



Research Article

Using chlorella vulgaris as a natural-textile dye

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ABSTRACT

The applications of algae are becoming more popular day by day. From biofuels to nutrients, cosmetics, pharmaceuticals, and most recently ink and textile dyes. Due to algae's high lipid content that works best as a substitute for petroleum-based products, and because it is carbon negative and eco-friendly, algae-based dyes can present a transitional solution to the environmental damages caused by the dyeing phases in the textile industry. Researchers have investigated the types, methods, applications, and efficiency of various algae species and types of dyes to serve in various coloring and printing applications. In this paper, we present the possibility of using microalgae as a natural dye for the textile industry. The microalgae studied were mixed species dominated by Chlorella Vulgaris. Pigments were extracted by acetone to create the natural dye which was used on a 100% cotton fabric using basic dyeing methods. A light-fastness test was subsequently performed, and the results indicated that the algae-colored fabric gained a value similar to those usually obtained with natural dyes.

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INTRODUCTION

With the growing awareness on the environmental damages caused by industrialization, and the increase in legislations that support carbon negative production and puts more limits on the use of harmful material or unmanaged waste disposal, researchers have become more interested in finding natural alternatives to synthetic material in almost every industry. In the textile industry, the dyeing stage represents the most dangerous to the environment due to the presence and high consumption of chemicals in each step and the difficulty to treat the resulted waste.

Natural dyes have proven to be successful substitutes for synthetic dyes in terms of color and quality. Only to produce such dyes, a large demand for landscape, water, and time is required; resulting in a challenge to withdraw convention-

al products in preference of natural alternatives. Dyes may be defined as substances that, when applied to a substrate, provide color by a process that alters, at least temporarily, any crystal structure of the colored substances [1, 2]. It is estimated that over 10.000 different dyes and pigments are used industrially, and over 7×10^5 tons of synthetic dyes are produced annually worldwide [3].

In recent years, significant interest has been developed in the commercial utilization of algae [4] due to its reliance on carbon dioxide and sunlight to grow, and because of its short growth rate and little need of landscape, in addition to its wide range of species that are majorly high in lipids, fatty acids, proteins, polysaccharides, and pigments [5]. Algae contains a broad range of photosynthetic pigments. Three main classes of photosynthetic pigments are: chlorophylls, carotenoids (xanthophylls and carotenes) and phycobilins.

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Table 1. Different color constituents in algae [20]

	Colored constituents in algae constituents (Major)	Category
Green algae	Chlorophyll b	Chlorophyta
Euglenoids	Chlorophyll b	Euglenophyta
Brown algae	Chlorophyll c 1, c2 and fucoxanthin	Phaeophyta
Yellow-brown or golden-brown algae	Chlorophyll c 1, c2 and fucoxanthin	Chrysophyta
Dinoflagellates	Chlorophyll c2, peridinn	Pyrrophyta
Cryptomonads	Chlorophyll 2, phycobillins	Cryptophyta
Red algae	Phycoerythrin, phycocyanin	Rhodophyta
Blue-green algae	Phycoerythrin, phycocyanin	Cyanophyta

Different algal groups are classified based on predominant pigments, such as chlorophyll b in green algae [6], fucoxanthin, and carotenoids in brown algae [7], and β -carotene and zeaxanthin in red algae [8]. Furthermore, acetone extraction is considered the most effective and ecofriendly approach for efficient extraction of natural dyes [9].

This research points out some of the basic methods of extraction of algal dyes and their potential to serve the textile industry by dyeing a natural fabric.

Textile Dyes

Before diving into the overall process, it is important to gain some background on the dyeing phase in the textile industry. As the coloring of fabrics is considered one of the key factors in the successful trading of textile products [10]. The current dyeing methods have been used for a long time which differ according to the desired fiber, print or color, and product quality such as water repelling, antistatic protection, fire retardants etc. In order to result with a colored fabric, it generally goes through three steps, preparation or pretreatment, dyeing and finishing [10, 11]. However, it is known that no dye can be applied to all existing fibers, and no fiber can be dyed by all known dyes [12].

