

**REMOVAL OF REACTIVE DYES BY USING
MICROALGAE; REACTIVE RED (120) AND REACTIVE
BLUE (198)**

**MİKROALG KULLANARAK REAKTİF BOYALARIN
GİDERİMİ; REAKTİF KIRMIZI (120) VE REAKTİF MAVİ
(198)**

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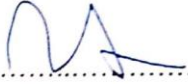
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ABSTRACT

REMOVAL OF REACTIVE DYES BY USING MICROALGAE; REACTIVE RED (120) AND REACTIVE BLUE (198)

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Toxic dyes constitute the largest part of textile wastes. In addition, these substances are soluble substances and may be present in large amounts in wastewater. In this research, *Chlorella vulgaris* is used for the biosorption of two most used textile dyes Reactive Red 120 and Reactive Blue 198. Main goal is identifying optimum conditions for biosorption of these dyes on to dry microalgae with using spectrophotometer. For this purpose, adsorption studies were conducted under different temperatures, different pH, initial dye concentrations and contact time. Optimum pH for *C. vulgaris* is found 2. The optimum biosorbent amount is 0.05 g. Then, initial dye concentration, optimum contact duration and temperature is examined. These results are respectively, 100 mg/ L, 30 minutes and 25 °C. When the optimum factors maintained, overall removal percentage is near %95 for Reactive Red 120. For Reactive Blue 198, the studies show that the adsorption on the *C. vulgaris* is best under pH=2, 25 °C. The adsorption on to *C. vulgaris* was completed in 30 minute. The optimum biosorbent was found as 0.05 g. The studies show that the removal rate for Reactive Blue was about %94 under these conditions when the 100 mg/L initial dye concentration was used.

Keywords: *Chlorella vulgaris*, reactive dyes, biosorption, dye removal, RR 120, RB 198

ÖZET

MİKROALG KULLANARAK REAKTİF BOYALARIN GİDERİMİ; REAKTİF KIRMIZI (120) VE REAKTİF MAVİ (198)

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Tekstil atıklarının en büyük kısmını toksik boyar maddeler oluşturmaktadır. Buna ek olarak, bu maddeler çözünabilir maddelerdir ve atık suda yüksek miktarlarda bulunabilir. Bu çalışmada, kuru *Chlorella vulgaris* mikroalgi kullanılarak Reaktif Kırmızı 120 ve Reaktif Mavi 198 biyosorpsiyonu için ideal şartlar araştırılmıştır. Giderim miktarları spektrometre ölçümleri ile tayin edilmiştir. Bu amaçla, adsorpsiyon çalışmaları farklı sıcaklıklar, farklı pH, başlangıç konsantrasyonu değişimi ve farklı temas süreleri değişimleri ile gerçekleştirilmiştir. Deneyler sonucunda, Reaktif Kırmızı 120 giderimi için en uygun pH 2, en uygun biyosorbent miktarı 0,05 g, ideal başlangıç boya konsantrasyonu 100 mg/ L, ideal bekleme süresi 30 dakika ve sıcaklık 25 °C bulunmuştur. Maksimum giderim %95 civarındadır. Aynı deneyler sonucunda Reaktif Mavi 198 giderimi için en uygun pH 2, en uygun biyosorbent miktarı 0,05 g, ideal başlangıç boya konsantrasyonu 100 mg/ L, ideal bekleme süresi 30 dakika ve sıcaklık 25 °C bulunmuştur. Maksimum giderim %94 civarındadır.

Anahtar Kelimeler: *Chlorella vulgaris*, reaktif boyalar, biyosorpsiyon, boya giderimi, RR 120, RB 198

CONTENTS

	<u>Page</u>
ABSTRACT	i
ÖZET	ii
CONTENTS	iii
TABLES	vi
FIGURES.....	vii
1. INTRODUCTION.....	1
2. DYE SPECIFICATIONS	4
2.1. Classification of Dyes According to Dyeing Properties	4
2.1.1. Acidic Dyes	4
2.1.2. Reactive Dyes	4
2.1.3. Direct Dyes	4
2.1.4. Alkaline Dyes	5
2.1.5. Disperse Dyes	5
2.1.6. Mordant Dyes	5
2.1.7. Ingrain Dyes	5
2.1.8. Metal Complex Dyes	5
2.1.9. Sulfuric Dyes	5
2.1.10. Solvent Dyes.....	6
2.1.11. Pigment Dyes.....	6
2.2. Classification of Dyes According to Their Solubility.....	6
2.2.1. Water Soluble Dyes	6
2.2.2. Dyes that Insoluble in Water	6
2.3. Classification of Dyes According to Chemical Structure	7
2.4. Dye Treatment	7
2.4.1. Biological Treatment of Dyes.....	7
2.4.2. Chemical Treatment of Dyes	8

2.4.3.	Physical Treatment of Dyes.....	10
2.4.4.	Microalgae	11
2.4.5.	Cyanobacteria	12
2.4.6.	Green Algae	12
2.5.	Dye Removal by Adsorption on the Microalgae	12
3.	MATERIAL AND METHOD	15
3.1.	Materials	15
3.1.1.	Microorganisms	15
3.2.	Dye Stuff.....	15
3.2.1.	Used Dyes and Their Chemical Properties.....	15
3.2.2.	Dye Stock	16
3.3.	Method.....	17
3.3.1.	Determination of Maximum Absorbance Values for Dyes by Using Spectrophotometer	17
3.3.2.	Calibrations of RR 120 and RB 198.....	17
3.4.	Determination of Dye Adsorption	17
3.4.1.	Biosorption Experiments	17
3.5.	Correlation Analysis	19
3.6.	Adsorption Isotherm Studies.....	19
3.6.1.	Langmuir Isotherm of Dye Removal.....	19
3.6.2.	Freundlich Isotherm to Dye Removal	20
4.	RESULTS.....	21
4.1.	Determination of Maximum Absorbance Values for Dyes	21
4.2.	Determination of Optimum Conditions for RR 120 Removal by <i>C. vulgaris</i>	21
4.2.1.	Optimum pH value for RR 120	21
4.2.2.	Initial Concentration of Biosorbent	22
4.2.3.	Temperature Effect on RR 120 removal.....	24
4.2.4.	Effect of different initial dye concentrations on RR 120 biosorption	25
4.2.5.	Different contact durations on RR 120 biosorption.....	26
4.3.	Determination of Optimum Conditions for RB 198 Removal by <i>C. vulgaris</i>	27
4.3.1.	Optimum pH value for RB 198	27
4.3.2.	Initial Concentration of Biosorbent	29
4.3.3.	Temperature Effect on RB 198 removal.....	30

4.3.4.	Effect of different initial dye concentrations on RB 198 biosorption	31
4.3.5.	Effect of different contact time on RB 198 biosorption	32
4.4.	Application of the obtained data to Adsorption Isotherms	34
4.5.	Adsorption Kinetics	38
5.	DISCUSSION and CONCLUSIONS.....	40
	REFERENCES	47
	CURRICULUM VITAE.....	52

TABLES

Table 3.1. Chemical Properties of RR 120 and RB 198.....	15
Table 4.1. Removal (%) at different pH values for biosorption of RR 120 dye.....	21
Table 4.2. Removal values (%) for different biomass amounts for RR120 biosorption	23
Table 4.3. % removal values at different temperatures for RR120 biosorption	24
Table 4.4. Removal values (%) at different dye concentrations for RR120 biosorption	25
Table 4.5. Removal values (%) at different contact times for RR120 biosorption	26
Table 4.6. Removal (%) at different pH values for biosorption of RB 198 dye.....	28
Table 4.7. Removal (%) for different biomass amounts for RB 198 biosorption	29
Table 4.8. % removal values at different temperatures for RB 198 biosorption.....	30
Table 4.9. Removal (%) for different initial dye concentrations for RB198 biosorption....	31
Table 4.10. Removal values (%) at different contact times for RB 198 biosorption	33
Table 4.11. Langmuir model parameters for Reactive Red 120 and Reactive Blue 198	35
Table 4.12. Freundlich model parameters for Reactive Red 120 and Reactive Blue 198...	37
Table 4.13. Pseudo-First order and Pseudo-Second order kinetics for RR 120.	38
Table 4.14. Pseudo-First order and Pseudo-Second order kinetics for RB 198	39
Table 5.1. Optimum conditions that provide maximum biosorption via <i>C. vulgaris</i> for RR 120	45
Table 5.2. Optimum conditions that provide maximum biosorption via <i>C. vulgaris</i> for RB 198	45
Table 5.3. Biosorptions of some dyes with different adsorbents according to other researches.....	46

FIGURES

Figure 3.1. Chemical formula of RR120	16
Figure 3.2. Chemical formula of RB 198	16
Figure 4.1. pH changes effects to dye removal that caused by <i>C.vulgaris</i>	22
Figure 4.2. Effects of biomass quantity on biosorption of RR 120	23
Figure 4.3. Effects of temperature changings on color removal by <i>C. vulgaris</i> on RR 120	24
Figure 4.4. Starting concentration on dye removal by <i>C. vulgaris</i> on RR 120	25
Figure 4.5. qe changes in terms of different durations on color treatment via <i>C. vulgaris</i> on RR 120.....	27
Figure 4.6. pH changes effects to dye removal that caused by <i>C.vulgaris</i>	28
Figure 4.7. qe changings due to biomass amount on RB 198 treatment	29
Figure 4.8. Effect of temperature on RB 198 removal by <i>C. vulgaris</i>	31
Figure 4.9. Effects of initial dye concentration changings on color removal by <i>C. vulgaris</i> on RB 198.....	32
Figure 4.10. Contact time impact on treatment by <i>C. vulgaris</i>	33
Figure 4.11. Langmuir isotherm for different dye concentrations for RR 120	34
Figure 4.12. Langmuir study with different dye concentrations for RB 198	35
Figure 4.13. Freundlich isotherm for different dye concentrations for RR 120.....	36
Figure 4.14. Freundlich isotherm for different dye concentrations for RB 198.....	37

1. INTRODUCTION

Textile industry is the one of the biggest industries and it needs lots of water and effluent discharged from this industry is highly polluted. Color parameter has been recently included (2011) in discharge limits from these industries in the Turkish legislation: Water Pollution Control Regulation (Su Kirliliği Kontrol Yönetmeliği, RG-24/4/2011-27914) The color standardization of discharge of effluents is 260 pt-Co for 24 hours composite sample and 280 pt-Co for 2 hrs composite sample in Turkey. Due to increasing environmental awareness and national and international legal constraints imposed on discharge of effluents, there is a need for using low budget alternative technologies for remediating industrial wastewaters.

Textile wastewater is features that high colorant, saltiness, heat, strong acidic and basic and high COD. These characteristics damage aesthetics and also have toxicological, pathogenic effects, causing hazardous influence in humanity.

In these years, many synthetic dyes are used extensively with advanced technology by almost all industries from food industry to pharmaceutical industry [2]. Natural or synthetic colorants are used in various industries such as apparel, tanneries, paints and pulp. It is reported that “10% to 15% of the dye used during the manufacturing of textile products are released into the environment worldwide annually” [3]. Significant quantities of synthetic dyes released from various sources around the world for many years and this problem causes environmental and health problems [3]. The discharge of these dyes to headwaters negatively affects flora-fauna and human life. Certain colourants causes allergies, dermal diseases, carcinoma and mutation in humankind and animals. Besides, these dyes are also caused a problem that is the absorption and reflection of the sunbeams. The main problem is, dyes causes forming bacteria, making it difficult to biodegrade in water. [4].

Reactive dyes are some of the synthetic colorants that fully soluble in water and their formation is poly aromatic [5]. Reactive dyes are widely used as a colouring however; 40% of these colours become waste because since the colour atoms can respond with the OH⁻ [6]. These types of colours degrading with difficulty in both aerobic and anaerobic conditions they are converted into carcinogenic aromatic amines by products [7].

The three most common groups of reactive dyes are azo, anthraquinone and phthalocyanine dyes [8]. The azo-dyes are commonly organic compounds prepared by

combining only one diazonium compound with a phenol or an aromatic amine [9]. The largest and most versatile classes of colours are used in many industries in the manner that textiles, sheet, fiber, laser coloring. Generally in coloring process, about 31-69% of the used azo-colours are hydrolysable and mixed with waste water [8]. In addition, improper disposal of waste dye in water is a big threat to public health [9]. For this reason, it is environmentally important to effectively remove azo dyes from waste water [10].

Various techniques can be applied to get rid of azo colours from wastewater [11]. The treatment of material profluent includes basically natural, physical and chemical strategies, which are frequently exceptionally expensive. These removal techniques include anaerobic/aerobic biological treatment, membrane nanofiltration, coagulation and flocculation, adsorption, photocatalytic analysis, chemical oxidation, catalytic ozonation [12], precipitation, ion exchange etc. However, most of the technologies cannot use because these are expensive and they causes seconder pollutions [13]. The dyes used in textile industry contain organic compounds with different functional groups, such as carboxylic (-COOH), amine (-NH₂), and azo (-N=N-) groups, which makes their treatment difficult by convential methods. .

Adsorption process is found to be the most suitable technique to eliminate pollutants from wastewater. Within this scope, biosorption is environmentally choice for the get rid of paint from industry. Adsorption has many economic and environmental benefits compared to other removal ways [6]. Biosorption is a physico-chemical process where contaminants are removed in wastewater via biosorbent. Many types of biomasses can be used for biosorption with high efficiency and cheap. Using cheap biomass material is one of the main advantages of this method. Various biosorbents have been used to remove contaminants from aqueous solutions by biosorption, such as fungi, bacteria and algae [14].

