with Chlorella sp. In one study, biosorption of cadmium (II) and nickel (II) binary mixture was investigated using *Chlorella Vulgaris* and compared with the single adsorption profiles [82]. Adsorption capacities and removal yields were reported with respect to mole ratio for each metal solution with different initial concentrations. They have reported that in the case of the mixture a decreased adsorption for each metal ion was observed with respect to individual tests at pH 4. This result can be explained by similar charge characters of these ionic compounds, rendering them competitive for adsorption process. Studies targeting biosorption of dye mixtures are rare but present with other types of biosorbents. One study reported Caulerpa lentillifera, a species of ulvophyte green algae, as biosorbent for the removal of dye mixtures consisting of Astrazon Blue FGRL (AB), Astrazon Red GTLN (AR), and Astrazon Golden Yellow GL-E (AY) [83]. Adsorption profiles of single dyes and their binary mixtures as AB+AR, AR+AY, and AB+AY were reported. Two dye mixtures, AB + AR and AB + AY, were prepared with a mass ratio of 1:1 (using 50 mg/L of AB, AR, and AY). Test samples contained 0.1, 0.5, and 0.9 g of dried C. lentillifera in 30 mL of the dye mixture. The algae adsorbent had higher affinity against AB than AY, particularly at lower quantities of adsorbent. However, at high adsorbent quantities, the removal percentages of AB and AY were not significantly different. Maximum removal yield of 94% was recorded in AB+AY binary dye mixture with 0,9 g of dried algae and initial concentrations of 0,9 mg/L for AB and 2,0 mg/L for AY.

A more recent study investigated the biosorption capacity of Ulva lactuca biomass against the combination of Pb^{2+} and carcinogenic Congo Red dye from binary solutions [84]. Ulva lactuca, with the common name sea lettuce, is a type of green algae. The study employing face-centered central composite design (FCCCD), resulted in highest removal

percentage of 97.89% for Congo Red dye and 98.78% for Pb^{2+} using 100 mg/L of Congo Red and 200 mg/L of Pb^{2+} solutions, 3 g/L algal biomass, initial pH of 6 and a contact time of 120 min.

Biosorption of mixtures of various ionic dyes using Chlorella Sp. microalgae was investigated in only one literature example, [85] but with several uncertainties in their methodology and conclusion. The design of that particular study targeted the removal of a mixture of nine ionic dyes all together, which definitely does not allow for an understanding or conclusion about how each individual dye's adsorption was affected by the presence of every other dye. But moreover, to the best of our knowledge, the methodology used for the detection of removal of dye mixture is definitely not adequate. Because the UV-VIS spectrophotometric detection of the mixture's post-adsorption dye concentrations was performed at one wavelength only. This approach cannot be expected to reveal the concentrations of several dyes which have maximum absorbance wavelengths that are distant from the selected wavelength. Individual dyes have maximum absorbances at 663 nm, 417 nm, 459 nm, 620 nm, 583 nm, 517 nm, 615 nm, 590 nm and 617 nm for Methylene Blue, Methyl Red, Methyl Orange, Toluidine Blue O, Crystal Violet, Safranin O, Bromothymol Blue, Bromophenol, and Malachite Green respectively. However, concentration for the mixture of these nine dyes was detected at 609 nm only, which undoubtedly leads to an inadequate conclusion about the removal yield.

Every dye has a particular absorbance value at its wavelength of maximum absorbance, which is the peak of its rather large absorption band. In a mixture of many dyes there are large overlaps between absorption bands, which result in shielding the peaks of absorption bands of individual dyes to some extent. Hence analyzing mixtures of many dyes in UV-VIS spectroscopy will intrinsically generate more inaccuracy as the number of dyes and corresponding absorption band overlapping increase. But independent of the number of dyes in a mixture, measurements should be performed at each maximum absorbance wavelength of every other dye, in order to obtain the best estimate about the concentrations. In current thesis, these crucial factors were considered carefully and measurements that lead to best estimations will be targeted.

2. AIM OF THE STUDY

Chlorella species is a well-known genus of microalgae, which has been studied not only for their potential in biomass production with high nutritional value, but also for biosorptive removal of aqueous micropollutants. In some cases, microalgae adsorb aqueous contaminants as effectively as activated carbon, which is an industrially produced sorbent for adsorption removal of contaminants from water or air. It is not adequately environmentally friendly as its production is energy intensive and costly. Therefore, microalgal adsorption techniques emerge as cost and energy effective alternatives in comparison to activated carbon.

In this study, the aim is to expand the study of Chlorella Sp. as the selected biosorbent towards more complex pollution models in particular dye mixtures of different chemical functionality, as a more realistic model of water contamination. In polluted waters, ionic contaminants are almost always found as a cocktail, which can alter their adsorption characteristics by microalgae in comparison to the case where there is only one single contaminant in the solution. Such a study also allowed examination of the possible synergistic or adverse effects of multiple pollutants being present as mixtures on the adsorption behavior of chosen microalgae and a proper type of activated carbon as a standard for comparison.

Dyes were chosen to construct a pollution model since they are widely used across industries and can be detected and quantified via inexpensive and versatile instrumental techniques such as UV-VIS spectrophotometry. Five ionic dyes and their mixtures with different properties were examined as adsorbates of the adsorption towards Chlorella Sp. and activated carbon. Selected dyes consist of a cationic dye Methylene Blue, zwitterionic dyes Methyl Orange and Congo Red and anionic dyes Isolan Dark Blue and Eriochrome Black T. All experiments were diversified by screening different concentrations and compositions of dyes and their mixtures, different levels of pH, and varying amounts of biosorbent. Hence the proposed wide variety of comparative screening of biosorption covered in this thesis work aims to provide valuable data for improving water treatment techniques and advancing biosorption literature. Not only biosorption profiles of selected

microalgae were monitored against mixtures of dyes as a more realistic but rarely studied aquatic contamination model, but also key factors and techniques were determined for reliable and comparable results in biosorption studies.

3. EXPERIMENTAL

3.1. Materials

Eriochrome Black T (C.I.14645- Mordant Black 11) was purchased from Merck KGaA, Methylene Blue (C.I. 52015- Basic Blue 9), Congo Red (C.I. 22120-Direct Red 28), Methyl Orange (C.I. 13025- Acid Orange 52) and sodium phosphate were purchased from Fisher Scientific. Isolan Dark Blue 2- SGL-03 (Acid Blue 193) was obtained from Dystar, Turkey. All dyes were used without further purification.

Chlorella biomass was purchased as Green Gem® Chlorella from Yaşamnet A.Ş.. Biomass was utilized by pestling and sifting to obtain a homogeneous fine powder. Scenedesmus sp. biomass powder which were used in Sections 4.1.3, 4.2.3, and 4.3.3 and laboratory grown Chlorella (strain CCAP 211/11B) used in Section 6 were cultivated by Beste Özgümüş in Professor Turgay Çakmak's laboratory in İstanbul Medeniyet University. Activated carbon, in powder form and Norit® SA2 type, was kindly donated by Cabot Corporation.

UV-2600 Shimadzu was used as UV-VIS spectrophotometer in all concentration analyses. Full UV absorption spectrums of dyes were obtained by UV-1600PC, VWR. MRC BT-350 Shaking Water Bath was used as the orbital shaker. Distilled water was obtained from NUVE-ND4. 0.22 μm, PVDF (Polyvinylidene difluoride) syringe filters was purchased from ISOLAB.

3.2. Adsorption of Single Dyes by Chlorella and Activated Carbon

3.2.1. Preparation of Dye Solutions and Adsorbent Suspensions for Batch Experiments

All experiments started with preparing the concentrated dye stock solutions at the concentrations of 68 mg/L or 136 mg/L by weighting 0.017 or 0.034 grams of powder dye in a 250 mL volumetric flask, respectively. A solution form of dyes was obtained by dissolving them in 1% (w/w) sodium phosphate containing distilled water and stirring at a

constant rate and room temperature for three hours except in the case of Isolan Dark Blue (IDB) dye. The period for the complete dissolution of IDB dye pigments was 24 hours as it was determined in preliminary experiments. After total dissolution of dyes, pH of dye stock solutions was adjusted to 7 by additions of 0.01 M, 0,1 M or 1 M of NaOH and HCl solutions.

Dye solutions were used for plotting the calibration curve or for adsorption tests that were performed in triplicate in the presence of suspensions of microalgae, or the activated carbon. The absence of possible precipitation of dyes were checked with centrifugation of the dye solution prior to experiments. Adsorbents were weighed in targeted quantities. pH of dye solutions in the presence of adsorbent suspension was checked or adjusted to targeted pH values prior to adsorption tests. All adsorption tests were performed in 15 mL plastic falcon tubes via constant shaking of the dye solution-adsorbent suspension at 150 rpm for one hour. A speed of 4700 rpm was used for the centrifugation of the suspensions for 15 minutes to acquire the supernatant. In most cases dye adsorbed activated carbon suspensions did not form a fully stable aggregate at the bottom of the falcon upon centrifugation, leaving very small amount of particle suspensions in supernatant. Therefore, separation of the supernatant from adsorbents were carried out with care in order to minimize the amount of solid particles remaining. Full scale UV absorbance spectrum of each dye at the concentration of 13.6 mg/L was obtained using UV/VIS spectrophotometer (UV-2600, Shimadzu) before every batch experiment. Measurement of remaining dye concentrations in supernatants were conducted at the same maximum wavelength obtained from the full UV absorption spectra of dyes. Blank solutions were prepared exactly as in the case of dye adsorption tests, through addition and centrifugation separation of adsorbents, with only exception being the absence of any dyes, in order to obtain the best possible baseline correction in UV absorption spectra and accurate measurements of dye concentrations of test samples.

3.2.2. Adjusting Parameters of Dye Adsorption Batch Experiments

Effects of storage temperature and duration for stock solutions of each dye was examined by keeping half of the stock solution at room temperature and the other half at 4°C for different durations. All solutions were kept in closed containers in the dark. pH (pH 7), concentration of the adsorbate (13.6 mg/L) and amount of the adsorbent (1 g/L), total volume

of solution (10 mL) were kept constant in comparative experiments. All solutions contained 1% phosphate buffering agent. Solutions for the calibration curve and dye adsorption tests involving UV absorption measurements were prepared by appropriate dilutions of the selected stock solution.

Effect of the initial concentration of dyes was investigated by mixing the same quantity of adsorbent (1 g/L) with different concentration of dye solution in the same flask size (15 mL). pH was adjusted to 7 and all solutions contained 1% (w/w) sodium phosphate buffering agent except IDB dye solutions, as IDB was determined to show better adsorption towards Chlorella without buffer in the solution.

Effect of pH on dye removal was examined by adjusting the pH to assigned values by additions of 0.01 M, 0.1 M, and 1 M NaOH or HCl solutions. At the obtained levels of pH, the absence of any precipitations were checked by centrifugation. At the end of adsorption period and before the UV absorption analysis, pH of supernatants was checked twice and recorded. Full UV absorption spectrum of each dye at the desired pH value was scanned and the maximum wavelength was determined for the particular experiment, in order to prevent any Bathochromic shift rising from acidity of the solution.

Effect of the biosorbent dosage of Chlorella on the dye removal was examined by changing the biomass quantity. Initial concentration (13.6 mg/L), pH of solutions (pH 7) and volume of test solution (10 mL) were kept constant for all samples. 1% (w/w) buffer was added to all dye and adsorption test solutions, except IDB.

3.2.3. Analysis of the Dye Solution Concentrations

All analyses of dye solution concentrations including the calibration solutions were conducted via UV/VIS spectrophotometer. Calibration curve of absorbance versus concentration was plotted from the known concentrations of each dye and corresponding absorbance values were recorded at the maximum wavelength of each dye. Equations of linear trendlines were obtained where the corresponding R^2 values were within the range of 0.9998 to 1.000. After mixing adsorbents with a known concentration of dye molecules and allowing the desired contact time for an effective adsorption, the supernatant containing the

non-adsorbed dye molecules was recovered by centrifugation at 4700 rpm for 15 minutes. Absorbance values of supernatant solutions were determined by UV/VIS spectrometer. Upon placement of absorbance values as y-value into the corresponding equation obtained from the calibration curve, the value of x variable gives the concentration of the non-adsorbed dye. In the exceptional case of Eriochrome Black T dye solutions, EDTA was added into supernatant dye solutions just before the measurements, for canceling the complexation effects of ions released from the biomass. Amount of EDTA added was taken into account as diluting factor in the final concentration of the dye. Figure 3.1 shows an example of a calibration curve with the related equation and R^2 value.

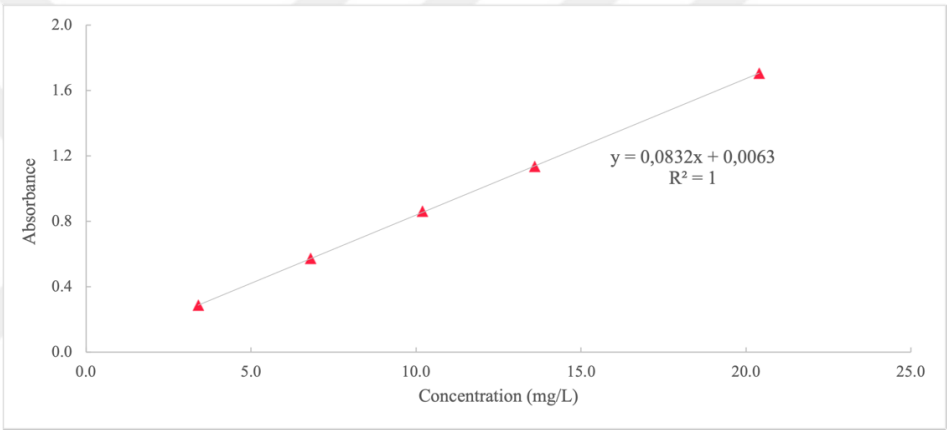


Figure 3.1. An example for the calibration curve of Methyl Orange dye solution with the equation of the trendline at the initial concentration of 13.6 mg/L.

Linearity of the trendline in the calibration plot comes from the direct proportionality between the value of absorbance and concentration according to the Beer-Lambert's Law (Equation 3.1) given as

$$A = \varepsilon \times b \times C, \tag{3.1}$$

where A is absorbance obtained from instrument, ε is the molar absorptivity coefficient ($M^{-1}cm^{-1}$), C is the molar concentration (M), and b is the optical length (cm). pH values of calibration solutions were always checked for consistency. The concentration of unadsorbed dye was calculated from the calibration equation and reported as final concentration. Values of removal capacities (q) of adsorbents that are reported in Sections 4.1.5, 4.2.5, 4.3.5, 4.4.5, 4.5.3, and 4.5.5, which represents the dye uptake per unit mass were calculated by

$$q = \frac{(C_{initial} - C_{final}) \times V}{m}, \tag{3.2}$$

where q is the adsorption capacity (mg/g), $C_{initial}$ (mg/L) and C_{final} (mg/L) are initial and final concentrations of the solution respectively, V (L) is the total volume of the solution and m (g) is the amount of adsorbent. Results for removal percentages of each dye reported in all sections were given by

$$Removal\ \% = \frac{(C_{initial} - C_{final})}{C_{initial}} \times 100, \tag{3.3}$$

where C_{final} (mg/L) is the final concentration found through the equation of calibration curve. Removal percentages was found by using Equation 3.3. Additionally, adsorption capacity of the biomass was calculated by using Equation 3.2.

3.3. Adsorption of Mixture of Selected Dyes by Chlorella and Activated Carbon

Dyes that were tested for their adsorption in mixtures was selected so there is none or minimum overlap between their UV absorption bands. All adsorption tests were conducted with 1 g/L of each adsorbent at pH 7 in 10 mL of total aqueous volume. 1:1 (w/w) mixture of dyes was prepared by mixing 0.5 mL of each dye solution from the corresponding 136 mg/L stock solutions. 2:1 (w/w) mixture of dyes were prepared by mixing 0.70 mL solution of the cationic dye Methylene blue and 0.35 mL solution of the tested dye using the corresponding136 mg/L dye stock solutions. For each targeted proportion of dyes in test media, calibration curve solutions were prepared at that particular ratio. All UV absorption measurements were done at multiple wavelengths that correspond to the maximum wavelengths of individual dyes in the mixture. Removal percentages of each dye in the mixture solution were calculated based on the measurement done at the wavelength of maximum absorption of the targeted dye.

4. ADSORPTIONS OF SINGLE DYES BY CHLORELLA AND ACTIVATED CARBON

Dyes have specific behaviors in their solution forms with respect to their particular structural features and properties of the medium. In this part, results of adsorption studies of five structurally distinct ionic dyes by mainly two different adsorbents, Chlorella and activated carbon, will be discussed.

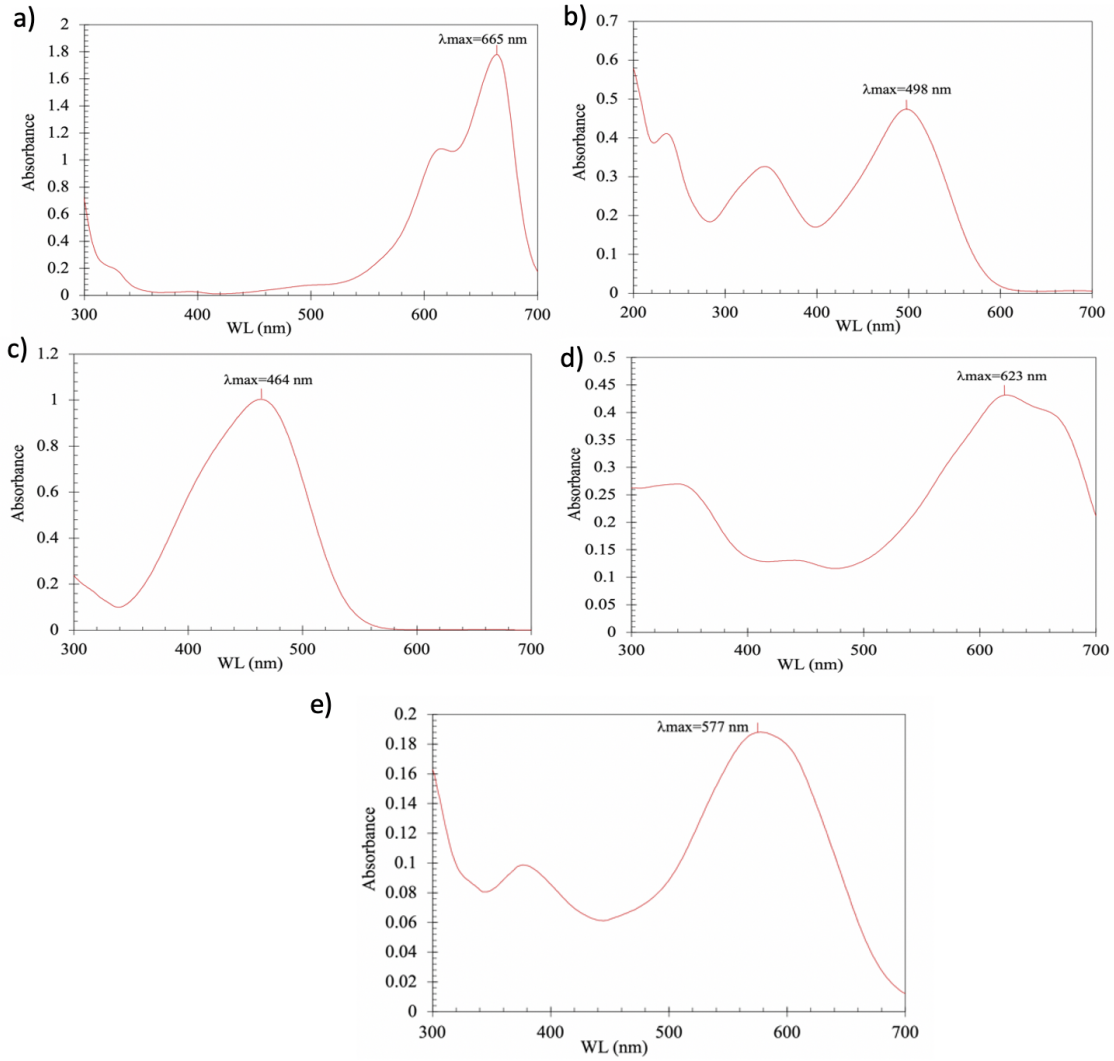


Figure 4.1. Full UV absorption spectrum of (a) Methylene Blue (MB), (b) Congo Red (CR), (c) Methyl Orange (MO), (d) Eriochrome Black T (EBT), (e) Isolan Dark Blue (IDB).

Basic approach is to monitor the decrease of dye concentration under different experimental parameters, by UV absorption measurements performed at corresponding maximum absorbance wavelengths of each dye (Figure 4.1).

4.1. Adsorption of Congo Red Dye by Chlorella and Activated Carbon

4.1.1. Effect of Storage Temperature and Duration of Congo Red Stock Solution on Subsequent Dye Adsorptions

In an effort to obtain greater control over the factors affecting the outcome of adsorption experiments, storage conditions for stock solutions of dyes were monitored. As a result, the use of freshly prepared Congo Red dye solutions was determined to play an important role in the accuracy of adsorption results. Age and storage temperature of Congo Red solutions clearly affected the adsorption behavior, especially onto Chlorella. On the other hand, determining the change in removal percentages for the aged dye solution allows for the monitoring of a more real-like pollution model since environmental pollutants typically spend a period in water bodies before any possible treatment, and they are hardly found as freshly dissolved chemicals.

As mentioned in Section 1.2, Congo Red (CR) can be considered a zwitterionic dye, as the molecule bears both positively charged and negatively charged functional groups separately. Various samples of CR dye stock solutions were prepared with different storage time and temperature. Other variables such as initial concentrations of adsorbent and adsorbate, pH, and presence of buffer were kept constant. To the best of our knowledge the adsorptive behavior of Chlorella and activated carbon against CR was not explored within the scope of different storage conditions of its stock solutions.

The removal through adsorption of CR by Chlorella was found to be 62.5% when the stock solution was freshly prepared. In the same experimental setup, the removal percentage by activated carbon was 92.6%. Then the concentrated stock solution (68 mg/L) of CR was stored at 4 ℃ and room temperature for one day (24 hr). The removal results of Chlorella and activated carbon (AC) for one day aged CR solution at 4 ℃ were 61.9% and 92.0% respectively.

However, for the one-day solution kept at room temperature removal results were 56.4% for Chlorella and 91.0%, for AC. After 13 days at 4 ℃, Chlorella removal dropped to 59.9% and activated removal was 91.1%. When the stock solution stayed at room temperature for 13 days, removal of Chlorella was 55.2% and activated carbon removal was 90.7% (Table 4.1).