By the main use of synthetic dyes, the textile industry has posed serious environmental risks with the generated wastewater that can be very difficult to treat and resist in nature for many years. Various types of synthetic dyes and chemicals are characterized by a very high pH and salinity values [13]. A percentage of 54% of the dye effluents in the world are produced by textile industries [14]. It's estimated that 17–20% of industrial water pollution is solely caused by the textile industry according to The World Bank [15].

The main materials used in producing synthetic dyes are coal tar and petroleum, which have negative impacts on the environment. The reason synthetic dyes are still favorable is because natural dyes usually cost higher because of the tedious extraction of colorants from raw materials, the low color value and long dyeing time. In addition, some natural dyes are fugitive and can need mordants to enhance their fastness characteristics. Natural dyes also work best with natural, not synthetic, fabrics [16].

Algae Dyes

Algae can be considered as microscopic plants that are invisible to the naked eye, but when grown in water with sufficient nutrients, changes the color of water to green, brown, red, or blue depending on the microalgae species [17]. Of the total of 200.000 to 800.000 species of algae, only tens of thousands are listed in the literature [18]. Algae is known to have high lipid content that ranges from 50 to 80 % in biomass [19] and contains photosynthetic pigments [20]. For high-yielding varieties, the oil content in the dry matter of algae can reach up to 50–60% [21]. Table 1 shows the color constituents in algae.

Its pigments have been successfully used in food dyes, cosmetics, and pharmaceutical industries. Algae dyes for the textile industry can be produced similarly by the same pigments. Considering algae's fast growth rate, variety of colors, main reliance on sunlight and carbon dioxide to grow, and availability worldwide, it can serve as the best source of natural dyes [22].

For the aforementioned reasons, this study was performed to prove the possibility of using microalgae as an ecofriendly alternative to synthetic dyes.

MATERIALS AND METHODS

The process of creating algal-based dyes goes through three main stages: Biomass Production, Pigment Extraction, and Dyeing. Each stage will be explained in further detail as follows.

Biomass Production

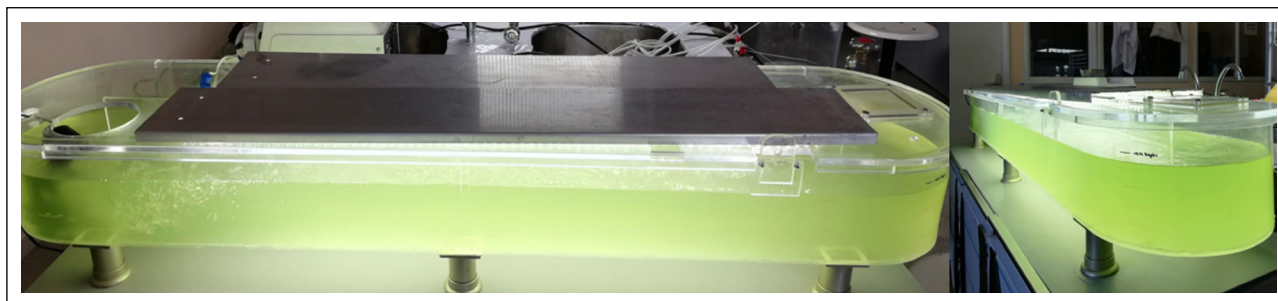
In this study, firstly, *Chlorella Vulgaris* microalgae species obtained from a strain of international collection at Yildiz Technical University's Environmental Engineering Laboratory were dominantly grown in a mixed-algae culture with a synthetic nutrient medium. Bold's Basal Medium (BBM) was used as the freshwater-synthetic culture medium. BBM medium consists of the nutrients given in Table 2. According to Nichols and Bold, 1965 [23].

The stock solutions used in the preparation of the BBM were prepared in corresponding volumes of balloon flasks covered with aluminum foil, each solution is then completed with pure water, mixed for 30 minutes, and finally set in the autoclave for 120 °C 20 minutes for sterilization [23].