Bioadsorbents for example “orange peel, banana peel [6], coir pith [51], rice husk [47], date pit [10], durian peel [34], broad bean peel [33], peanut hull [67], Citrullus lanatus rind, mussel shells [18], Lady finger (*Abelmoschus esculentus*) [43], *Phragmites australis* [36] *Persea* species [59]” are successfully used to treat dyes from wastewater.

Biosorption of colourants can be accomplished via not only living, but also non-viable algae. *C. vulgaris* is a member of the green algae. It contains not only protein, but also polysaccharides, lipids and vitamins. These incorporate different useful bunches such as carboxyl, hydroxyl, sulfate, phosphate, and other charged bunches that can be dependable for color authoritative [17]. Microalgae are also widely used in industry for biosorption.

In this thesis, biosorption of Reactive Red (He9n) and Reactive Blue (He3r) via using dry biomass *Chlorella vulgaris* will be studied. These are azo dyes and also they are widely used in leather industry. The impact of distinctive operational factors like, pH [18], temperature [18], contact time [18], adsorbent dosage [18] and initial dye concentration [18] has been studied in a batch biosorption studies. The adsorption isotherms and adsorption kinetics of the non-liveable *Chlorella vulgaris* has been also studied.

It is also aimed to determine the effects various conditions thus pH changings, biomass amount, beginning concentration, duration and temperature on biosorption.

2. DYE SPECIFICATIONS

The fixed color particles that contained in the dyes contain mixed unsaturated aromatic components. Chemical structure of color particles is different from each other. Dyes are usually found in dense, soluble, durable structures [1].

There are many kinds of dye types also; the chemistry, structure and bonding of each dye are different from each other. If we look at structural diversity while classifying dyes, the basic structure of the molecule will be taken into account, as well as the chromatic and chromogenic moieties. If we look their material structures, dyes have two kinds; synthetic and natural. However, if we choose discolouration structures, we have azo, indigoid and also cationic and anionic. On the other hand, there are three types of industrial dyes. These are protein textile, cellulose textile and synthetic textile.

2.1. Classification of Dyes According to Dyeing Properties

2.1.1. Acidic Dyes

These are the most common colorants and it contains above two thousand types. Their general formula is SO_3^- and Na^+ . Their class name comes from their production types. These dyes washed in acidic showers and they all are from organic acid salt. Furthermore, they have a big industrial prescription.

2.1.2. Reactive Dyes

Reactive colorants are the foremost utilized sort of colors in coloring wool, silk. The main mechanism is binding reactive sides of molecule. Covalent bindings are the way of binding. Common features of reactive dyes include chromophore moiety, a molecule or group that provides a reactivity resolution.

2.1.3. Direct Dyes

These colours are kind of Sodium and carboxylic acids. They have anionic color side and they are part of azo dyes. Direct dyes are carried out directly into cellulose and wool and they use connection method with fiber strands that is van der Waals bonds. These dyes are easy to find and much cheaper than the other type of dyes. It is usually used for simple fiber, leather, silk, paper, etc. paintings.

2.1.4. Alkaline Dyes

Cationic compounds or organic bases have the form of hydrochlorides. They attach to fibrillas and contain the cationic group on the colored side. Their structure is electron taker so they react materials that have anionic molecules. However, reactive dyes are more practical and more colorful than alkaline dyes. Therefore, alkaline dyes lose their importance. On the other hand, they are more durable to the sunlight. For this reason, they are used as orlon coloring. Also, they have strong fiber bonds.

2.1.5. Disperse Dyes

These types of dyes are found in early 1900's. They used for coloring cellulose acetate and cellulose triacetate. They have low molecule weight. These dyes, which contain amino and hydroxyl groups, constitute the oldest class in the dyeing of synthetic fibers. They are widely used for coloring Polyamide, acrylic, polyvinyl chloride, polyolefin and modacrylic.

Disperse dyes cannot be soluble in water. Also they have non-ionic hydrophobic fibers. They are 1400 different kind of disperse dye. They used mostly polyester colorization.

2.1.6. Mordant Dyes

They have both acidic and alkaline groups in their construction. Mostly, they used for coloring paper, silk and leather etc.

2.1.7. Ingrain Dyes

Ingrain dyes are the main element of making azo dyes. In other words, azo dyes came from ingrain dyes. Naftol-As and phthalocyanine dye are also included in ingrain dye class. These dyes are very durable to light and chlorine.

2.1.8. Metal Complex Dyes

It is a mix of azo dyes and nickel, copper, chrome and also cobalt. It is used as a colorization material in cotton, leather and wool industries.

2.1.9. Sulfuric Dyes

It is used for dyeing cellulose fibers. While colorization process, sulfuric dyes are role playing in oxidation and reduction reactions. Moreover, these dyes are cheap and easy to use so that it is widely used in industry.

2.1.10. Solvent Dyes

They are mostly polar compounds of colors. They are used for coloring melting materials, such as plastics. However, it is not used in textile industry.

2.1.11. Pigment Dyes

Pigment dyes have two categories. These are organic and inorganic types. However, mostly organic types are used. They bind fibers via synthetic resin. Their main mechanism is simple. After the fibers and fabric absorbed, they will become dissolvable in aqution. These colours are mostly used in coloring ink.

2.2. Classification of Dyes According to Their Solubility

They are two types of dyes according to their solubility. These are soluble in water dyes and water insoluble dyes.

2.2.1. Water Soluble Dyes

Dyes can be soluble in water. On the other hand, it cannot be soluble. However, some dyes are soluble in water with certain added groups. They are 3 types of soluble dyes.

Anionic water-soluble dyes: Acidic and direct dyes are in this group. They contain sulfonic and carboxylic acid salts.

Cationic water-soluble dyes: These type of dyes show alkaline specifications. When they mix with acid, they produce salt.

Zwitter water-soluble dyes: These dyes have both acidic and alkaline characteristics. They produce salt in between each other. In alkaline environment, they act like anionic dye.

2.2.2. Dyes that Insoluble in Water

There are 5 different types of water nonsoluble dyes;

Substrate-soluble dyes: They are mixed in water with very thin suspension. Dispersion dyes applicable in these types and in this group.

Organic Solver-soluble dyes: These dyes are in solvent dyes group. They are used with spray and they are soluble with only organic solver.

Temporary soluble dyes: They cannot dissolve in water. However, after the reduction reactions, they become soluble. In fiber, they become non-soluble with oxidation reactions.

Polycondensation dyes: Dyes which are caused by the formation of substances with large contents in between with each other or with other types of molecules.

Dyes generated in the fiber: Azo dyes are in this class. They become from chemical reaction that two different molecules.

2.3. Classification of Dyes According to Chemical Structure

If we want to talk about their chemical structure, we can classify them with their coloring chromogenic side. Colours are classified in pursuant of their chemical conformation this way;

- Azo Dyes
- Polymethine Dyes
- Arylmethine Dyes
- Carbonyl Dyes
- Sulfur Dyes
- Azo-annulane Dyes
- Nitro-Nitrosate Dyes

2.4. Dye Treatment

Dyes are using in various industries. Because of that, dye removal is important situation. The most especial criterias are making the low cost, and high proficiency. There are 3 treatment methods:

1. Biological
2. Chemical
3. Physical

2.4.1. Biological Treatment of Dyes

Biosorption

Biological treatment means using biological ways for remove pollution and making contaminated areas safe. In this way, algae, plants, bacteria and fungi are used. Moreover, it does not matter that they are dead or alive. Their main treatment process is biosorption. In this process, pollutant is hold on to dead or alive cell's surface. It is observed that, dead biological materials are very effective for adsorption [2]. It is less costly and more efficient for removing dye than other methods. Also, dead materials are advantageous for big

volume of wastewaters because bioadsorbents cannot be affected from toxic compounds [3].

Biosorption is the most reliable way for remove both organic and inorganic contaminating particles. Moreover, there are many studies about dye removal with using biosorption. They are show that using different kinds of biomasses have different results. Furthermore, efficiency is changed with some parameters.

Aerobic Treatment

It is biologically difficult to reduction waste in activated sludge systems commonly used for industrial wastewater treatment. In addition, water soluble basic and azo dyes enter the biological reaction with microorganisms to adsorb some of the dye for removal.

Anaerobic Treatment

At the beginning of this treatment, bacteria convert organic nutrients (fat, protein, carbohydrates, etc.) into components that have low molecular weight. Bacteria use these products to make acetate, carbon dioxide and hydrogen. Then these bacteria are degradate $C_2H_3O_2^-$ and CO_2^- .

This type of treatment is mostly used for biosorption activity for watersoluble azo colourants. In fact, they are need for extra carbon resource. After the destruction of azo bonds, aromatic amine is produced.

Biodegradation

Organic pollutants absorbed by microorganisms. Then, in their cytoplasm, pollutants have some chemical and enzymatic reactions and become safe. With this mechanism, dangerous pollutants stay in microorganism and some safe products go out of cell.

2.4.2. Chemical Treatment of Dyes

Oxidation

This method is the most used way for removing dye from wastewater. After the oxidation, the aromatic chain will be broken in dye and it is become wasted.

Ozone

Ozone's history about treatment is started in 1970's. Ozone is used for removing phenol, pesticide, and aromatic hydrocarbon. The biggest advantage of ozone is it is used in gas form. However, it is expensive. Also if ozone is used for treatment, process has to be repeated [4].

Photochemical

In this method, hydrogen peroxide is activated by ultraviolet to decompose into hydroxyl types, H₂O and CO₂. In this method of chemical oxidation of organic matter, inorganic and organic acids, organic aldehydes and halides are activated according to the size of the reaction.

Using Sodium Hypochlorite

In this method, Cl molecules will make bonds to dyes amino group. Then azo bonds will break easily and faster. However, using sodium hypochlorite is not suitable for disperse dyes. Because, some toxic molecules will be produced due to rising chlorine levels. Thus, it is not very effective in industry.

Fenton Separators

This method is the appropriate method for the treatment of toxic wastewater. It has 2 different ways. While the first method is coagulation, the second method is to pre-oxidize. The disadvantage of this method is that the mud is formed at the bottom by the agglomeration of the reagent and dye molecules. Unfortunately, this is an expensive method for cleaning the mud. Acid, direct, mordant and reactive dyes often cause agglomeration in this process, and therefore this method is not very suitable for these type of dyes.

Electrochemical Catabolism

In the electrochemical method, the reactor consists of an anode, a cathode, a conductive electrolyte and a power supply. In this method, carried out in the mid-1990s, the charge on the cathode causes the reduction of the oxidation, while the charge on the surface increases the oxidation state. In this method, chemical is not used, there is an effective and economical paint cleaning feature and also there is no sludge accumulation. However, the

possibility of the formation of dangerous compounds makes this method disadvantageous. Electricity costs are normal and use is convenient.

Coagulation

This is clumping of particles, typically in a colloidal. Coagulation of coagulant substances at appropriate pH is called coagulation by combining colloidal and suspended solid materials in waste water. Colloidal molecules and chemically formed particles will form at the bottom of the coil.

Flocculation

This is the process of combining small particles into flocs by mixing the waste water at a suitable speed. In order to increase the purification process, clay, calcite, polyelectrolyte and activated silica are included to the blend and the method will be completed. In order the settling, rapid mixing, slow mixing and sedimentation basins can be applicable.

2.4.3. Physical Treatment of Dyes

Adsorption

Adsorption is the most in demand treatment way in recent years. It is very effective especially wastewaters that contains too much contaminants. Furthermore, its efficiency is very high and treated water is high quality. Moreover, it is very cheap way. However, adsorption is bittersweetly. Its efficiency is variable due to some factors. These are pH, heat levels, duration, biomass interactivity, biomass s/v ratio etc.

In this method, activated carbon is the most commonly used way. Active carbon method is effective against cationic, acidic and mordant dyes. On the other hand, it is not effective against direct, pigment, vat and disperse dye adsorptions. The most used activated carbon material is peat and it is used as adsorbent. It is very effective for treating heavy metals and polar organic compounds in wastewater.

Materials such as tree cracks, natural clay, corn cobs and rice husks are cheap and good adsorbents. Molecular structure and solubility are conditions that significantly affect the cleaning mechanism.

Removal using adsorption is depends physico-chemical factors such as colour sorbent friction, pH, contact time and particle size. However, disperse, pigment and cube dyes are

not adsorbed enough because of their low solubility in water. Researches show that natural carbons have good adsorption effect on synthetic dyes. However, sorbents that contain natural carbon are not used in industry due to their high cost. The reason for this, high concentration of wastewater needs too much carbonic sorbent.

Pulp, natural clay, peanut shell, active alumina, alkali mud, bauxite and dolomite are very effective for acidic dye adsorption. Studies have shown that, while adsorption process with these materials, increasing pH will increase adsorption quality in acidic dyes. The results gained from this studies are, mechanism for removing dye in wastewater is usually ion exchange [5].

Ion Exchange

In this method, anionic and cationic dyes are separated from wastewater with high efficiency. Also ion exchange is commonly used in industry. If we talk about its advantages and disadvantages, after the treatment process, solvent can be used over and over again. However, it is too expensive method for dye removal [6].

Membrane Filtration

Membrane Filtration is the one of the ways of eliminating colour from wastewater. Furthermore, membrane filtration method is durable to temperature changes, pH changes, chemical environment and microbial effects. However, it is not always advantageous. For example, membrane can be plunge easily. Also it is expensive.

2.4.4. Microalgae

Microalgae can be prokaryotic and eukaryotic and they have photosynthetic reactions in their cell. Cyanobacteria were formerly known as blue-green algae. However, later studies shown that they are prokaryotic microorganisms. On the other hand, Chlorophyta is known as mono cellular photosynthetic green algae.