Table 4.1. Removal percentages (%) of Congo Red at different storage durations and temperatures of stock solution, measured at 498 nm.

Biosorbent	**Fresh**	**1 day at 4℃**	**1 day at RT**	**13 days at 4℃**	**13 days at RT**
Chlorella	62.5	61.9	56.4	59.9	55.2
Activated Carbon	92.6	92.0	91.0	91.1	90.7

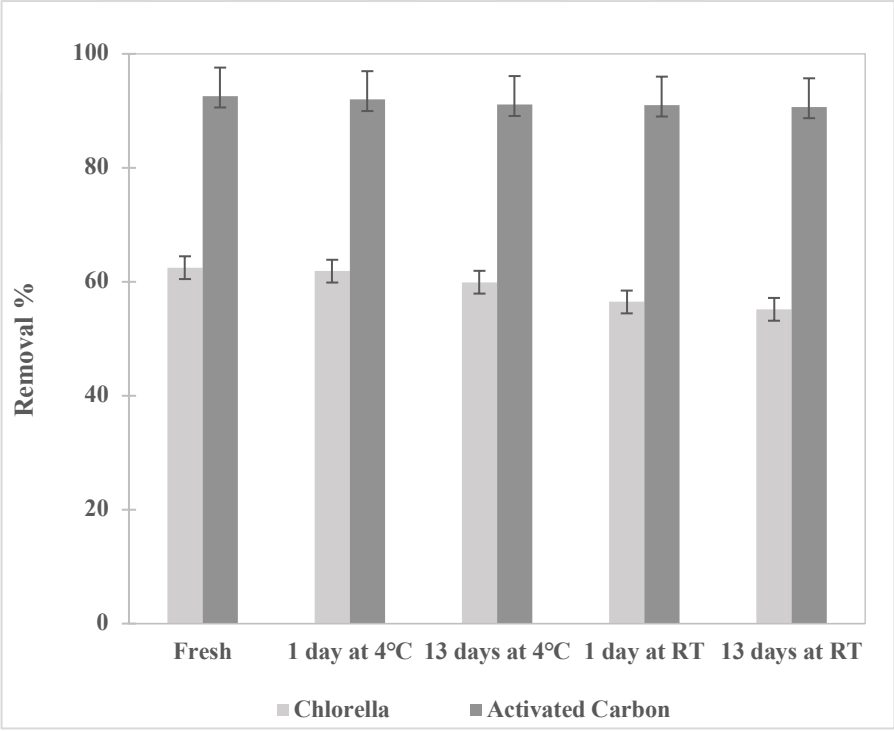


Figure 4.2. Congo Red removal percentages by changing storage conditions of dye stock solution.

Results showed that storing the CR stock solution in a cooler environment didn't significantly affect the removal of CR by Chlorella. However, storing the solution at room temperature for 13 days had an adverse effect on the removal of about having 5-6% less than the fresh one (Figure 4.2). On the other hand, by altering storage temperature and time, decolorization of CR solutions by activated carbon did not experience a discernible decline. This suggested that changes in solution age and temperature—two important environmental parameters determining the characteristics of water soluble micropollutants—had no effect on the removal of CR dye molecules from the aqueous solution by AC. In an attempt to investigate possible changes in Congo Red solution behavior, full scale UV spectra were obtained for solutions that were kept under different storage conditions.

Table 4.2. Peaks (nm) and absorbance values from the full-scale UV spectra of fresh and aged CR dye solutions with 13,6 mg/L initial concentration and pH 7.

Fresh		**13 days at 4°C**		**13 days at RT**	
Peak (nm)	**Absorbance**	**Peak (nm)**	**Absorbance**	**Peak (nm)**	**Absorbance**
498	0.534	498	0.533	498	0.518
345	0.385	345	0.386	345	0.385

The full spectrum of freshly obtained Congo Red dye solution consists of two peaks in the 300-700 nm UV spectrum range, which are at 345 nm and 498 nm. While monitoring the stock solution through time and under different temperatures, the maximum wavelength of the CR solution did not display any shift and remained at 498 nm under conditions presented in Table 4.2. Absorbance values of the freshly prepared dye solution didn't have a significant shift compared to absorbance values of the dye solution stored at 4°C for 13 days at both wavelengths. In contrast, the absorbance value of the dye solution stored at RT for 13 days underwent a decrease in its absorbance value at 498 nm (Table 4.2). The reflection of this decline on the removal percentage was 7.3% lower than the removal of freshly prepared solution. The peak at 345 nm had no alteration in all conditions. Results showed no sign of self-aggregation or photochemically relevant structural changes in Congo Red dye stock solutions at the given concentration due to changing storage conditions, except in the case of the oldest (13 days) stock solution. A decrease in the absorbance value

at 498 nm for the most aged solution may arise from the light degradation of the dye molecules [86] or moisture spoils the dye molecules [87].

4.1.2. Effect of Buffer on Dye Removal for Congo Red

In biosorption processes, other dissolved components, especially ionic compounds have the potential to alter the mechanism, which can then affect the removal of targeted compound adversely or positively. All dye-biosorbent interactions were investigated for the effect of the ionic sodium phosphate buffering agent that is used to stabilize the pH. All other experimental conditions were kept constant during this investigation. pH stabilization was achieved readily at 7.2 with the use of a buffering agent in the solution. However, the dye solution without the buffering agent required a pH adjustment through additions of 0.1M or 1M solutions of NaOH.

Table 4.3. Effect of sodium phosphate buffering agent on dye removal, measured at 495 nm (CR (+): with buffer, CR (-): without buffer).

Buffer Status	**C_0 (mg/L)**	**Removal % by Chlorella**	**pH**
CR (+)	13.6	64.6	7.2
CR (-)	13.6	38.8	7.2

Congo Red adsorptive removal by Chlorella with sodium phosphate buffering agent in the dye solution was 64.6%, whereas the removal dropped to 38.8% without the buffering agent. Results showed that the existence of sodium phosphate buffering agent in the dye solution of CR improved the biosorption (Table 4.3). Phosphate buffer stabilized the pH at around 7.2. CR-Chlorella biosorption was recorded under the same conditions as in Section 4.1.1. Removal of the freshly prepared CR dye solution by Chlorella was 62.5% in those experimental results; however, it was noted as 64.6% in Table 4.1. This difference is considered within acceptable experimental error limits as small changes in humidity and temperature, along with personal or instrumental errors, can cause such variations of a few percent in results. Removal of CR by activated carbon was also analyzed in the presence and absence of the buffering agent. Unlike the case of Chlorella as an adsorbent, the presence or absence of buffering agent did not show any effect on the dye adsorption by AC.

As a remark, it was noted that pH values of solutions treated with AC suspension tended to shift towards higher values (8-9) throughout the adsorption experiment, even though the solution was initially adjusted to pH 7. However, because the AC removal capacity was not found to be affected by changes in pH, increasing pH was not accepted as a contributor to the effect of the presence of the buffering agent.

4.1.3. Effect of Initial Congo Red Dye Concentration on Removal

Concentrated Congo Red dye stock solution (136 mg/L) was prepared freshly and used by diluting into 1.7, 3.4, 13.6, 34.0, and 102.0 mg/L concentrations.

Table 4.4. Congo Red removal percentages of solutions with different initial concentrations, measured at 495 nm and pH 7.

C_0 (mg/L)	Removal % by Chlorella	Removal % by Activated Carbon
1.7	80.1	95.2
3.4	75.1	93.7
13.6	62.2	90.6
34.0	50.4	89.3
102.0	39.0	88.7

Initial dye concentration determines the number of surrounding molecules around biosorbents thus it is known to affect the biosorption process. Results showed that the removal percentage declined from 80.1% to 39.0% by increasing the initial concentration from 1.7 to 102.0 mg/L (Table 4.4). Activated carbon showed a similar but less pronounced trend for the initial dye concentration dependence of the dye removal while exhibiting superior CR removal than Chlorella at all initial CR concentrations. The removal by AC at 1.7 mg/L initial dye concentration resulted in dye removal of 95.2%, and at 102.0 mg/L, CR removal dropped to 88.7%. Changes in initial dye concentrations did not have an equally significant effect on dye removal by AC as opposed to Chlorella.

In concentrated dye solutions, Congo Red adsorption onto Chlorella was affected much more adversely than towards activated carbon. On the other hand, the gap between the

decolorizations of Congo Red by Chlorella and AC was much lower in dilute dye solutions, and at 1.7 mg/L, both biosorbents reached the highest removal of the dye. In Figure 4.3, the removal by Scenedesmus was carried out in addition to Chlorella and activated carbon. Scenedesmus removals were 50.0%, 43.0%, 36.0%, 34.2%, 10.5% at corresponding concentrations from 1.7 to 102.0 mg/L respectively. Scenedesmus exhibited much weaker biosorption with Congo Red, especially in concentrated dye solutions.

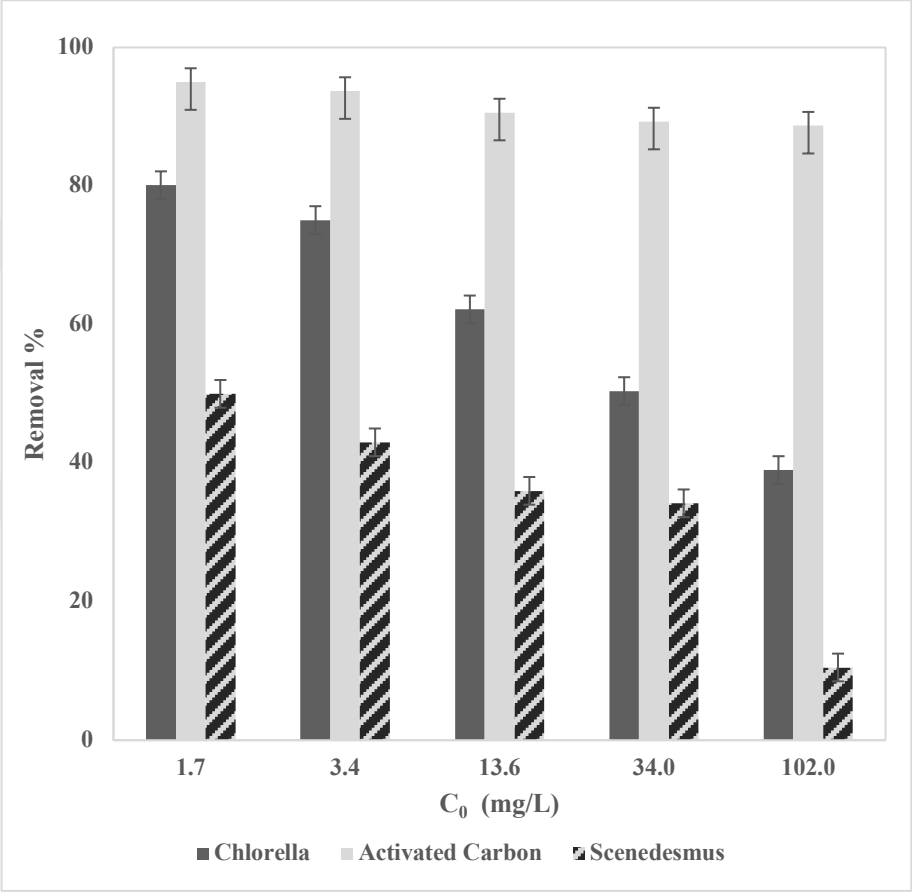


Figure 4.3. Removal of Congo Red plotted against initial dye concentrations (Measured at 495 nm).

The lower absorption of CR by Scenedesmus probably comes from the chemical composition of its cell wall and cell membrane. Our results may suggest that a smaller variety of functional groups are involved in adsorption in the case of Scenedesmus species in comparison to Chlorella. The cell wall of *C. Vulgaris* consists of glucosamine derivatives in addition to monosaccharides, glucose, galactose, and mannose. However, Scenedesmus

consists of only monosaccharide derivatives, glucosamine is not found [42]. Ionic functional group variety of cell composition in Chlorella is more prosperous than for Scenedesmus. This factor may enable Chlorella to adsorb a higher number of dye molecules than Scenedesmus.

It is relevant to examine biosorption in the experiments involving lower initial dye concentrations since dyes are more commonly found as relatively low concentration pollutants, micropollutants, in aqueous environments, and wastewater effluents [54]. UV/VIS analyses are restricted by the limit of detection of the instrument. The lowest limit of detection (LOD) was determined by measuring the absorbance in solutions with concentrations from $0.7X10^{-2}$ to 3.4 mg/L (Table 4.5).

Table 4.5. Dye concentrations versus absorbance values used in the calibration curve for Congo Red solutions (Measured at 498 nm. Italics represents undetectable concentrations).

Concentration (mg/L)	Absorbance
0.007	*0.003*
0.034	*0.002*
0.054	*0.002*
0.068	*0.002*
0.136	*0.001*
0.204	*0.003*
0.238	0.005
0.340	0.008
0.680	0.021
1.360	0.040
3.400	0.111

Initial CR concentrations of $0.7X10^{-2}$ and $20.4X10^{-2}$ mg/L were not detectable through UV-VIS light absorbance since absorbance values in that interval stayed almost the same despite the change in concentrations. According to Beer-Lambert's Law, as concentration increases, absorbance should increase. From $23.8X10^{-2}$ to $34.0X10^{-2}$ mg/L, absorbance had

the same increment as the concentration increase. Thus, the lowest LOD for Congo Red dye started at approximately $23.8X10^{-2}$ mg/L. Full spectra were scanned in the 300-700 nm range to examine the dye behavior at selected concentrations. As seen in Table 4.6, undetectable concentrations had only the left peak at ≈330 nm with seemingly background absorbance values, while the maximum peak of CR at 498 nm disappeared at CR concentrations below $6.8X10^{-2}$ mg/L. Thus, detection of the dye was not possible. Therefore, the detection limit started at $23.8X10^{-2}$ mg/L concentration. In Table 4.4, the lowest initial concentration was taken as 1.7 mg/L because the final concentration following partial removal of the dye should also be within the detection limits. 80.1% of the initial concentration of 1.36 mg/L falls within the LOD interval.

Table 4.6. UV-VIS spectrum data of Congo Red dye solution (Italics represents undetectable concentrations).

Concentration (mg/L)	Peak (nm)	Absorbance
0.007	*330*	*0.015*
0.034	*330*	*0.015*
0.054	*330*	*0.016*
0.068	*335*	*0.016*
0.238	495	0.004
	335	0.019
0.340	495	0.009
	339	0.024
1.360	495	0.040
	345	0.049
3.400	495	0.108
	345	0.100

Within the approach of determination of the removal percentages, one question may arise regarding the peak observed at lower wavelengths (330-350 nm). The removal percentage calculated based on absorbance change of the 330-350 nm peak was determined

to decrease from 72.8% to 43.6%, by increasing the concentration of solution from 1.7 to 34.0 mg/L respectively (Table 4.7).

Removal percentages calculated through absorbance measurements from two different wavelengths region are given in Table 4.7 against different initial dye concentrations. In Table 4.6 removal percentages are obtained from the maximum wavelength of 495 nm because it reflects the concentration dependent light absorption behavior of Congo Red with the best precision, in compliance with previous literature examples. In the case of the lower wavelength peak as the detection site, changes in wavelengths from 330 to 350 nm (Table 4.6 and 4.7) came from fluctuations in light absorbance behavior of Congo Red. These slight shifts in maximum wavelength were determined to be ineffective in the resulting values of removal percentages.

Table 4.7. Congo Red removal percentages by Chlorella at two wavelengths.

	Removal % by Chlorella	
C_0 (mg/L)	**330-350 nm**	**498 nm**
1.7	72.8	79.4
3.4	67.3	71.8
13.6	52.3	62.4
34.0	43.6	47.5

Looking at calibration curves, linear lines are observed to be almost in the same direction (Figure 4.4). Similarities in removal yields (Table 4.7) and calibration curves (Figure 4.4) come from the fact that in the full spectrum of Congo Red (Figure 4.1b) there are two peaks within the 300-700 nm range and the peak at 498 nm has slightly more elevated absorbance. This is also why 498 nm is accepted as the selected maximum wavelength for concentration measurements.

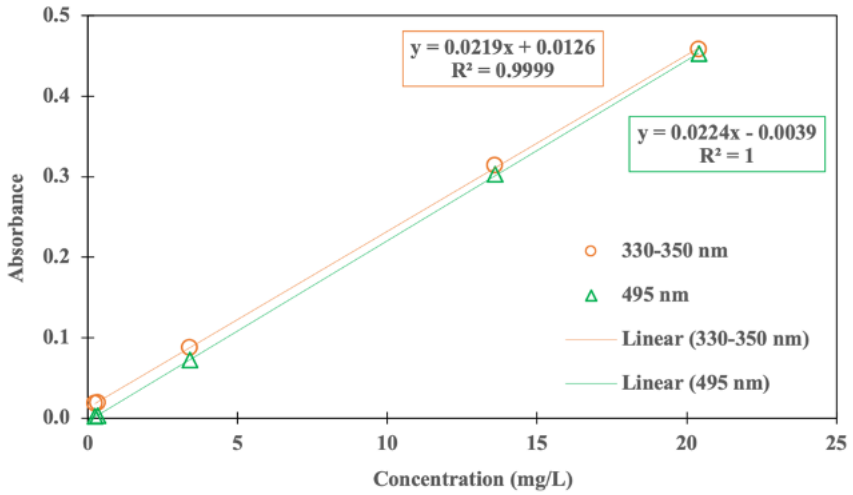


Figure 4.4. Calibration curve of CR at its peaks in the full UV absorption spectrum.

4.1.4. Effect of pH on Congo Red Dye Removal

Electrostatic interactions of ionic molecules is supposed to be a very important factor in the biosorption mechanism, thus presence of ionic compounds in the medium has the potential to influence the biosorptive removal. Thus far, the pH of the dye solution was kept constant near 7, in all experiments. In this section, the effect of changes in pH in biosorption process of Congo red is examined while all other variables were kept constant.

Table 4.8. CR dye removal percentages at different pH values (with a fixed initial CR concentration of 13,6 mg/L).

~ pH	Removal % by Chlorella	λ_{max} (nm)
6.0	84.6	499
6.5	74.0	496
7.0	62.2	495
8.0	52.4	494

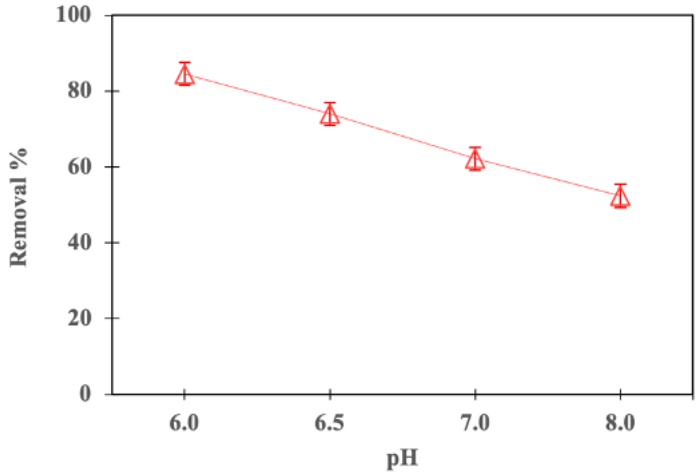


Figure 4.5. Trend of CR dye removal at different pH values.

Congo Red dye solutions with pH values 6.0, 6.5, 7.0, and 8.0 were subjected to adsorption tests involving Chlorella and activated carbon under previously established procedures. The removal percentages were 84.6%, 74.0%, 62.2%, and 52.4% respectively (Table 4.8). Figure 4.5 shows that the removal of CR by Chlorella is inversely proportional with pH of the solution.

Possible precipitations of the dye due to pH changes were carefully checked before each adsorption experiment, to avoid any inaccuracy. CR forms a precipitate near the pH of 2, possibly because of the coalescence of dye molecules through dominant π–π stacking dye interactions [88]. Congo Red has $-NH_2$ and $-COO^-Na^+$ functional groups that's why it is considered as a zwitterionic dye. When pH is changing, some structural alterations of Congo Red depending on the pH of the solution are shown in Figure 4.6.

When the solution is acidic enough, the azo linkage of the CR molecules get protonated and occurs azonium form (Figure 4.6b). The resonance form of it given in Figure 4.6c. Thus, the conjugated structure turns into quinonoid form due to the electron movements with resonance. The lowest selection of pH is 6.0 because phosphate buffer loses its ability under pH 5.6. In section 4.1.2. buffer effect was under investigation and results showed that removal of Congo red in the absence of buffering agent was dramatically lower. Thus, below pH 5.7, the solution acts like there are no buffer and Congo Red removal can not be

accurately compared to higher pH range. This expectation was confirmed when removal percentages were recorded as 46% at pH 5.6 and 84.6% at pH 6. Additionally, partial protonation of CR molecules is possible near pH 5.5 which can affect adsorption behavior (Figure 4.6). Utilizing another buffer that works at lower pH might show the exact removal percentage of Congo Red at pH values lower than 6.0, however such investigation was not included within the scope of the current work.

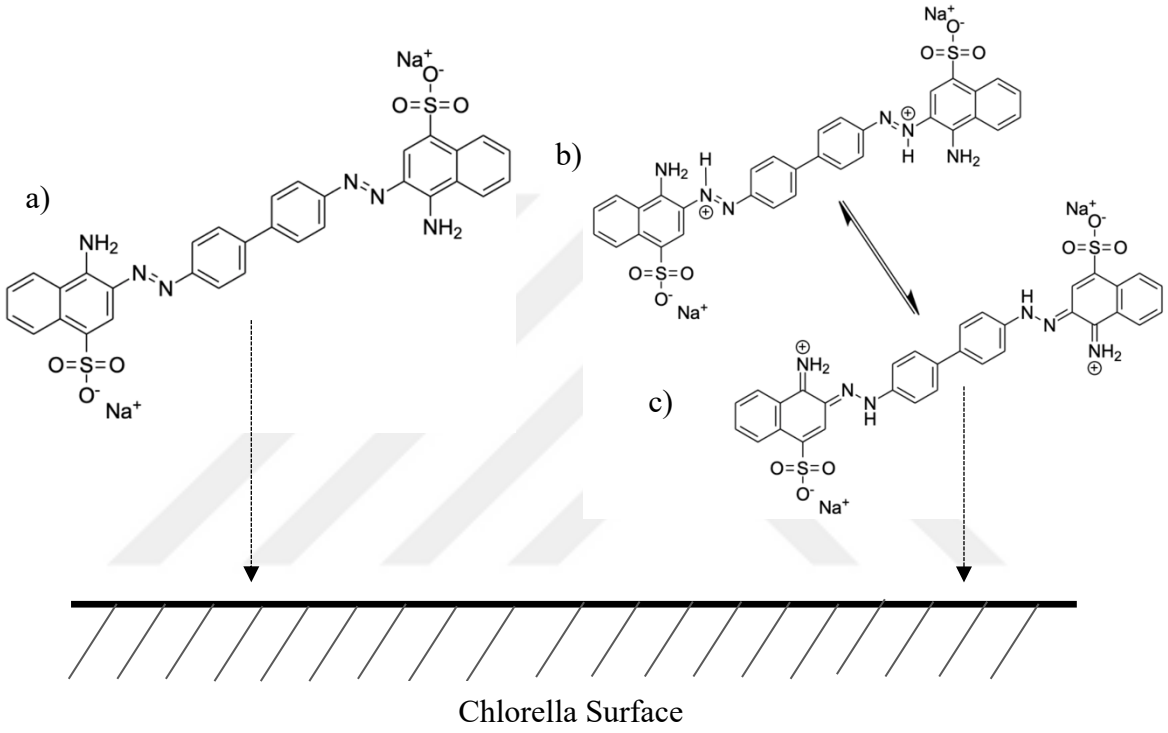


Figure 4.6. (a) Chemical structure of CR at pH 7, (b) azonium form of CR when the pH of the solution is acidified near 5.5, (c) resonance form of azonium dye structure [89].