Table 2. Chemicals used in the preparation of the BBM culture media [23]

Stock solution number	Name of chemical	Formula	Weight (g)	Distilled water (ml)
1	Di potassium hydrogen orthophosphate	K_2HPO_4	1.875	250
2	Potassium dihydrogen orthophosphate	KH_2PO_4	4.375	250
3	Magnesium sulfate	$MgSO_4 \cdot 7H_2O$	1.875	250
4	Sodium nitrate	$NaNO_3$	6.250	250
5	Calcium chloride	$CaCl_2 \cdot 2H_2O$	0.625	250
6	Sodium chloride	$NaCl$	0.625	250
7	Tetrasodium EDTA	$EDTA-Na_4$	5.000	100
	Potassium hydroxide	KOH	3.100	
8	Ferrous sulfate	$FeSO_4 \cdot 7H_2O$	0.498	100
	Sulfuric acid (1 mL=1.84 g)	H_2SO_4	0.184 g	
9*	Boric acid	H_3BO_3	1.142	100
10	Zinc sulfate	$ZnSO_4 \cdot 7H_2O$	0.353	25
11	Manganese chloride	$MnCl_2 \cdot 4H_2O$	0.058	25
12	Copper sulfate	$CuSO_4 \cdot 5H_2O$	0.063	25
13	Cobalt nitrate	$Co(NO_3)_2 \cdot 6H_2O$	0.020	25
14	Sodium molybdate	$Na_2MoO_4 \cdot 2H_2O$	0.048	25

*: Should be heated at 50–60 °C to dissolve. All chemicals should be weighed with aluminum foil.

**Figure 1.** Microalgae production and growth processes in the bioreactor.

This process was repeated numerous times in order to fill the algae bioreactor that had a 50-liters volume in total, extra volumes were also made for the smaller algae samples that were constantly under control and required addition of BBM every week.

For the growth processes, available mixed-microalgae culture dominated by *Chlorella Vulgaris* were combined with sufficient amounts of media in the bioreactor as shown in Figure 1 for large production.

The growth rate and species were constantly monitored by measuring the Suspended Solid Matter's SSM concentration daily, and detecting the species under the microscope, the growth of algae in the bioreactor over time can be shown in Figure 2.

Following the daily measurements, and when the Suspended Solid Matter's SSM concentration reached approximately 0,5 mg/L, the samples were concentrated by a centrifuging machine to begin the drying phase.

Pigment Extraction

In the drying phase, semi-liquid biomass was obtained from microalgae species, which were concentrated by centrifuging at 4000 RPM for 10 minutes in the ROTO-FIX 32. This biomass was spread on a tray and placed in an oven to dry at 40 °C for 6 hours.

Afterwards, the obtained dry biomass was crushed in a mortar as in Figure 3 to create a fine powder of algae.

Soxhlet extraction was applied as the pigment extraction method from the microalgae powder. In this method, 5 gr of dry biomass' pigment was extracted using acetone as a solvent, and then the solvent was collected with a rotary evaporator [4]. Following the extraction process, the colored supernatant part was separated from the biomass wastes by filtration and/or centrifuging. After the separated supernatant was concentrated, it was stored for use in the dyeing process.

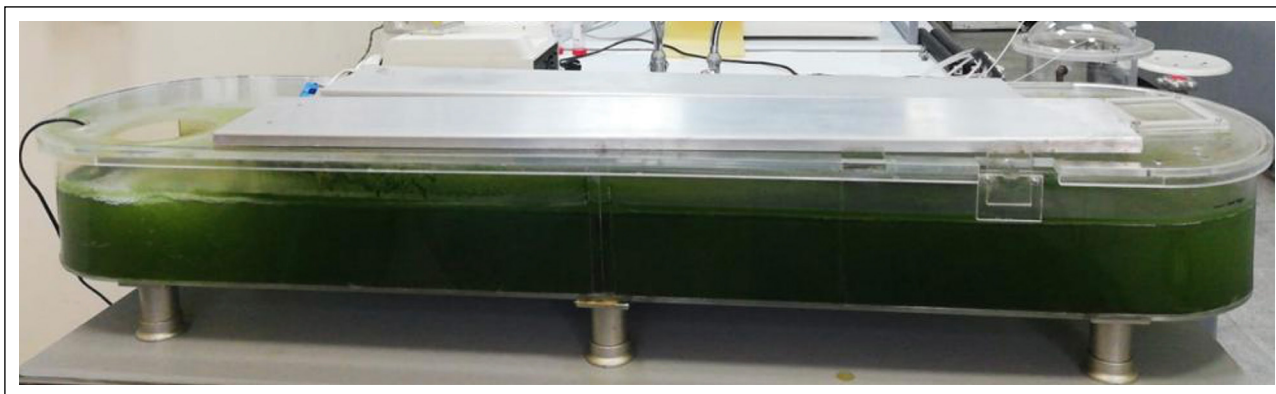


Figure 2. Biomass production.