In algiculture, the aim of large-scale algae breeding is to develop efficient product with less expenditure. Each algal species exhibits its ideal development in culture environments where specific conditions are inherent. For example, *Spirulina* needs high pH and bicarbonate. On the other hand, *Chlorella* needs nutrient – rich environment.

2.4.5. Cyanobacteria

Cyanobacteria are prokaryotic bacteria. It is found nearly 3.5 billion years ago. They can stay alive at high temperatures, high light areas at low CO₂ concentrations and at also cold areas. Therefore, they can be found anywhere in world.

Most of the cyanobacteria are aerobic so that they are photosynthetic. They can be found in oceans, too. They have chlorophyll-a and carotenoid in their cell, so that they can adapt different wave length of lights. Therefore, they can produce energy faster.

2.4.6. Green Algae

Green algae are found in nearly all regions of the earth. They usually found in shallow areas of fresh water, moist soil and seas. They also contain chlorophyll a and chlorophyll b, and for this reason, they have many similarities with high plants.

They can be single and multicell and for their construction, they can be tall, siphoned or paranmatic tall.

Chlorella vulgaris

C. vulgaris is rich in B12 vitamins and contains magnesium, phosphorus, iodine and zinc. It is green because of its chlorophyll. Moreover, cells are usually 5-8.5 μ in diameter and they are unmixed reproductive species. Because the cell wall is made of cellulose molecule, it is very hard and its shape is rounded. It is the living species that contains the most chlorophyll in nature. The amount of protein it contains is around 50-60% [7].

2.5. Dye Removal by Adsorption on the Microalgae

Dotto and others are studied that; the biosorption of the nanoparticles of the *Spirulina plantesis* in the food colorings with different acidity values and temperatures. Maximum removal capacity for FD&C Red no. and 40 ve Acid Blue 9 had been discovered in order of 468,7 mg/g and 1619,4mg/g at pH 4 and 298K. This process has been determined as exothermic in consequence of decreasing Adsorption capacity with the increasing temperature [8].

For removal of the Reactive Black B (RBB), Junnarkar and Pandhi used “*P.chryso sporium*, *Aspergillus sp.* *Trichoderma sp.*”. Maximum adsorption capacity was maintained at pH 3 for each three adsorbent has been reported[9].

Removing the Tartrazine and Allura Red by biomass of *Spirulina platensis* have been studied by Dotto and friends. The highest effectiveness is occurred in temperature of 298 °K at the range of 363 - 469 mg/g [10].

Aksu et al. used dead *C. vulgaris* biomass for biosorption of Remazol Black B, Remazol Red RR and Remazol Golden Yellow RNL reactive dyes [11]. The optimum pH is found 2 for each three dyes [11]. Maximum biosorption capacity was maintained at 35°C for Remazol Black B, 25°C for Remazol Red RR and Remazol Golden Yellow RNL[11].

Removal of Tectilon Yellow 2G (TY2G) by *Chlorella vulgaris* has been studied by Acuner and Dilek. The removal rate of dye concentrations for 50, 200, 400 mg/L has been calculated as %69, %66 and %63 respectively [12].

AO 7, BR 46 and BB 3 dyes were used for biosorption by Khataee and others in their studies. The best removal capacities were achieved for Acid Orange 7 at pH 4 as % 42, for BR 46 and BB 3 at pH 10 as % 91,6 and % 87,15 respectively. Optimum amount of biomass and dye concentration were found as 1,0g/L also 45g/L [13].

Mitrogiannis and others perform an experiment about removal of Methylene Blue (MB) via *Arthrospira platensis* biomass. The duration of the equilibrium is achieved within 60-120 minutes. The biosorption capacity for Methylene Blue (MB) was found that, it rises with increasing color concentration and diminish with rising heat [14].

Güngörmedi et al. investigated the removal of RR 198 dyes using dry biosorbent of *T. versicolor* ATCC 200801. According to the results, optimum conditions were figured as high acidity, biosorbent concentration of 0.4g, starting concentration 75 mg / L, 20 min duration and 35 ° C temperature [15].

Nadeem and others are worked with *Spirulina platensis* to remove Crystal Violet (CV) over solution. Most efficient removal percentage for Crystal Violet occurred 79% at pH 6. Equilibrium contact time was found to be 60 minutes. Rate of evacuation diminished with expanding color concentration. The highest adsorption was observed at 81% at 10 mg / L [16].

Aziam and others research the adsorption of AB 129 with almond skin. The effects of pH, starting concentration, biomass amount, duration and temperature on AB 129 recovery

were examined. At high acidity (2), contingency was found 14 minutes, the adsorbent amount at 0.4 g and the percent recovery found that 98% [17].

Naraghi and others are studied the removal of Acid Orange 7 dye with their Kenyan tea in their work. The highest yield of color removal achieved at pH 2, 120 minutes, 50mg/L starting concentration and 98g/L biomass amount [18].

In their study, Junxiong and colleagues examined the adsorption of MB and RR 4 by sludge belongs to biological coke treatment plant. It can be said that pH is very important variable for biosorption. As the pH increases, Methylene Blue removal increases while the Reactive Red 4 removal decreases. Maximum Reactive Red 4 removal with protonated sludge is 73.7mg/g at pH1 and near 235 mg/g at Methylen Blue at pH9 [19].

Aksu and Çağatay examined the removing the Gemazol Turquoise Blue-G azo-type colorant ions on *R.arrhizus* using a continuous packed bed adsorption technique. With that method, the highest retention was found at a concentration of 0.5g/L biosorbent, 45 ° C temperature, pH 2 and 773.0 mg / g at an initial dye concentration of 812.6 mg / L [11].

3. MATERIAL AND METHOD

In this research, the main purpose is removing Reactive Red (120) and Reactive Blue (198) via using *C. vulgaris* biomass. Different conditions that effect dye biosorption such as pH changes, biomass quantity, dye concentration changes, temperature changes and contact duration changes will be examined.

3.1. Materials

3.1.1. Microorganisms

In this study, *C.vulgaris* will be used as biomass and also it is used dead dry powder. It is obtained from Nurbal Şifa Merkezi in İstanbul.

3.2. Dye Stuff

In this study, removal of two reactive dye which are highly used in textile industry are investigated and these dyes are Reactive Red (120) and Reactive Blue (198).

3.2.1. Used Dyes and Their Chemical Properties

Chemical structures of RR 120 and RB 198 are given in Table 3.1. Also, the formulas are given in Figure 3.1 and Figure 3.2.

Table 3.1. Chemical Properties of RR 120 and RB 198

Dye name	Reactive Red 120	Reactive Blue 198
Abbreviation	RR 120	RB 198
Dye Type	Reactive	Reactive
λ_{max}	500 nm	600 nm
Weight	1469.97 g/mol	1304.80 g/mol
CAS number	61951 – 82 - 4	124448 - 55 - 1
EC Number	263 - 351 - 0	219 - 949 - 9
Moleculer Formula	$C_{44}H_{24}Cl_2N_{14}Na_6O_{20}S_6$	$C_{41}H_{30}C_{14}N_{14}Na_4O_{14}S_4$

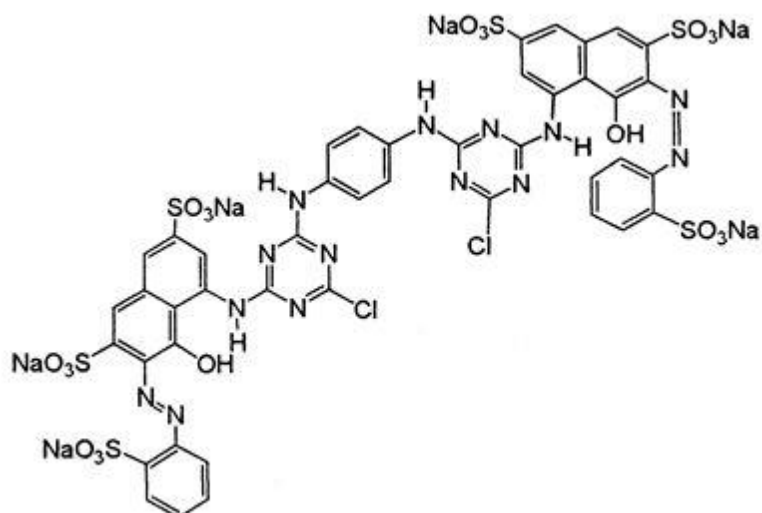


Figure 3.1. Chemical formula of RR120

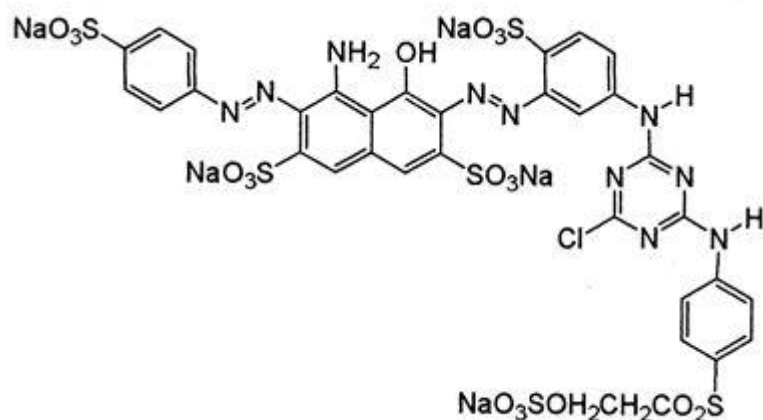


Figure 3.2. Chemical formula of RB 198

3.2.2. Dye Stock

For identifying colour removal from aqueous solution, optimum pH, biomass concentration, dye concentration, temperature and contact time parameters are examined. Because of that, 5.000 mg/L (0.5g/50 mL) stock solutions of these dyes were prepared and used in these studies. For dye biosorption experiments, %1 of stock is taken and completed to 10 mL. In this research, each experiment was run in parallel.

3.3. Method

3.3.1. Determination of Maximum Absorbance Values for Dyes by Using Spectrophotometer

The absorbance values of concentrations are measured by UV-V12 Spectrophotometer. Measurements of the dyes were performed by recording the absorbance values between 400 nm to 600 nm of the dye solutions. Experiment solutions are elaborate via only distilled water – dye stock.

3.3.2. Calibrations of RR 120 and RB 198

Calibration curves were obtained with the absorbancy of solutions of prepared mixtures at concentrations of 10, 25, 50, 75 and 100 mg / L. The maximum absorbance value for RR 120 is 500 nm, whereas for RB 198 is 600 nm.

Table 3.2. Maximum Absorbance Values of Dyes

Dye Symbol	Dye Name	Max. Absorbance Value
RR 120	Reactive Red 120	500 nm
RB 198	Reactive Blue 198	600 nm

Therefore, the measurement was done according to the absorbances given in table 3.2.

3.4. Determination of Dye Adsorption

In this study, the activity of dye removal in water (%) is calculated by the following formula.

$$\text{Dye Removal (DR) \%} = [(C_o - C_f) / C_o] \times 100$$

C_o: Initial Absorbance Value

C_f: Final Absorbance Value

For first absorbance value, the value is used that is obtained from only dye solution.

3.4.1. Biosorption Experiments

Investigation of the optimum pH value for dye removal

For identifying optimum pH level, pH changed between 2.0 and 9.0 [11]. pH was adjusted by using NaOH, 1N and also by HCl, 1N. In this experiment, *C. Vulgaris* biomass amount is 0.05 g, dye concentration is 100 mg/L. Also temperature is set to 25 °C. Samples are

shaken for 30 minutes at 100 rpm. Then, samples are centrifuged at 5000 rpm for 15 minutes. Finally, for RR 120, absorbance value is measured at 500 nm in spectrophotometer. For RB 198 wavelength is set to 600 nm.

Optimum amount of Microalgae biomass

For the effect of microalgae biomass on the biosorption on RR 120 and RB 198, initial mass of biosorbent quantities between 0.05-0.25 mg/L are studied[11]. In this experiment, pH value was set to 2. Starting dye stock is 100 mg/L. The studies were conducted at room temperature (25 °C). The, samples were shaken at 100 rpm for 30 minutes. Then, centrifugal machine is used at 5000 rpm for 15 minutes. Absorbance values of solutions were measured at 500 nm for RR 120 and 600 nm for RB 198 by spectrophotometer.

Different initial dye concentrations on dye removal

For investigating impact of initial dye concentration, dye concentrations were changed between 50 mg/L and 200 mg/L [11]. These tests were conducted in pH 2, 25 °C. Then samples were shaken at 100 rpm for 30 minutes. The samples will be centrifuged at 5000 rpm for 15 minutes. Before spectrophotometric measurements, absorbance values of collected samples will be measured at 500 nm for RR 120. For RB 198 wavelength is set to 600 nm. Finally, dye removal will be calculated.

Effects of different contact durations on dye removal

In this study, biomass contact duration was varied between 15 minutes to 120 minutes For investigating impact of contact time for dye treatment [11]. In this experiment, pH is 2. Colour stock is 100 mg/L and temperature is 25 °C. Furthermore, *C. Vulgaris* biomass amount was 0.05 g. Later, samples will be shaken at 100 rpm for 15 to 120 minutes. Then, samples will be centrifuged at 5000 rpm for 15 minutes. After all, absorbance values of collected samples will be measured at 500 nm for RR 120. For RB 198 wavelength is set to 600 nm. For accurate result, data was collected every 10 minutes.