4.1.5. Effect of Biosorbent Dosage of Chlorella on Congo Red Dye Removal

Another important variable affecting biosorption is the quantity of the biosorbent which represents the available interaction sites on the algae where dye molecules could interact and bind to. In addition to removal percentage of Chlorella, the adsorption capacities representing the amount of adsorbed dye molecules per unit mass of the biosorbent was calculated.

Table 4.9. Removal percentage of CR at different biosorbent dosages of Chlorella at pH 7.0 and C_0 of 13,6 mg/L.

Biosorbent Dosage (g/10 mL)	**Removal %**	**Adsorption Capacity (mg/g)**
0.005	37.0	9.9
0.010	62.2	7.5
0.050	74.9	2.0
0.100	89.9	1.2
0.250	98.3	0.5

In previous adsorption experiments involving Congo red, a 0.001 g amount of biosorbent were used. In the current set of biosorbent dosage experiments and the quantity of Chlorella was changed within the range of 0.005 to 0.250 grams. All other experimental variables were kept constant in accordance with standards determined in previous adsorption experiments.

According to Table 4.9, the removal percentage of Congo Red increased as the amount of Chlorella increased while adsorption capacity was in reverse order. 0.005 grams of Chlorella had 37.0% removal percentage while 0.250 grams had 98.3%. Upon initial observation, the assumption might be that removal yield and biomass dosage is directly proportional. However, the adsorption capacity was observed to be 9.9 mg/g when the amount of biomass is 0.005 grams. For 0.250 grams, the adsorption capacity decreases to 0.5 mg/g. As it is seen in Figure 4.6, a sharp decrease in the value of adsorption capacity occurred when the biomass amount is increased from 0.005 grams to 0.050 grams. This result shows that microalgal dose and adsorption capacity are inversely proportional. For both y-axis variables in Figure 4.7, there are no significant change beyond a biosorbent dosage of 0.100 grams, which suggests that beyond such dosage biosorbent approaches a level of saturation.

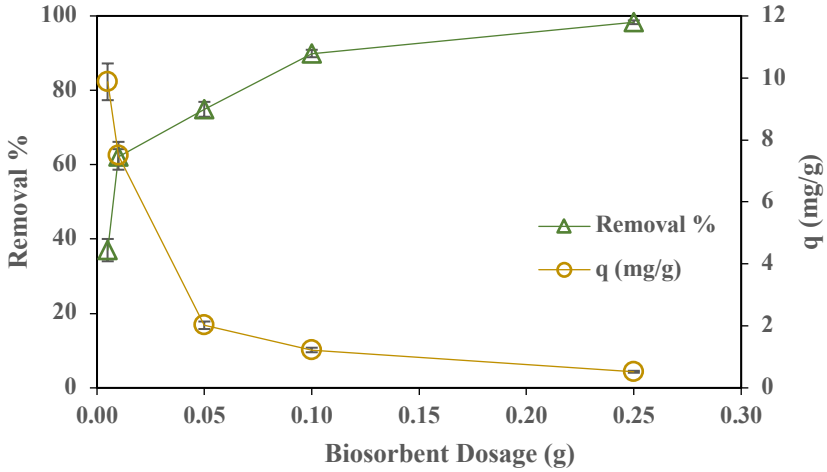


Figure 4.7. Change in adsorption capacity, q (mg/g), and removal % versus biosorbent dosage of Chlorella, at initial CR concentration of 13.6 mg/L.

Overall, these results showed that the removal percentage increases when the number of adsorption sites of the biomass rises for a constant initial dye concentration (13.6 mg/L). Ionic dye molecules interact with various functional sites of the microalgal biomass surfaces until the biosorption reaches near complete dye removal. On the other hand, the adsorption capacity which shows the amount of surface attached dye molecules per unit biomass (mg/g) has an inverse proportion to increasing microalgal dose. This inverse proportionality may suggest that increasing amount of biosorbent does not necessarily lead to an increase in the number of active collisions between dye molecules and biomass, even under constant initial dye concentration and volume of solution. The observation of agglomeration of biosorbent, especially at the bottom of the test flask at higher biosorbent dosages, definitely an important contributing factor to decreasing adsorption capacities [90].

4.2. Adsorption of Methyl Orange Dye by Chlorella and Activated Carbon

4.2.1. Effect of Storage Temperature and Duration of Methyl Orange Stock Solution on Subsequent Dye Adsorptions

The second zwitterionic dye studied in this thesis work in addition to Congo red is Methyl orange (MO). Structures of Congo red and Methyl orange are quite similar especially in terms of functional groups. However, MO has a lower molecular weight. Since these types

of dyes contain ionic and conjugated aromatic groups, their solution behavior can be affected from environmental conditions due to possible intermolecular interactions and aggregations. In this section, the effect of storage temperature and duration of MO solutions on subsequent MO removal efficiencies is investigated. To the best of our knowledge the adsorptive behavior of Chlorella and activated carbon against MO was not explored within the scope of different storage conditions of its stock solutions.

Decolorization of Methyl orange was given in Table 4.10 as 4.3% by Chlorella and 97.8% by activated carbon when the stock solution of MO was prepared freshly. The gap in removal percentages between two adsorbents have the highest value among all selected dyes. Elimination of the MO dye was clearly observed to be insufficient under standard conditions (pH 7, 10 mg Chlorella, and C_0 of 13,6 mg/L) by the microalgae. Therefore, the effect of storage time and temperature on adsorption of MO was studied through a rather limited number of experiments. Dye solution that was kept at 4 ℃ for two days resulted in a removal of 4.1% by Chlorella and 95.6% by activated carbon.

Table 4.10. Removal percentages (%) of Methyl orange at different storage durations and temperatures of stock solution, measured at 464 nm, at pH 7.

Biosorbents	**Fresh**	**2 days at 4 ℃**
Chlorella	4.3	4.1
Activated Carbon	97.8	95.6

Changes in storage temperature and duration had no appreciable impact on the decolorization of MO solutions by activated carbon and Chlorella. This indicated that the removal of MB dye molecules from the aqueous solution by AC and Chlorella was unaffected by changes in solution age and temperature, two crucial environmental factors controlling the properties of water soluble micropollutants.

Majority of MO molecules remained in the aqueous medium after the contact with the biosorbent. Such a level of inefficient biosorption of MO by Chlorella is expected to be better understood following further investigations about the effects of buffering agent, pH, initial dye concentrations and biosorbent dosage as will be discussed in following sections.

4.2.2. Effect of Buffer on Methyl Orange Dye Removal

The low removal percentages of Methyl Orange by Chlorella under standard experimental conditions bring about the question of the effect of buffer molecules on biosorption because anionic phosphate buffer molecules may intervene with the biosorption of MO. In the case of Congo Red, the presence of the buffering agent in the solution was shown to have a great effect on the extent of adsorption.

Methyl orange was removed by 4.1% using Chlorella with sodium phosphate buffering agent presenting in the solution, whereas 2.9% removal was recorded without the buffering agent in the solution (Table 4.11). Activated carbon was very successful in adsorption removal for both cases, with or without buffer, as observed for Congo Red (Figure 4.8). In the absence of phosphate buffer, it was difficult but possible to stabilize the pH at 7 for dye-Chlorella liquor. However, AC suspension comes with a strong influence towards higher pH levels in the absence of buffer, leading to typical pH values of around 9.0. However, it must be noted that this level of basicity of the solution is not expected to have any significant effect on the biosorption of MO by activated carbon as will be discussed in section 4.2.4 elevated pH levels do not show any effect on MO adsorption on AC.

As mentioned in Section 4.1.2, removal of another zwitterionic dye, Congo Red, was affected negatively by the absence of buffer in the solution. Absence of buffer in MO and CR dye solutions resulted in a significant decrease, as much as halves, in removal percentages.

Table 4.11. Effect of sodium phosphate buffering agent on dye removal, measured at 665 nm (MO (+): with buffer, MO (-): without buffer).

Buffer Status	**C_0 (mg/L)**	**Removal % by Chlorella**	**Removal % by AC**
MO (+)	13.6	4.15	98.4
MO (-)	13.6	2.94	95.6

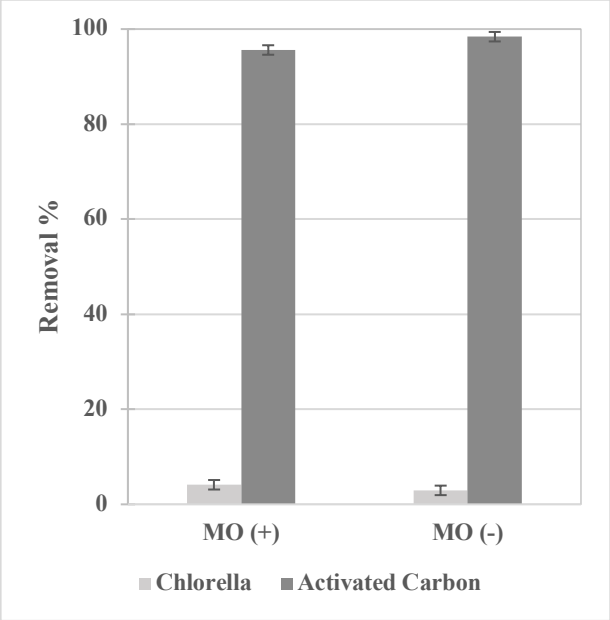


Figure 4.8. Effect of the presence of sodium phosphate buffering agent on MO dye removal.

4.2.3. Effect of Initial Methyl Orange Concentration on Dye Removal

Dye removal percentages of adsorbents were calculated from the Equation 3.3 included the difference between initial and final concentrations. In this section, the role of initial Methyl Orange concentration on biosorption was studied.

The role of the initial concentration of Methyl Orange on the effectiveness of biosorption onto Chlorella and activated carbon was examined between 3.4 and 34 mg/L concentrations at pH 7.0. Decolorization percentage of MO decreased from 5.2 to 4.2 with increase in initial concentration of dye solution with Chlorella (Table 4.12). Adsorptive removal of MO using activated carbon was well suitable in all tested concentrations, noting that the removal percentage of AC decreased slightly in more concentrated solutions, suggesting a possible saturation on adsorptive sites of AC against MO. In conclusion, the gap between Chlorella and AC towards decolorization of MO was very large at all concentrations, and Chlorella did not seem to be a suitable adsorbent at the given biosorbent dosage at pH 7 (Figure 4.9). The reason behind the very low adsorption of MO onto Chlorella can be suggested to be related to the particular chemical structure and zwitterionic nature of MO, where close proximity of anionic and cationic sites may induce a repelling effect against

ionic adsorptive sites of Chlorella. Additionally, biosorption of MO by Scenedesmus biomass was examined and removal percentage was 2.7% at the pH value of 7.

Table 4.12. Removal percentages of MO at different initial concentrations, measured at 465 nm and pH 7.0.

C_0 (mg/L)	Removal % of Chlorella	Removal % of Activated Carbon
3.4	5.2	98.6
13.6	4.4	97.3
34.0	4.2	93.2

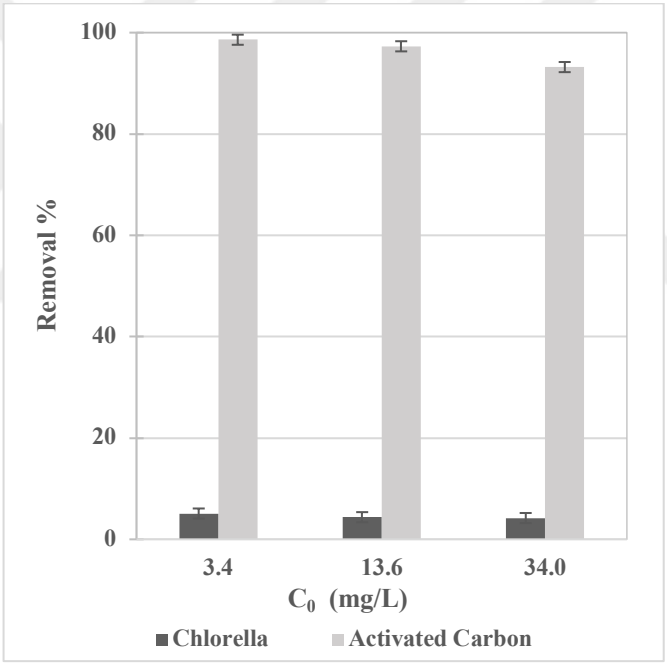


Figure 4.9. Removal of MO plotted against initial dye concentrations, measured at 465 nm.

4.2.4. Effect of pH on Methyl Orange Dye Removal

Biosorption can be a highly pH dependent process since ionic characters of adsorbate and biosorbent may be altered, also altering electrostatic interactions leading to adsorption. Adjustment of pH to the same value in every experiment is an important factor for higher reliability of results. Charge of ionic sites on the microalgal surfaces and Methyl Orange

change with changing pH. Methyl Orange has a color change between the pH values of 3.0 and 4.4. The color is red below 3.0 and yellow above 4.4, with an end point at pH 3.5 [91].

Table 4.13. Removal percentages of MO at different pH values, measured at 465 nm at initial MO concentration of 13.6 mg/L.

Chlorella		**Activated Carbon**		**λ_{max}(nm)**
~ pH	**Removal %**	**~ pH**	**Removal %**	
2.0	35.9	4.0	99.4	508
2.5	55.8	4.5	97.3	508
3.5	64.9	7.0	97.0	508
5.0	6.6	9.0	98.0	465
6.0	3.6			465
7.0	4.6			465
8.0	0.1			465

Methyl Orange biosorptive removal by Chlorella was recorded as 4.6% at pH 7.0 and further decreased as the pH value was increased to pH 8.0. At around pH 8.0, Chlorella dye removal capability was almost zero whereas AC provided an average removal of 97.5%. As seen in Figure 4.10, activated carbon was successful to remove MO dye molecules from the solution at any level of pH within the experimental range of 4.0 to 9.0. On the other hand, in acidic conditions between pH 6.0 and 5.0, decolorization of MO in the solution by Chlorella didn't have any significant change. However, a huge jump in removal percentage was recorded when pH was decreased from 5.0 to 3.5, resulting in 64.9% removal of MO (Table 4.13). Because end point of the dye is at pH 3.5, structural change caused by the protonation of the compound, also causing the color change from orange to red, is suspected to lead to distinctly better adsorbate character (Figure 4.11).

A bathochromic shift on UV spectrum was observed when the solution was highly acidic (at pH 2.0, 2.5 and 3.5), where the maximum peak shifted to a longer wavelength (Table 4.13). This shift arises from the changes in the extent of conjugation in the molecular structure as seen in Figure 4.11.

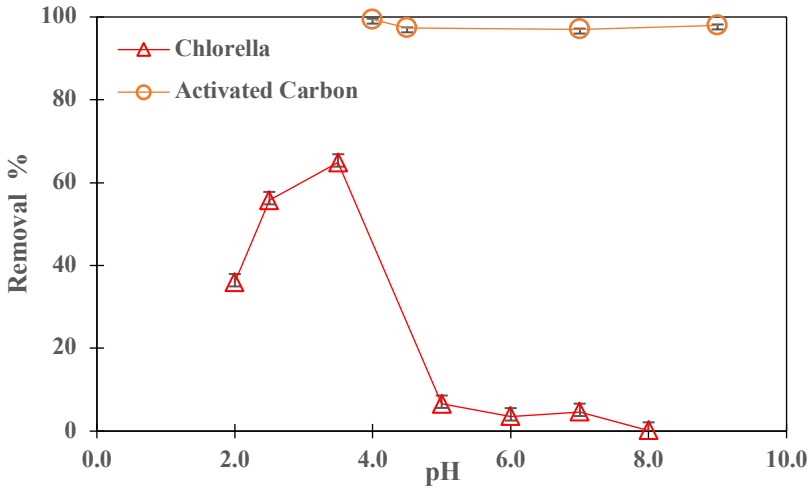


Figure 4.10. Trend of removal percentages of MO by Chlorella and activated carbon at different pH values.

The deprotonated form of MO dye molecule (Figure 4.10a) changes into a quinonoid structure (Figure 4.11c and Figure 4.11d) where light absorption and emission of electrons are altered on n–π* transition, at the pKa point of 3.5 [92].

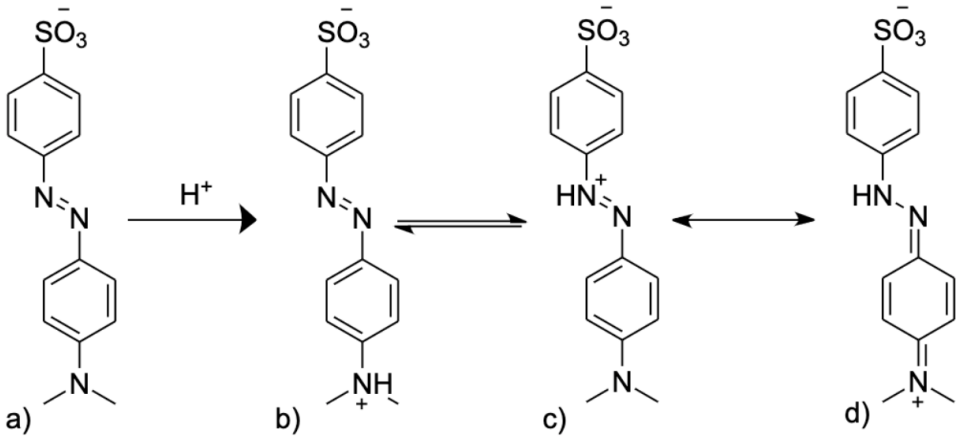


Figure 4.11 Chemical change of Methyl orange structure by pH a) anionic form b) monoprotonated zwitterionic structure of MO (ammonium tautomer) c) zwitterionic form of azonium tautomer d) the resonant form of. Sodium ions are neglected [93].

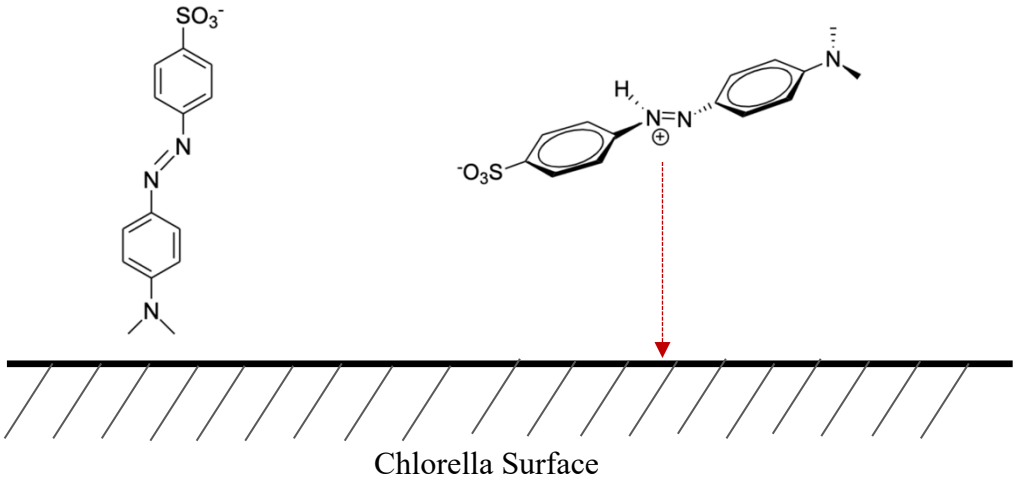


Figure 4.12. Representative MO orientations in biosorption with Chlorella a) at pH 7 and b) when acidity of the solution increases [94].

In between pH values of 5.0 and 7.0, structure of dye molecules do not undergo a significant change that can lead to better biosorption by Chlorella; thus, removal percentages were similarly low. As hydrogen ion concentration rises in the solution, protonation of MO occurs on the secondary amine moiety as seen on Figure 4.11b. At the end point of the MO solution, which is pH 3.5, molecular structures shown in Figure 4.11b, c, and d occur [93]. The protonation generates the zwitterionic forms of MO dye, probably increasing the binding capacity of dye molecules onto negatively charged sites of Chlorella [95]. Polar-π interactions, H-bonds, and other possible attractive forces may lead to a change from the weak surface interaction between Methyl Orange and Chlorella towards better by changing the MO structure into the azonium form. (Figure 4.12).

4.2.5. Effect of Biosorbent Dosage of Chlorella on Methyl Orange Dye Removal

In previous sets of experiments involving Methyl Orange the biosorbent dosage of Chlorella was fixed at 0.01 gram. In this section, the aim is to find the response of Methyl Orange biosorption onto Chlorella to changes in the quantity of biomass while keeping all other experimental variables constant. In addition to removal percentages, adsorption capacities (q) were also calculated as a mean to determine the amount of removed adsorbate per unit mass of biomass.

Table 4.14. Removal percentages and adsorption capacities (q) of Methyl Orange at different biosorbents dosages of Chlorella at pH 7.0 and C_0 of 13,6 mg/L.