Figure 3. Crushing of dried biomass in a mortar.

Coloring Process

The dyeing or coloring process experiment was carried out by using acetone for pigment extraction, with a pH 8.4, and 10 gr of %100 cotton fiber, the fiber was heated under 55 °C and set in a mixer for around 1 hour. This method was modified and adapted from the European Sea Colors Project [24]. The mordanting process to fix the dye to the product was not performed, as mordanting was disregarded in this study. Since chlorophyll is the photosynthetic pigment found in the plant cell's chloroplast with its specific form chlorophyll-a, also a general photosynthetic pigment that plays an important role in photosynthesis [25], the amount of pigment extracted from the microalgae biomass was measured spectrophotometrically depending on the amount of chlorophyll-a presented on the fabrics.

Light Fastness Analysis

The color quality of the dyed fabric was determined by light fastness analysis carried out according to the ISO 105 B02 1994 method, through service procurement.

RESULTS AND DISCUSSION

Obtained Species

As previously mentioned, mixed microalgae culture dominated by *Chlorella Vulgaris* was used in the bioreactor and was monitored daily using a light microscope.

Figure 4 shows images of mixed species of microalgae obtained from the microscope.

The image shows *Chlorella Vulgaris*, *Nannochloropsis*, and traces of *Spirulina*.

Light Fastness

When exposed to sunlight, dyed fabrics can fade or change color over time. The durability of color on such materials against light is called light fastness [26]. Light fastness measurements are interpreted with the standard dyed blue scale made on 8 wools and separated according to their fastness. The dyed wool scale, along with the tested fabric, which are both exposed to light for the same amount of time, consist of 1–8 points for 8 appropriate standardly dyed samples for

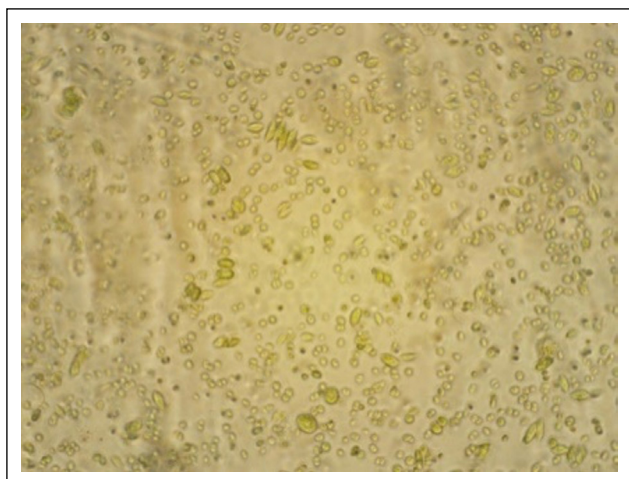


Figure 4. Mixed species under the light microscope.



Figure 5. Dyeing results of using an algae pigment.

light fastness. Number ‘1’ indicates low light fastness whereas number ‘8’ indicates high fastness [26]. Nearly all natural dyes have a lightfastness below British Standard (BS) grade 5 and most have a fastness below 4 [27].

Figure 5 shows the resulting light-green color of dyeing the cotton fabric. The sample gained a result of “2”, which is in the range of 1–8 and deemed normal for usual organic dyes. This result was given by a private laboratory in İstanbul, Türkiye.

CONCLUSION

According to this study, the rich content of chlorophyll in microalgae gives it a high potential to be used as a natural dye resource. Although terrestrial plants can provide abundant amounts of chlorophyll, microalgae are much faster to grow, and require less resources and land to produce. Nevertheless, more research is required for the best methods of application of natural dyes in order to achieve similar quality to synthetic dyes.

The future of algae-based dyes is very promising as they are carbon negative and very safe to produce. The study showed preliminary results of successful coloring of fabric using pigments extracted from microalgae, nonetheless, further research on optimum algae species, variety of colors, and color fastness should be done.

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DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

CONFLICT OF INTEREST

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

ETHICS

There are no ethical issues with the publication of this manuscript.

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