Effects of different temperature values of dye removal

In order to determine temperature effects on dye removal, 25 °C, 30 °C, 40 °C and 50 °C is tested [3]. In this experiment, *C. Vulgaris* biomass amount was 0.05 g, initial dye concentration was 100 mg/L. Samples were shaken in the shaker for 30 minutes at 100 rpm.

3.5. Correlation Analysis

This analysis is utilized to measure the affiliation between two persistent factors. The purpose is to see how the subordinate variable (Y) changes when the free variable (X) changes. As a result, whether there is a linear relationship between these variables and if there is a correlation coefficient of this relation.

- If r equals to -1 , it means full negative linear relationship
- If r equals to $+1$, it means full positive linear relationship
- If r equals to 0 , it means there is no relationship

According to value of correlation coefficient (r), relationships can be described like below.

- $0.00 - 0.25$ Very low relationship
- $0.26 - 0.49$ Low relationship
- $0.50 - 0.69$ Medium relationship
- $0.70 - 0.89$ High relationship
- $0.90 - 1.00$ Very high relationship

3.6. Adsorption Isotherm Studies

In this system, adsorption / biosorption equilibrium and kinetics are studied. For determine biomass capacity, adsorption isotherm have to be generated. In other words, adsorption isotherm is an equation between biomass and dye amount. Usually, the adsorption isotherm is obtained by bringing the solution containing the adsorbed material at different concentrations into the equilibrium with the known amount of adsorbent.

3.6.1. Langmuir Isotherm of Dye Removal

This isotherm describes single-layer adsorption obtained on a homogeneous adsorption. Several assumptions have been made for the Langmuir model.

- It is suggesting, the adsorption is constant also that substance molecules retained on the biomass are not displaced. In other words, biomass is assumed that solid single layer.
- There are receiving points on the surface of the adsorbent and these points are similar in terms of energy. So that, the adsorption energy is constant and the molecules of the material holding on the biomass are not displaced.
- Assume absence of subsequent interactive relation of molecules being adsorbed.

Langmuir model is seen below.

$$q_e = \frac{q_s b C_e}{1 + b C_e}$$

After linearization;

$$\frac{C_e}{q_e} = \frac{1}{q_s b} + \frac{C_e}{q_s}$$

In this equation; q_e is sum of biosorbed colour ions (mg / g) on the biosorbent at the time of equilibrium, C_e is the concentration of the dye ion remaining in the solution in equilibrium, q_s is the maximum amount of substance biosorbed in the unit weight of the biosorbent to form a single layer on the surface. Also, b is the adsorption equilibrium constant.

3.6.2. Freundlich Isotherm to Dye Removal

Another of the adsorption isotherm equations is the Freundlich adsorption equation, which has been widely used for many years. Freundlich equality is an empirical equation that defines many adsorption data. The Freundlich isotherm is generally used for adsorption for liquid solutions as well as for adsorption of gases [20].

The Freundlich isotherm recognizes that the adsorption heat is logarithmically reduced by the surface cover and it is expressed below.

$$q_e = K_f C_e^{1/n}$$

This equation is linearized by taking the logarithms of both sides of equation.

$$\log q_e = \log K_f + \frac{1}{n} \log C_e$$

In this equation;

C_e : Substance concentration in solution after adsorption (mg/ L)

q_e : Amount of adsorbed material on the unit adsorbent (mg/ g)

K_f : Adsorption capacity

n : Measure of adsorption severity

4. RESULTS

4.1. Determination of Maximum Absorbance Values for Dyes

For both RR 120 and RB 198, 400 nm, 500 nm and 600 nm wavelengths were tested. As a result, depending on a spectrophotometer, 500 nm wavelength was found as maximum absorbance value for RR 120. On the other hand, 600 nm is chosen for RB 198.

4.2. Determination of Optimum Conditions for RR 120 Removal by *C. vulgaris*

4.2.1. Optimum pH value for RR 120

One of the most important factor that affect the dye removal capacity is the initial pH of the dye solution. For this reason, the effect of initial pH value on dye biosorption was investigated. In this experiment, *C. Vulgaris* biomass amount is 0.05 g and dye concentration was 100 mg/L at 25 °C. For identifying optimum pH level, initial pH is varied between 2.0 and 9.0. Outcomes are displayed in table 4.1 and figure 4.1.

Table 4.1. Removal (%) at different pH values for biosorption of RR 120 dye

pH	qe (mg/g)	Dye removal (%)
2	100.06	95.05
4	82.27	78.15
6	71.72	68.13
8	60.15	57.14
9	53.21	50.54

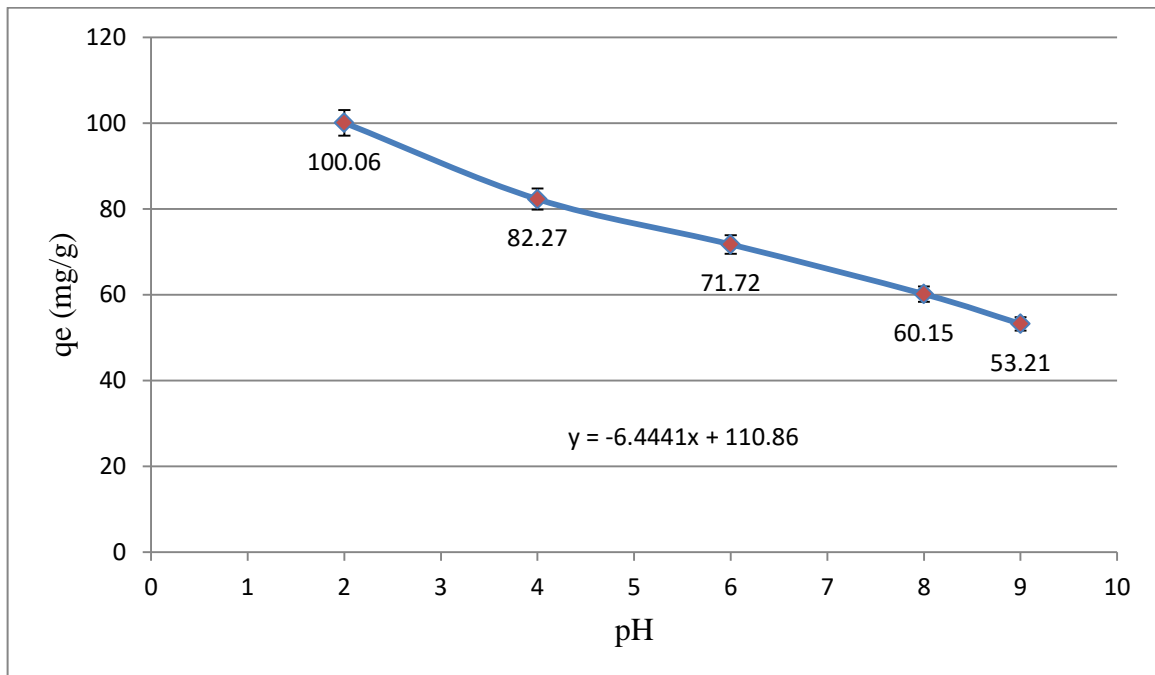


Figure 4.1. pH changes effects to dye removal that caused by *C.vulgaris*

The dye removal decreased with the increasing pH. Maximum removal efficiency was achieved at pH=2. However, when the pH level is approximately 9, dye removal was minimum. (50.5%) (Table 4.1, Figure 4.1). As a result of that, the optimum pH level for RR 120 biosorption was found 2. Consequently, for other experiments, pH was set to 2.

In consequence of correlation, negative correlation (-0.995) between the RR 120 removal and the pH values was determined. For this reason, statistically, this correlation is meaningless.

4.2.2. Initial Concentration of Biosorbent

In this experiment, for investigating the effect of microalgae biomass amount on dye biosorption, *C. Vulgaris* biomass amount is changed 0.05g to 0.25. Other variables are held steady (100 mg/L,pH2, 25°C)

Table 4.2. Removal values (%) for different biomass amounts for RR120 biosorption

Biomass Amount (g)	qe (mg/g)	Dye removal (%)
0.05	100.06	95.05
0.10	99.77	94.78
0.15	98.74	93.80
0.20	95.81	91.01
0.25	93.93	89.23

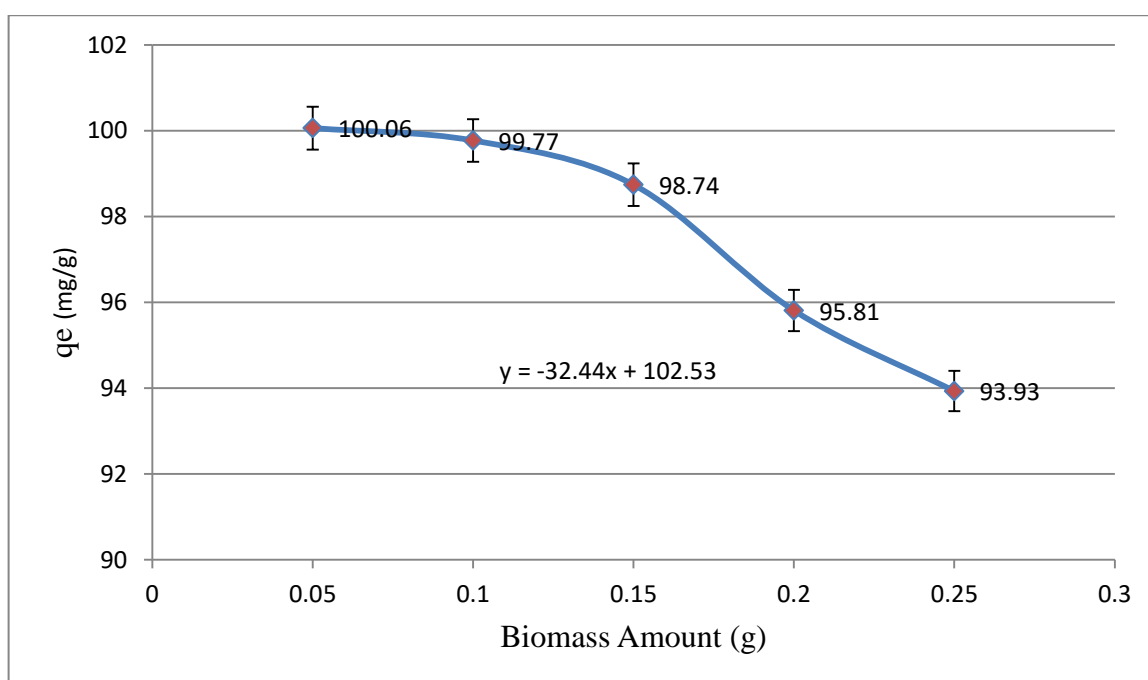


Figure 4.2. Effects of biomass quantity on biosorption of RR 120

Table 4.2 and Figure 4.2 shows that, when the biomass amount is increasing, dye removal activity is slightly decreases. However, decrease rate is not much. Accordingly, maximum dye removal has been done in when the *C. vulgaris* is 0.05 g. On the other hand, minimum dye treatment is done when the biomass amount is 0.25 or higher.

In consequence of correlation, negative correlation (-0.941) between the RR 120 removal and the biomass amount was determined. For this reason, statistically, this correlation is significant. The differences between dye removal depending on biomass concentration is about 7 % between 0.05 mg/l to 0.25 mg/L biomass.

4.2.3. Temperature Effect on RR 120 removal

In this study, biosorption studies were tested under different temperatures to investigate the impact at heat levels on colour treatment efficiency. Therefore, 25°C, 30°C, 40°C and 50°C tested.

Table 4.3. % removal values at different temperatures for RR120 biosorption

Temperature (°C)	qe (mg/g)	Dye removal (%)
25	100.06	95.05
30	99.48	94.50
40	95.81	91.01
50	92.93	88.28

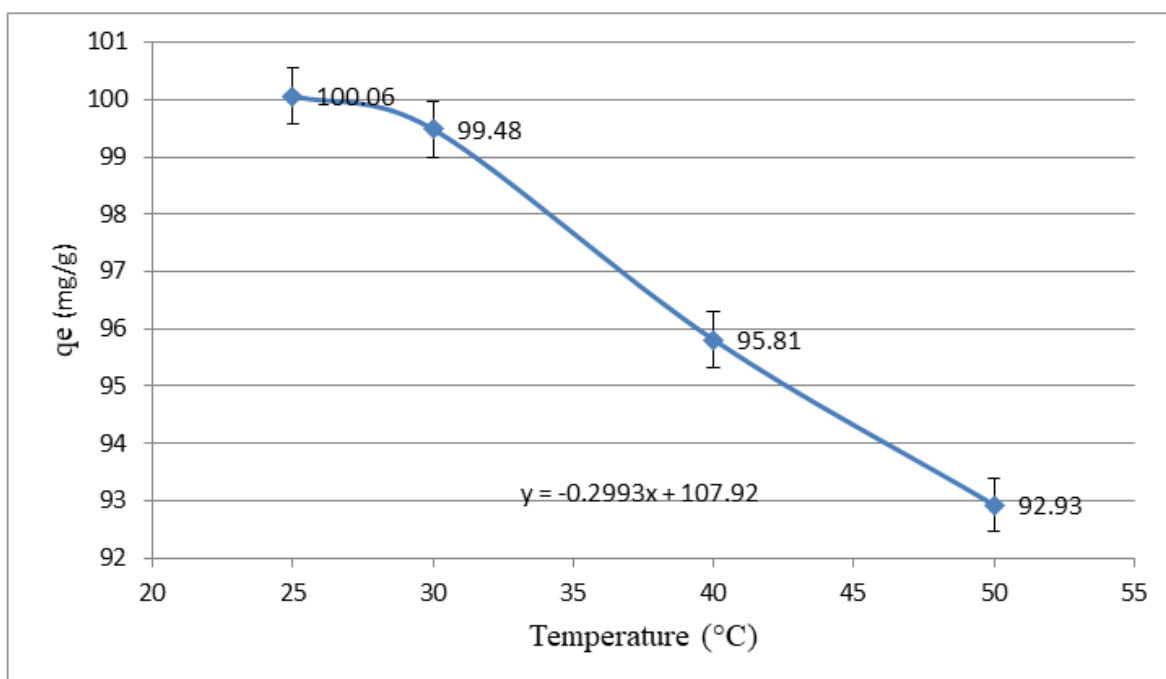


Figure 4.3. Effects of temperature changings on color removal by *C. vulgaris* on RR 120

It is shown that, when the temperature is increasing, dye removal rate decreases. The maximum biosorption achieved at 25°C. After the 25°C, dye removal activity is decreasing. However, the decrease was not significant.