Biosorbent Dosage (g/10 mL)	**Removal %**	**Adsorption Capacity (mg/g)**
0.005	1.8	0.21
0.010	5.8	0.58
0.050	22.0	0.59
0.100	46.6	0.63
0.250	69.5	0.38

Biosorbent dosages of Chlorella that were used in this set of experiments were 0.005 g, 0.010 g, 0.050 g, 0.100 g, and 0.250 grams. The effect of this increment of biomass to the fraction of removed MO from the solution was 1.8%, 5.8%, 22.0%, 46.6%, and 69.5%, respectively (Table 4.14). When biosorbent dosage of Chlorella was increased from 0.010 g to a mass that is a five times higher value in the same volume of the solution, uptake of MO molecules from the solution changed from 5.8% to 22.0%. However, the effect of increase in biomass quantity to the value of adsorption capacity, 0.58 to 0.59, was not even close to such magnitude. Adsorption capacity (q) of Chlorella increased from 0.21 to 0.63 mg/g when the amount of biosorbent is raised to 0.100 g. After that point, uptake of MO molecules per unit mass of microalgae decreased to 0.38 mg/g, at 0,250 g biosorbent dosage.

The amount of removed MO molecules from the solution by Chlorella increased constantly throughout the range of increasing selected dosages whereas amount of removed MO molecules per unit mass of Chlorella declined after the point of 0.1 grams of biosorbent (Figure 4.13).

The reversal in the trend of adsorption capacity revealed that the increase in the number of removed MO dye molecules was not in the same magnitude as the increase in the value of biosorbent dose. As quantity of biosorbent was 10 times higher than the lowest tested dose 0.005 grams, while the flask volume was kept constant, the adsorptive interactions of dye molecules with surface molecules of microalgae were supposedly not as effective and the resulting value of the adsorption capacity was not even nearly fivefold higher. After a certain

value of microalgal dose, capacity of Chlorella to remove MO dye molecules from the solution decreased, probably due to MO dye molecules in the solution did not being able to bind to all available sites of microalgae [96]. As suggested in the previous study about Congo Red biosorption, the aggregation of microalgae at higher dosages of suspended biomass may very possibly lead to excess aggregation which would then block certain part of adsorptive surface of microalgae, partially blocking the biosorption process. Nevertheless, removing 69.5% of MO molecules with 0.25 grams of microalgal dose at the given initial concentration (13.6 mg/L) demonstrate the importance of biosorbent dosage, especially for a rather poor adsorbate for Chlorella such as MO.

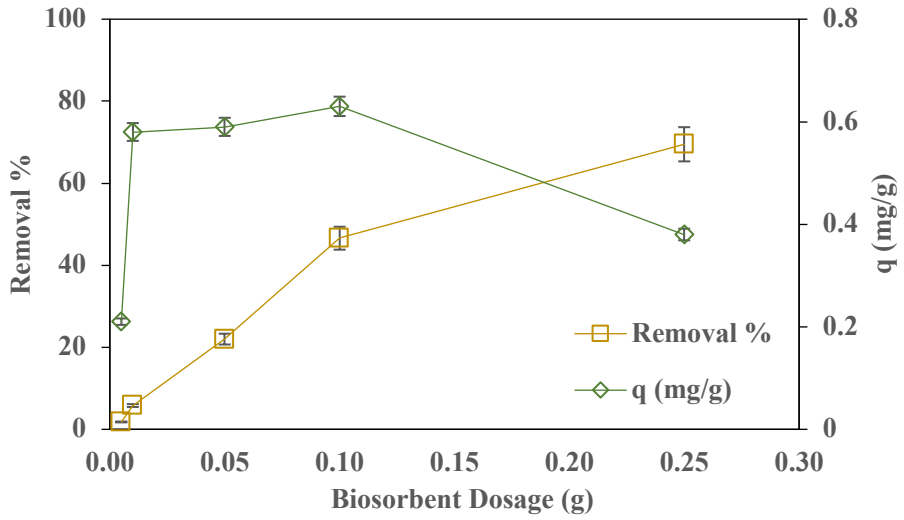


Figure 4.13. Adsorption capacity, q (mg/g), and removal % versus biosorbent dosage at a fixed amount of initial concentration of 13.6 mg/L.

4.2.6. Enhancements for Methyl Orange Dye Uptake towards Chlorella by Changing pH and Biosorbent Dosage Together

Until now, variations on the removal percentage of Methyl Orange by pH and biosorbent dosage were examined as two separate factors. Since removal percentage of MO was the lowest among all dyes, tested within this thesis work, possible enhancements on its removal procedure were targeted by combining two critical variables, pH of the medium and biosorbent dosage, keeping the initial dye concentration constant at 13.6 mg/L.

Methyl Orange dye uptake was first investigated with 0.01, 0.03, 0.10, and 0.25 grams of Chlorella biosorbent dosage at various level acidity and in neutral media (Table 4.15). Results were in accordance with conclusions of Section 4.2.4 as acidity of the solution enhances the binding of Methyl Orange to Chlorella. Furthermore, in acidic medium, increased biosorbent dosage exhibited a more pronounced effect for decolorization of dye solution. A rise in removal percentage from 63.0% to 82.3% was achieved when microalgal dose was raised from 0.01 g to 0.03 g in the biosorption experiment at the pH of 3.5. When the weight of microalgae used in an experiment was 0.1 g, that is 10 times higher than 0.01 g, removal percentage reached 86.2% at pH 5.5. When the quantity of Chlorella was 0.25 grams, the highest value of the MO removal was provided as 92.5% at the pH of 6.0, while 69.5% of MO dye molecules were retained from the neutral solution by Chlorella. In conclusion, the relatively weak adsorption capacity of Chlorella towards Methyl orange was improved by both decreasing acidity of the solution and increasing the microalgal dosage together.

Table 4.15. Removal percentage of Methyl Orange at different biosorbent dosage of Chlorella.

Biosorbent Dosage (g/ 10mL)	pH	Removal % by Chlorella	λ_{max} (nm)
0.01	3.5	63.0	508
	7.0	5.8	465
0.03	3.5	82.3	508
	7.0	-	-
0.10	5.5	86.2	465
	7.0	46.6	465
0.25	6.0	92.5	465
	7.0	69.5	465

4.2.7. Role of Contact Time on Methyl Orange Dye Uptake by Chlorella at Different pH Values

In adsorption experiments, contact time represents the required time allowed for the interaction between the adsorbate and the adsorbent while agitating the suspension via an

orbital shaker. The effect of contact time on dye removal was examined at different pH values.

Table 4.16. Removal percentages (%) of MO by time and pH.

		Removal % by Chlorella			
λ_{max} (nm)	**pH**	**Contact Time (hours)**			
		2	**24**	**48**	**168**
508	2.5	58.8	57.8	57.6	48.9
465	5.0	6.7	6.3	5.4	4.9
465	7.0	4.3	4.2	4.1	2.2

Adsorptions of Methyl Orange by Chlorella at pH of 2.5, 5.0, and 7.0 were examined by agitating the solutions for 2, 24, 48, and 168 hours on orbital shaker. As expected, adsorptive behavior of Chlorella towards MO was the highest at the pH value of 2.5, but at two hours contact time. However, as the contact time gets longer towards seven days (168 hours), the removal percentage decreased from 58.8 to 48.9% (Table 4.16). Solutions at pH values of 5.0 and 7.0 showed the same trend by time where longer contact time did not bring better adsorption and best adsorption was obtained at two hours, the shortest contact time tested (Figure 4.14).

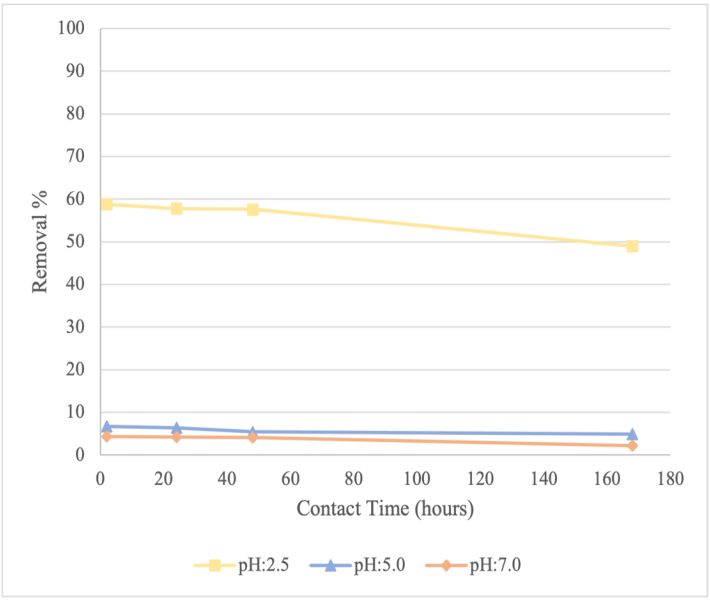


Figure 4.14. Removal % of Methyl Orange by contact time at different pH values.

The reason for decreasing removal percentages of MO by increasing contact time most probably arises from desorption of MO dye molecules throughout time. Dye molecules attached to the surface of the microalgae were also possibly disrupted by the kinetic motion of orbital shaker; thus, number of free dye molecules in the solution escalated as contact time between the adsorbate and the adsorbent got longer.

4.3. Adsorption of Methylene Blue Dye by Chlorella and Activated Carbon

4.3.1. Effect of Storage Temperature and Duration of Methylene Blue Stock Solution on Subsequent Dye Adsorptions

Methylene Blue is a cationic dye having secondary and tertiary amine moieties on its molecular structure. In order to obtain accurate results in adsorption experiments, the effect of storage temperature and duration of MB stock solutions on subsequent MB removal efficiencies by Chlorella and activated carbon was investigated. MB uptake by different biosorbents was shown that negatively charged adsorbents could effectively remove this dye [56]. Yet, to the best of our knowledge the adsorptive behavior of Chlorella and activated carbon against MB was not explored within the scope of different storage conditions of its stock solutions.

Table 4.17. Removal percentages (%) of Methylene Blue at different storage durations and temperatures of corresponding stock solution, measured at 665 nm, at pH 7.

Biosorbents	Fresh	1 day at 4°C	2 days at 4°C	1 day at RT	2 days at RT
Chlorella	92.2	88.5	88.5	86.6	86.4
Activated Carbon	99.5	99.3	99.0	98.4	98.0

Removal of MB from freshly prepared dye solution by Chlorella was 92.2%, somewhat nearly as successful as activated carbon that removed 99.5% of MB under the same conditions. The adsorptive capacity of Chlorella towards Methylene Blue was the highest amongst all five tested dyes. Removal of MB nevertheless decreased as dye stock solution is aged for longer times whether it was kept at 4°C or room temperature. Storing the MB dye stock solution for one day at 4°C decreased the decolorization percentage in

solutions diluted from such stock, with Chlorella as adsorbent, from 92.2% to 88.5% (Table 4.17). The same trend was observed for the stock solution that was kept for two days under the same conditions (88.5%). When the flask was kept at room temperature for one and two days, the decolorization of MB solutions dropped to 86.6% and 86.4%, respectively. Decolorization of MB solutions by activated carbon did not undergo a notable decrease by changing temperature and duration of storage. This meant that eliminating MB dye molecules from the aqueous solution by AC was not affected by fluctuations in solution age and temperature as critical environmental factors affecting properties of water soluble micropollutants.

Results in Figure 4.15 show that the adsorption capability of Chlorella is comparably close to activated carbon when the adsorbate is Methylene Blue. The decrease in removal percentage between freshly prepared dye solution and other conditions is not negligible. It is clearly seen that MB dye stock solutions for biosorption experiments should be prepared freshly, to obtain best removal efficiencies. This conclusion has reflections on real aqueous chemical pollutants where the aging time of the dissolved aqueous pollutant may become more difficult to remove by biosorption based techniques. Nevertheless, under all storage conditions, activated carbon eliminated aqueous MB dye molecules with high efficiency.

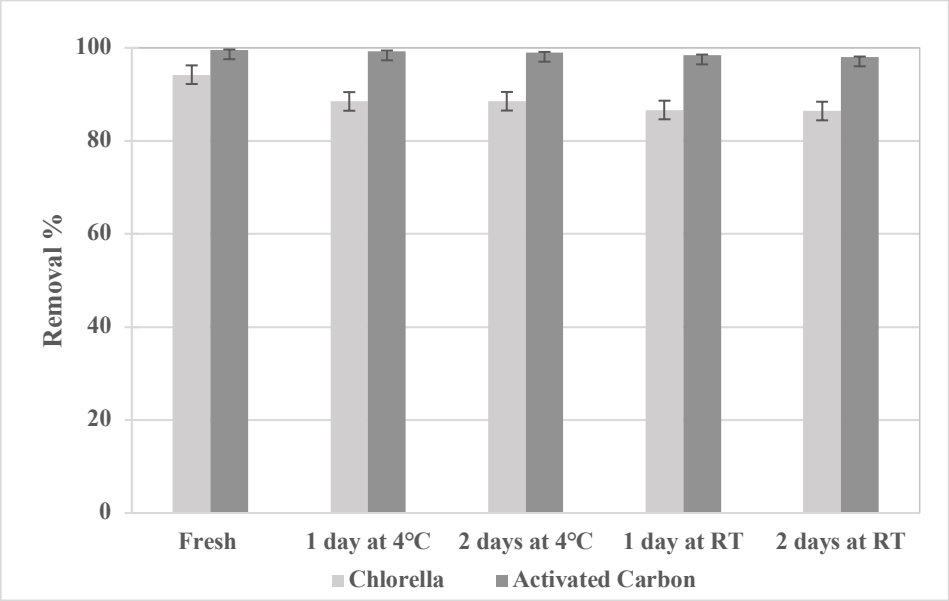


Figure 4.15. MB removal percentages at different storage conditions of dye stock solution.

4.3.2. Effect of Buffer on Methylene Blue Dye Removal

One of the spectator ions which might have an effect on the biosorption mechanism between adsorbate and adsorbent is buffering molecule. Phosphate buffer was examined in previous sections about its influence on the adsorption outcomes between the selected adsorbents, *C. Vulgaris* and activated carbon and selected dyes. Phosphate ion carries a negative charge on its oxygens, and it has the potential to interact with ionic dye molecules or adsorbent surfaces, which may possibly lead to alterations in the biosorption process. The effect of the anionic phosphate molecule on the removal of MB by Chlorella and activated carbon will be presented in this section.

In Table 4.18, decolorization of MB solution by Chlorella in the absence of the buffering agent in the solution is seen to be slightly higher than in the presence of the buffering agent in MB solution. Chlorella adsorbed 90.6% of MB dye molecules from the solution with the buffering agent while dye solution without buffer had a dye removal of 91.8% by Chlorella.

Table 4.18. Effect of sodium phosphate buffering agent on adsorption measured at 665 nm (MB (+): with buffer, MB (-): without buffer).

Buffer Status	C_0 (mg/L)	Removal % by Chlorella	Removal % by AC
MB (+)	13.6	90.6	98.5
MB (-)	13.6	91.8	96.4

The gap between the results of MB (+) and MB (-) can be considered insignificant and following batch experiments were all included the phosphate buffering agent in the medium as the buffer brings convenience to adsorption experiments by allowing stability of solution pH. In the case of Congo Red (Section 4.1.2.), there was a big difference between the presence and absence of the buffer in the solution. CR is a bigger dye molecule than MB (Figure 1.2) and a certain configuration of CR was adsorbed by 64.6% by Chlorella at standard conditions (Table 4.1). With CR dye, the presence of a small amount of phosphate buffering agent may have helped the mechanism positively because it probably stabilized a favorable form of Congo red rendering it more adsorbable towards Chlorella. On the other

hand, MB is adsorbed much more effectively than CR by Chlorella and the presence or absence of phosphate ions do not change the adsorption behavior of MB dye molecules onto the microalgae. Additionally, existence of phosphate ions in the dye solution did not show any significant effect on the MB dye uptake by AC, as it was the case in Congo Red and Methyl Orange dyes.

4.3.3. Effect of Initial Methylene Blue Dye Concentration on Removal

Number of dye molecules in an aqueous solution, i.e. the initial dye concentration is one of the most important factors affecting the dye uptake of the adsorbent. In this section, various initial concentrations were used to unleash the effect of initial concentrations on dye removal by microalgae species *C. Vulgaris* and Scenedesmus, and activated carbon, as a standard industrial adsorbent.

Bioremoval of Methylene Blue from the solution by Chlorella and Scenedesmus were 95.9% and 84.9% with the initial concentration of 3.4 mg/L, respectively. Activated carbon was the most successful adsorbent among all the initial concentrations with removing 99.8% of MB dye from the medium at C_0 of 3.4 mg/L (Table 4.19).

Table 4.19. Methylene Blue removal percentages at different initial concentrations, measured at 665 nm and pH 7.

C_0 (mg/L)	Removal % by Chlorella	Removal % by AC	Removal % by Scenedesmus
3.4	95.9	99,8	84.9
6.8	94.6	99,6	84.3
10.2	93.6	99,6	83.5
13.6	92.0	98.5	83.0
20.4	90.7	98.0	82.2
34.0	90.5	96.2	80.0
102.0	89.5	90.0	65.3

When the number of dye molecules was about 30 times higher than the 3.4 mg/L solution, decolorization percentage of MB solution by Chlorella dropped from 95.9% to 89.5% while a decrease was observed from 99.8% to 90.0% for the removal of MB by

activated carbon. These results show that, Chlorella can be as successful as AC in adsorptive removal from concentrated MB dye solutions. As seen in Figure 4.16, removal percentages of Chlorella and AC at the initial concentration of 102.0 mg/L is almost equal. On the other hand, Scenedesmus had the lowest dye removal yields when compared to Chlorella and AC for selected initial dye concentrations.

Initial dye concentration of the solution determines the mechanism of adsorption onto biosorbents. Dye molecules surrounds around the adsorbent with the interactions such as H-bonds, or ionic electrostatic interactions [97]. The reason of exhibiting lower removal percentages for Scenedesmus was discussed in the Section 4.1.3 and explained as that it does not consist of glucosamine derivatives in its cell wall structure which may decrease the variety of intermolecular interactions. As seen in Figure 4.16, microalgal biosorbent *C. Vulgaris* had a competing adsorptive capacity towards MB dye solution in comparison to AC, especially in concentrated solutions (102.0 mg/L). Since surface of Chlorella is prone to be negatively charged, the cationic dye MB was successful to be adsorbed by the microalgae. AC is industrially produced adsorbent, and it has various functional groups on its structure to conduct effective interactions with various adsorbates [97]. Here, it can be seen that a natural alternative of AC is comparably successful to remove the MB dye molecules from water, especially in high dye concentrations.

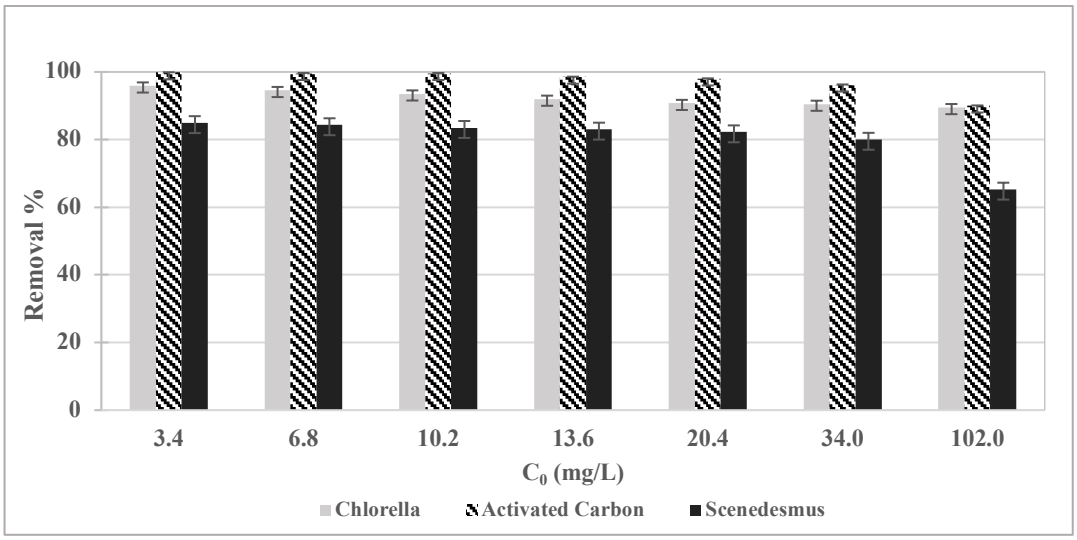


Figure 4.16. Removal of Methylene Blue by various adsorbents plotted against initial dye concentrations (Measured at 665 nm).

4.3.4. Effect of pH on Methylene Blue Dye Removal

In aqueous solutions, pH stabilization is an essential factor in evaluating the adsorption results accurately. Since adsorption mechanism leans on the charge interaction between the functional sites of the adsorbates and the adsorbents, every fluctuation that might change the ionic composition of the dye solution needs to be considered such as the effect of the efect of a buffering agent.

Table 4.20. Methylene Blue removal percentages by Chlorella and activated carbon at different pH levels, measured at 665 nm at constant initial concentration of 13.6 mg/L

Chlorella		**Activated Carbon**	
~ pH	**Removal %**	**~ pH**	**Removal %**
5.0	84.2	6.5	99.3
6.0	89.6	7.0	98.5
7.0	92.5	8.0	98.2
8.0	92.3	9.0	97.7

Methylene Blue dye solutions were adjusted to the pH values of 5.0, 6.0, 7.0, and 8.0 for the biosorption with Chlorella by carefully checking the absence of any precipitations at these pH levels. Congo red and Methyl orange zwitterionic dyes had an increasing trend on removal percentages as the value of pH was lowered. (Section 4.1.4 and 4.2.4) Here, the cationic dye, Methylene Blue, showed a different trend where its uptake percentages by Chlorella increased with the rise in the pH values. Decoloring of MB dye solution by Chlorella was 84.2% at pH 5.0 while removal of 92.3% MB dye molecules was achieved at pH 8 (Table 4.20). Removal percentages of MB by activated carbon in relation to pH level showed a very small pH effect with an inversely proportional relationship. As the basicity of the dye solution-AC suspension was increased from the pH of 6.5 to 9.0, removal percentage of AC decreased from 99.3% to 97.7%. Towards the pH value of 8.0, the MB dye uptake by Chlorella and AC almost converge at the removal percentage of 95.0 approximately (Figure 4.17).

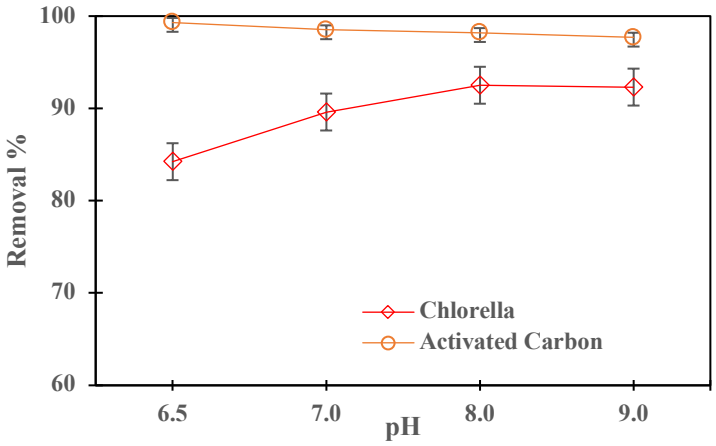


Figure 4.17. Trend of pH effect on MB adsorption by Chlorella and activated carbon.

4.3.5. Effect of Biosorbent Dosage of Chlorella on Methylene Blue Dye Removal

An alternative representation for the dye removal capability of adsorbents is adsorption capacity. It gives the number of removed dye molecules per unit mass of the biosorbent or adsorbent (mg/g).

0.01, 0.05, 0,10, and 0.25 grams of Chlorella was used in biosorption experiments of this section. In addition to decolorization percentages of Methylene Blue, adsorption capacities of different microalgal doses with MB solution were calculated for each dye removal test (Table 4.21).

Table 4.21. Removal percentages (%) and adsorption capacities (q) of MB at different biosorbent dosages of Chlorella at pH 7.0 and C_0 of 13,6 mg/L.

Biosorbent Dosage (g/10 mL)	Removal %	Adsorption Capacity (mg/g)
0.01	92.0	12.1
0.05	96.7	2.6
0.10	98.4	1.3
0.25	98.9	0.5

Removal percentage of MB by Chlorella had an increasing trend as the quantity of microalgal dose was increased since available sites for the adsorbate molecules onto the adsorbent was increased providing better attachment. However, adsorption capacity of Chlorella had an inverse proportion as the amount of biomass rises within the same volume of test flask (Figure 4.18). Overall results of the biosorbent dosage effect on MB dye removal were parallel with the previous cases of dyes Congo Red and Methyl Orange. Similarly, the reverse relation between adsorption capacity and microalgal dose may arise from the insufficient active collision between dye molecules and biomass under fixed initial dye concentration and volume of aqueous medium.

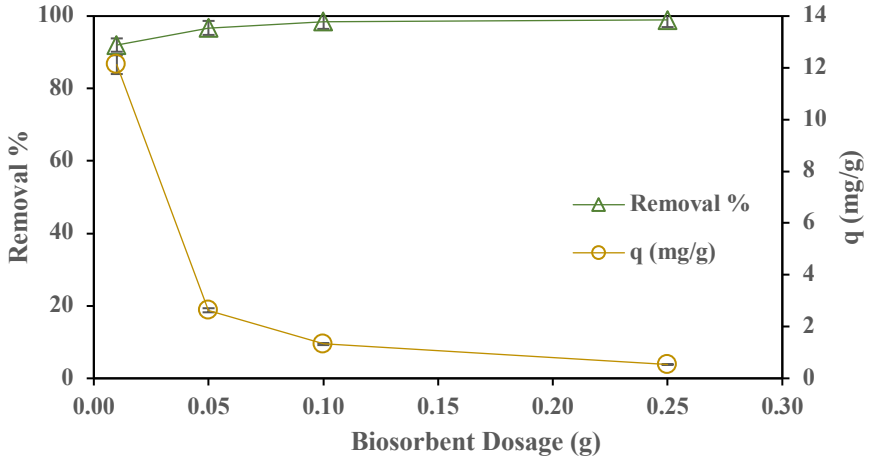


Figure 4.18. Trend of biosorbent dosage of Chlorella on adsorption capacities (q) and removal percentages (%).

4.4. Adsorption of Eriochrome Black T Dye by Chlorella and Activated Carbon

4.4.1. Effect of Storage Temperature and Duration of Eriochrome Black T Stock Solution on Subsequent Dye Adsorptions

Eriochrome Black T (EBT) is classified as an anionic azo dye with nitro, sulphonate, and hydroxy moieties (Figure 1.2d). Environmental conditions (light and temperature) may change the structural behaviors of the conjugated system of this dye. Here, the effect of storage conditions of aqueous EBT dye stock solution on adsorption outcome was revealed

by tracking the changes in the removal percentages of EBT dye solutions at a fixed amount of initial dye concentration and pH of the solution.

Table 4.22. Removal percentages (%) of Eriochrome Black T at different storage durations and temperatures of corresponding stock solution, measured at 623 nm, at pH 7.

Biosorbents	**Fresh**	**1 day at 4°C**	**1 day at RT**	**3 days at 4°C**	**3 days at RT**	**5 days at 4°C**	**5 days at RT**
Chlorella	77.6	73.6	62.5	70.9	45.2	61.6	40.7
Activated Carbon	85.7	80.7	75.5	75.1	50.8	50.0	-

In Table 4.22, adsorption removal of Eriochrome Black T is shown to be determined as 77.6% by Chlorella and 85.7% by activated carbon, when the stock solution of the dye was prepared and used freshly. It is noted that even for a freshly prepared stock solution, EBT dye uptake by AC was not as high as the removal of other four dyes in this study. After the dye stock solution was kept at 4°C (refrigerator) for 1 day, removal results were shifted to 73.6% for Chlorella and 80.7% for AC. The decreasing trend in the removal percentages of EBT by Chlorella continued down to the lowest result, 40.7%, when the dye stock solution was stored at room temperature (RT) for five days. AC dye removal effectiveness was also observed to be decreasing down to 50.0% for the dye stock solution stored at 4°C for five days. In a final experiment, a two months aged EBT stock solution was tested and the removal percentage by Chlorella was near 40%, similar with the result of adsorption using the stock solution kept at room temperature for 5 days.

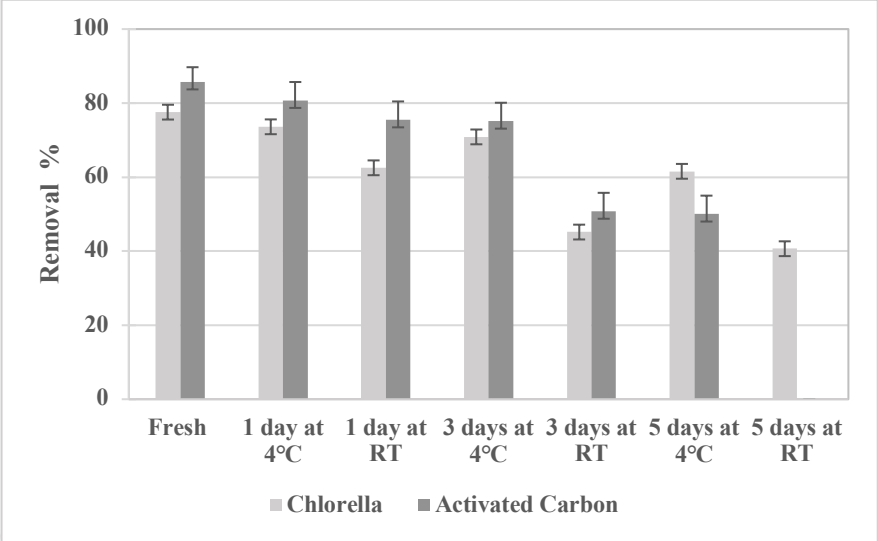


Figure 4.19. EBT removal percentage at different storage conditions of dye stock solution.

Influence of the stock solution's storage time and temperature on EBT decolorization from the solution demonstrated that EBT stock solution should be used freshly for best results in adsorption tests (Figure 4.19). The drop in the removal percentages of EBT from freshly prepared to aged dye stock solutions was greater than that of other dyes. The reason might be structural changes on dye molecules by time and temperature. An Eriochrome Black T azo dye molecule carries a nitro group ($-NO_2$) substituted to a benzene ring (Figure 1.2d). The only nitro substituent on conjugated dye molecule was on EBT among other selected dyes in this study. Nitro groups are known as their strong electron withdrawing substituents on aromatic moieties [98,99]. Whether it is due to this aspect related to the possibility of structural changes on the nitro moiety or not there is a considerably strong effect of storage time and temperature on the adsorption of EBT, which is not mentioned in any literature report to the best of our knowledge. Below, some spectral changes in the UV spectra of EBT was given in terms of the shifts in the wavelengths and absorbance values of the solutions containing only 13.6 mg/L dye liquor, measured for different storage time and temperature (Table 4.23).

Table 4.23. Peaks (nm) and absorbance values from the full- scale UV spectra of fresh and aged EBT dye solutions with 13.6 mg/L initial concentration and pH 7

Fresh		3 days at 4°C		3 days at RT	
Peak(nm)	**Absorbance**	**Peak(nm)**	**Absorbance**	**Peak(nm)**	**Absorbance**
623	0.332	624	0.304	616	0.280
440	0.164	440	0.144	440	0.150
340	0.290	340	0.292	340	0.320

4.4.2. Effect of Buffer on Eriochrome Black T Dye Removal

To find the optimal condition for the adsorption tests of Eriochrome Black T, the effect of the use of phosphate buffering agent was examined. Ionic molecules in the dye solution might possibly have an impact on the adsorption mechanism between the adsorbate and the adsorbents.

Table 4.24 Effect of phosphate buffering agent on EBT dye biosorption, measured at 623 nm and at pH 7 (EBT (+): with buffer, EBT (-): without buffer).

Buffer Status	C_0 (mg/L)	Removal % by Chlorella
EBT (+)	13.6	72.0
EBT (-)	13.6	70.7

Bioremoval of Eriochrome Black T by *C. Vulgaris* was 72.0% when the phosphate buffering agent is in the test solution whereas EBT uptake by the biomass was 70.7% in the absence of the buffering agent in the dye-microalgae solution (Table 4.24). pH, biosorbent dosage, and initial concentrations were kept constant while presence of the buffer compound was changing. The disparity between the removal results of EBT (+) and EBT (-) was insignificant; thus, no distinct undesirable condition was acquired in the existence or extinction of the buffering agent. Phosphate buffer was continued to be applied in dye adsorption tests to provide pH stability throughout experiments.

4.4.3. Effect of Initial Eriochrome Black T Dye Concentration on Removal

Along with a fixed volume of aqueous medium and adsorbent dosage, multifarious number of dye molecules in the test solution amends the removal of Eriochrome Black T by, *C. Vulgaris* and activated carbon. In this section, effect of initial EBT dye concentration on adsorption was examined by diluting the freshly prepared concentrated stock solution (136 mg/L) into concentrations of 2.0, 3.4, 5.4, 13.6, 34.0, 102.0 mg/L.

Table 4.25. Eriochrome Black T removal percentages at different initial dye concentrations, measured at 623 nm and pH 7.

C_0 (mg/L)	Removal % by Chlorella	Removal % by AC
2.0	95.2	93.2
3.4	95.1	92.3
5.4	90.9	90.1
13.6	73.9	85.4
34.0	56.1	83.3
102.0	40.0	78.0

As expected, the removal percentage of EBT by adsorbents is decreasing whilst the initial concentration of the solution is increasing. Results demonstrated that percentage of dye uptake by Chlorella declined from 95.2% to 40.0% by increasing the initial concentration from 2.0 to 102.0 mg/L. When AC was the adsorbent in the experiments, percentage of EBT removal dropped from 93.2% to 78.0% for the same increase of the initial number of dye molecules (Table 4.25). Decolorization of the anionic dyes by activated carbon from the solution was just slightly better than removals by Chlorella at diluted initial concentrations, 2.0, 3.4, and 5.4 mg/L.

The gap between the removal percentages of EBT by the AC and Chlorella from the solution was higher in concentrated solutions than in diluted solutions. In Figure 4.20, the gray column represents the adsorption by AC. The first three value of initial dye concentrations in the solution indicates that Chlorella has the potential to compete with the adsorptive behavior of AC in the case of a possible remediation of EBT polluted water.

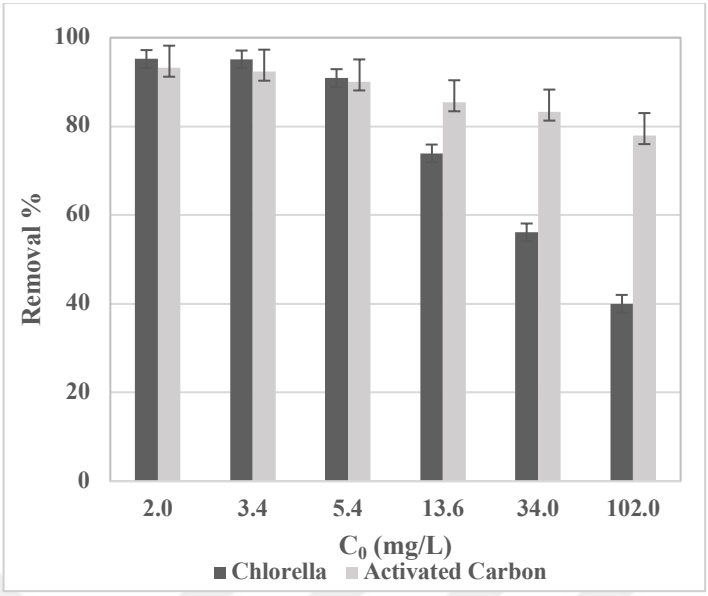


Figure 4.20. Removal of EBT plotted against various initial dye concentrations, measured at 623 nm.

As in the Section 4.3.3, remediation of cationic Methylene Blue containing water, by Chlorella and AC was investigated for the effect of the changing initial dye concentration. In Figure 4.16, the value for MB dye uptake behavior of AC and Chlorella were close to each other in concentrated solutions (102.0 mg/L), a reversed trend was observed in the case of EBT. Removal effectiveness of Chlorella and AC against EBT dye is comparable with each other in lower initial dye concentrations. This can be a practically relevant point because dyes are more commonly found at fairly low concentrations, as micropollutants in aqueous environments, and wastewater effluents. Therefore, analysis of lower dye concentrations via UV/VIS spectroscopy was performed in order to determine the lowest limit of detection (LOD) of the instrument, by measuring the absorbance of the dye solutions with concentrations from 0.24 to 3.4 mg/L (Table 4.26).

In the UV spectrum of Eriochrome Black T (Figure 4.1d) solution, three peaks were seen in the 300-700 nm spectral range, which are at almost 340, 440, and 623 nm. The maximum wavelength of the EBT dye solution is assigned as 623 nm where batch removal experiments for EBT adsorption were conducted through measurements at that wavelength [100].

Table 4.26. Dye concentrations and their relevant absorbance values at two peak absorbances in the EBT spectra (Italics represents the undetectable concentration).

Concentration (mg/L)	**Absorbance at 623 nm**	**Absorbance at 340 nm**
0.24	*0.000*	*0.019*
0.34	0.005	0.022
0.68	0.011	0.028
1.36	0.024	0.043
3.40	0.065	0.082

In the range of 0.24 to 3.40 mg/L, a set of low concentration solutions were analyzed (Table 4.26). The maximum peak of EBT dye solution at 623 nm was undetectable at the concentration of 0.24 mg/L. Increasing the dye concentration from 0.34 to 0.68 mg/L, the absorbance values were also duplicated meaning that Beer- Lamberts law was obeyed. Therefore, detection limit of the instrument was near the concentration value of 0.34 mg/L. In Table 4.25, the minimum initial dye concentration for the experiments was assigned as 5.4 mg/L because after achieving 90.9% removal of EBT dye by Chlorella and 90.1% for AC, the final concentrations analyzed by the instrument was almost 0.5 mg/L which falls within the LOD interval. That's why, the removal results at the initial dye concentrations of 2.0 and 3.4 mg/L were found by extrapolation of the calibration curve.

Table 4.27. Eriochrome Black T removal percentages at different wavelengths.

	Removal % by Chlorella	
C_0 (mg/L)	**620 nm**	**629 nm**
2.0	95.2	95.1
3.4	95.1	94.9
13.6	75.5	75.2
34.0	55.0	54.9

The maximum wavelength of EBT dye solution shifted from 620 nm to 629 nm in some specific cases. Before every experiment, λ_{max} was determined by scanning the solution

and corresponding maximum wavelength was used for that set of experiments. Therefore, the removal percentage of EBT by Chlorella at different initial dye concentrations were measured at different corresponding maximum wavelengths, namely at 620, and 629 nm (Table 4.27). Results show that fluctuations on the light absorbance behavior of EBT dye did not have an effect on the removal percentages by Chlorella. Calibration curves were constructed by the absorbance values of solutions with different concentrations at given wavelengths on Table 4.27. Trendlines coming from the absorbance measurements of dye solutions at 620 and 629 nm are in the same direction as seen in Figure 4.21.

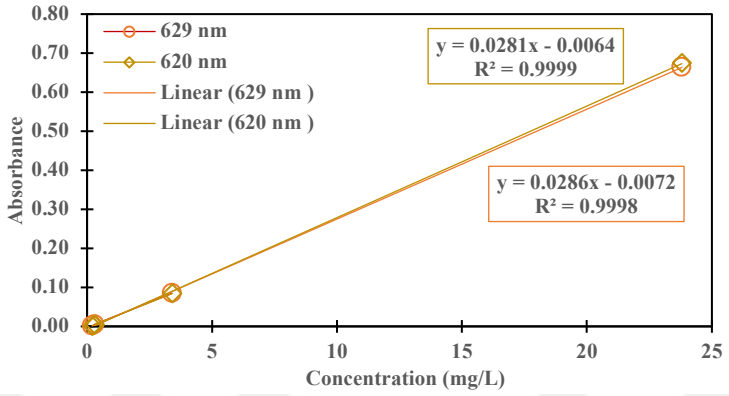


Figure 4.21. Calibration curve of EBT at its peaks in UV spectra.

4.4.4. Effect of pH on Eriochrome Black T Dye Removal

Eriochrome Black T is an indicator with dark blue color solid powder form. In aqueous solution, its color is blue at pH of 7 or higher. In the presence of Ca^{+2} and Mg^{+2} ions it turns into red color by complexation with these ions. In adsorption experiments, deionized water was used to prevent such complexation in the solution, however it was observed that after the centrifugation part of the adsorption experiments in the presence of Chlorella biomass, the color of the supernatant was red with a pH of 7 or higher. This could mean that microalgae biomass may release ions that EBT forms complexes with, which brings great difficulty for the concentration measurements as actual absorbance values of supernatants shift considerably, along with light absorbance characteristics of dye molecules. In order to prevent such troublesome complexation of the EBT with ions originating from biomass, EDTA (Ethylenediaminetetraacetic acid) was added into the supernatants resulting from

adsorption experiments, to chelate the ions in the solution and release EBT molecules back to the solution. The quantity of EDTA added into the supernatants was taken into account as dilution factor at final calculations. Here, the effect of pH on dye removal was studied based on the pH adjustments during adsorption experiments. On the other hand, the pH was recorded to be around 10 due to the addition of EDTA, which was performed after the experiments when supernatant was separated from the adsorbent biomass.

Table 4.28. EBT removal percentages at different pH levels and at constant initial concentration (13.6 mg/L).

Chlorella		**Activated Carbon**		**λ_{max}(nm)**
~ pH	**Removal %**	**~ pH**	**Removal %**	
3.0	95.9	3.5	95.7	629
6.0	92.8	4.5	93.4	620
7.0	77.2	7.0	84.4	620
8.0	73.7	9.0	69.5	620
11.0	52.3	10.0	55.1	557

As an industrially produced adsorbent, activated carbon showed a removal percentage higher than 90-95% at the pH value of 7 in the adsorption experiments with Congo Red, Methyl Orange, and Methylene Blue dyes. Here, EBT dye uptake by AC was 84.4% at pH 7 and it decreased with increasing pH of the solution whilst acidic environment boosted the adsorption of EBT by AC up to 95.7% (Table 4.28). The absence of any possible precipitations of the dye were checked in every set of experiments.

As a natural biosorbent, *C. Vulgaris* showed an increasing trend in removal percentages by elevation in the acidity of the suspension. At the pH value of 3.0, microalgae adsorbed 95.9% of dye molecules from the solution whereas under basic conditions down to pH of 11.0, EBT removal by Chlorella was as low as 52.3%.

As the basicity of the dye solution is increased, EBT dye molecules are expected to convert into their deprotonated structure. Functional sites of the adsorbents were also deprotonated and so that electrostatic repulsions may be expected to occur, resulting in the

lower removal effectiveness of the adsorbents, where almost 50% of the EBT dye remain non-adsorbed. In Figure 4.22, dye uptake trends of adsorbents are clearly altered depending on pH level, but both curves show decreasing removal with increasing pH.

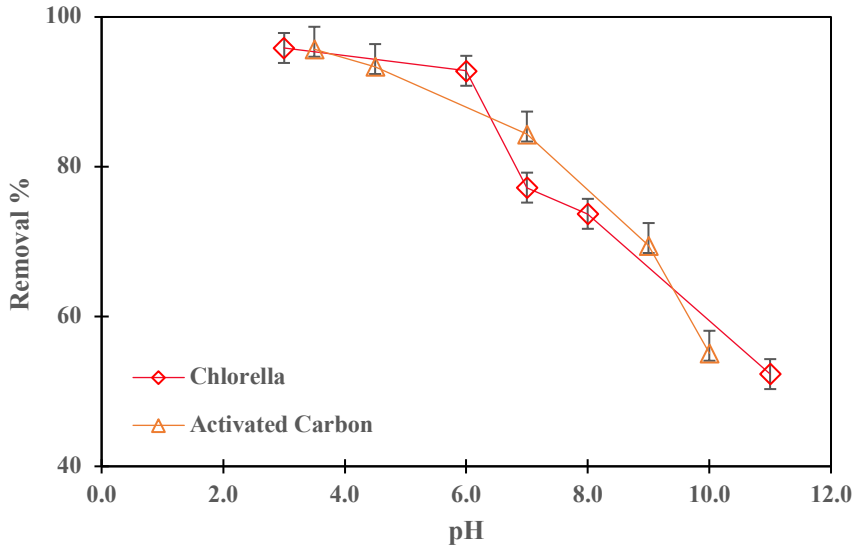


Figure 4.22. Removal percentages of EBT by Chlorella and AC at various pH values at a fixed concentration (13.6 mg/L).

4.4.5. Effect of Biosorbent Dosage of Chlorella on Eriochrome Black T Dye Removal

As mentioned before, adsorption mechanism for those dyes and adsorbents employed in this study is expected to occur through electrostatic interactions. Any change in the aqueous test medium have the potential to affect this process, therefore in this section alterations on microalgal dose was studied by keeping all other variables constant.