In consequence, negative correlation (-0.993) between the RR 120 removal and the temperature was found. For this reason, this correlation was found to be statistically significant.

4.2.4. Effect of different initial dye concentrations on RR 120 biosorption

The effects of different dye concentrations to biosorption of RR 120 dye were examined. For this purpose, dye concentration varied between 25 and 150 mg / L with 0.05 g of biosorbent, 25 °C and pH=2.

Table 4.4. Removal values (%) at different dye concentrations for RR120 biosorption

Dye concentrations (mg /L)	qe (mg/g)	Dye removal (%)
25	93.69	89.01
50	95.81	91.01
100	100.06	95.05
150	99.48	94.50

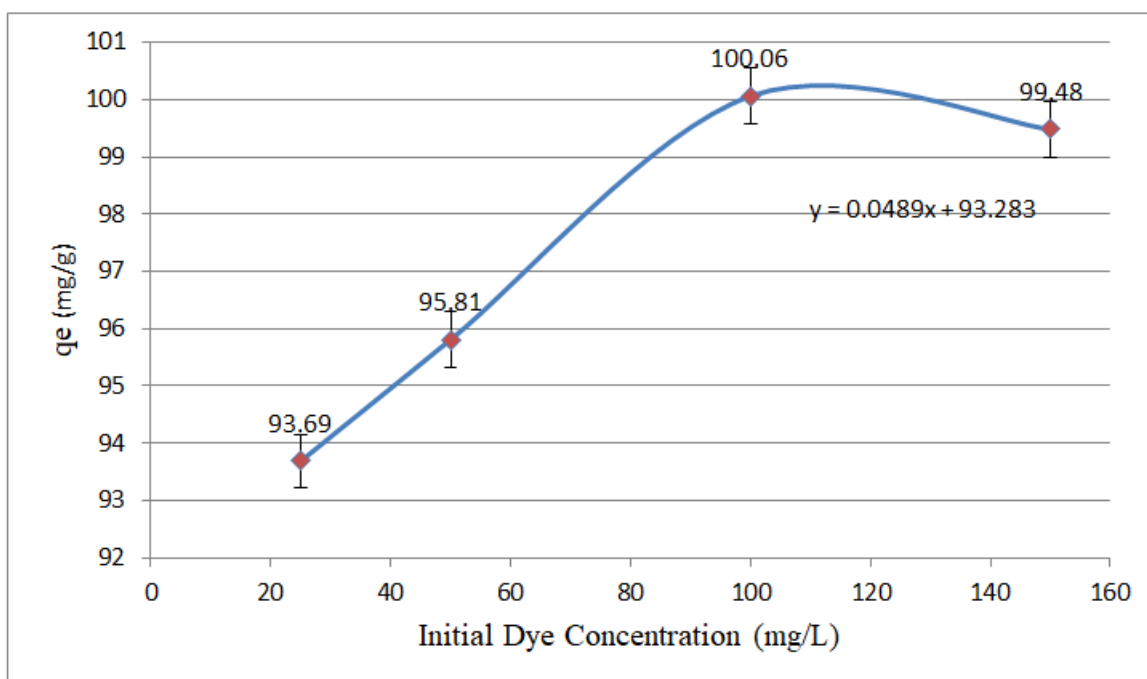


Figure 4.4. Starting concentration on dye removal by *C. vulgaris* on RR 120

As seen on the Figure 4.4, increasing initial concentration causes increasing % dye biosorption. Maximum adsorption achieved at 100 mg/ L dye concentration. However,

while the concentration increased, dye removal rate decreased after 150 mg/ L. As a result, 100 mg/ L was used during the studies as the optimum initial dye concentration.

In consequence, negative correlation (-0.568) between the RR 120 removal and the initial dye concentration was found. For this reason, this correlation was found to be statistically not significant.

4.2.5. Different contact durations on RR 120 biosorption

Different contact periods on RR 120 dye removal were examined. Studies were carried out; *C. Vulgaris* biomass amount was 0.05 g, dye concentration 100 mg/L. and 25 °C. and at pH=2. The biosorption studies were conducted up to 15 minutes to 120 minutes for examine the impact of duration parameter for dye removal.

Table 4.5. Removal values (%) at different contact times for RR120 biosorption

Contact time (min)	qe (mg/g)	Dye removal (%)
15	89.03	84.57
30	100.06	95.05
45	100.98	95.93
60	101.72	96.63
75	102.13	97.02
120	102.23	97.11

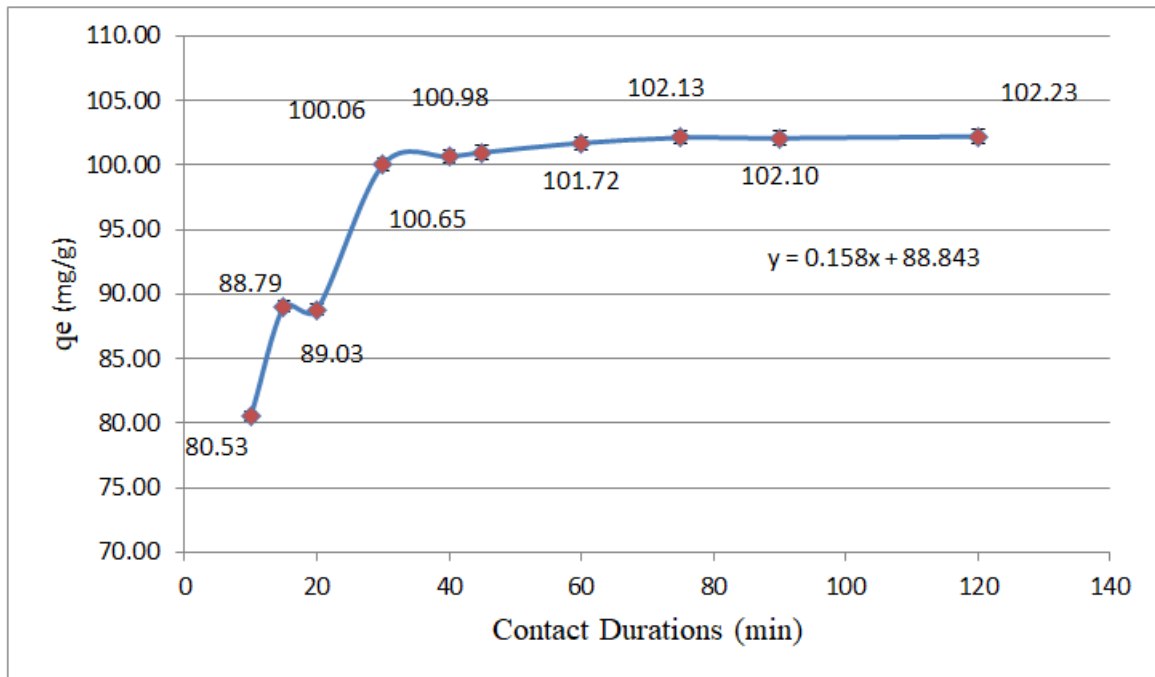


Figure 4.5. q_e changes in terms of different durations on color treatment via *C. vulgaris* on RR 120

When the percentage of dye removal and the time chart are examined; increasing trend is seen in dye removal due to the increase in time from the beginning. However, adsorption process acceleration is going slowly according the change of contact duration. Results show that, most of the dye treatment process is completed at 30 minutes. At 60 minutes, dye removal activity reaches the equilibrium. After 60 minutes, there are some minor changes happens in dye removal. However, it is not very significant. (Table 4.5, Figure 4.5)

In consequence, medium positive correlation (0.663) between the RR 120 removal and contact duration was determined. For this reason, this correlation was found to be statistically significant.

4.3. Determination of Optimum Conditions for RB 198 Removal by *C. vulgaris*

4.3.1. Optimum pH value for RB 198

In this experiment, the effect of initial pH value on dye biosorption was investigated. All the factors are set to standart except pH. *C. Vulgaris* biomass amount is 0.05 g, dye concentration is 100 mg/L and temperature is 25 °C. For identifying optimum pH level, pH is varied between 2.0 to 9.0.

Table 4.6. Removal (%) at different pH values for biosorption of RB 198 dye

pH	qe (mg/g)	Dye removal (%)
2	99.73	94.74
4	85.28	81.02
6	69.48	66.00
8	52.98	50.32
9	50.34	47.82

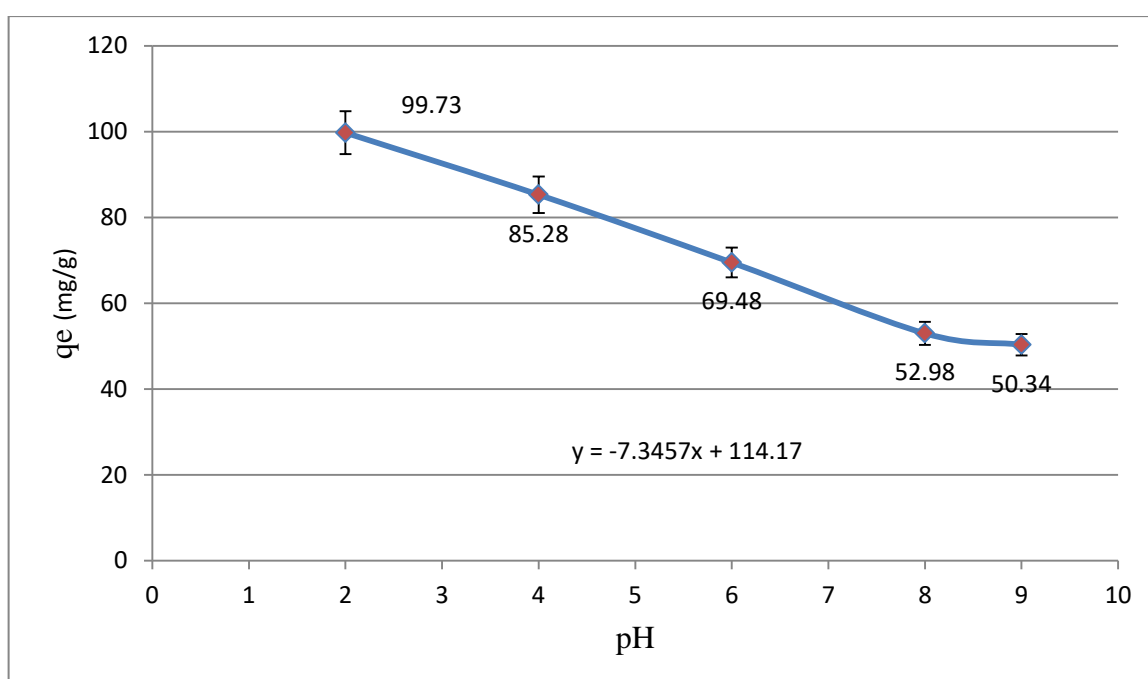


Figure 4.6. pH changes effects to dye removal that caused by *C.vulgaris*

Experiments shows that, when the pH levels increase, dye removal decreases. Maximum removal efficiency is achieved at pH =2 levels. However, when the solution pH is increased to 9, dye removal become minimum. As a result of that, pH 2 is found as the optimum pH level for RB 198 biosorption. Consequently, for other experiments, pH is set to 2.

In consequence of correlation, highly negative correlation (-0.996) between the RB 198 removal and the pH values was found. For this reason, statistically, this correlation is regarded as significant.

4.3.2. Initial Concentration of Biosorbent

In this experiment, for investigating the effect of microalgae biomass amount on dye biosorption, *C. Vulgaris* biomass amount is changed 0.05g to 0.25 (Dye concentration 100 mg/L, temperature=25 °C. Also, pH=2).