Biosorption of the anionic dye EBT from the aqueous solution was 73.9% for Chlorella and it reached to a 100% complete removal when the microalgae dose was 0.25 grams. At that dosage, adsorption of the dye by the unit of biomass was decreased from 8.3 to 0.5 mg/g (Table 4.29). As seen in the Figure 4.23, removal percentage and adsorption capacity has an inverse proportionality as it would be expected. This trend may possibly arise from the blockage of available adsorption sites as the aggregation of microalgae is expected to more prevalent at higher dosages of suspended biomass, as mentioned in Sections 4.1.5 and 4.2.5.

Table 4.29. Removal percentages (%) and adsorption capacities (q) of EBT at different Chlorella dosage at pH 7 and C_0 of 13,6 mg/L.

Biosorbent Dosage (g/10 mL)	Removal %	Adsorption Capacity (q)
0.010	73.9	8.3
0.025	98.5	5.4
0.050	99.6	2.9
0.100	99.8	1.3
0.250	99.9	0.5

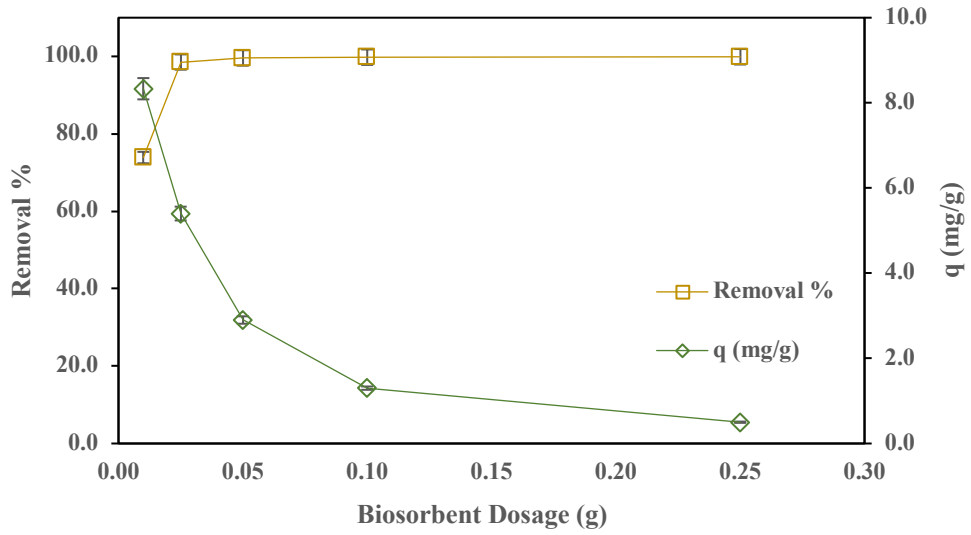


Figure 4.23. Adsorption capacity, q (mg/g), and removal percentage at a constant initial concentration (13.6 mg/L).

4.5. Adsorption of Isolan Dark Blue by Chlorella and Activated Carbon

4.5.1. Effect of Storage Temperature and Duration of Isolan Dark Blue Stock Solution on Subsequent Dye Adsorptions

Isolan Dark Blue 2-SGL-03 (Acid Blue 193) is a 1:2 metal complex with di-sulphonated dye (Figure 1.2e). In comparison to the other four dyes of the study (CR, MO,

MB, and EBT), there is a very limited number of adsorption studies involving IDB and more specifically there is not any literature reports related to the adsorption of IDB by Chlorella, to the best of our knowledge. Here, results of the study about the effect of storage conditions of aqueous IDB stock solution on subsequent dye removal by adsorption onto the Chlorella *Vulgaris* and activated carbon will be discussed.

Firstly, removal of Isolan Dark Blue from aqueous solution by Chlorella was tested against it different storage conditions. Its removal was found to be 42.6% by Chlorella and 80.2% by AC when a freshly prepared dye stock solution was used in adsorption experiments.

Table 4.30. Removal percentages (%) of Isolan Dark Blue at different storage durations and temperatures of corresponding stock solution, measured at 577 nm, at pH 7, with 1% sodium phosphate buffer.

Biosorbents	**Fresh**	**1 day at 4°C**	**2 days at 4°C**	**1 day at RT**	**2 days at RT**
Chlorella	42.6	41.4	38.5	40.6	40.2
Activated Carbon	80.2	77.5	77.1	71.5	70.4

Among all five dyes of the study, the lowest removal percentages obtained by AC were recorded with IDB dye. As the age and temperature of dye stock solutions were changed, the removal percentage by Chlorella decreased from 42.6% to 38.5%, the latter being the stock solution kept at 4°C for two days.

Additionally, dye uptake by AC decreased from 80.2% to 70.4% as the dye stock solution was kept at room temperature for two days (Table 4.30). When the dye stock solution was stored at refrigerator (4°C) for one or two day(s), IDB dye uptake by AC changed about 3% while storing the stock solution at room temperature for one or two day(s) prior to adsorption tests by AC resulted in 10% decrease when compared to adsorption experiments done with freshly prepared stock solution.

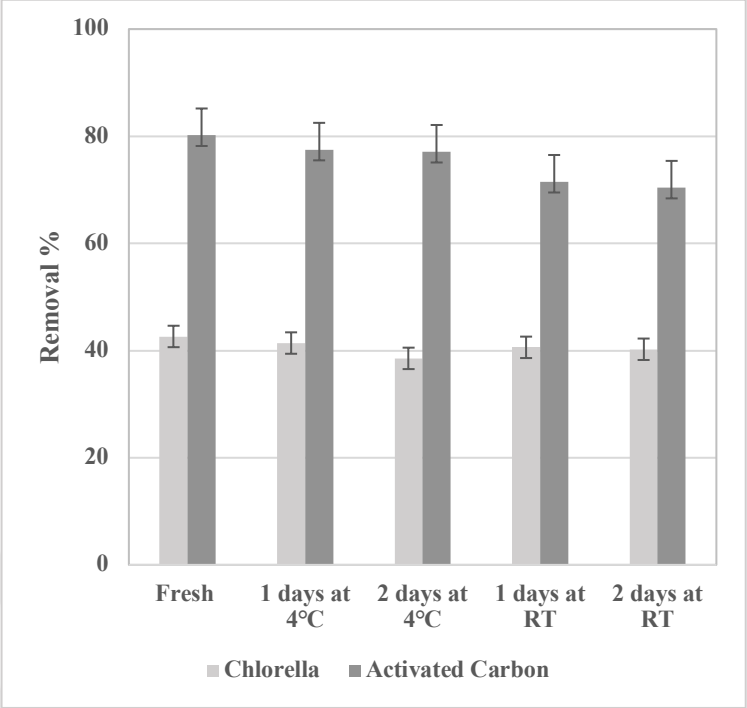


Figure 4.24. IDB removal % by Chlorella and activated carbon for dye solutions stored at different conditions.

However, IDB removal results by Chlorella was decreased about 1-2% when the concentrated dye stock solution stored at room temperature or 4°C for one or two day(s). Also as seen in Figure 4.24, removal results of AC were more influenced by fluctuations on storage conditions than Chlorella.

4.5.2. Effect of Buffer on Isolan Dark Blue Dye Removal

Solutions of the anionic dye, Isolan Dark Blue, were prepared with and without the sodium phosphate buffering agent in the solution and tested in adsorption experiments with Chlorella and activated carbon.

As seen in Table 4.31, IDB dye uptake by Chlorella in the presence of the buffering agent in the solution was determined to be 40.4% while it was 51.4% in the absence of the buffering agent in the test media. Phosphate ions might interfere the biosorption of IDB possibly by hindering electrostatic interactions between the anionic dye and Chlorella. On

the other hand, dye removal of AC was altered from 81.2% to 69.7% when the buffering agent was not used (Table 4.31)

Table 4.31. Effect of sodium phosphate buffering agent on adsorption measured at 578 nm (IDB (+): with buffer, IDB (-): without buffer).

Buffer Status	**C_0 (mg/L)**	**Removal % by Chlorella**	**Removal % by AC**
IDB (+)	13.6	40.4	81.2
IDB (-)	13.6	51.4	69.7

The number of dye molecules (13.6 mg/L) and the pH (7.2) in the aqueous media were kept constant. The existence of phosphate buffering agent had opposing effects on IDB dye removal by Chlorella and activated carbon. Buffering agent in the solution increased the dye uptake by Chlorella whereas absence of the phosphate molecules in the media suppressed the IDB dye removal by activated carbon (Figure 4.25).

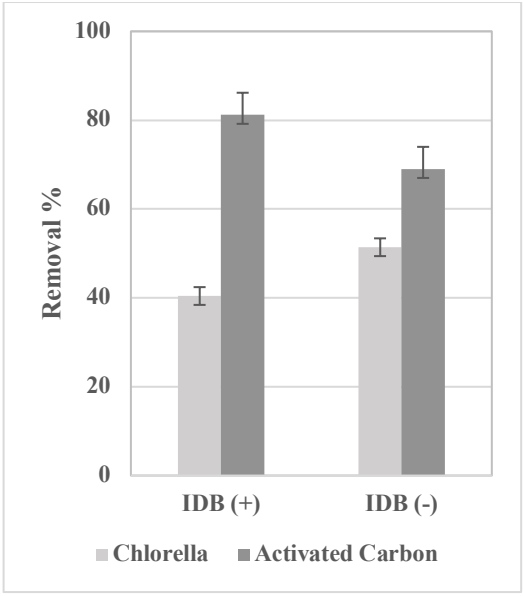


Figure 4.25. Effect of the presence of sodium phosphate buffering agent on IDB dye removal.

The absence of buffer causing a decrease in the dye uptake by AC was an unexpected result considering previous results obtained with the four other dyes of the study. Additionally, the adsorption of IDB, in the presence of buffer in the media, was not as superior as adsorptions of other dyes by activated carbon. As a result of these findings, following experiments in this section were performed in the absence of the buffering agent, as Chlorella performed better biosorption without the sodium phosphate buffer in the media.

4.5.3. Effect of Initial Isolan Dark Blue Concentration on Dye Removal

Concentration of dye molecules at the beginning of the adsorption process determines the percentage of dye removal following different trends. In adsorption experiments of this section, aqueous solutions with 3.4, 6.8, 13.6, 23.8, and 34.0 mg/L initial IDB concentrations were prepared by diluting the stock solution (68 mg/L) for monitoring the effect of initial dye concentration on IDB removal.

Table 4.32. Removal percentages (%) of IDB and adsorption capacities (q) of adsorbents at different initial concentrations, measured at 577 nm and pH 7.0, in the absence of buffering agent.

C_0 (mg/L)	Removal % by Chlorella	Removal % by Activated Carbon	$q_{Chlorella}$ (mg/g)	$q_{Activated\ Carbon}$ (mg/g)
3.4	74.7	41.8	2.2	1.4
6.8	66.5	58.9	3.8	3.8
13.6	50.5	68.5	5.5	9.3
23.8	43.6	(71.1)	9.8	17.1
34.0	41.0	(70.2)	11.8	8.3

The result of IDB dye uptake by Chlorella increased from 41.0% to 74.7% as the initial concentration was decreased from 34.0 to 3.4 mg/L (Table.32). The trend of removal results by Chlorella against different initial concentrations was similar to results with other dyes, in other words IDB removal percentages by Chlorella increased as the number of the molecules in the dye solution-microalgae suspension was decreased. In more concentrated IDB solutions removal became gradually less effective against increasing number of adsorbate.

Surprisingly, IDB dye removal results by activated carbon showed a different trend than in other dyes, which is a decreasing trend of removal percentages from 71.1% to 41.8% as the initial dye concentration was decreased from 23.8 to 3.4 mg/L. Any possible concentration induced aggregation might play a role in increased adsorption of IDB onto AC. When the initial concentration of IDB gets larger, increasing dye-dye interactions may lead to specific molecular orientations of dye molecules, which may also lead to possible molecular aggregations, favoring the adsorption by AC. At initial concentrations larger than 23.8 mg/L, decolorization of the dye by AC declined from 71.1% to 70.2%, or it can be considered to remain approximately same. A decline on removal by increasing concentrations can be expected because a fixed quantity of the adsorbent dosage would have a certain capacity to adsorb dye molecules until it reaches a saturation of the adsorbing sites (Figure 4.26). Additionally, the minimum concentration for the detection of absorbance of IDB dye molecules via UV/VIS spectrophotometer was determined to be 0.68 mg/L.

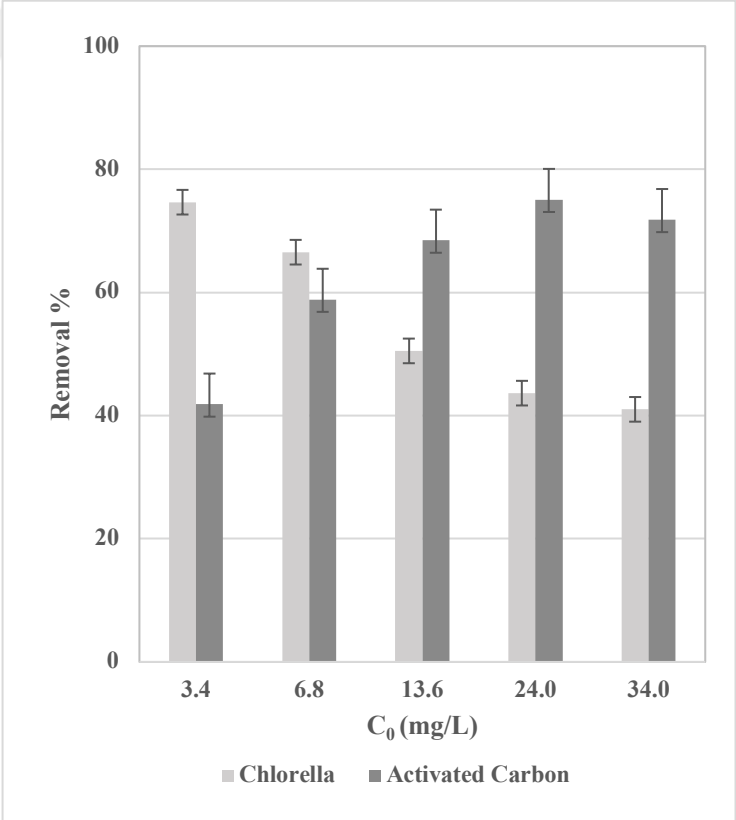


Figure 4.26. Removal percentages at different initial dye concentrations.

4.5.4. Effect of pH on Isolan Dark Blue Dye Removal

Changes in pH level have the potential to change the adsorption interaction between the adsorbate and the adsorbent in an aqueous solution. Since pH of the solution affects the charges and functionalities of ionic moieties, the effect of pH on Isolan Dark Blue (IDB) removal was examined. IDB dye solution has 577 nm as the maximum wavelength in the UV absorption spectrum. Changing the pH of the solution did not show any significant shift in λ_{max} in UV adsorption spectrum of IDB, in accordance with two literature examples [101,102] whereas shifts in λmax were observed in experiments with each one of the other four dyes in the study.

Table 4.32. IDB removal percentages at different pH levels, measured at constant initial concentration (13.6 mg/L) and in the absence of buffering agent.

Chlorella		**Activated Carbon**	
~ pH	**Removal %**	**~ pH**	**Removal %**
4.0	94.4	6.0	57.1
5.0	86.3	6.5	63.0
7.0	51.4	7.0	70.3
8.0	7.3	7.5	74.2
9.0	5.9	9.0	67.9
		9.5	67.1
		10.0	58.0

Biosorption of Isolan Dark Blue by Chlorella biomass decreased drastically from 51.4% to 5.9% when basicity of the suspension is increased from the neutral pH value to 9.0. From pH 4.0 to 7.0, there is also a decrease in removal from 94.4% to 51.4% (Table 4.33). Adsorption of IDB by activated carbon does not have drastic changes with changing pH as in the cases of other four dyes. It reaches its maximum dye decolorization extent at ph 7.5. In all adsorption tests, dye solutions were used in the absence of a buffering agent, otherwise dye removal by AC would be expected to reach higher removals according to results on Table 4.24. Near pH 6.5 both adsorbents seem to have approximately same removal effectiveness (Figure 4.27). Highest IDB removals were observed at pH 4.0 and 5.0 by

Chlorella, at the range of 86-95%, an extent that was not possible to reach using AC at any pH value tested.

Structure of IDB dye consists of two chelating agents forming complex with Cr (III) cation (Figure 1.2e). Acidic pH is expected to drive the doubly sulphonated dye towards protonation which may then favor the adsorption by the microalgae as the protonation of ionic sites can reduce the water solubility of molecules, also minimizing possible anion-anion repulsions. pH induced electrostatic interaction between IDB and microalgae is maximized at lower pH values. Similar observations were seen in literature reports [103–106].

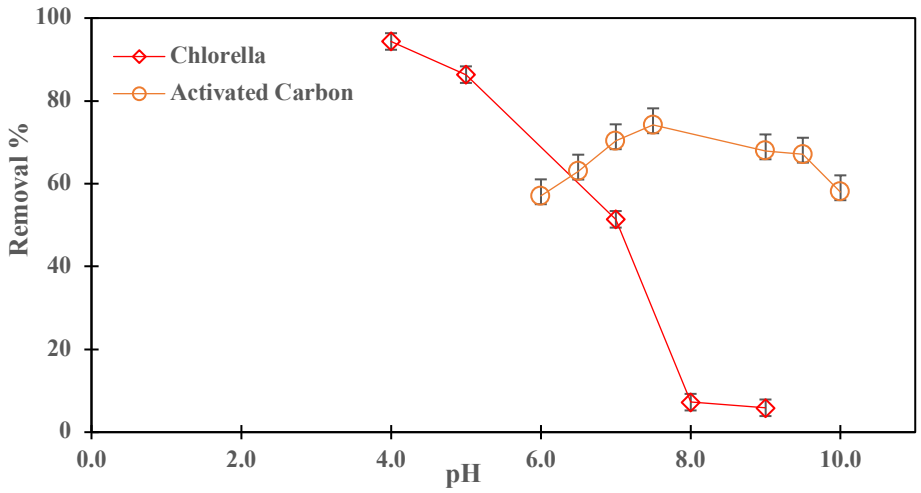


Figure 4.27. IDB removal percentages by Chlorella and activated carbon at different pH values.

4.5.5. Effect of Biosorbent Dosage of Chlorella on Isolan Dark Blue Dye Removal

Isolan Dark Blue adsorption on Chlorella was tested by using two microalgal dose, 0.01 and 0.10 grams. Resulting removal percentages and adsorption capacities of these two dosages are presented in Table 4.34.

Table 4.33. Removal percentages and adsorption capacities of Chlorella at two different dosages, without buffer in the solution.

Biosorbent Dosage (g/10 mL)	Removal % by Chlorella	q (mg/g)
0.01	52.4	5.6
0.10	93.5	1.2

When the microalgal dose is 10 times higher in the solution, IDB removal by Chlorella increased from 52.4% to 93.5% where dye uptake per unit biomass decreased from 5.6 to 1.2 mg/g (Table 4.34). The reason for lower removal percentage at lower biosorbent dose is possibly the same as in the case other dyes, which is the saturation of available adsorption sites on the microalgae at fixed amount of dye molecules. On the other hand blockage of adsorption sites due to suspended microalgae agglomeration may explain the decrease in adsorption capacity [107].

93.5% of dye removal was achieved with 0.1 g of biomass; thus, higher values of biosorbent dosage were not examined for this section. Activated carbon was used at an adsorbent dosage of 0.01 and 0.005 grams in 10 mL buffered solutions, as its dye removal was found to be higher in the presence of sodium phosphate buffer (Table 4.31). 81.2% dye uptake from the buffered dye solution was accomplished with 0.01 grams of activated carbon powder. Removal percentage decreased to 79.4%, when the dose of the adsorbent was 0.005 grams. The change between these two results is negligible suggesting that lesser amount of AC powder can be used for the removal of Isolan Dark Blue dye.

4.6. Comparison of Results from All Single Dye Adsorption Experiments

The effect of storage conditions of stock solutions on dye removal for each dye in the study was examined. Removal percentages by Chlorella and AC for each dye where their stock solutions were used freshly, i.e. without any delay between solution preparation and adsorption tests, are given in the Table 4.35. These results were obtained as an average of at least three experimental trials.

Table 4.34. Removal percentages (%) of each dye with freshly prepared stock solutions at pH 7.

Adsorbents \ Dyes	Methylene Blue	Congo Red	Methyl Orange	Eriochrome Black T	Isolan Dark Blue
Chlorella	92.2	62.5	4.3	77.6	42.6
Activated Carbon	99.5	92.6	97.8	85.7	80.2

Here the effect of sodium phosphate buffering agent is presented comparatively for all five dyes used in adsorption experiments by *Chlorella* at the pH value of 7. These results established that presence of the buffering agent in the test solution affected the bioremoval of CR, MO, and EBT positively whereas IDB exhibited better bioremoval by Chlorella without buffering agent in the test solution. Results in both buffer status were fairly similar in the bioremoval of MB. Therefore buffering agent was used in the adsorption experiments involving MB, CR, MO, and EBT. Adsorption experiments of IDB were conducted without adding the buffering agent to test solutions since there is almost 10% difference between the removals of IDB in buffered and non-buffered solutions (Table 4.36).

Table 4.35. Removal percentages (%) of each dye by Chlorella with and without the sodium phosphate buffering agent in the solution.

Buffer Status \ Dyes	Methylene Blue	Congo Red	Methyl Orange	Eriochrome Black T	Isolan Dark Blue
With buffer	90.6	64.6	4.1	72.0	40.4
Without buffer	91.8	38.8	2.9	70.7	51.4

Table 4.37 shows removal percentages of each dye at the lower initial dye concentration of 3.4 mg/L while Table 4.38 shows removal percentages of each dye at higher initial dye concentration of 102.0 mg/L, except for MO and IDB. The maximum initial dye concentration was 34.0 mg/L for these two dyes.

Results imply that at constant initial dye concentrations, removal percentages of MB (a cationic dye) by Chlorella are comparable to those obtained by activated carbon. Removals of EBT and IDB (anionic dyes) from diluted dye solutions by Chlorella are more effective than removals by activated carbon, especially for IDB. For the decolorization of

CR and MO (zwitterionic dyes), activated carbon is a more powerful adsorbent than Chlorella, especially in smaller initial dye concentrations (Table 4.37).

Table 4.36. Summary for removal percentages (%) of each dye when the initial dye concentration was 3.4 mg/L at pH 7.