Table 4.7. Removal (%) for different biomass amounts for RB 198 biosorption

Biomass Amount (g)	qe (mg/g)	Dye removal (%)
0.05	99.73	94.74
0.10	99.39	94.41
0.15	97.91	93.01
0.20	95.00	90.24
0.25	93.23	88.57

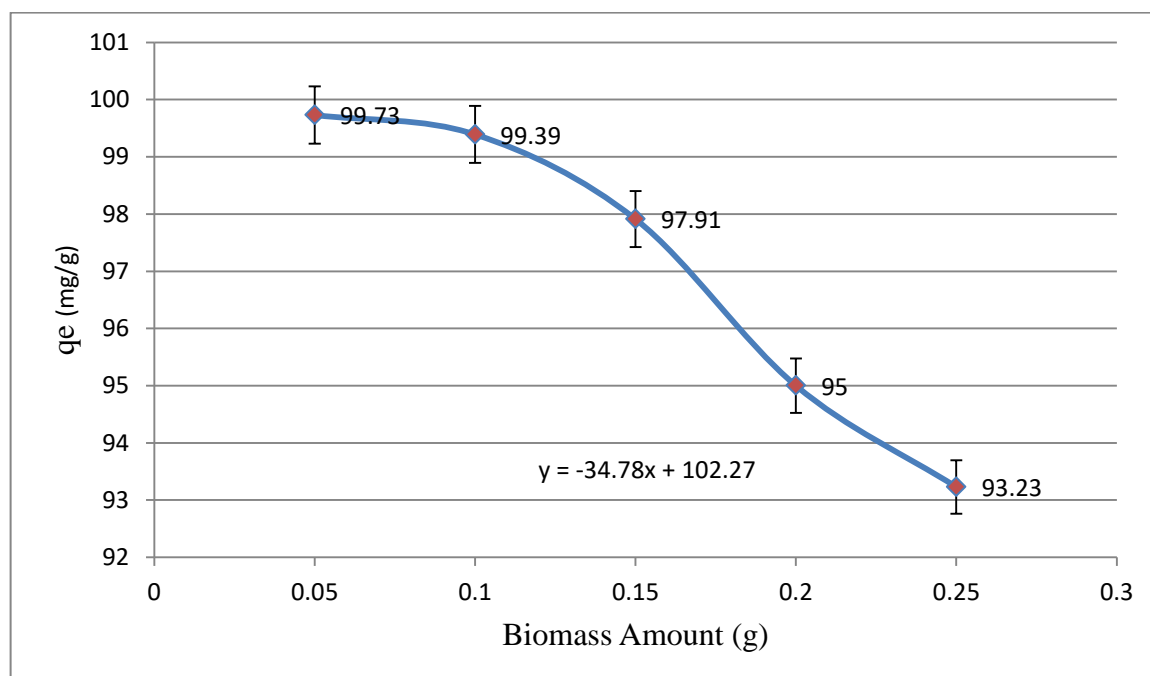


Figure 4.7. qe changings due to biomass amount on RB 198 treatment

These results show that, when the biomass amount increases, dye removal decreases slightly. However, the decrease rate is not high (about 6 %). Accordingly, maximum dye removal has been achieved when the *C. vulgaris* biomass amount is 0.05 g.

In consequence of correlation, negative correlation (-0.969) between the RB 198 removal and the biomass amount was determined. For this reason, statistically, this correlation is significant.

4.3.3. Temperature Effect on RB 198 removal

In this study, biosorption studies were tested under different temperatures to investigate the effect at temperature on dye removal efficiency. Therefore, the studies were conducted at 25 °C, 30 °C and 40 °C and 50 °C.

Table 4.8. % removal values at different temperatures for RB 198 biosorption

Temperature (°C)	qe (mg/g)	Dye removal (%)
25	99.60	94.62
30	96.21	91.40
40	93.50	88.82
50	83.59	79.41

It is shown that, when the temperature is increasing, dye removal rate decreased. The maximum biosorption achieved at 25°C. After the 25°C, dye removal activity is decreasing. However, the decrease was not significant.

In consequence, negative correlation (-0.967) between the RB 198 removal and the temperature was found. For this reason, this correlation was found to be statistically significant.

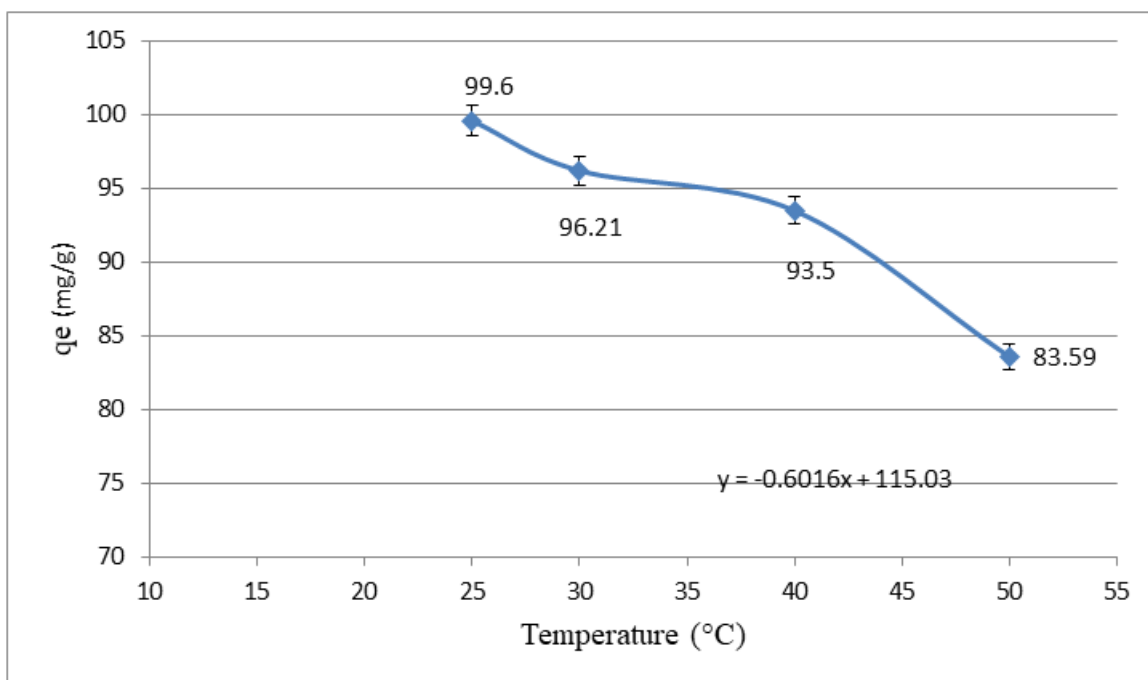


Figure 4.8. Effect of temperature on RB 198 removal by *C. vulgaris*

4.3.4. Effect of different initial dye concentrations on RB 198 biosorption

In this study, the effects of different dye concentrations on the removal of RB 198 dye was examined. For this purpose, dye concentration varied between 25 and 150 mg / L with 0.05 g of biosorbent, 25 °C and pH=2.

As seen on the figure 4.9 and Figure 4.9, when the initial dye concentration increases, % dye removal is also increasing. Maximum adsorption achieved at 100 mg/ L dye concentration. However, while the concentration increased, dye removal rate decreased in 150 mg/ L. As a result, 100 mg/ L was used during the studies as the optimum initial dye concentration.

Table 4.9. Removal (%) for different initial dye concentrations for RB198 biosorption

Dye concentrations (mg /L)	qe (mg/g)	Dye removal (%)
25	95.13	90.37
50	96.21	91.39
100	99.73	94.74
150	99.42	94.45

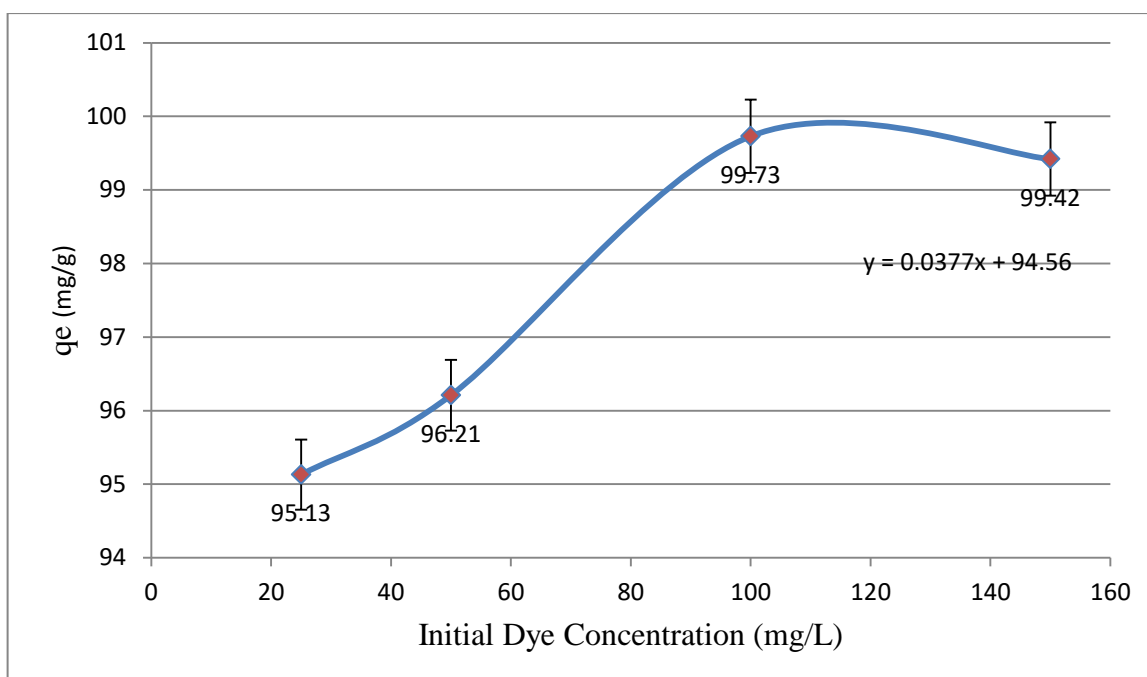


Figure 4.9. Effects of initial dye concentration changings on color removal by *C. vulgaris* on RB 198

In consequence, medium negative correlation (-0.557) between the RB 198 removal and the initial dye concentration was determined. For this reason, this correlation was found to be statistically meaningless.

4.3.5. Effect of different contact time on RB 198 biosorption

In this study, the effect of different contact periods on RB 198 dye removal was examined. The studies were carried out, *C. Vulgaris* biomass amount was 0.05 g, dye concentration 100 mg/L. and 25 °C. and at pH=2. The biosorption studies were conducted up to 15 minutes to 120 minutes for examine the impact of duration parameter for dye removal.

Table 4.10. Removal values (%) at different contact times for RB 198 biosorption

Contact time (min)	qe (mg/g)	Dye removal (%)
15	95.17	90.41
30	99.74	94.75
45	100.13	95.12
60	100.39	95.36
75	100.96	95.91
120	101.03	95.98

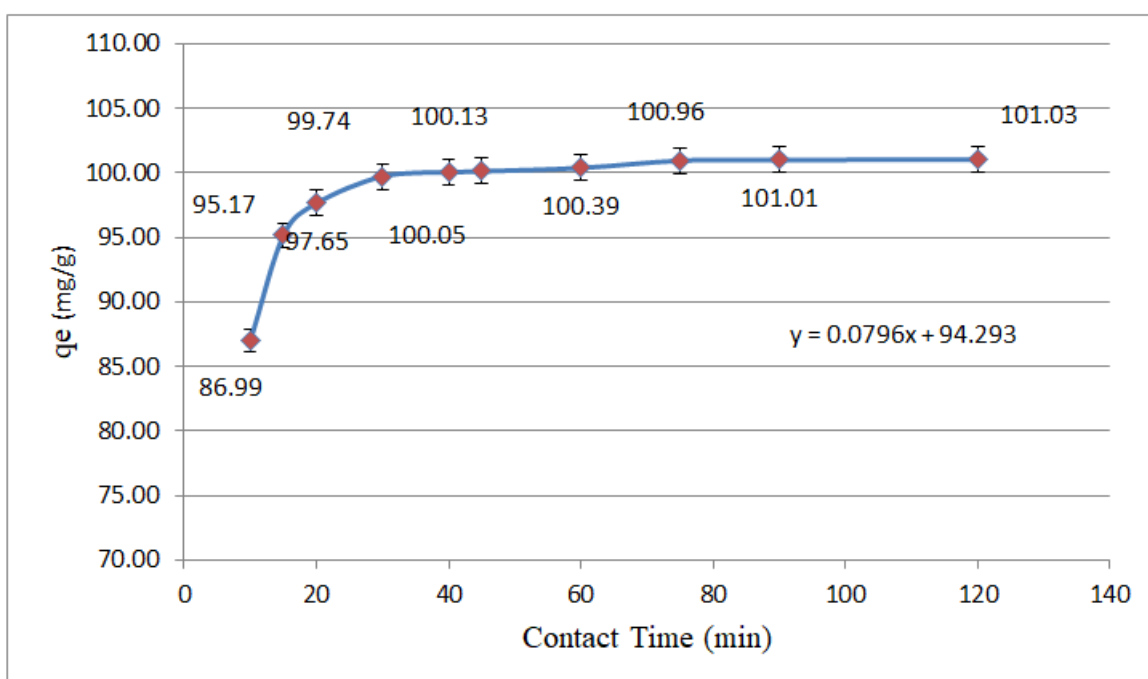


Figure 4.10. Contact time impact on treatment by *C. vulgaris*

When the percentage of dye removal and the time chart are examined; increasing trend was observed in dye removal due to the increase in contact time. However, adsorption process the rate of adsorption decreases depending on the contact duration. Results show that, most of the dye treatment process is completed at 30 minutes. At 60 minutes, dye removal activity reaches the equilibrium. After 60 minutes, the change in dye concentration was not significant (Table 4.10, Figure 4.10).

In consequence, medium positive correlation (0.737) between the RB 198 removal and contact duration was determined. For this reason, this correlation was found to be statistically significant.

4.4. Application of the obtained data to Adsorption Isotherms

Obtained results are applied to Langmuir and Freundlich equilibrium isotherm models. Good fitting equilibrium models were investigated and the model constants were calculated.

Langmuir Isotherm

Figure 4.11 shows the C_e / q_e values versus the solution equilibrium concentration obtained for the Langmuir isotherm.