Dyes / Adsorbents	Methylene Blue	Congo Red	Methyl Orange	Eriochrome Black T	Isolan Dark Blue
Chlorella	95.9	75.1	5.2	95.1	74.7
Activated Carbon	99.8	93.7	98.6	92.3	41.8

Table 4.37. Summary for removal percentages (%) of each dye when the initial dye concentration was 102.0 mg/L (For MO and IDB, 34.0 mg/L).

Dyes / Adsorbents	Methylene Blue	Congo Red	Methyl Orange	Eriochrome Black T	Isolan Dark Blue
Chlorella	89.5	39.0	4.2	40.0	41.0
Activated Carbon	90.0	88.7	93.2	78.0	70.2

In concentrated dye solutions dye uptake by Chlorella decreased, however activated carbon exhibited higher potency, for all five dyes. In concentrated dye solutions, adsorption of MB by Chlorella is comparable to adsorption by AC and remarkably adsorptions of IDB and EBT by Chlorella are better than activated carbon.

In conclusion, Chlorella might be a promising biosorbent for the remediation of water bodies in elimination of compounds with sufficient structural similarities to EBT and IDB, noting that micropollutants in aquatic environments are commonly found in concentrations that are similar or lower than lower concentrations of dyes tested in this study.

It can be suggested that ionic moieties on dye structures being different for five dyes of the study, result in different trends on the effect of pH of the solution on dye removals. Removal of the cationic dye, MB, increases when the basicity of the solution increases while removal of other four dyes by Chlorella showed a decreasing trend with increasing pH (Figure 4.28a). Maximum dye removals by Chlorella were recorded at pH values of 7.0 for MB (92.5%), 6.0 for CR (84.6), 3.5 for MO (64.9%), 3.0 for EBT (95.9%), and 4.0 for IDB (94.4%). Maximum dye removals by AC were recorded at pH values of 6.5 for MB (99.3%),

4.0 for MO (99.4%), 3.5 for EBT (95.7%), and 7.5 for IDB (74.2%) (Figure 4.28b). By changing the pH value of the test solution, dye removal of IDB was achieved as 94.4% by Chlorella which means that it is more efficient than the dye removal by AC.

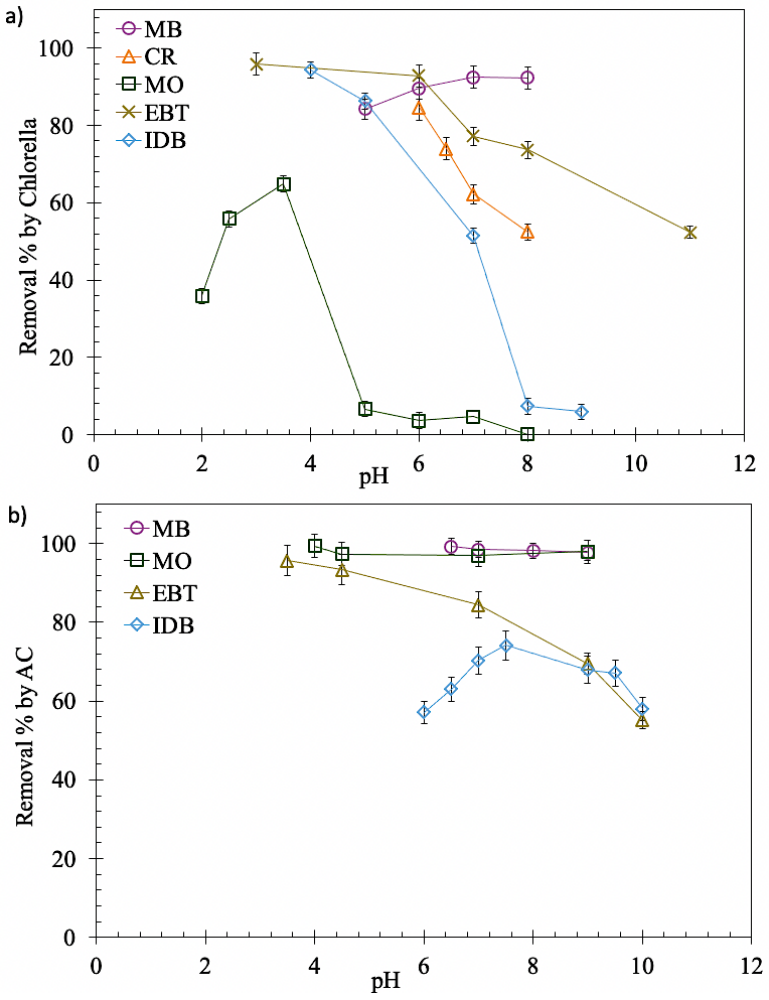


Figure 4.28. Removal percentages of each dye by (a) *Chlorella* and (b) activated carbon at different pH levels of aqueous dye solutions.

Biosorbent dosage of the biomass was varied to examine changes in dye removals by Chlorella, while all other experimental variables were kept constant. For all five dyes, increasing the microalgal dose increases the removal of the dye, all the way to complete removal, except in the removal of MO. As removal of MO from the test media was found out to be relatively weak by Chlorella, a 0.25 grams of biomass in 10 mL of solution only achieved a 69.5% decolorization. Remarkably, when the other four dyes were treated by

Chlorella at that same high dosage (0,25 g/10 mL), almost 100% removals were reached (Figure 4.29).

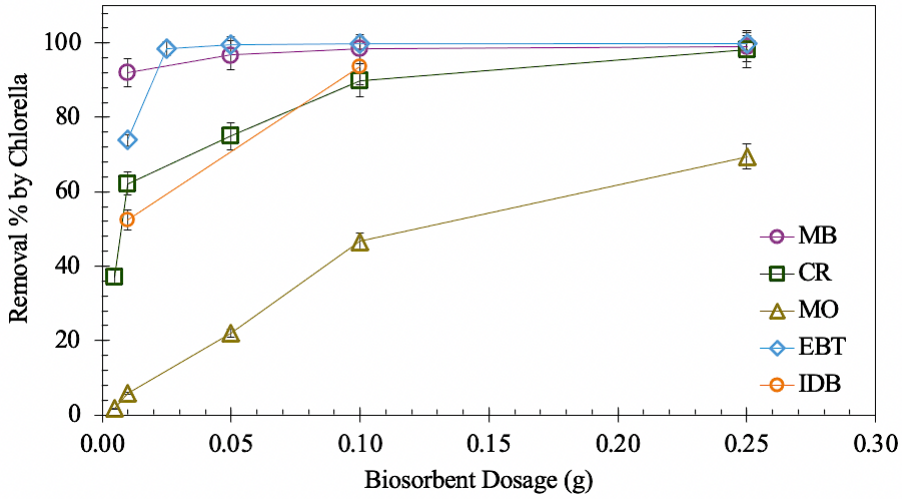


Figure 4.29. Summary for removal percentages (%) of each dye by *Chlorella* at different dosages of Chlorella.

5. ADSORPTIONS OF SELECTED DYE MIXTURES BY CHLORELLA AND ACTIVATED CARBON

5.1. Adsorption of Methylene Blue and Methyl Orange Dye Mixture

As it was discussed in sections 4.3.1 and 4.2.1, average removal percentages of single dyes Methylene Blue (MB) and Methyl Orange (MO) at pH 7 by 1 g/L Chlorella were 92 ± 3% and 4 ± 3%, and by 1 g/L activated carbon 96.0 ± 1% and 98.0 ± 1% respectively. Low adsorptions of MO by Chlorella under above stated conditions called for finding alternative conditions for better removal, and lower pH and higher biosorbent dosages were shown to achieve that goal. Additionally, the presence of other soluble compounds, such as buffering agent, or maybe more interestingly other dyes was expected to have an effect on MO adsorption. In fact in real aquatic pollution, pollutants coexist in great diversity, which would definitely be expected to affect their various behaviors, including adsorption. Therefore testing the adsorption behaviors of mixtures of dyes was expected to provide valuable insight for practical applications of biosorption in water remediation. Additionally adsorption study of mixtures may provide a possibility of better removals of poorly adsorbing dyes such as MO. The cationic MB is the best adsorbate for Chlorella amongst all five dyes of the study. As dyes become anionic or zwitterionic, adsorptive removals fall. It was postulated that removal of MO may be increased by expanding the charge interactions by the involvement of MB dye molecules that effectively cover the biomass, possibly modifying the biosorbent surface more prone to MO adsorption. MB dye molecules could play a bridge mechanism to remove the MO molecules by supportive electrostatic forces. In following corresponding adsorption experiments with dye mixtures, activated carbon was continued to be used as a standard for comparison. Selected dyes, MB and MO, have maximum wavelengths that are not overlapped in their full spectrum of dye mixture solution as shown in Figure 4.1. UV absorbance values of the mixture solution were analyzed at 665 nm for MB and 464 nm for MO. They were combined as 1:1 or 2:1 (w/w) mixture for the calibration curve and adsorption test solutions. Figure 5.1 shows the calibration curve of 1:1 MB-MO mixture at pH 7 and relevant equations are given on the curve.

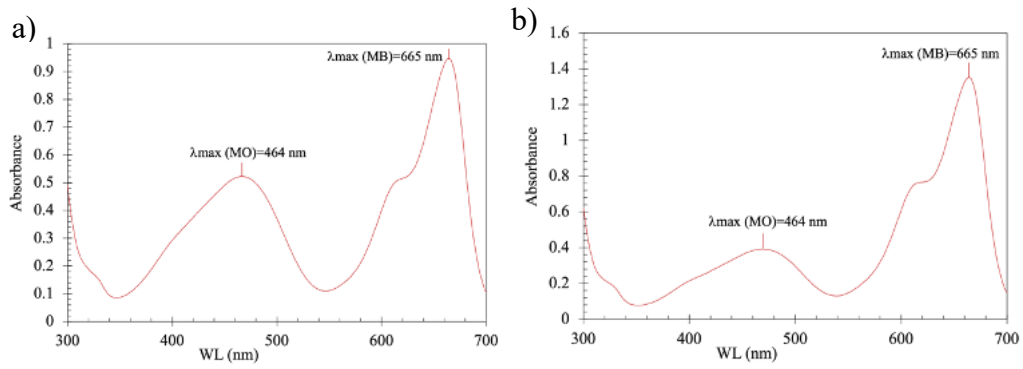


Figure 5.1. (a) UV spectrum of 1:1 MB-MO mixture, (b) UV spectrum of 2:1 MB-MO mixture.

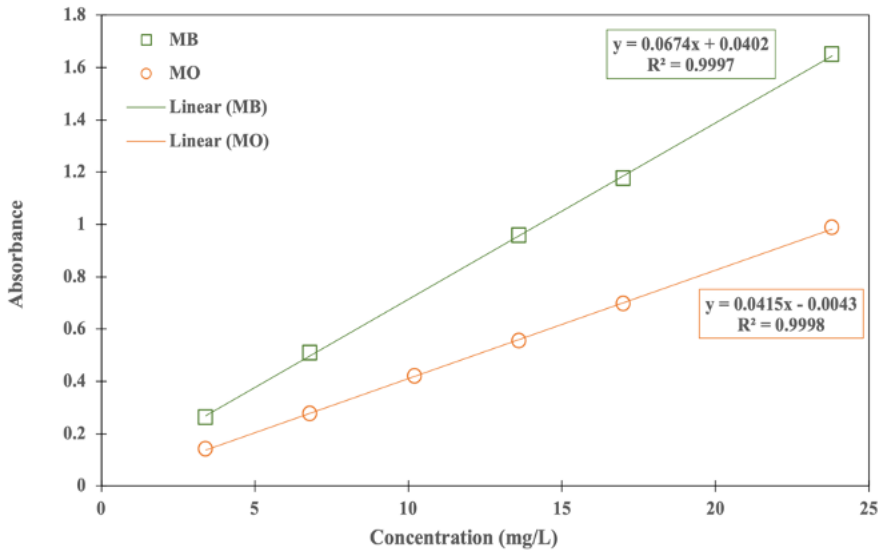


Figure 5.2. Calibration curve for 1:1 mixture of MB and MO.

In both 1:1 and 2:1 w/w MB:MO ratios, the decolorization of MB by *Chlorella* decreased slightly from 90.2% to 83.8% and 82.7% at 665 nm respectively, in comparison to single dye adsorption of MB. On the other hand, MO decolorization in both ratios slightly increased from 3.3% to 4.5% and 10.3% respectively (Table 5.1). Calibration curves were obtained by using the multiple wavelength format in the UV instrument at relevant λ_{max} values (Figure 5.2). When the amount of the cationic dye, MB, was in excess with respect to MO, twice as much, it had an increasing effect on the removal percentage of MO, when compared to 1:1 mixture. This may be show that increasing coverage of Chlorella by cationic

sites (amine and sulfide groups) of MB improve the adsorption of MO. The biomass gains excessive positive charge, possibly enabling the zwitterionic dye to interact more with the biosorbent. Also, the non-attached side of the cationic MB molecule may exhibit electrostatic interaction with the negatively charged side of MO molecule in the solution, increasing the number of possible attachment sites for MO. In comparison to the synergistic effect of MB on MO removal by chlorella, the presence of MO in the solution influenced the MB removal yield adversely. This may probably be due to MO occupying the available biosorption sites of the biomass. However, results showed that mixing MB-MO dyes was not effective in increasing the MO removal yield as much as expected. It leads to a slight increase in the removal of MO but a decrease in removal of MB. In the case of activated carbon as the biosorbent, it exhibited higher removal yields than *Chlorella* in adsorption of single dyes and mixture of dyes.

Table 5.1. Removal percentages of single dyes and mixed dyes of MB and MO at different mass ratios in the solution.

	Single Dye		1:1 Mixture of Dyes		2:1 Mixture of Dyes	
Adsorbents	MB	MO	MB	MO	MB	MO
Chlorella	90.2	3.3	83.8	4.5	82.7	10.3
Activated Carbon	98.4	97.0	95.2	97.5	93.3	98.9

Additionally, an experiment is set to determine the effect of successive biosorption on dye removal by treating the microalgae first with MB and then MO, without mixing dyes at the same time. The idea behind is the same with mixing dyes simultaneously, using the best adsorbing cationic dye to modify the biomass surface and improve the adsorption of otherwise poorly adsorbing dye, MO. The cationic dye, Methylene Blue, has higher removal percentages and the zwitterionic dye, MO, has lower removal percentages than other dyes with Chlorella. The successive biosorption might increase the removal of MO by the effect of positive charges of MB and its strong interaction with microalgal surface. However, experiments showed no synergistic or adverse effects of pre-adsorption of MB to the removal of MO, via successive biosorption. The removal percentage of MO from the mixture solution was 5.5 ± 3% at pH 7 with 10 mg/10 mL biomass, while single biosorption of MO was 4.7 ± 3%, an almost equal removal under the same conditions.

5.2. Adsorption of Methylene Blue and Congo Red Dye Mixture

With the same approach described in Section 5.1, the second dye mixture that was employed consists of the cationic dye Methylene Blue (MB) along with Congo Red (CR), the other zwitterionic dye of the study. The effect of number of moles of dyes per unit volume on the removal should also be considered for better comparisons, as Congo Red with relatively higher molecular weight than MO comes with greater discrepancy when weight ratios of dyes are used.

Single dye biosorption of MB and CR dyes towards Chlorella was found to be 90.2 ± 3% and 62.3 ± 3% respectively where activated carbon removal values for these two dyes were 95.0 ± 1% and 90.0 ± 1% respectively. The initial aim was to find out the change in MB and CR removal efficiencies with Chlorella and activated carbon when they are in a mixture solution as opposed to their individual biosorption. In the full UV spectrum of the mixture media (Figure 5.4), selected dyes did not have overlapped peaks, which enabled the removal percentage to be calculated more accurately for each dye; 665 nm is the maximum wavelength for MB while 477 nm and 483 nm are the maximum wavelength for CR in 1:1 and 2:1 dye mixture, respectively. Calibration curve was obtained with different concentration ratios of both dyes in the mixture and relevant equations were given in Figure 5.3.

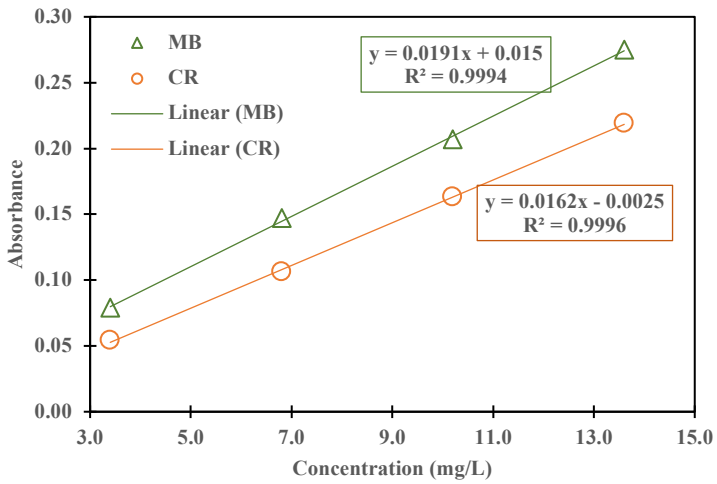


Figure 5.3. Calibration curve of 1:1 volume mix of MB-CR dyes.

Full UV spectra of different mixture compositions were given in Figure 5.4. The mixture consists of MB and CR in equal weight ratios (1:1 Mixture Adsorption) and in another experimental set, the weight of MB was twice MO (2:1 Mixture Adsorption). Same procedure was applied to the adsorption experiments by activated carbon.

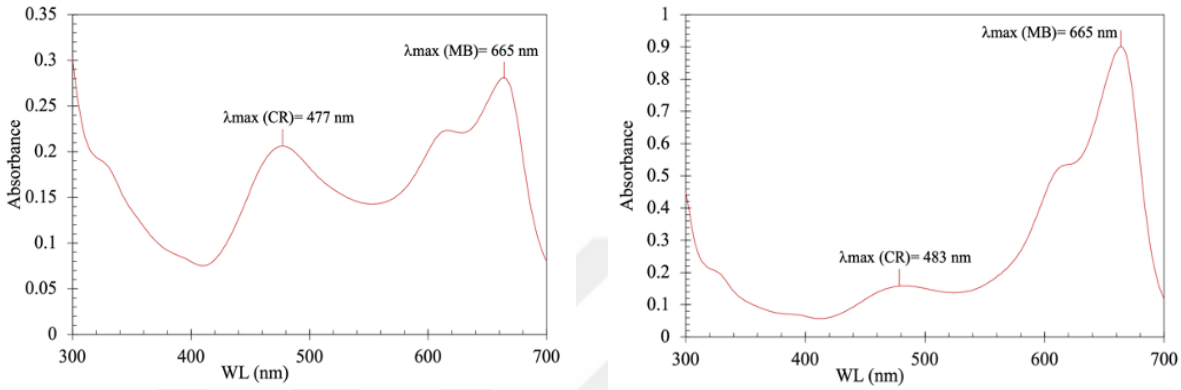


Figure 5.4. UV spectra of (a) 1:1 MB-CR mixture, (b) 2:1 MB-MO mixture.

Unlike MB-MO mixture biosorption, blending MB and CR in an aqueous solution had an adverse effect for both dye with Chlorella. Methylene Blue removal percentage decreased from 90.2% in 1:1 MO-MB mixture, to 83.2% with 1:1 Congo Red in the dye mixture. Likewise, 2:1 (w/w) dye mixture resulted in a drop of the MB removal percentage from 90.2% to 82.3% with Congo Red instead of MO.

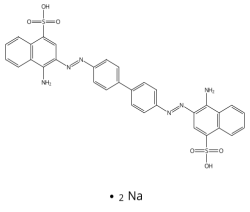
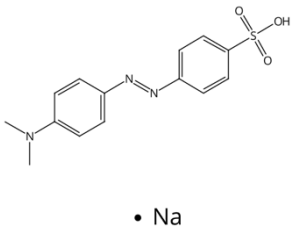
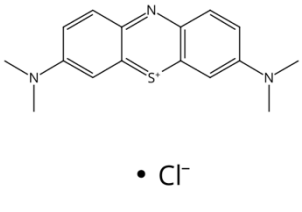
Table 5.2. Removal percentages of single dyes and mixed dyes of MB and CR at different mass ratios in the solution.

	Single Dye		**1:1 Mixture of Dyes**		**2:1 Mixture of Dyes**	
Adsorbents	MB	CR	MB	CR	MB	CR
Chlorella	90.2	62.3	83.2	11.7	82.3	10.3
Activated Carbon	98.4	92.7	98.1	92.1	93.3	93.3

The reason for this decrease could be the same as for the case of MB-MO mixture set (Section 5.1), available sites for the biosorption of MB on Chlorella could be filled with

Congo Red molecules. The decrease in the CR removal in the aqueous mixture was sharper than MB which is from 62.3% to 11.7% in 1:1 w/w dye mixture solution ratio. 2:1 MB-CR mixture showed a sharp decrease in the removal of CR from 62.3% to 10.3% (Table 5.2).

Table 5.3. Properties of Congo Red, Methyl Orange, and Methylene Blue.

Properties	**Congo Red (CR)**	**Methyl Orange (MO)**	**Methylene Blue (MB)**
IUPAC Name	Disodium 4-amino-3-[[4-[4-[(1-amino-4-sulfonato naphthalen-2-yl)diazenyl]phenyl]phenyl]diazenyl] naphthalene-1-sulfonate	4-diMethylaminoazobenzene-4'-sulfonic acid sodium salt	3,7-bis(Dimethylamino)-phenothiazin-5-ium chloride
Molecular Mass (g/mol)	696.08	327.33	319.85
Molecular Formula	$C_{32}H_{22}N_6Na_2O_6S_2$	$C_{14}H_{14}N_3NaO_3S$	$C_{16}H_{18}ClN_3S$
Chemical Structure			
Maximum Wavelength	498 nm	464 nm	665 nm

Decolorization of Congo Red-Methylene Blue mixture solution by Chlorella was more negatively influenced than the mixture of Methyl Orange with Methylene Blue. The biosorption of MO was better from the mixture than single form. On the other hand the

biosorption of CR in the presence of MB was much weaker than as single dye. Since CR is a heavier and bigger molecule than MO, number of moles of CR are less than MO at the same mass concentration. On the other hand MB has a much similar molecular weight, and size in comparison to MO (Table 5.3).