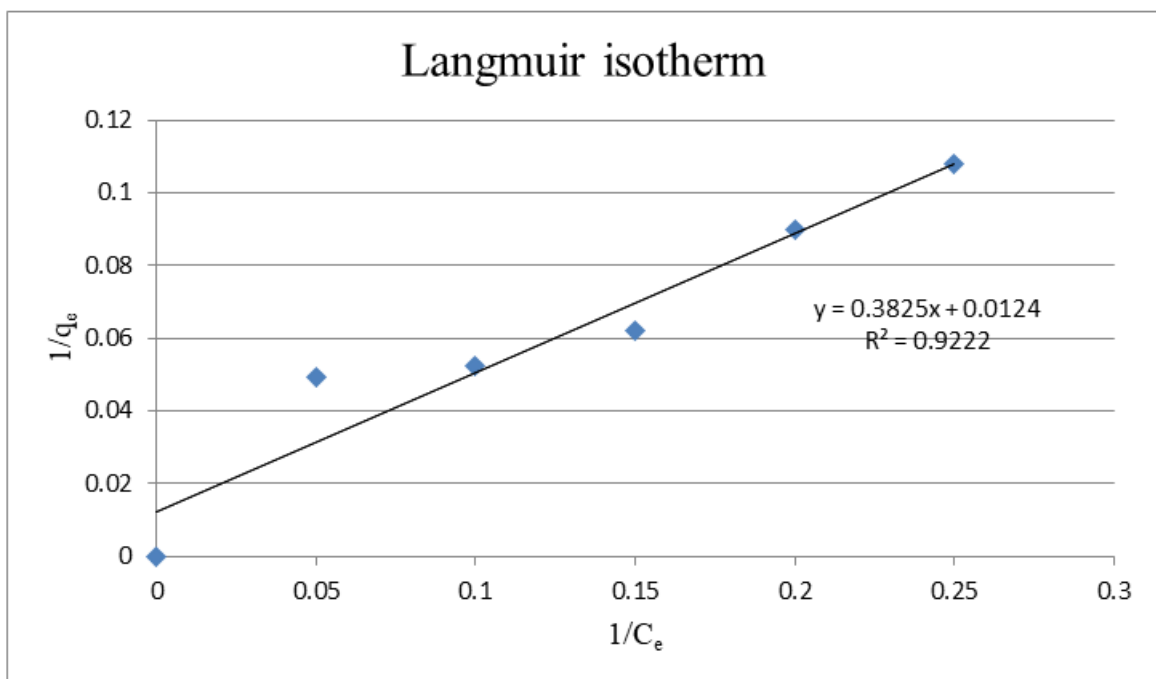


Figure 4.11. Langmuir isotherm for different dye concentrations for RR 120

As seen in Figure 4.11, test outcomes with all color concentrations of RR 120 are in great assention with the Langmuir isotherm. Correlation coefficient R^2 was 0.922 which implies that there is a coexistence of monolayer adsorption and heterogenous surface conditions.

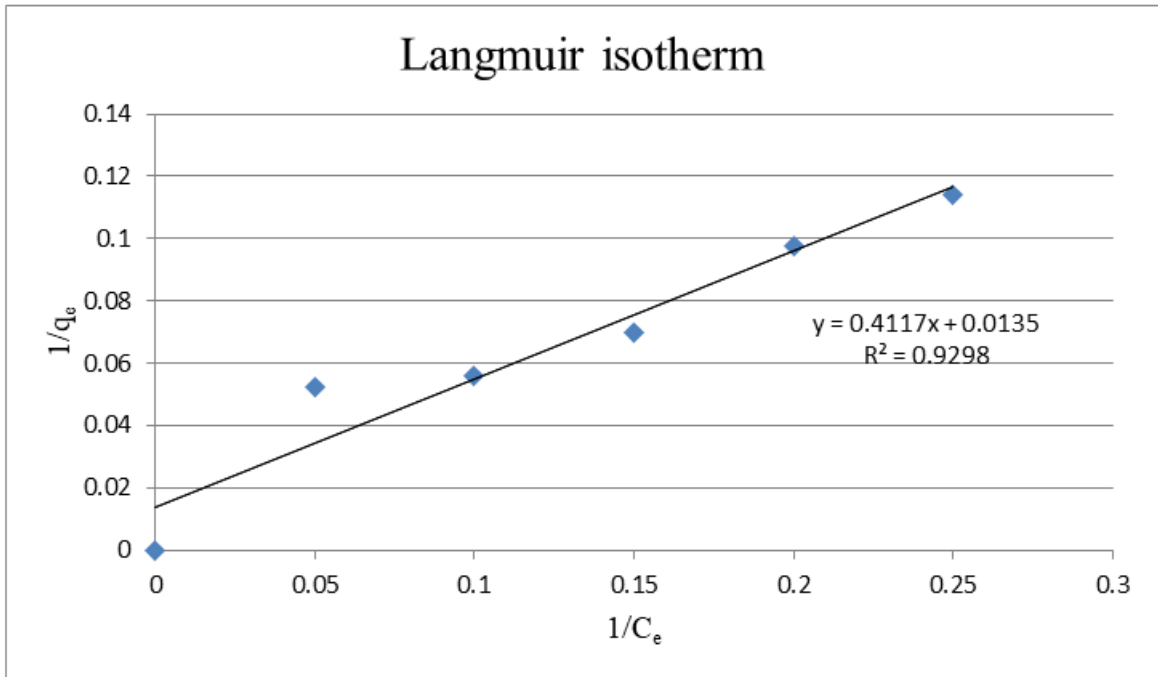


Figure 4.12. Langmuir study with different dye concentrations for RB 198

As seen in Figure 4.12, test outcomes with all color concentrations of RB 198 are in great assention with the Langmuir isotherm. Correlation coefficient R^2 were 0.929 which implies that there is a coexistence of monolayer adsorption and heterogenous surface conditions.

Table 4.11. Langmuir model parameters for Reactive Red 120 and Reactive Blue 198

Dye	b	q_e	R^2
RR 120	0.009	55.593	0.922
RB 198	0.008	42.721	0.929

Freundlich Isotherm

Figure 4.13 shows the $\log (q_e) / \log (C_e)$ values versus the solution equilibrium concentration obtained for the Freundlich isotherm.

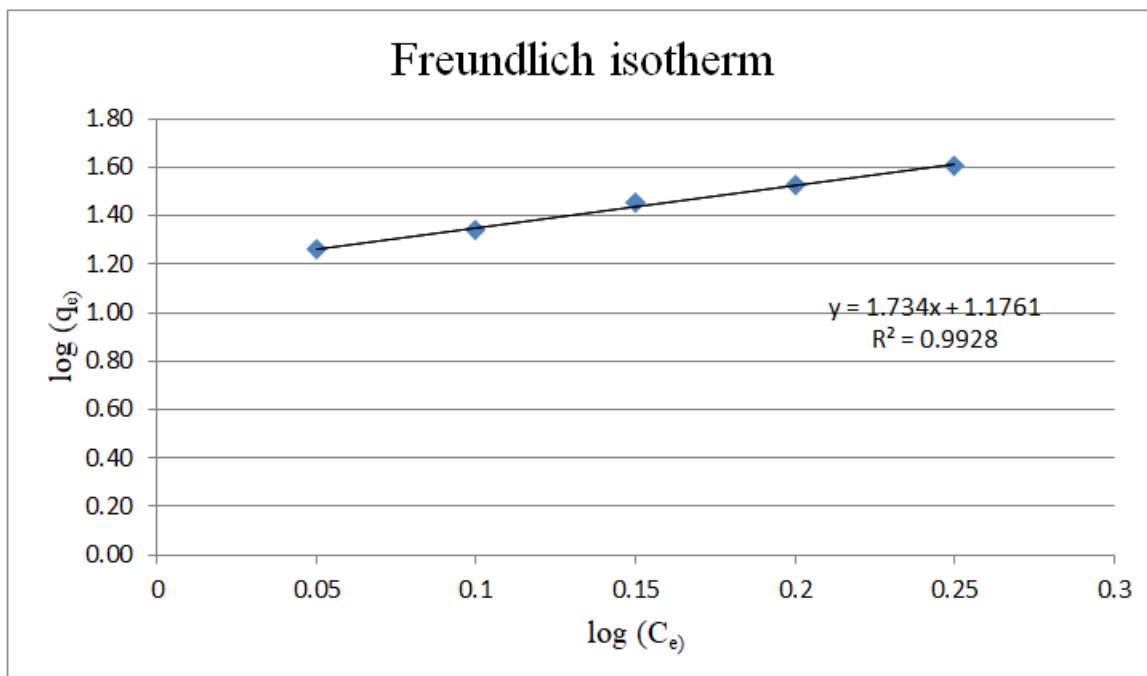


Figure 4.13. Freundlich isotherm for different dye concentrations for RR 120

As seen in Figure 4.13, experimental results obtained with all dye concentrations of RR 120 are in good agreement with the Freundlich model. The correlation coefficient R^2 of Freundlich isotherm were 0.993.

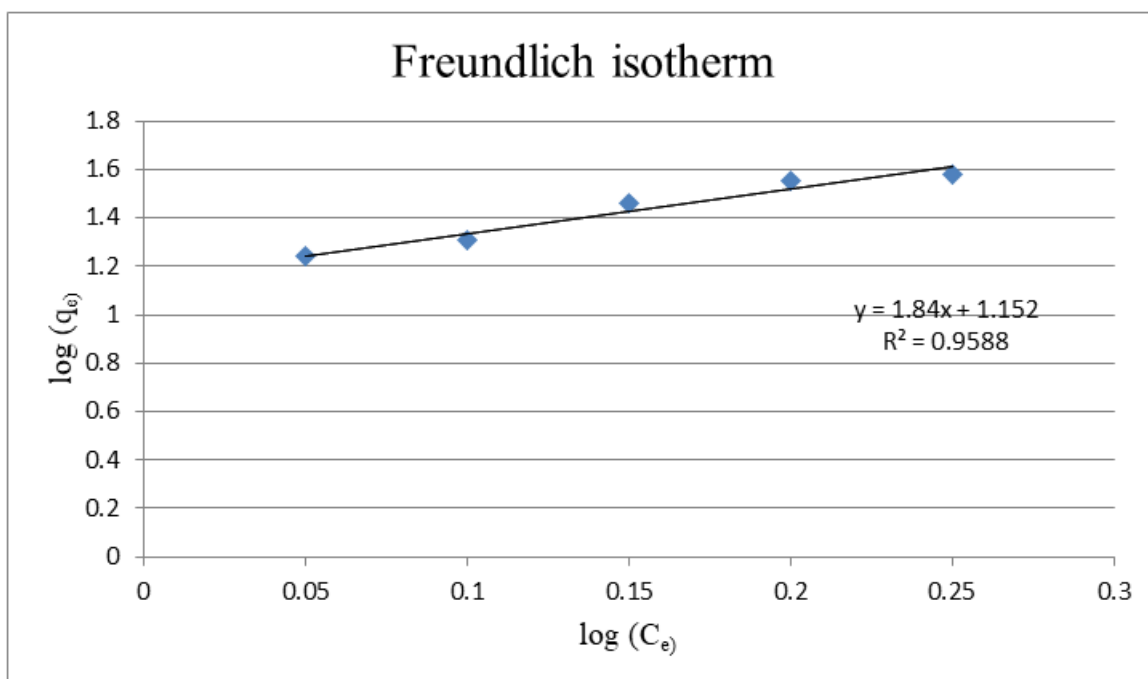


Figure 4.14. Freundlich isotherm for different dye concentrations for RB 198

As seen in Figure 4.14, experimental results obtained with all dye concentrations of RB 198 are in good agreement with the Freundlich model. The correlation coefficient R^2 of Freundlich isotherm was 0.958.

Table 4.12. Freundlich model parameters for Reactive Red 120 and Reactive Blue 198

Dye	n	K_f	R^2
RR 120	1.60	1.176	0.992
RB 198	1.40	1.152	0.958

As seen in Table 4.12, both of the RR 120 and RB 198 are good agreement with Freundlich and Langmuir isotherm model. However, differences between Freundlich and Langmuir isotherms are 7% and 2% respectively. That means, Freundlich isotherm is fitted reasonably well with the experimental data. Both of the dyes have heterogeneous surface on the other hand; good fitting Langmuir designed for homogenous surface and reflective monolayer. That means the Freundlich is an experimental condition and another frame of Langmuir

that can be connected to multilayer adsorption. Expect that the surface of the adsorbent is heterogeneous and dynamic locales and their energies disperse exponentially [21].

4.5. Adsorption Kinetics

In order to examine the mechanism of reactions, pseudo-first order and pseudo-second order kinetics are adopted to investigate the adsorption process.

Pseudo – First Order

The pseudo-first order equation is expressed below:

$$\log(q_e - q_t) = \log q_e - k_1 t / 2.3$$

In this equation, q_e and q_t are the dye adsorption capacity at equilibrium state at time t (min) and also k_1 is the constant of this kinetic. Pseudo-first order parameters are given in the Table 4.13 and Table 4.14.

Pseudo – Second Order

The pseudo-second order equation is expressed below:

$$t/q_t = 1/k_2 q_e^2 + 1/q_e t$$

In this equation, k_2 is the rate constant of pseudo second order mechanism. The parameters of pseudo-second order are shown in the next tables.

Table 4.13. Pseudo-First order and Pseudo-Second order kinetics for RR 120.

Dye Conc. (mg/L)	Pseudo – First Order			Pseudo – Second Order		
	R^2	$q_{1,e}$	$k_1 * 10^2$	R_2^2	$q_{2,e}$	$k_2 * 10^3$
25	0.903	32.58	13.45	0.999	33.40	11.65
50	0.919	46.75	12.77	0.999	39.58	10.35
100	0.956	59.25	7.21	0.999	42.72	6.78
150	0.941	67.58	3	0.999	60.51	5.44

Table 4.14. Pseudo-First order and Pseudo-Second order kinetics for RB 198

Dye Conc. (mg/L)	Pseudo – First Order			Pseudo – Second Order		
	R^2	$q_{1,e}$	$k_1 * 10^2$	R_2^2	$q_{2,e}$	$k_2 * 10^3$
25	0.904	34.78	12.13	0.999	43.66	14.39
50	0.917	37.40	9.71	0.999	46.94	12.78
100	0.924	47.50	6.49	0.999	52.37	9.46
150	0.846	53.66	2.21	0.999	60.65	6.21

For each dye, all of the initial concentrations are examined at 25 °C and for 30 minutes. The first order kinetic coefficient is decreased with the increasing initial dye concentration. Furthermore, the correlation coefficients are also lower than Pseudo-Second Order. It is known that the biosorption experiments are defined with Pseudo-Second Order kinetics with better coefficients for dye removal.

Table 4.13 and Table 4.14 shows that, the dye removal of kinetic data for the biosorption of RR 120 and RB 198 have good linear relationships. This meant that the Pseudo-Second Order model is suitable for the biosorption of lower molecular-weight adsorbates on smaller biosorbent particles [22].

5. DISCUSSION and CONCLUSIONS

In this thesis, under the optimum conditions, removal of RR 120 and RB 198 with using *C.vulgaris* is examined. Adsorption tests are carried out for determine the ideal conditions for color expulsion under different environmental conditions like pH, optimum heat, initial dye concentrations and biomass amount and test duration. Studies were conducted about 2 different reactive dyes and these are RR 120 and RB 198.

One of the foremost critical components that influencing adsorbent capacity in waste is pH. The removal rate and quality of biosorption is based on pH level of sample, because pH changings value causes change in ionization of the adsorptive molecule of adsorbent [23].

In the first phase of the experiments, optimum pH value is determined. Different pH values such as 2, 4, 6, 8 and 9 is tested. For both dyes, dye removal activity decreased with the increasing pH. For RR 120, dye removal rates were, % 95, % 78, % 68, % 57, %50 respectively. Likewise, for RB 198, dye removal rates were % 94, % 81, % 66, % 50, %47 at pH 2, 4, 6, 8, 9 respectively. For both dyes, maximum biosorption is observed at pH 2. As a result, pH 2 is found as the optimum pH for both RR 120 and RB 198 removal by *C.vulgaris*.

This change is usually observed at acidic conditions. At acidic pH levels (pH = 2-3) the COOH, OH⁻ and amino groups are protonated. *C. vulgaris* is positively charged because they carry protonated amine or hydroxyl groups. In aqueous solutions, the anionic dyes are negatively charged due to sulfonate (SO₃⁻) groups [35]. Thus, due to the electrostatic interactions between the positively charged adsorbent and the dye anions increased, removal activity is increased, too. As the pH increases, the negative charge density increases, while the positive charge density decreases. Moreover, the negatively charged surface in the adsorbent will not support the adsorption of dye anions due to electrostatic repulsion. As a result, pH value affects the biosorption of the dye [23].

Daneshvar and others have argued that, the electrostatic attraction mechanism between the anionic dyes in the acidic solutions and the cationic surface of the biomass, leads to a high efficiency in the dye biosorption below pH 4 [24].

Marzbali and others studied that, Direct Yellow 12 biosorption via using *S. platensis*. They obtained maximum removal at pH 2 [25].

Cardoso et al. show that removal of RR 120 using *Spirulina platensis* and activated carbon in strong acid and strong basic (2-10). They found that highest efficient pH level as 2, as in the case of the present study [26].

Mona et al. examined the biosorption of Reactive Red 198 with *Nostoc linckia*. Also they studied the effects of initial dye concentration, temperature and contact time. As a result, max adsorption rate conducted in 2 (%93.5) [27].

Ozer and others have studied the biosorption of azo dyes with *Spirogyra rhizopus*. As a result, they found that optimum pH condition is between 2 and 3 [28].

Kousha and others have studied the adsorption of AB 1 about pH range 2-6 using *N. zanardini*, *S. glaucescens* and *S. marginatum* and determined that the optimum pH value to be 2 [29].

Similar results were obtained from other experiments, optimum pH of the dye biosorption was found at 2-4. As it is seen, these results are parallel to present work. As a result of pH studies with *C. vulgaris* biomass, the optimum pH value was found to be 2.

Tests are continued with to decide the effective biomass quantity. Because of that, values of 0.05 - 0.25 g of *C. vulgaris* were used. It is found that the percentage of adsorption decreased with increasing biomass concentration.

For both dyes, dye removal activity is decreased with the increasing biomass amount. For RR 120, dye removal rate for 0.05-0.25 g/L *C. vulgaris* biomass, 89-95% removals were obtained. Likewise, for RB 198, dye removal rate changed between 88-94 for the same amount of adsorbent. For both dyes, maximum biosorption is achieved with minimum biomass amount (0.05).

The biomass amount is an important operational factor in determining the adsorbent capacitance in the event of operation. Usually, the percentage of biosorption rises correlatively with biomass amount.

However, using less amount of biomass is more economical. Low percentage of biosorption efficiency can be occur because of unsaturation of remaining biomass during the adsorption process. Another explanation of this situation is, the agglomeration of biomass particles observed during the studies causeg decreasing biomass surface area. Consequently, this causes a decreasing q_e in terms of biomass.

Khaniabadi et al. examined the amount of biomass in activated carbon prepared by *aloe vera* leaves. They tried amount of biomass in the range of 0.5-2.0 g. As a result of that, they found optimum biomass amount of 0.5 g [30].

In this thesis, the optimum amount of biomass was found to be 0.5 grams for both dyes for *C. vulgaris*. Therefore, this result is similar to previous studies.

In the third phase of the experiments, different initial dye concentration is examined. Such as 25 mg/L, 50 mg/L, 100 mg/L and 150 mg/L are tested. For both dyes, dye removal increased up to 100 mg/L. However, after 100 mg/L, dye removal decreased.

Adsorption capacity is increased while initial dye concentration is going up from 25 to 100mg/L. However, dye removal activity decreased to 150 mg/L.

Under the similar conditions for the removal rates for RR 120, dye removal rate for 005-0.25 g/L *C. vulgaris* biomass, 89-95% removals were obtained. Likewise, for RB 198, dye removal rate changed between 90-95%. According to increasing initial concentration. Maximum adsorption is done in 100 mg/L and for RR 120 and RB 198 was %95 and %94.8 respectively.

The adsorption amount for dye removal depends strongly on the initial dye concentration. The effect of the initial dye concentration depends on relationship between surface area of biomass and colour concentration. In general rate of color evacuation diminishes with expanding introductory color concentration, which is due to the immersion of the adsorption regions on the biomass surface.

In Dakhil study, three different adsorbents were tested for adsorption of Methylene Blue (MB). Maximum percent removal for all three adsorbents was approximately %90 at 100 mg / L [31].

Al-Homaidan and others have investigated the biosorption of copper with *Spirulina platensis* biomass in their work. The highest treatment capacity was found about 91% at 100mg/L [43].

The outcomes are in agreement with the previous experiments. Studies of initial dye concentrations with *C. vulgaris* biomass showed a maximum adsorption percentage of 100 mg / L as a result.

The experiments are continued with the contact time was tested. In this study, 15, 30, 45, 60, 75 and 120 minutes are tried. The main objective of this experiment is finding equilibrium time. For RR 120, removal rates are between 84% and 97%. On the other hand, for RB 198, the results are between 90% and 95%.

These results show that, for both dyes, the optimum equilibrium contact time is 30 minutes. Dye removal rate increased with time. However, after 30 minutes, adsorption still continues but rate is too slow and the change in removal rate was not significant.

Knowing the duration of the equilibrium contact is critical factor in designing of biosorption processes. Before equilibrium state, biosorption is fast. However, the reaction rate decreases after the equilibrium.

The beginning fast biosorption may be a result of the dynamic area abundance on the biosorbent surface at the primary level of contiguity. Adsorption percentage is gradually decreasing at the next contact level due to the decreasing active areas [32]. In physical adsorption, most of the removal activity happens in a very short time. When the outer surface reaches saturation, the dye molecules adsorb to the porous structure of the biomass [33].

Balarak and others used red mud to remove Acid Red 18. Biosorption volume increased with increasing duration and reached equilibrium about 75 minutes [34].

Balarak et al. examined adsorption of AB113 dye with *Lemna minor* biosorbent. As a result, they reported that biosorption happens rapidly and approached balance near 60 minutes [35].

Joghatayi and others investigated the removal of Reactive Blue 19 with the dead *Azolla filiculoides* biomass. They showed that the adsorption was fast at the start and reached the equilibrium in 75 minutes [36].

In our experiment, for both dyes, adsorption reached equilibrium in 30 minutes. Before 30 minutes, removal rate was high. However, after the 30 minutes to 120 minutes, adsorption was nearly same as 30 minutes namely. The change was not significant.

Temperature impact is another critical physico-chemical experiment condition since the temperature will alter the biomasses biosorption capacity. One of the most important factors that causes biosorption decrease through the temperature rise is, decrease of the

interaction between the biomass and color particles. Also, the reducing of physical bond between the positive sides of the colour and adsorbent with increasing temperature is another reason [37].

Last part of the experiments, pH set to 2, biomass amount to 0.05 g and the initial dye concentration to 100 mg/L and contact time to 30 minutes. To find exact optimum temperature, following values were tested. For both dyes, the effects of 25°C, 30°C, 40°C and 50°C were tested on dye biosorption. For Reactive Red, the results are between 88% and 95%. On the other hand, for RB 198, the results are between 88% and 94%.

As mentioned before, for RR 120 and RB 198, 25 °C is found as the optimum temperature for dye removal. It was observed that, biosorption activity decreased with increasing temperature. This means these reactions are exothermic.

Madhav et al. examined the adsorption of AO7 via untreated sugar cane. Temperature effect on adsorption is examined at 30-75°C. Maximum removal happens at 30°C.

Mohan et al. makes experiments about biosorption of DB1:1 with inanimate *Spirogyra sp* biomass. The effect of ambient temperature on biosorption is examined at temperatures of 10, 20, 30, 40 and 50°C. As a result, the highest biosorption was observed at 30°C.

Findings in these studies are parallel to the results of this thesis. The optimum temperature for *C. vulgaris* adsorbent is 30 ° C.

The optimum conditions obtained for both dye types are given in Table 5.1 and 5.2. As a result of this thesis, *Chlorella Vulgaris* biomass is found as effective in biosorption of RR 120 and RB 198 in textile waste waters. Also previous studies support these results. However, some conditions must be provided for effective adsorption. These conditions are pH level, temperature, biomass amount, duration and initial dye concentration. As seen in the tests, minimal changes can be disrupting the rate of dye removal.

Table 5.1. Optimum conditions that provide maximum biosorption via *C. vulgaris* for RR 120

OPTIMUM CONDITIONS FOR RR 120 BIOSORPTION	
Optimum pH	2
Optimum biomass (g)	0.05
Optimum initial dye concentration (mg/L)	100
Optimum contact time (min)	30
Optimum temperature (°C)	25
Maximum Dye removal percentage (%)	95.05

Table 5.2. Optimum conditions that provide maximum biosorption via *C. vulgaris* for RB 198

OPTIMUM CONDITIONS FOR RB 198 BIOSORPTION	
Optimum pH	2
Optimum biomass (g)	0.05
Optimum initial dye concentration (mg/L)	100
Optimum contact time (min)	30
Optimum temperature (°C)	25
Maximum Dye removal percentage (%)	94.74

The results of previous studies are given in the Table 5.3. in comparisons with the findings of the present study. As seen from the Table, organic materials are very effective for removal of both acidic and reactive dyes. Removal rate can face up to 99 percent. However, meeting the optimum conditions can be expensive and not-feasible except laboratory conditions. Meeting the ideal pH levels are not cost effective, but also not environment friendly. Decreasing the pH to 2 in large scales of textile wastewater can be costly. Also the process produces high amount of acidic waste. Moreover, keeping the temperature at 25°C is challenging in textile wastewater. However, the use of *Chlorella Vulgaris* is effective in meeting the discharge standars for color.

Table 5.3. Biosorptions of some dyes with different adsorbents according to other researches

Adsorbents	Dye Name	Percentage of Removal (%)	References
Pine cone	Congo red	13.45–18.96	[38]
Modified Alumina	Crystal violet	58–99	[39]
Kaolin	Crystal violet	75–97	[40]
Fly ash	Methylene blue	45.16–96	[41]
Modified sawdust	Methylene blue	34.4–96.6	[42]
Cashew nutshells	Congo red	56.3–99.3	[43]
Chlorella Vulgaris	Remazol Black	57–92	[11]
Chlorella Vulgaris	Remazol Red	52–92	[11]
Chlorella Vulgaris	Remazol Golden Yellow	18–80	[11]
Chlorella Vulgaris	Reactive Red 120	50–95	-
Chlorella Vulgaris	Reactive Blue 198	47–94	-

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UjuVTBsNA iCHkyW3WO BTfL5wnC1 mmrWiEC1b muGHn3oCD w5Sh48cuG
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tkOi8h5nc jVn5Meg2Q c3YP5h7oF lb7J2HpYT InLfLO8pu uCtYWLjjx.”

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