5.3. Adsorption of Methylene Blue and Isolan Dark Blue Dye Mixture

In previous sections, the cationic dye Methylene Blue was mixed with two different zwitterionic dyes, Methyl Orange and Congo Red, with MB showing higher removal behavior among all five dyes studied with Chlorella. Remaining two dyes Eriochrome Black T and Isolan Dark Blue (anionic dyes) were considered with respect to their maximum absorbance wavelengths not to overlap with the maximum absorbance wavelength of Methylene Blue (665 nm). Eriochrome Black T (EBT) has a maximum peak at around 620 nm and Isolan Dark Blue reaches a maximum peak at 578 nm. The peak of EBT shows a higher overlap of absorbance band with MB in an aqueous mixture. In the UV/VIS spectroscopy, when maximum peaks nest together, actual absorbance values of each dye in the mixture gets less certain. Incident beam passes through the analyte solution, excitation occurs from the ground state to an excited state when analyte absorbs light. This jump releases some energy, and the detector records this energy as absorbance values at that wavelength. If those wavelengths are adjacent in a binary or tertiary dye mixture, the result is detected as a combination of absorbances, hindering the determination of actual removals for each dye. Therefore, Isolan Dark Blue was selected as the anionic dye to be combined with MB in aqueous solution.

In single dye biosorption, Isolan Dark Blue resulted in 50.5% removal and MB resulted in 90.2% removal by Chlorella. On the other hand, AC was capable to remove 96.4% of MB and 68.5% of IDB. When they are in 1:1 (w/w) dye mixture, the adsorption of MB in the presence of the IDB dye molecules in the solution decreased to 62.4% by Chlorella and 84.8% by AC (Table 5.4). Such an extent of decrease in the removal percentages is significant, revealing a clearly negative effect of mixing MB and IDB on adsorptions by Chlorella. IDB dye uptake by AC was not affected by the presence of MB while IDB dye uptake by Chlorella decreased to 39.7% in the presence of MB molecules. In the case of 2:1

adsorption where MB quantity is twice of IDB quantity in the solution, AC dye uptake is similar with the results of 1:1 mixture. However, IDB dye uptake by Chlorella in the presence of the MB dye molecules increased to 54.9%, which can still be considered a weak adsorption. Overall, mixing IDB and MB had adverse effect on their removal percentages where dyes are probably competing for adsorption sites.

Table 5.4. Removal percentages of single dyes and mixed dyes of MB and IDB at different mass ratios in the solution.

	Single Adsorption		**1:1 Mixture Adsorption**		**2:1 Mixture Adsorption**	
Adsorbents	MB	IDB	MB	IDB	MB	IDB
Chlorella	90.2	50.5	62.4	39.7	82.9	54.9
Activated Carbon	96.4	68.5	84.8	68.9	84.3	66.5

5.4. Adsorption of Methylene Blue, Congo Red, and Methyl Orange Dye Mixture

In this part, the cationic dye (MB) was mixed with two zwitterionic dyes, MO and CR forming a ternary mixture. Although these latter dyes have overlapping maximum absorbance wavelengths, remaining concentration of the combination of these two dyes can be monitored. Two major peaks were found in the full UV spectrum of the ternary mixture solution, 464 nm and 665 nm. The first arises mostly from Methyl Orange and then Congo Red dye and the latter from Methylene Blue dye. Three dyes were combined in equal weights. It should be noted that mole ratios are different. As MO has a lower molecular weight than CR (Table 5.3), its mole ratio is higher than CR in the mixture and it is expected to dominate the spectrum as molecular absorbance of each dye are somewhat comparable.

Therefore similar results are expected and obtained to those of Section 5.1. However, as mentioned in Sections 5.1 and 5.2, MB was adversely affected by the presence of both MO and CR. Here, the removal percentage of MB is lower than in experiments of previous sections about binary mixtures. MB removal in single dye biosorption is 90.1% but in 1:1:1 (w/w/w) mixture, its removal falls down to 44.5% (Table 5.5). The decrease in MB removal may arise from competitive biosorptions of both CR and MO dye molecules with MB; however, the decolorization of MO or CR alone could not be determined because of overlapped peaks of MO and CR in UV spectrum. Change in the removal percentage of MO

and CR together by both *Chlorella* and activated carbon did not show a significant change in ternary mixture with MB as opposed to binary mixtures or single dye adsorptions.

Table 5.5. Removal percentages of the dyes as in single form and mixture form of MB, MO, and CR at different mass ratios in the solution.

	Removal % by Chlorella		**Removal % by Activated Carbon**	
Adsorption Status	MB	MO	MB	MO
Single	90.1	3.3	98.0	97.0
Mixture	44.5	4.3	83.5	92.5

6. ADDITIONAL INVESTIGATIONS INTO DYE ADSORPTIONS

6.1. Selection of Chlorella Sp. Source for Dye Decolorization Tests

Chlorella Vulgaris strain CCAP 211/11B (Laboratory Chlorella) was grown in our laboratory, and it was used in adsorption experiments in a previous thesis work from our laboratory. In the current thesis work, a commercial alternative of the Chlorella was evaluated and utilized for the adsorption experiments.

The adsorption of the particular commercial Chlorella (Green Gem® Chlorella, Yaşamnet) was tested initially to make a comparison to laboratory- grown Chlorella. Activated carbon was also tested as a reference adsorbent to ensure reliability of experimental results.

Table 6.1 Removal percentages of selected dyes by Chlorella species from different sources, at initial concentrations of 13.6 mg/L and 102.0 mg/L (except Methyl Orange).

	Eriochrome Black T Removal %		**Congo Red Removal %**		**Methyl Orange Removal %**
C_0 (mg/L) / Adsorbents	**13.6**	**102.0**	**13.6**	**102.0**	**13.6**
Laboratory Chlorella	65.8	29.0	60.8	36.3	2.6
Commercial Chlorella	75.6	40.7	62.3	40.3	6.8
Activated Carbon	85.0	75.8	90.0	88.9	93.7

Comparative dye adsorption tests were done by using two different Chlorella and activated carbon. Eriochrome Black T, Congo Red, and Methyl Orange were used as adsorbates. Removal of Eriochrome Black T was 65.8% by laboratory grown Chlorella and 75.6% by commercial Chlorella at the initial concentration of 13.6 mg/L. At the initial concentration of 102.0 mg/L, commercial one was still superior with a removal of 40.7%, whereas laboratory grown Chlorella retained 29.0% of the dye from the solution. Dye uptake potential of Congo Red by laboratory grown Chlorella (60.8%) and commercial microalgae (62.3%) were close to each other when the initial concentration was 13.6 mg/L. When the

amount of CR dye molecules at the beginning of the adsorption was raised to 102.0 mg/L, removals were decreased to 36.3% and 40.3% for laboratory grown and commercial Chlorellas respectively. Decolorization of Methyl Orange solution was the lowest among all three dyes tested thus only initial concentration of 13.6 mg/L was investigated where 2.6% and 6.8% removals were obtained by laboratory grown and commercial Chlorellas respectively (Table 6.1).

Below, the graph (Figure 6.1) reveals the clear difference between the two biosorbents regarding their adsorption profiles towards the selected dyes. Dye removals by activated carbon were the best ones among these three adsorbents. The removal gap between two microalgae species was not significantly high, but the better dye adsorbing Chlorella, the commercial was shown to be a better candidate for adsorption tests.

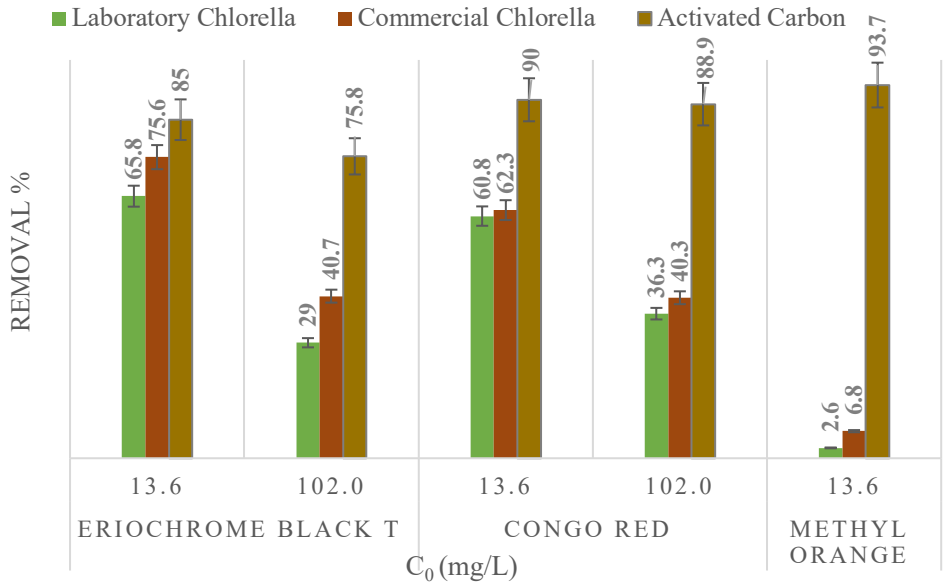


Figure 6.1. Removal percentages of selected adsorbents towards selected dyes.

Overall results showed that commercial Chlorella had higher removal yields than laboratory-grown biomass. Regarding with the adsorption profiles of the biomasses, commercially available type of the microalgae was employed in all experiments included in this thesis work. The use of the commercial microalgae largely eliminates the possibility of bacterial contamination, potentially allowing for more precise and diligent experiments. In

addition, traditional microalgae cultivation is somewhat time consuming. It requires a sterile environment, long growth periods followed by extraction of crude biomass, continuing with thrice washing with distilled water to eliminate remaining BBM solution. Also, cultivation of laboratory-grown microalgae requires a lyophilization step to dry and disintegrate the living algae to obtain a biomass. However, commercial Chlorella also required 1-2 times of washing to get rid of some of soluble substances such as extracellular polymeric substances (EPS). UV spectra of the raw and washed commercial Chlorella suspensions reveal differences (Figure 6.2). When the commercial biomass was not rinsed with distilled water, its blank supernatant of 0.01 grams in 10 mL water show a set of absorbance peaks in UV spectrum. 259, 417, 668 nm wavelengths were assigned in the corresponding UV spectrum. The peak at 259 nm was the strongest possibly rising from soluble polysaccharides with specific functional groups such as esters, carbonyls, carboxylates, and, amines [108]. Also, one type of polysaccharide, chitin molecules give a peak at 266 nm [109]. There (Figure 6.2), 259 nm might represent the peak of chitin molecules as Chlorella is known to contain chitin in its cell wall as microfiber unit (Section 1.1). Monosaccharides of glucose, sucrose, and xylose also give peaks at near 270 nm [110] and these molecules are also found in Chlorella's cell wall (Section 1.1). The other distinct peaks at 417 nm and 668 nm may in part arise from Chlorophyll- A found in large quantities in Chlorella [111].

As seen in Figure 6.2, rinsing 0.01 grams of commercial Chlorella powder with distilled water 1-2 times helped to remove these EPSs which are released from the biomass in the solution. Figure 6.2 (red line) shows no distinct peak at around 259 nm which may mean polysaccharides and monosaccharides were almost completely removed by rinsing procedure. The absorbance value of the peak at 668 nm is almost the same at both procedures, (red and black line) while the absorbance value at the peak at 417 nm decreased after rinsing the biomass of Chlorella. All results showed that rinsing the commercial Chlorella decreases the amount of soluble EPS molecules in supernatants; that is why it was employed in adsorption experiments of all experimental Sections. Through simple washing it was possible to minimize any possible interactions between dye and EPS molecules in the solution. Nevertheless, in order to examine the effect of these EPS on dye removal, adsorption tests were performed with Methylene Blue.

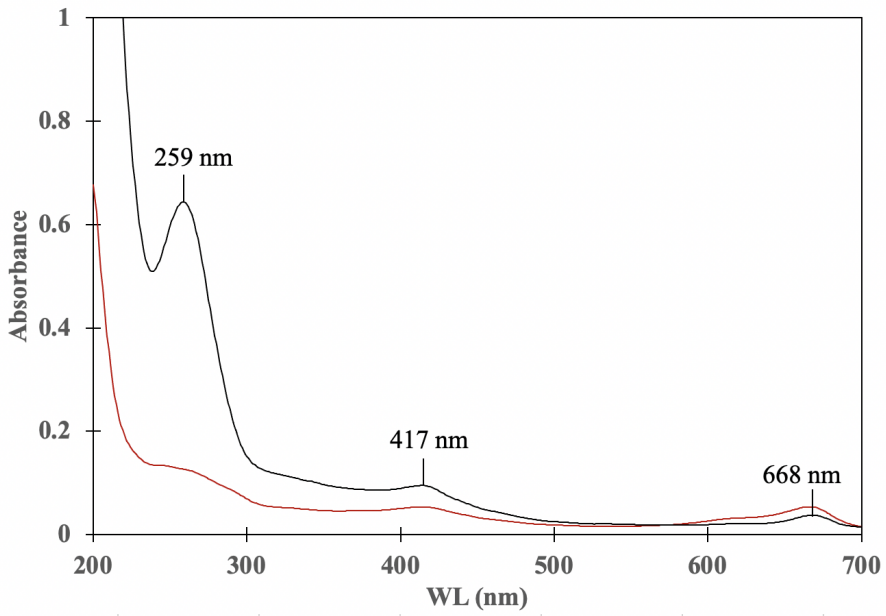


Figure 6.2. Supernatant of the blanks for raw Chlorella (black line) and washed Chlorella (red line).

Dye removals were analyzed for both form of Chlorella suspension, with and without the prior washing of the biomass. Methylene Blue was selected as adsorbate and experiments were conducted with initial dye concentration of 13.6 mg/L, adsorbent concentration of 1 g/L, at pH 7. Results were correlated with what was expected prior to tests. EPS interfered with the removal of MB dye molecules by the biomass. When Chlorella powder was rinsed with distilled water and contaminated supernatant was removed after centrifugation, 90.1% of MB dye removal was achieved upon adsorption. Whereas the raw, unwashed Chlorella adsorbed 84.5% of MB dye molecules.

6.2. Effect of Filtration on Dye Solutions

In all experiments, a proper type of activated carbon was employed as a standard adsorbent for aqueous pollutants to provide a comparative study with better reliability. The very small particle size of activated carbon samples was observed to cause some fraction of suspended activated carbon particles remain suspended even after powerful and lengthy centrifugations, at the stage of separation of insoluble adsorbate from the remaining dye solution. In such cases microfiltration was tried for eliminating any suspended particles

which may interfere with UV/VIS adsorption measurements. This is a critical step in determining final dye concentrations accurately however on the other hand microfiltration membranes were observed to adsorb a fraction of the dissolved dye, at various extents depending on the molecular structure of the dye. Hence different types of aqueous solution filters, such as PVDF, alcohol-rinsed PVDF, or cotton, were screened for their dye adsorption profile for each type of dye used in the study, in order to minimize any error in concentration change measurements.

Table 6.2. Absorbance values of dye solutions before and after the filtration at different initial concentrations.

	Absorbance Values						
	Methyl Orange		Congo Red		Eriochrome Black T		Methylene Blue
C_0 (mg/L) / **Filter Status**	**1.7**	**13.6**	**1.7**	**13.6**	**1.7**	**13.6**	**13.6**
Non-Filtered	0.133	1.014	0.033	0.242	0.023	0.133	1.852
PVDF-Filtered	0.087	0.899	0.007	0.016	0.003	0.076	1.639
Alcohol-Rinsed PVDF Filtered	-	0.892	-	0.058	-	0.075	-
Cotton-Filtered	-	0.94	-	-	-	-	0.47

Initial dye concentrations were adjusted to 1.7 mg/L and 13.6 mg/L considering that when the biomass adsorbs some part of dye molecules in the solution, the remaining solution, the supernatant has much lower concentration than the initial solution. Thus, a relatively lower concentration solution was also examined in the presence and absence of the filtration. Dye solutions were analyzed directly with UV/VIS spectrophotometer at concentrations of 1.7 (except MB) and 13.6 mg/L, and the relevant absorbance values were reported as 'Non-Filtered' in Table 6.2. Then polyvinylidene difluoride (PVDF) filter was used for these solutions and results were given as 'PVDF-Filtered' where absorbance values were observed to be decreased after PVDF filtration for all selected dyes. Dye molecules seem to bind to the filter possibly by electrostatic interactions at different extents depending on the type of dye.

When the PVDF filter was used absorbance of Methyl Orange was decreased to an extent of 34.6% and 11.3%, for initial dye concentrations of 1.7 and 13.6 mg/L respectively. Hypothetically, the cationic moiety ($-NH_2$) within the zwitterionic structure of MO may cause stronger interactions with the fluoride sites of the PVDF filter through hydrogen bonds [112]. Additionally, absorbance of the other zwitterionic dye of the study, Congo Red, showed declines in its UV absorbance to an extent of 78.8% and 93.4% after the microfiltration, at initial dye concentrations of 1.7 and 13.6 mg/L respectively. Increasing the number of dye molecules in the solution expectedly affected the interaction between dye molecules and the filter material. CR (696.08 g/mol) is a bigger molecule than MO (327.33 g/mol) with similar ionic functionalities, however. Size of dye molecules would be expected to cause comparable effects in retention on the PVDF filter (0.22 μm). Absorbance, i.e. concentration of the anionic dye Eriochrome Black T was strongly affected by the filtration with decreases in absorbances at the extent of 86.9% and 42.8%, at initial dye concentrations of 1.7 and 13.6 mg/L respectively. When the quantity of the EBT dye molecules is lower in the solution, they seem to remain more at the filter. Lastly, the absorbance of Methylene Blue declined by 11.5% when PVDF filter was used before the analysis for the 13.6 mg/L dye solution. The cationic nature of this dye molecule could be causing smaller retention by the filter in comparison to Congo Red. MB (319.85 g/mol) has similar molecular size to MO (327.33 g/mol) but with different ionic functionalities. Both were retained at an extent of near 11.5% in 13.6 mg/L dye solution. So, type of the ionic moiety of the dye did not seem to affect the interaction with the molecular meshes of the filter; size of the compounds seems to have stronger effects on the interaction leading to retention, as seen in the case of CR.

Isopropyl alcohol was passed through the PVDF filter to wet the filter as a mean of modification. Change in absorbance values representing the change in concentration as a result of filtration, at 13.6 mg/L initial concentrations of MO and EBT solutions, was not as significant with PVDF filter. For CR dye solution, unwashed form of the filter adsorbed higher fraction of dye molecules than alcohol-rinsed PVDF filter, at 13.6 mg/L initial dye concentration. Lastly, cotton was used as a filter for solutions of MO and MB. Retention of MO with cotton was less than other filtration methods where 74.6% of MB molecules were attached to the cotton filter. Cotton is composed of macromolecules of cellulose. -OH groups on the cellulose units may probably interact with cationic MB dye molecules whilst zwitterionic molecules didn’t show that much electrostatic interaction due to repulsive

interactions of the anionic moieties on its structure. Because cotton filter is not designed to exhibit certain mesh size membrane structure a discussion about the effect of the size of molecules in retention is not done.

In conclusion, filters were not used in adsorption experiments of this thesis. It can be critical to find a suitable non-adsorptive type of filter at any study involving use of filters in analyses of concentration of molecules, which can bind to filter.

7. CONCLUSION

At the beginning of the study, single dye adsorptions of selected dyes (Methylene Blue (MB), Congo Red (CR), Methyl Orange (MO), Eriochrome Black T (EBT), and Isolan Dark Blue (IDB)) by Chlorella and activated carbon were examined in batch experiments. Storage conditions of dye stock solutions, pH of the solution, adsorbate concentrations and adsorbent dose were examined for their effects on adsorption in Section 4 to find the best dye removal conditions towards the biosorbents. All conditions other than the experimental factor in question were kept constant to have only one changing variable.

Storage conditions of each dye stock solution were studied by keeping them at room temperature and at 4 °C for various time spans. It was concluded that dye stock solutions of MB, CR, MO, EBT, and IDB should be prepared freshly and operated in adsorption experiments as soon as it is prepared to obtain the maximum dye removal percentages (Table 4.35).

Effect of the buffering agent on dye removals had same trend for MB, CR, MO, and EBT dyes which can be stated as sodium phosphate buffering agent having a positive effect in the dye adsorption by Chlorella. Yet, IDB dye was adsorbed better when the buffering agent was absent in the solution (Table 4.36).

Another set of batch experiments were performed for the investigation of change in dye removal percentages depending on initial dye concentrations. Adsorbents were treated with various initial dye concentrations in a fixed test volume and their dye removal capabilities were determined. The trend of dye removals was the same for all dyes, when the initial dye concentration is increasing, dye removal by Chlorella decreases (Table 4.37 and Table 4.38).

Next set of experiments revealed the behavior of single dyes in adsorption process by changing the pH of the solution. Removal of the cationic MB dye by Chlorella was increased as the basicity of the solution was increased. Removal of zwitterionic MO and CR dyes and anionic EBT and IDB dyes by Chlorella decreases as the pH of the solution increases. Dye

adsorption by AC had different trends than Chlorella for MO and MB. Yet, EBT and IDB dye removals decreased when the basicity of the solution increases. (Figure 4.28). In addition, all dye removals were increased when the biosorbent dosage of the microalgae is increased (Figure 4.29).

Before adsorption experiments for the mixture of dyes, all parameters for the best adsorption results of the individual dyes were reported. All dyes were used in the adsorption tests in freshly prepared solutions. All dyes except IDB showed higher removal yields by Chlorella with the presence of the phosphate buffering agent in test solutions. Over 90% removal of MB, CR, EBT, and IDB dyes by Chlorella were achieved either by increasing the biosorbent dosage to 0.25 grams or decreasing the initial dye concentrations, at the same pH. Yet, Chlorella was determined to be very ineffective in removing MO dye from the solution by changing only one variable. Increasing the microalgal dose from 0.001 to 0.250 grams resulted in an increase in removal percentage from 4.6% to 69.5%; but, at the same time, if pH of the solution becomes acidic, it enhances the removal of MO dye by Chlorella significantly. The MO dye uptake of Chlorella was 92.5% when the microalgal dose is 0.25 grams and pH of the solution was 6.0.

Mixture of dyes were treated with adsorbents as a simple model for polluted aquatic environments, where pollutants are found as a cocktail. The high removal yields of the cationic dye (MB) towards Chlorella was utilized in adsorption experiments by combining with anionic (IDB) and zwitterionic dyes (CR and MO). Section 5 reveals that the decolorization of MB dye solution by Chlorella in the presence of other dyes was diminished in all experiments involving mixtures of dyes, where other dyes in the mixture did not show any relevant change in their adsorptions except CR. Single dye adsorption of CR was 62.3% by Chlorella. Removal of CR in the MB-CR dye mixture (2:1 % w/w) by Chlorella decreased to a significantly low 10.3%.

Lastly, additional experiments showed the importance of the choice of Chlorella source. Chlorella species from different sources were shown to adsorb at different extents; in our experiments a commercial Chlorella was shown to perform better and used in all experiments.

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